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Enhanced removal of naproxen and carbamazepine from wastewater using a novel countercurrent seepage bioreactor immobilized with *Phanerochaete chrysosporium* under non-sterile conditions

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HIGHLIGHTS

- Fungal reactor was stably operated under non-sterile condition.
- Countercurrent seepage mode reactor enhanced carbamazepine and naproxen removals.
- High carbamazepine removal efficiency of ~80% was achieved.
- Naproxen was removed to undetectable level.

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ABSTRACT

A countercurrent seepage bioreactor immobilized with *Phanerochaete chrysosporium* was continuously operated under non-sterile conditions to treat a synthetic wastewater spiked with naproxen and carbamazepine (1000 μg/L each) for 165 days. There were no serious bacterial contaminations occurred during the operational period. Naproxen was always removed to the undetectable level regardless of the experimental conditions, while the average removal efficiency for carbamazepine, a well-known recalcitrant pharmaceutically active compound, reached around 80%. The excellent removal performance was mainly attributed to the application of countercurrent seepage mode and the cardhouse fabric of the carriers, which provided the high efficiency in the transfer of oxygen and nutrients inside the bioreactor. From the fungal immobilization combined with the temperature adjustment, the fungal activity including the enzyme production was protected as well as the bacterial contamination inside the reactor was suppressed effectively.

1. Introduction

Micropollutants mainly consist of pharmaceuticals, personal care products, steroid hormones, industrial chemicals, pesticides, and many other emerging compounds (Luo et al., 2014). They are usually present at the low concentration level of ng/L to μg/L in the environment, but still have the biological activity and pose a significant threat to the aquatic environment. The variety and trace amounts of these micropollutants bring a challenge to the water and wastewater treatment technology. The conventional biological treatment such as the activated sludge process and even the membrane bioreactor (MBR) technology have been shown only able to remove the soluble organic compounds effectively but without a good or stable performance in removing those biologically persistent micropollutants such as naproxen and carbamazepine (Yang et al., 2013a). Consequently, many micropollutants would pass through the wastewater treatment process and end up in the aquatic environment. Naproxen and carbamazepine are pharmaceutically active compounds (PhACs), frequently detected around the world (Luo et al., 2014). The removal efficiencies for naproxen (a non-steroidal anti-inflammatory drug) at the conventional wastewater treatment plants (WWTPs) have been reported to vary from negative to >70% (Joss et al., 2006). For carbamazepine (an antiepileptic drug), the removal efficiency at the conventional WWTPs even using the MBR has mainly been <10% (Zhang and Geiβen, 2008).

Many studies have shown that white-rot fungus is capable of degrading some micropollutants recalcitrant to the bacterial degradation, including diclofenac, carbamazepine, naproxen, and ibuprofen, through the nonspecific extracellular enzyme system and/or the intracellular enzyme system such as cytochrome P450

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(Jelic et al., 2012; Joss et al., 2006; Zhang and Geiβen, 2010). The key issue for applying the white-rot fungus technology in practice is to design and establish a suitable white-rot fungus reactor. The existing ones mainly include packed bed bioreactor, fluidized bed bioreactor, trickle-bed bioreactor, stirred tank bioreactor, rotating biological contactor, and MBR (Cruz-Morató et al., 2013; Jelic et al., 2012; Nguyen et al., 2013; Novotný et al., 2012; Pakshirajan and Kheria, 2012; Rodarte-Morales et al., 2012; Yang et al., 2013b). The high removal efficiencies for micropollutants were achieved with these bioreactors but mostly under sterile conditions. Few studies have been done in terms of removing micropollutants through a continuous bioreactor in the long-term operation under non-sterile conditions. Yang et al. (2013b) have used an MBR inoculated with *Trametes versicolor* to continuously remove bisphenol A and diclofenac from the synthetic wastewater under non-sterile conditions, but the removal performances achieved in the bioreactor were much less than the ones obtained from the pure fungal culture in batch tests. They attributed the lower removal performances to the continuous loss of enzyme and the bacterial contamination occurring in the reactor. Nguyen et al. (2013) used an MBR containing a mixed culture of bacteria and white-rot fungus to remove 30 micropollutants including naproxen and carbamazepine but also observed a significant drop of target compounds removal performances in the continuous bioreactor compared to the batch test. During 100 days of continuous operation, even though naproxen was almost removed, the removal efficiency for carbamazepine was only around 20%. A significant decline in the removal performance of the continuously operated bioreactor under non-sterile conditions would commonly occur, compared to the performance under sterile conditions, regardless of micropollutants types. This decline is generally caused by the overgrowth of bacteria existing in the bioreactor under non-sterile conditions, which would impose an inhibition on both fungal growth and enzyme production, resulting in the bioreactor performance deterioration (Yang et al., 2013a; Zhou et al., 2013). Therefore, it is crucial to create an environment where fungi can dominate while inhibiting the bacterial growth. Nilssona et al. (2006) attempted to create a completely sterile environment in the bioreactor through purifying the in/out air using a sterile air filter but found it was quite difficult to maintain the sterile condition. Jin et al. (1999) claimed the bacterial contamination could be effectively inhibited by increasing the temperature to above 40 °C or decreasing the pH to below 3.5, but also found the low pH had negative effects on protein synthesis and enzyme production. In comparison, Libra et al. (2003) found the bacterial growth could not be inhibited even at pH as low as 3.0 and suggested to use the nitrogen-limited culture medium and the fungal immobilization to suppress the bacterial growth, which was also reported by Zhou et al. (2013). They suggested the immobilization could enhance the fungal biomass as well as its stability, longevity, and enzyme production, keeping them competitive with the contaminating bacteria. They also proposed to use ozone for the selective disinfection since fungi are considered more resistant to ozonation than bacteria. Li et al. (2015) also showed the addition of 8.25% sodium hypochlorite at the ratio of 1:100 (v/v) into the fungal reactor inhibited the bacterial contamination under the non-sterile condition. On the other hand, Dhouib et al. (2006) added the ground white-rot fungus mycelium into the bioreactor every two weeks because a large amount of fungal biomass was considered able to decrease the negative effect of bacteria. Some fungal species have been found to possess a stronger resistance against the bacterial contamination. Nilssona et al. (2006) found the decolouration efficiency of *Pleurotus flabellatus* was not affected by the presence of bacteria in a fungal reactor. In general, the bacterial contamination issue occurring in the fungal reactor under the non-sterile condition has still not been counter-measured effectively. The white-rot fungus reactor should be effective in fungal immobilization and diffusion/transfer of nutrients and metabolites, including enzymes and reaction products, and it is important for the bioreactor to be operated steady for a long term under non-sterile conditions.

