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Biodegradation of three tetracyclines in river sediment

Bea-Ven Chang *, Yen-Lin Ren

Department of Microbiology, Soochow University, Taipei, Taiwan

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ABSTRACT

Tetracyclines (TCs), including tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC) are widely used in human and veterinary medicine. We investigated the aerobic degradation of three TCs in river sediment from southern Taiwan. The degradation rates in the sediment were in the order of CTC > OTC > TC. Sediment samples were separated into fractions with particle size from 2–50 to 500–2000 μm. Sediment fractions with smaller particle sizes demonstrated higher degradation rates. The sediment particle size affected the amount of the microbial community present, as did the various treatments in sediment. Degradation of TCs in sediment was enhanced by the addition of enzyme extract from spent mushroom compost of *Pleurotus eryngii* and was higher with extract-containing microcapsules than suspended enzyme extract. The addition of extract-containing microcapsules enhanced the removal rates of the three TCs, and 2 additions of TCs maintained the extract-containing microcapsule activity in sediment in bioreactor. The system to immobilize TCs we developed is stable and could be used in bioremediation applications.

1. Introduction

Tetracycline antibiotics (TCs), such as tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC) are broad-spectrum antimicrobial agents widely used in human therapy, animal husbandry, and aquaculture. Some studies have investigated the existence of TC antibiotics in sediment (Kim and Carlson, 2007; Li et al., 2008; Zhang et al., 2011). The release of antibiotics into the environment is of considerable concern because persistent antibiotic residue may lead to the development of antibiotic-resistant bacteria (Baquero et al., 2008).

Environmental factors that may influence the degradation of toxic chemicals in the environment include pH, temperature, oxygen availability, and nutrients (Fan et al., 2004). The size of sediment particles is also of fundamental importance. With small particles, the specific surface area is large and features low porosity, which favors the settling of microorganisms, thus promoting microbial degradation. Conversely, with large particles, the specific surface area is small, and the number of potential sites for microbial activity is reduced (Delhomenie et al., 2002). TC antibiotics are toxic to microalgae and aerobic bacteria (Halling-Sorensen, 2000; Halling-Sorensen et al., 2002), but we have few studies on the biodegradation of TCs in sediment.

White rot fungi can be a biological alternative for removal of toxic chemicals because it can produce three main extracellular enzymes: laccase, lignin peroxides (LiP), and manganese peroxidase (MnP) (Lee et al., 2004). *Pleurotus eryngii* is a saprophytic mushroom that is taxonomically related to the fungi *Basidiomycota*,*Agaricomycetes*, *Agaricales*, *Pleurotaceae*, and *Pleurotus*. *P. eryngii* can produce laccase, MnP, and the H_2O_2-generating enzyme aryl-alcohol oxidase (Munoz et al., 1997). Spent mushroom compost (SMC), a waste product of the mushroom industry, contains many residual enzymes such as protease, cellulase, hemicellulase, LiP, MnP, and laccase (Lau et al., 2003). The use of SMC as a source material for ligninolytic enzymes is a win–win strategy because SMC is easily obtained and could reduce mushroom growers' disposal costs for bioremediation. These features offer potential advantages for bioremediation and biological detoxification of toxic compounds (Li et al., 2010).

Immobilization of enzyme is increasingly being used in biotechnology processes for biochemical conversion and bioremediation. Immobilized enzyme is useful for various transformation processes because as compared with the free form, immobilization exploits the catalytic properties of enzymes, for better removal efficiency (Nicolucci et al., 2011). A high-voltage electrostatic system to immobilize enzyme extract in alginate microcapsules was used to remove nonylphenol and TCs from wastewater (Hsu et al., 2013). However, microencapsulation is not commonly used to remove toxic chemicals from sediment.

The climatic characteristics of subtropical regions foster diverse microbial communities in sediment. Many studies have used

* Corresponding author. Tel.: +886 228806628; fax: +886 228831193.
E-mail address: bvchang@scu.edu.tw (B.-V. Chang).

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PCR-denaturing gradient gel electrophoresis (PCR-DGGE) to examine the effect of pollutants on microbial communities in sediment (Tian et al., 2008; Yen et al., 2009; Yuan et al., 2010). However, we lack studies using the PCR-DGGE technique to characterize TC-degrading microbial communities in river sediment.

We investigated the use of a high-voltage electrostatic system to immobilize enzyme extracts in alginate microcapsules for removing TCs in river sediment. With batch experiments, we examined the effects of various factors on the aerobic degradation of TCs and explored the microbial community changes in river sediment. With bioreactor experiments, we evaluated the removal of TCs after three separate additions of TCs in sediment. The target compounds were TC, OTC, and CTC.

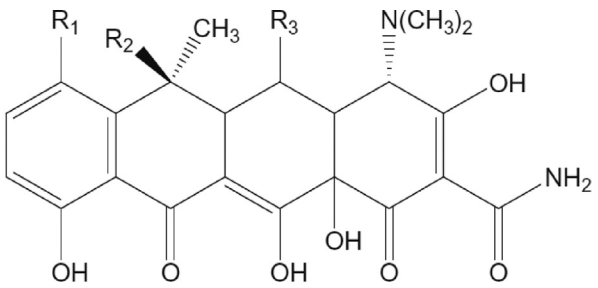
2. Materials and methods

2.1. Chemicals

TC, OTC, and CTC of 97.0% purity were from Sigma Chemical Co. (St. Louis, MO). Solvents were from Mallinckrodt (Paris, KY); all other chemicals were obtained from Sigma Chemical Co. Individual stock solutions of TCs dissolved in methanol were established at 1000 mg L^{-1}, then diluted with 0.05 M HCl before use. The chemical structures of TC, OTC, and CTC are shown in Fig. 1.

