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JES JOURNAL OF ENVIRONMENTAL SCIENCES www.jesc.ac.cn

Structural and metabolic responses of microbial community to sewage-borne chlorpyrifos in constructed wetlands

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ARTICLE INFO

Article history:
Received 27 January 2015
Revised 8 July 2015
Accepted 20 July 2015
Available online 8 February 2016

Keywords:
Constructed wetland
Chlorpyrifos
BIOLOG
DGGE

ABSTRACT

Long-term use of chlorpyrifos poses a potential threat to the environment that cannot be ignored, yet little is known about the succession of substrate microbial communities in constructed wetlands (CWs) under chlorpyrifos stress. Six pilot-scale CW systems receiving artificial wastewater containing 1 mg/L chlorpyrifos were established to investigate the effects of chlorpyrifos and wetland vegetation on the microbial metabolism pattern of carbon sources and community structure, using BIOLOG and denaturing gradient gel electrophoresis (DGGE) approaches. Based on our samples, BIOLOG showed that Shannon diversity (H') and richness (S) values distinctly increased after 30 days when chlorpyrifos was added. At the same time, differences between the vegetated and the non-vegetated systems disappeared. DGGE profiles indicated that H' and S had no significant differences among four different treatments. The effect of chlorpyrifos on the microbial community was mainly reflected at the physiological level. Principal component analysis (PCA) of both BIOLOG and DGGE showed that added chlorpyrifos made a difference on test results. Meanwhile, there was no difference between the vegetation and no-vegetation treatments after addition of chlorpyrifos at the physiological level. Moreover, the vegetation had no significant effect on the microbial community at the genetic level. Comparisons were made between bacteria in this experiment and other known chlorpyrifos-degrading bacteria. The potential chlorpyrifos-degrading ability of bacteria in situ may be considerable.

Introduction

Chlorpyrifos is one of the chlorinated organophosphate (OP) pesticides that have been used for pest control in agriculture since the 1960s (Maya et al., 2011). The half-life of chlorpyrifos is usually 60 to 120 days in soil, but it can range from 2 weeks to over 1 year depending on the illumination intensity, soil type, temperature and other factors (Anwar et al., 2009). Long-term usage of chlorpyrifos has caused agricultural non-point source pollution, and increased risk to the quality of aquatic environments (van Dijk and Guicherit, 1999; Spalding et al., 2003; Leu et al., 2005). Accumulation of chlorpyrifos in water bodies could cause potential damage (such as carcinogenicity, neurotoxicity, and reproductive and developmental toxicity) to both aquatic organisms and humans (Jorgenson, 2001; Robles-Mendoza et al., 2009). Thus, the demand for cost-effective methods to remove chlorpyrifos in pesticide wastewater is increasing.

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http://dx.doi.org/10.1016/j.jes.2015.07.020

Constructed wetlands (CWs) have been proven to be an effective management practice to reduce aqueous concentrations of pesticides (Moore et al., 2009) and to control pesticide mitigation (Schulz and Peall, 2001; Moore et al., 2007; Budd et al., 2011). The degradation of pesticides in CWs mainly takes place by photolytic degradation, substrate sorption, plant uptake, and microbial degradation processes (Zhang et al., 2014). The efficiency of different microbial degradation pathways has also been studied. Numerous studies have concentrated on the selection of specific pesticide-degrading bacteria in different pesticide-polluted samples (Lakshmi et al., 2009; Sasikala et al., 2012). Nevertheless, research on the effect of pesticides on microbial community dynamics should not be neglected. This can provide background information for pesticide testing (Engelen et al., 2003).

In addition to microbial degradation, plant uptake is another major method that can decrease chlorpyrifos. Also, there have been a large number of studies focused on the interaction of plants and microbes being treated with chlorpyrifos (Fang et al., 2009; Xie et al., 2010). The roots of plants could provide a stable environment and suitable attachment points for rhizosphere microorganisms, and root exudates could also provide energy and carbon sources for the growth of microbes (Faulwetter et al., 2009).

In this article, the short-term response (30 days) of microbial community structure was examined before and after the addition of chlorpyrifos in both vegetated and non-vegetated CWs. Both BIOLOG (Garland and Mills, 1991) and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) techniques (Muyzer, 1999) were used to reveal the basic features of the microbial community, with respect to metabolism and genotypic structure, respectively (Bushaw-Newton et al., 2012). The aim of this research was to explore the response of microbes in rhizosphere substrates of constructed wetlands being treated with chlorpyrifos at the physiological and genetic levels.

