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Short communication

Effects of biopellets composed of microalgae and fungi on pharmaceuticals present at environmentally relevant levels in water

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ABSTRACT

Removal of seven pharmaceuticals (acetaminophen, carbamazepine, diclofenac, metoprolol, naproxen, ranitidine and sulfamethoxazole) from water was investigated using three different microbial treatments with: (1) the microalga *Chlorella vulgaris*, (2) the fungus *Aspergillus niger* and (3) biopellets composed of both microorganisms. The three-day experiment was performed under laboratory conditions and pharmaceuticals were spiked at the environmentally relevant concentration of 10 $\mu g\,L^{-1}$. The biopellets and fungal treatments resulted in significantly lower ranitidine concentration compared with the initial value. Also, treatment with biopellets resulted in significantly lower final ranitidine concentrations compared to those found after control and microalgal treatments. Low removal rates were obtained for other substances, possibly because the amount of microbial biomass used was 16–500-fold lower than that normally used in activated sludge processes in wastewater treatments plants. Thus, the pharmaceutical removal potential, elimination potential and performance of biopellets should be further investigated at higher biomass concentrations.

1. Introduction

Recent studies have shown that current wastewater treatment processes only partly remove pharmaceuticals (Jones et al., 2005; Pereira et al., 2015). Thus, these substances are frequently detected in aquatic ecosystems and organisms (Daneshvar et al., 2010; Hedgespeth et al., 2014) and in groundwater and drinking water (Vulliet and Cren-Olivé, 2011; Milić et al., 2013). Biotransformation is suggested to be one of the main processes responsible for pharmaceutical removal (22–99%) in wastewater treatment plants (WWTPs; Gao et al., 2012), together with sorption onto sewage sludge (Falås et al., 2013). Still, adsorption to active carbon (AC) is presently one of the most promising methods to increase removal of pharmaceutical residues at wastewater treatment plants (WWTPs; Serrano et al., 2011; Kyzas et al., 2015). Unfortunately, active carbon requires high amounts of energy to produce, may display unpredictable removal capacities and after usage the exhausted AC filter generates toxic by-products with resulting disposal problems (Gadd, 2009; Luo et al., 2014). Thus, more sustainable and efficient wastewater treatment methods based on biological treatment techniques are needed to remove pharmaceutical residues and other organic contaminants. Successful removal of pharmaceuticals from wastewater by microalgae and fungi has been reported in numerous studies (More et al., 2010; de Godos et al., 2012; Jelic et al., 2012; Escapa et al., 2015). Removal is reported to occur mainly via adsorption, biotransformation and degradation, the latter two due to release of non-specific enzymes (de Godos et al., 2012; Santos et al., 2012). The aims of the present study were to: (1) evaluate the potential of two different microorganisms, a microalga and a fungus, for removing pharmaceuticals from water, and (2) assess whether synergistic effects between microbial species in pharmaceutical removal can result in new types of sustainable wastewater treatment systems. For the purposes of the latter aim, a modified version of the method described by Zhang and Hu (2012), developed to aid harvesting of microalgae, was used to construct biopellets from microalgae and fungi to remove pharmaceuticals from water. As a first step, the study was carried out in a pure culture medium with spiked concentrations of seven pharmaceutical substances from different substance groups.

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2. Methods

2.1. Selected active pharmaceutical substances

The pharmaceuticals were selected on the basis of high frequency of detection in aquatic environments, low removal rate in WWTPs and high consumption in Sweden (Zhang et al., 2008; Official Statistics of Sweden, 2013). Acetaminophen, carbamazepine, diclofenac, metoprolol, naproxen, ranitidine and sulfamethoxazole and the internal standards (ISs) carbamazepine-D_{10}, diclofenac-$13C_6$, and naproxen-D_3 were purchased from Sigma–Aldrich. For chemical analysis, gradient grade methanol (MeOH) and acetonitrile (ACN) were purchased from Sigma–Aldrich (Steinheim, Switzerland). Stock standard solutions were prepared in MeOH and stored at −18 °C. Theoretical concentration of each pharmaceutical in the stock solution ranged between 8 and 11 mg L^{-1}.

2.2. Microorganisms

The microalga used in the study was *Chlorella vulgaris* strain 211/11B from CCAP-SAMS (Culture Collection of Algae and Protozoa, The Scottish Association for Marine Science, Scotland), which was cultivated in BG-11 medium under sterile conditions (Zhang and Hu, 2012). To start an algal culture, 400 mL of BG-11 solution were inoculated with 40 mL of a *C. vulgaris* culture taken from a previous 4-day algal culture inoculated in the same manner. The culture was kept in a greenhouse at 20 °C, a photoperiod of 16 h and illumination of 100 μmol m^{-2} s^{-1} (PAR). The cultures were aerated (0.3 vvm) to prevent the algal cells from settling. A stock solution containing 7.5×10^8 cells L^{-1} was made using BG-11 medium after determining number of algal cells in a Bürkner chamber.