2.2. Sampling and medium

Sediment samples were collected from the Erren River, one of the most heavily contaminated streams in Taiwan (TEPA, 1997). The sampling site (22.55°14.32′N, 120.11°12.9′E) is downriver and is well known from previous studies of aquatic pollutants (Chang et al., 2014). Sediment samples (0–15 cm) were collected by use of a soil core during low tide. Detailed descriptions of the sampling site were previously published and are given in our previous study (Chang et al., 2014). The concentrations of TC, OTC, and CTC in the samples were 1.72, 2.09, and 1.90 mg kg^{-1}, respectively. The samples were first air-dried, and the particle size of the sediment refers to the original particle size. Sieves of various sizes were then used to separate samples into fractions with particle diameters 50–105, 105–500, and 500–2000 μm. The <50-μm fraction was further milled into powder by using an agate mortar and pestle. Powders were ultrasonically dispersed in double-deionized water, and the fraction of particles 2–50 μm was collected by sedimentation in accordance with Stokes' law (Yuan et al., 2010).



TCs	R_1	R_2	R_3
TC	H	OH	H
OTC	H	OH	OH
CTC	Cl	OH	H

Fig. 1. Chemical structure of tetracyclines (TCs).

The medium used in the degradation experiments contained the following (in g L^{-1}): K_2HPO_4, 0.0653; KH_2PO_4, 0.0255; $Na_2HPO_4{\cdot}12H_2O$, 0.1338; NH_4Cl, 0.0051; $CaCl_2$, 0.0825; $MgSO_4{\cdot}7H_2O$, 0.0675; and $FeCl_3{\cdot}6H_2O$, 0.00075.

2.3. Enzyme preparation

SMC of *P. eryngii* was produced in obtained from a mushroom cultivation factory in Chiayi, Taiwan. The enzyme was extracted from SMC with use of 600 mL sodium acetate buffer (pH 5.0), and 120 g SMC was extracted from *P. eryngii* for 3 h at 4 °C. The samples were centrifuged (10,000 × *g* × 10 min), and the supernatant was partially purified by precipitation with ammonium sulphate and dialysis. Because SMC is a potential source of ligninolytic enzymes such as laccase, we measured laccase activity in SMC (Liao et al., 2012). Enzyme activity for the extract was 885.4 ± 17.1 U L^{-1}. The material was stored at −20 °C.

2.4. Preparation of microcapsules

Alginate solution was made by dissolving sodium alginate (4 wt.%) in 0.9 wt.% sodium chloride with stirring for 1 h at room temperature. Enzyme extract solution was then added into the alginate solution. The final concentration of alginate was 2.0 wt.% in the mixture solution. The solution was prepared by dissolving calcium chloride (1.5 wt.%) with stirring at room temperature. An electrostatic droplet generator was used to prepare capsules (Hsu et al., 2013). The mixture was drawn into a 10-mL syringe fitted with a needle (23 G) and attached to a syringe pump that provided a steady solution flow rate of 25.2 mL h^{-1}. The positive electrode of the high-voltage electrostatic system was connected to the needle, and the negative electrode was placed in the collection solution 10 cm away from the needle tip. The size and shape of microcapsules were characterized under a Leitz Laborlux II phase-contrast biological microscope (Germany). The magnitude of the voltage generated between the needle and the gelling bath were important in determining alginate droplet size. The applied potential and gel bead diameter were inversely related. We used 12-kV potential to form microcapsules of 250-μm in diameter in the following experiments.

2.5. Experimental design

The experiments involved 125 mL serum bottles containing 45 mL medium and 5 g sediment, to which was added 100 mg kg^{-1} of a mixture of the three TCs (100 mg kg^{-1} each of TC, OTC, and CTC). We measured the effects of the following factors on the degradation of TCs in sediment: TC concentration (25, 50, 100, 200, and 500 mg kg^{-1}); presence of the three TCs individually (100 mg kg^{-1} each of TC, OTC, and CTC) and simultaneously (100 mg kg^{-1} each of TC, OTC, and CTC); and the addition of suspended enzyme extract (5 mL) or extract-containing microcapsules (5 g). Inoculated controls were treated with TCs in sediment. Sample bottles were incubated at 30 °C on a rotary shaker in the dark. Sterile controls were autoclaved at 121 °C for 30 min on two consecutive days. Each treatment was performed in triplicate. Aqueous samples were periodically collected from the two samples to measure the concentration of residual TCs, then underwent PCR-DGGE.

The experimental apparatus was a 2.5-L glass bioreactor (inner diameter, 18 cm; height, 30 cm) as previously described (Huang et al., 2012). It was aerated by an air diffuser, and materials were agitated by use of a stirrer. Reactors were filled with 800 mL medium, 50 g extract-containing microcapsules, 50 g sediment, and a 100-mg kg^{-1} mixture of the three TCs (100 mg kg^{-1} each of TC, OTC, and CTC). When the initial 100 mg kg^{-1} of three TCs had decreased to below detection limits, the second 100 mg kg^{-1} of TCs