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Biodegradation of sulfonamide antibiotics in sludge

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HIGHLIGHTS

- This study investigated sulfonamides biodegradation in sludge.
- The removal rates of sulfonamides of were enhanced by addition of spent mushroom compost at six times.
- *Acinetobacter* and *Pseudomonas* were major bacteria involved in sulfonamides degradation in sludge.

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ABSTRACT

Sulfonamide antibiotics are widely used in human and veterinary medicine. This study assessed the degradation of three sulfonamides (100 mg kg^{-1} each of sulfamethoxazole, sulfadimethoxine and sulfamethazine) and changes in the microbial communities of sewage sludge. Sulfamethoxazole degradation was enhanced by spent mushroom compost (SMC), SMC extract, and extract-containing microcapsules in the sludge. The degradation of sulfonamides in sludge and SMC mixtures occurred in the order of sulfamethoxazole > sulfadimethoxine > sulfamethazine. Bioreactor experiments revealed that the sulfonamides removal rates in sludge with SMC were greater than those in sludge alone. The sulfonamides removal rates were enhanced by the addition of SMC for six time additions. The sulfonamides concentrations were 200 and 500 mg kg^{-1} for the first to third additions and the fourth to sixth additions, respectively. With the high correlations between TOC and the proportions of sulfonamides remaining in sludge, sulfonamides may be mineralized to a greater extent with SMC in sludge than in sludge alone. Four bacterial genera were identified from the different settings and stages of the bioreactor experiments. *Acinetobacter* and *Pseudomonas* were major bacterial communities that were responsible for sulfonamide degradation in sludge.

1. Introduction

Antibiotics have been recently been investigated as a source of emerging environmental contaminants. These antibiotics may exert selective pressure that favours resistant bacteria (Schwartz et al., 2003), which are a major public health concern due to the increased occurrence of associated clinical infections. Sulfonamide antibiotics (SAs) exhibit a wide spectrum of action against most gram-positive and many gram-negative microorganisms. SAs inhibit the proliferation of bacteria by acting as competitive inhibitors of *p*-aminobenzoic acid in the folic acid metabolism cycle (Sukul and Spiteller, 2006). SAs that are excreted by humans or animals can enter wastewater treatment plants (WWTPs) through the sewage system (Ingerslev and Halling-Sørensen, 2000). Conventional wastewater treatment facilities are unable to efficiently remove SA residues (Barber et al., 2009). SAs have been found in the sludges of most WWTPs (Gobel et al., 2005; Nieto et al., 2010). Three SAs, i.e., sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfamethazine (SMZ), have been detected in the sludge of a WWTP in Taiwan (Yang et al., 2011). Microbial degradation has been proposed to serve as one major process for the removal of antibiotics from contaminated sludge.

Pleurotus eryngii is one of the most widespread white rot fungi in the world (Lee et al., 2004). Spent mushroom compost (SMC) is a waste product of the mushroom industry (e.g., *P. eryngii* cultivation factories) and contains many residual enzymes that can be used to degrade many organic toxic chemicals (Lau et al., 2003; Li et al., 2010). SMC contains bacteria that can degrade many organic toxic chemicals, such as nonylphenol, tetrabromobisphenol-A and tetracycline (Hsu et al., 2013; Chang and Ren, 2015).

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Metagenomic studies with next-generation sequencing allow for the study of microbial diversity in environmental samples with genus-level resolution (Xu et al., 2012; Yang et al., 2015a, b). However, little is known about the microbial communities that are involved in SAs degradation in sludge. The aim of this study was to compare the degradations of SMX according to various additives, various sludge-to-SMC ratios, and the individual or simultaneous presence of three SAs. The degradation rates of SAs and the bacterial community changes in the sludge in bioreactor were also evaluated. The target compounds were SMX, SDM, and SMZ.

2. Materials and methods

2.1. Chemicals

SMX, SDM and SMZ of 99.0% purity were obtained Sigma Chemical Co. (St. Louis, MO). The solvents were from Mallinckrodt (Paris, KY), and all other chemicals were from Sigma Chemical Co. Individual stock solutions of SMX, SDM and SMZ were dissolved in 0.03 M NaOH to establish concentrations of 5000 mg L^{-1} and then diluted with water before use.

2.2. Sampling and medium

The sludge was a semi-solid slurry that was produced as sewage sludge from the Dihus wastewater treatment plant in Taipei, Taiwan. The SMX, SDM, and SMZ concentrations in sludge were 5.9, 9.4, and 5.2 mg kg^{-1}, respectively. The basal medium contained the following (in mg L^{-1}): K_2HPO_4, 65.3; KH_2PO_4, 25.5; $Na_2HPO_4 \cdot 12H_2O$, 133.8; NH_4Cl, 5.1; $CaCl_2$, 82.5; $MgSO_4 \cdot 7H_2O$, 67.5; and $FeCl_3 \cdot 6H_2O$, 0.75. The pH of the basal medium was adjusted to 7.2 before autoclaving at 121 °C for 20 min.