1. Materials and methods

1.1. Experimental systems and operating conditions

Six sets of vertical flow CWs were made from polyethylene (PE) buckets with a height of 600 mm and a diameter of 250 mm, which were equally separated into two groups on vegetated and non-vegetated CW systems. Each system was filled with river sand (1–5 mm) with a height of 300 mm, and only the vegetated group was planted with three uniform *Iris pseudacorus*. The river sand was passed through a sieve to remove the large particles, and then washed with tap water in order to remove the small particles. Therefore, the remaining river sand was 1–5 mm in diameter. The *I. pseudacorus* used in this experiment was obtained from the cultivation base of vegetation of the institute of hydrobiology. Fig. 1 shows the ichnography of the simulative vertical flow CWs. After an acclimatization stage of 15 days, the experimental stage lasted for 30 days. In the acclimatization stage, the artificial sewage, which was composed by 7–8 mg/L of total nitrogen (TN), 0.2–0.4 mg/L of total phosphorus (TP), and 60–70 mg/L of chemical oxygen demand (COD), was discharged into the CWs. In the experimental stage, chlorpyrifos was added in the artificial sewage until its concentration reached 1 mg/L. Chlorpyrifos (purity 99.9%) was purchased from Sigma-Aldrich Company. The hydraulic loading was 25.48 mm d^{-1}. Water samples were collected on days 4, 7, 17 and 29 after the chlorpyrifos was added. The chlorpyrifos removal rates on days 4, 7, 17 and 29 in the vegetated systems amounted to (93.97 ± 1.62)%, (96.57 ± 1.46)%, (96.14 ± 1.71)% and (95.72 ± 0.64)%, respectively, and in the non-vegetated systems amounted to (87.65 ± 3.77)%, (96.12 ± 1.28)%, (94.33 ± 1.03)% and (91.67 ± 2.32)%, respectively.

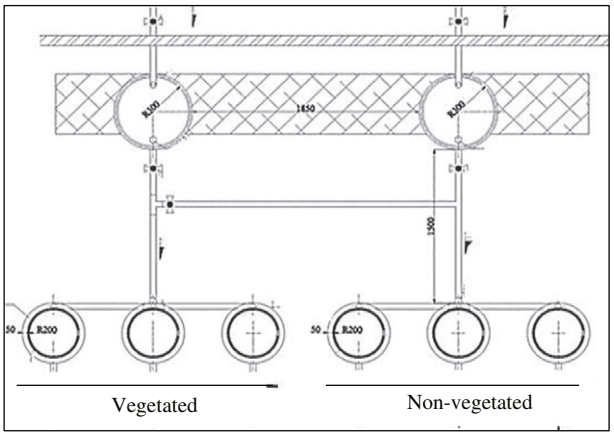

Fig. 1 – Ichnography of simulative vertical flow CWs.

1.2. Substrate sample collection

Samples were collected from the rhizosphere substrate, and each substrate sample was collected from 5 uniformly distributed spots of the surface layer (0–10 cm) from one randomly selected bucket, and then mixed evenly. Substrate samples were taken at the beginning and the end of the experimental stage, and stored at −80°C prior to analysis. The samples collected at the beginning and end of the experimental stage in vegetated systems were designated Pbs and Pas respectively, while those in non-vegetated systems were designated non-Pbs and non-Pas respectively.

1.3. BIOLOG ECO microplate experiment

The BIOLOG ECO microplates contained 31 carbon sources of different groups: 12 kinds of carbohydrates (CHs), 6 kinds of amino acids (AAs), 5 kinds of carboxylic acids (CAs), 4 kinds of polymers (PMs), 2 kinds of amines (AMs), and 2 kinds of phenolic compounds (PCs). A 250 mL conical flask containing 10 g substrate and 100 mL of sterile saline was shaken for 30 min (200 r/min) at room temperature. The supernatant was diluted 25 times, and 150 μL of the diluted bacterial suspension was added to each well of a BIOLOG ECO microplate. The microplate was incubated at 30°C in darkness. Optical density (OD) was measured with SpectraMax M5 at 590 nm (color and turbidity) and 750 nm (turbidity) wavelengths every 12 hr until no more growth in OD value could be observed.