



Removal of emerging contaminants using spent mushroom compost

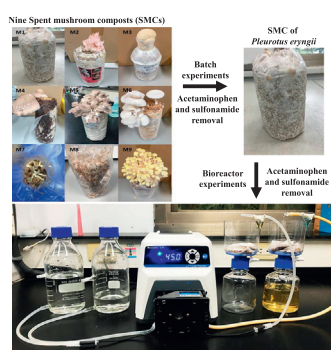
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HIGHLIGHTS

- Spent mushroom composts were tested for acetaminophen and sulfonamide removal.
- Fungal enzymes in SMC were identified by metaproteomic analysis.
- Acetaminophen and sulfonamide removal in wastewater were simulated by bioreactor.

GRAPHICAL ABSTRACT



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ABSTRACT

Acetaminophen and sulfonamides are emerging contaminants. Conventional wastewater treatment systems fail to degrade these compounds properly. Mycoremediation, is a form of novel bioremediation that uses extracellular enzymes of white-rot fungi to degrade pollutants in the environment. In this study, spent mushroom compost (SMC), which contains fungal extracellular enzymes, was tested for acetaminophen and sulfonamides removal. Among the SMCs of nine mushrooms tested in batch experiments, the SMC of *Pleurotus eryngii* exhibited the highest removal rate for acetaminophen and sulfonamides. Several fungal extracellular enzymes that might be involved in removal of acetaminophen and sulfonamides were identified by metaproteomic analysis. The bacterial classes, Betaproteobacteria and Alphaproteobacteria, were revealed by metagenomic analysis and may be assisting with acetaminophen and sulfonamide removal, respectively, in the SMC of *Pleurotus eryngii*. Bioreactor experiments were used to simulate the capability of *Pleurotus eryngii* SMC for the removal of acetaminophen and sulfonamides from wastewater. The results of this study provide a feasible solution for acetaminophen and sulfonamide removal from wastewater using the SMC of *Pleurotus eryngii*.

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1. Introduction

Human activities generate a large number of pollutants. Many chemical pollutants are widespread in household, hospital, livestock and aquaculture wastewaters. Emerging contaminants (ECs) are defined as

“chemical substances that have no regulation, are suspected to affect the environment or whose effects are unknown” (Ahmed et al., 2017; Barrios-Estrada et al., 2018a; Deblonde et al., 2011). These pollutants include persistent organic pollutants, POPs (Ren et al., 2018), endocrine disrupting chemicals, EDCs (Barrios-Estrada et al., 2018a) and pharmaceuticals and personal care products (PPCPs) (Bayen, 2012). There are many types of PPCPs, with a significant proportion of antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs). Sulfonamide

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antibiotics have been detected in the wastewater of most WWTPs (Nieto et al., 2010). Three sulfonamides, i.e., sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfamethazine (SMZ), have been detected in the sludge of a WWTP in Taiwan (Yang et al., 2011). Anti-inflammatory drug contaminants were also common in wastewater systems (Radjenović et al., 2009). The concentrations of acetaminophen were approximately 7.1–11.4 mg L⁻¹. Acetaminophen exists at concentrations of 1–10 µg L⁻¹ in effluent from wastewater treatment plants (Gómez et al., 2007). Acetaminophen can exist for a considerable period of time in an aqueous environment (Yamamoto et al., 2009). Acetaminophen and sulfonamides cannot be removed by the conventional procedures of wastewater treatments (Geng et al., 2016). These compounds penetrate the adjacent aquatic systems from the municipal wastewater treatment plant, which in turn impacts the environmental ecosystems (Li et al., 2014; Li et al., 2015; Yang et al., 2011; Yang et al., 2012).

Biodegradation and bioremediation are techniques that exploit the ability of microorganisms to breakdown or transform environmental pollutants (Asgher et al., 2008; Haritash and Kaushik, 2009). Previous studies have found that fungi can destroy and decompose many substances, such as wood, paper, textiles and plastics (Maqbool et al., 2016; Treu and Falandysz, 2017; Yang et al., 2017). White-rot fungi contain an extracellular ligninolytic enzyme system that includes laccase, lignin peroxidase and manganese ion peroxidase (Christian et al., 2005). Laccase is the major enzyme in the ligninolytic enzyme system. It has been used in the removal of many emerging contaminants that are difficult to decompose, such as endocrine-disrupting compounds and dye-based industrial pollutants (Asgher et al., 2014; Bilal et al., 2017a; Bilal et al., 2017b; Barrios-Estrada et al., 2018b). Studies of the application of white-rot fungi extracellular enzymes for mycoremediation have focused on the enhancement of fungal culture and the production of laccases. In particular, genetic engineering techniques were used to improve fungal strains to enhance their contaminant degradation ability (Asgher et al., 2014; Fan et al., 2011; Gao et al., 2010). However, there are certain disadvantages to the use of purified laccases for mycoremediation: (1) high cost, (2) difficulty of producing large quantities of purified enzymes, (3) instability of enzymes in wastewater, (4) difficulties of enzyme regeneration and reutilization and (5) lack of fungal commensal bacteria that assist degradation (Chatha et al., 2017).