2.3. Preparation of the SMC, SMC extract and extract-containing microcapsule

The SMC of *P. eryngii* was produced at a mushroom cultivation factory in Chiayi, Taiwan. The SMC extract (SE) was extracted from the 120 g SMC via the use of 600 mL sodium acetate buffer (pH 5.0) for 3 h at 4 °C. The samples were centrifuged (10,000 g × 10 min), and the supernatants were partially purified by precipitation with ammonium sulfate and dialysis. SMC is a potential source of ligninolytic enzymes (Li et al., 2010). Most ligninolytic fungal species produce constitutively at least one laccase isoenzyme and laccases are also dominant among ligninolytic enzymes in the environment (Baldrian, 2006). We measured the laccase activity in the sludge with SMC, MC, and SE.

The laccase activity was measured by spectrophotometry at 405 nm. The reactive mixture contained 0.5 mL of enzyme supernatant, 0.25 mL of 100 mM glycine buffer (pH 3.0), and 0.25 mL of 4 mM ABTS. The enzyme reactive mixtures were incubated at 25 °C for 5 min before measurement. For the evaluation of the laccase activity, one activity unit was defined as the amount of enzyme necessary to oxidize 1 μmol of substrate per min. Each value presented in this paper represents the mean of three replicates.

Alginate solution was made by dissolving sodium alginate (4 wt.%) in 0.9 wt.% sodium chloride with stirring for 1 h at room temperature. SE was then added to the alginate solution. The final concentration of alginate was 2.0 wt.% in the mixture solution. An electrostatic droplet generator was used to fabricate the capsules (Hsu et al., 2013). The mixture solution was drawn into a 10-mL syringe fitted with a 23G needle and attached to a syringe pump that provided a steady solution flow rate. The solution was pumped through the needle at a fixed feed rate (25.2 mL h^{-1}) and fixed voltage (12 kV) into a gently agitated aqueous solution of calcium chloride (1.5 wt.%) to form an extract-containing microcapsule (MC) of 250 μm in diameter for the experiments.

2.4. Experimental design

The batch experiments involved 125-mL serum bottles containing 40 mL medium, 5 g sludge, 5 g SMC and the SAs. We first compared three additives (i.e., SMC, SE and MC) and various sludge-to-SMC ratios (i.e., 1:0.5, 1:1, 1:1.5 and 1:2) in terms of the degradation of SMX (200 mg kg^{-1}) in the sludge. We also compared the three SAs (200 mg kg^{-1} each of SMX, SDM and SMZ) that were present individually or simultaneously in the sludge-SMC mixtures. Inoculated controls were treated with sludge as the inoculum and incubated with shaking at 30 °C in the dark. Sterile controls were prepared by autoclaving the sludge with SMC, SE or MC at 121 °C for 20 min on 3 consecutive d. Each treatment was performed in triplicate. Samples were periodically collected to measure the residual SAs.

The bioreactor was designed as previously described (Chang and Ren, 2015) and was aerated via the use of an air diffuser. The materials were agitated with a stirrer. Bioreactor A was filled with 1800 mL medium and 200 g sludge. Bioreactor B was filled with 1600 mL medium, 200 g sludge, and 200 g SMC. The three SAs were added, and when the SMX decreased to below the detection limit (ND), the three SAs were added again. The SA concentrations were 200 and 500 mg kg^{-1} for the first to third additions and the fourth to sixth additions, respectively. The entire experiments experiment lasted 82 d. Samples were taken periodically for analyses of the residual SAs, total organic contents (TOCs) and microbial communities.

2.5. Chemical analysis

SA determination was performed with an Agilent 1260 HPLC system equipped with a 4.6 × 250-mm column (Zorbax Eclipse Plus C18, Agilent) with a photodiode array detector monitoring at 270 nm. The mobile phase was acetonitrile and water (with 0.1% formic acid) at 70%:30%. Flow rate was 1 mL/min. The 0.5 mL aqueous phase samples were collected, and equal volumes of extraction solution were added with water (containing 0.1% formic acid): acetonitrile: methanol = 10:3:1, vortexed well and centrifuged at 10,000 rpm for 5 min. The supernatant was filtered with a 0.22-μm filter into a vial for HPLC. The recovery percentages for SMX, SDM and SMZ were 97.5%, 97.4%, and 96.4%, respectively. The detection limits for SMX, SDM, and SMZ were all 0.1 mg L^{-1}. The TOCs were measured using a TOC Analyzer (OI Analytical 1030W, USA).

2.6DNA extraction, PCR, and pyrosequencing

Total DNA was extracted from each experimental sample using the PowerSoil DNA Isolation Kit (MO BIO Laboratories). Partial 16S rRNA genes containing variable V5–V8 regions were amplified from the extracted DNA. The sequence for the 5′ primer included a 454 pyrosequencing adaptor, a unique 4-mer tag for each sample and 787F (5′-ATTAGATACCCNGGTAG-3′) for the 16S rRNA genes. The sequence of the 3′ primer included a 454 pyrosequencing adaptor and 1391R (5′-ACGGGCGGTGWGTRC-3′) for the 16S rRNA genes. The PCR reactions were performed as we have previously described (Yang et al., 2015a). Pyrosequencing was performed at the Genome Center of the National Yang-Ming University, Taiwan, ROC with a GS Junior System (Roche Diagnostics Corp., CT, USA). The sequence data produced in this study are provided as Supplementary material.

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