The fungus used in the study was the filamentous fungus *Aspergillus niger* ATCC® 16888™ from the American Type Culture Collection. Fungal spores were cultivated on Petri plates with potato dextrose agar (PDA) at room temperature for 10 days. The spores were harvested using glucose (20 g L^{-1}) applied directly on the PDA plates and the fungal solution produced was filtered through a nylon filter (mesh size 100 μm). Number of spores in the solution was determined using a Bürkner chamber and a stock solution containing 7.5×10^7 spores L^{-1} was made. To induce the formation of biopellets, 300 mL of sterile distilled water were mixed with 300 mL of microalgal stock solution (suspended in BG-11 medium) and 300 mL of fungal stock solution (suspended in 20 g glucose L^{-1}).

2.3. Experimental design

Treatments included control without biomass and treatments with three types of microbial biomass, i.e. microalga (2.5×10^8 cells L^{-1}, 50 ± 16 mg dry weight L^{-1}), fungus (2.5×10^7 spores L^{-1}, 25 ± 8 mg dry weight L^{-1}) and biopellets (microalga + fungus; 75 ± 12 mg dry weight L^{-1}). Four replicates were used in each treatment. In all treatments, the theoretical starting concentration of each pharmaceutical substance ranged between 8 and 11 μg L^{-1} and that of glucose was 6.7 g L^{-1}. Initial amounts of microorganism biomass used in this study were similar to those commonly used in commercial applications for production of algae biofuels or reported by other researchers for the pre-cultivation and co-cultivation of microalgae and fungi (Zhang and Hu, 2012; Xie et al., 2013). A treatment time of 3 days was selected, based on the general time that it takes for biopellets to form. The control treatment consisted of 1/3 BG-11 solution, 1/3 glucose and 1/3 pharmaceutical solution; the algal treatment consisted of 1/3 algal stock solution (suspended in BG-11 medium), 1/3 glucose and 1/3 pharmaceutical solution; the fungal treatment consisted of 1/3 BG-11 medium, 1/3 fungal stock solution (suspended in 20 g glucose L^{-1}) and 1/3 pharmaceutical solution; and the biopellet treatment consisted of 1/3 algal stock solution (suspended in BG-11 medium), 1/3 fungal stock solution (suspended in glucose) and 1/3 pharmaceutical solution. Initial pH in all treatments was adjusted to 4.0 using 1 M HCl and/or NaOH. All treatments were performed in Erlenmeyer flasks containing 200 mL aliquots. The flasks were placed on a horizontal shaker (100 rpm) at room temperature without additional light in the laboratory for 3 days. All treatments, including the control, were filtered through GF/F filters (WVR collection, CAT-no 516-0348) before analysis. These filters have been reported to not adsorb pharmaceutical compounds (Falås et al., 2013). In the present study this result was also confirmed for the tested pharmaceuticals in preliminary tests preparing the analysis (data not shown).

2.4. Analysis and statistics

Dry weight of biomass in all treatments was estimated by filtration through pre-dried and pre-weighed GF/F filters. The filtered water was used for analysis of pharmaceutical compounds. Pharmaceuticals were analysed by solid phase extraction, with sample pH adjustment and using Oasis HLB cartridges, and instrumental analysis was performed using HPLC-MS/MS (Agilent 6460) interfaced with an electrospray ionisation source in positive-ion mode ((+)ESI). Statistical analysis of the data obtained was performed using Minitab (version 16 for Windows). Effects of treatment type on final concentration were evaluated using one-way ANOVA followed by Tukey's multiple comparison test. To examine significant differences between initial concentrations and final concentrations in the different treatments, a *t*-test was employed. The significance level in all cases was set to $p < 0.05$.

3. Results and discussion

There was an increase in biomass after treatment (final biomass 129 ± 27 mg L^{-1} for fungus; 91 ± 25 mg L^{-1} for microalga and 123 ± 14 mg L^{-1} for biopellets) and a visible formation of biopellets (Fig. A.1., Appendix A). The final pH value in each treatment was 3.8 ± 0.0 for the control; 4.1 ± 0.0 for the microalga, 4.9 ± 0.2 for the fungus and 4.8 ± 0.3 for biopellets. Generally, there was no significant difference between the initial and final concentration of the different pharmaceuticals in the control treatment (Fig. 1). Thus, this result showed that pH adjustment, presence of inorganic salts and glucose, and filtration did not have any significant effect on pharmaceutical removal from water. Still, for diclofenac, the control treatment resulted in significantly lower final concentration compared with the initial control concentration (Fig. 1). Still, the relative removal was low, only 8% of the initial control concentration. Noutsopoulos et al. (2015) showed that the relative removal of diclofenac in wastewater was much higher at pH 6 (90% removal) compared to pH 10 (10% removal). Thus, for the present study, one possible explanation for the lower concentration of diclofenac after the control treatment was the lower final pH (3.8 ± 0.0) compared to the pH in the other treatments (4.1 ± 0.0 for microalga, 4.9 ± 0.2 for fungus and 4.8 ± 0.3 for biopellet). Only ranitidine concentrations were significantly lower when treated with biopellets or fungus compared with the initial concentration (Fig. 1). Relative removal of initial ranitidine concentrations was $50 \pm 19\%$ and $20 \pm 13\%$, for the biopellets and fungus treatments, respectively. The final ranitidine concentration in the biopellet treatment was significantly lower than the one after the control and microalgal treatments, whereas the fungal treatment was not