In recent years, mushrooms have typically been grown using plastic bags with a mixture of wood shavings, soybean meal, corn meal and rice bran. According to statistics, the production of 1 kg mushroom produces 5 kg of spent mushroom compost (SMC) (Lau et al., 2003). Improper handling of large amounts of SMC may result in environmental pollution. As these mushrooms are white-rot fungi, their SMC contains ligninolytic enzymes, and residual mycelia can continue to secrete enzymes (Ball and Jackson, 1995; Trejo-Hernandez et al., 2001). In recent years, the use of low-cost agricultural waste for the solid-state fermentation of white-rot fungus-specific extracellular enzyme has had good results. Studies using solid-state cultures of four *Pleurotus* spp. to decompose aromatic hydrocarbons and remove the polycyclic aromatic hydrocarbons suggest that *Pleurotus eryngii* works best (Rodríguez et al., 2004). Moreover, Chang and Chang (2016) found that the SMC of *Pleurotus eryngii* and *Pleurotus ostreatus* were effective in removing organic poisonous compounds with the SMC of *Pleurotus eryngii* having superior removal efficiency.

In this study, the removals of acetaminophen and sulfonamides with 9 mushroom SMCs were tested. The metaproteomics method (LC-MS/MS) was used to analyze the extracellular enzyme compositions of SMCs. The metagenomics method (next generation sequencing) was used to analyze the changes in the bacterial community in SMC extracts before and after acetaminophen and sulfonamide removal. Finally, the bioreactor experiments were conducted to simulate the procedures of acetaminophen and sulfonamide removal in wastewater by SMC.

2. Materials and methods

2.1. Sampling and spent mushroom compost of mushrooms

Wastewater samples were collected from the effluent of the Dihus domestic sewage-treatment plant, Taipei (GPS coordinates: 25.072647 121.510654). The characteristic values of the effluent wastewater pH, COD, conductivity, TSS, BOD and total solids were 6.7, 14.2 mg L⁻¹, 62 mS m⁻¹, 36.1 mg L⁻¹, and 10.2 mg L⁻¹ and 0.87 g L⁻¹, respectively.

Spent mushroom composts (SMCs) of nine mushrooms (M1: *Pleurotus eryngii*, M2: *Pleurotus djamor*, M3: *Hericium erinaceus*, M4: *Lentinus edodes*, M5: *Pleurotus ostreatus*, M6: *Hypsizygus marmoreus*, M7: *Agrocybe aegerita*, M8: *Auricularia auricula-judae*, M9: *Pleurotus citrinopileatus*) were obtained from mushroom cultivation farms in Nantou, Taiwan (Fig. 1A).

2.2. Chemicals

Chemicals, solvents and the four target compounds acetaminophen and three sulfonamides: sulfamethoxazole (SMX), sulfamethazine (SMZ), and sulfadimethoxine (SDM), of 99.0% purity were obtained from Sigma-Aldrich (Sigma-Aldrich Co. LLC). The structural formulae of the four target compounds are shown in Fig. 1B and C.

2.3. Experimental design

The SMC extract was prepared via a method described in a previous publication (Yang et al., 2016a, 2016b). Briefly, 120 g SMC and 600 mL sodium acetate buffer (pH 5.0) were used to extract extracellular enzymes of mushrooms for 3 h at room temperature. The extracts were centrifuged (10,000g, 10 min), and the supernatants were used for batch experiments. Laccase activity was measured as described previously (Liao et al., 2012).

Batch experiments were performed using 125-mL flask bottles containing 50 mL SMC extract and 10 mg L⁻¹ of acetaminophen or three sulfonamides (10 mg kg⁻¹ each of SMX, SDM and SMZ). Sample bottles were incubated on a shaker (120 rpm) at 25 °C in the dark. Each treatment was performed in triplicate. The procedure was repeated 3 times. Samples were taken periodically to analyze the residual acetaminophen or three sulfonamides.

Adsorption experiments were performed using a 125-mL flask containing 10 g SMC, 40 mL sterile water, and acetaminophen (5, 10, 20, 50, 100 mg L⁻¹) or three sulfonamides (5, 10, 20, 50, 100 mg L⁻¹ each of SMX, SDM and SMZ). Each treatment was performed in triplicate. The sample bottles were incubated for one day on a shaker (120 rpm) at 25 °C in the dark. The suspension was centrifuged (5870g, 30 min) and the supernatant was collected. The supernatant was filtered through a 0.22-µm Millipore filter, and the residual acetaminophen or sulfonamide concentration was analyzed. The adsorption isotherm for the acetaminophen or sulfonamides collected in this study fit well with the linear adsorption isotherm, i.e., $S = k_d C$, where S is the concentration of the substrate on the solid phase, C is the concentration of the substrate in the aqueous phase, and k_d is the adsorption constant.

The reactor set-up was shown in Fig. 2A. Bioreactor experiments of acetaminophen removal were performed as shown in Fig. 2B. Initially, the reactors were treated RO (reverse osmosis filtration) water that was circulated through the reactor for 24 h. The procedure of inoculum circulation through reactors was repeated until the reactor reached steady state. After the steady state was reached, the reactors with hydraulic retention time (HRT) of either 2 h or 30 min were continuously fed with either RO water or wastewater containing 2 mg L⁻¹ acetaminophen that had been flowed through 50 g of SMC using a peristaltic pump as shown in Fig. 2A. The reactors had a total volume of 500 mL. The glass reactors had an internal diameter of 10 cm and length of 10 cm. The temperature in the bioreactor was maintained at 25 °C. 500 mL RO or wastewater with 10 mg L⁻¹ acetaminophen was flowed

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