Cunninghamia is one of the main tree species in southern China and due to its loose structure and low density and fast growing and inexpensiveness, it was used as the carrier material for the fungal immobilization in this study. On the other hand, while the submerged culture has been the most common way when the white-rot fungus applied in studies for the wastewater treatment, the countercurrent seepage mode bioreactor is proposed in the current study to solve the incompatibility between natural conditions and industrial applications. Therefore, the objective of this study was to establish a white-rot fungus reactor, which would be continuously operated under the non-sterile condition, to remove carbamazepine and naproxen from synthetic wastewater. Through the fungal immobilization on sawdust, it was expected to create the conditions to ensure the better transfer of oxygen and nutrients. The operational mode was determined and the related parameters were further optimized. The kinetics of two target micropollutants biodegraded by the enzyme produced by *P. chrysosporium* was also studied.

2. Methods

2.1. Microorganism

The white-rot fungal strain *P. chrysosporium* BKM-F-1767 was purchased from the Guangdong Microbiology Culture Center, Guangdong Institute of Microbiology (China). The strain was transferred onto a fresh potato dextrose agar (PDA) slant. The PDA medium contained 20 g glucose, 3 g KH_2PO_4, 1.5 g $MgSO_4$, 0.1 g $CaCl_2$, 8 mg thiamine, and 20 g agar in one liter of 20% potato extract, as recommended by the white-rot fungus supplier with some modifications. The strain was stored at −18 °C until use.

2.2. Immobilization

After cultivated at 30 °C in an incubator (BGZ series, Boxun Co., Ltd, China) for one week, *P. chrysosporium* spores formed on the surface of PDA plate were scraped out and diluted in sterile water to prepare the spore suspension with the concentration of 1.5×10^7 spores/mL. *Cunninghamia lanceolata* flakes (wood sawdust; 10–20 mm length × 5–10 mm width × 0.2 mm thickness) at 1.0 g/L were added into the liquid medium in flasks. Cunninghamia is one of the main tree species in southern China. Due to its loose structure, low density, fast growing, and inexpensiveness, it was used as the carrier material for the fungal immobilization in this study. The liquid medium was prepared according to Kirk et al. (1978) with modification, containing 2 g KH_2PO_4, 0.5 g $MgSO_4$, 0.1 g $CaCl_2$, 10 g glucose, 0.2 g ammonia tartrate, 1 mg thiamine, and 0.2 g veratryl alcohol in one liter of tap water. The pH of the medium was adjusted to 4.5 using 50 mM sodium tartrate buffer. The flasks were then placed into a LDZX-50KBS vertical heating pressure steam sterilizer (Shenan Medical Co., Ltd, China) to autoclave (121 °C, 20 min). After cooling to the ambient temperature, the prepared spore suspension was inoculated into each flask at the ratio of 1:40 (v/v). The flasks were then placed onto a ZHWY-200H incubator shaker (Zhichen Co., Ltd, China) for the fungal cultivation at 120 rpm and 30 °C. One week later, *Cunninghamia* flakes immobilized with *P. chrysosporium* were filled into the bioreactor and the remained liquid medium was disposed.