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Removal of endocrine disrupting compounds from wastewater by microalgae co-immobilized in alginate beads

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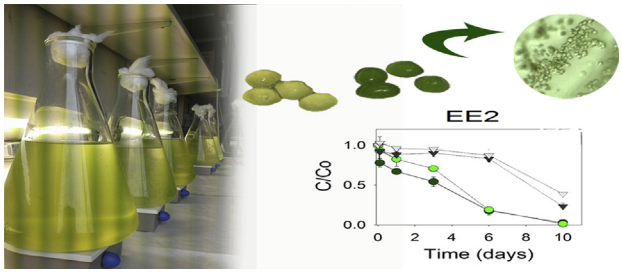
HIGHLIGHTS

- The effect of co-immobilized microalgae on the removal of EDCs has been evaluated.
- Immobilizing the microalgae increased the removal of NH_4-N and TP.
- The kinetic removal rates of EDCs ranged from 0.013 to 2.131 d^{-1}.
- Co-immobilizing microalgae increased the removal of some EDCs.
- NH_4-N and TP concentration decay correlated with the EDC concentration decay.

GRAPHICAL ABSTRACT



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ABSTRACT

Microalgae systems have been found to be efficient for removing microcontaminants from wastewater effluents, but the effectiveness of immobilized microalgae for removing endocrine disrupting compounds (EDCs) has not yet been addressed. This paper assesses the effect of free and immobilized microalgae on removal efficiency for 6 EDCs by mixing them in 2.5 L reactors with treated wastewater. The experimental design also included control reactors without microalgae. After 10 days of incubation, 64 and 89% of the NH_4−N and 90 and 96% of total phosphorous (TP) had been eliminated in the free microalgae and immobilized microalgae reactors, respectively, while the control reactors eliminated only 40% and 70% of the NH_4−N and TP, respectively. Both the free and immobilized microalgae reactors were able to remove up to 80% of most of the studied EDCs within 10 days of incubation. Free microalgae were found to increase the kinetic removal rate for bisphenol A, 17-α-ethinylestradiol, and 4-octylphenol (25%, 159%, and 41%, respectively). Immobilizing the microalgae in alginate beads additionally enhanced the kinetic removal rate for bisphenol AF, bisphenol F, and 2,4-dichlorophenol. This study shows that the use of co-immobilized microalgae-based wastewater treatment systems increases the removal efficiency for nutrients and some EDCs from wastewater effluents.

1. Introduction

Endocrine disrupting compounds (EDCs) are exogenous chemicals that can disrupt hormonal signaling systems. EDCs are ubiquitous in the environment and include pesticides, flame retardants, pharmaceuticals, and chemicals in personal care products and housewares (Khalil et al., 2014). Since conventional wastewater treatment plants (WWTPs) are not designed to treat these types of contaminants, many of these compounds occur at different concentrations in natural water bodies (Carballa et al., 2004), where they may exert ecotoxicological effects even at relatively low concentrations (Henry and Black, 2008; Muñoz et al., 2009). Among the

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better researched compounds, 17α-ethinylestradiol (EE2) has been found to produce feminization in male fish (fathead minnow, *Pimephales promelas*) at very low concentrations (5–6 ng L^{-1}) and, ultimately, the near extinction of this fish from surface water bodies containing this pollutant (Kidd et al., 2007). Bisphenol A (BPA) induces feminization in *Xenopus laevis* tadpoles, and 4-octylphenol (OP) has high potential estrogenic effects on the same amphibian (Levy et al., 2004; Huang et al., 2005). Therefore, it is of great importance to reduce their discharge into the aquatic environment.

Nowadays there are different available tertiary-treatment wastewater technologies capable of efficiently removing EDCs, from advanced oxidation to constructed wetland systems (Liu et al., 2009), but little if know about the use of microalgae technologies for that purpose. Microalgae-based wastewater treatment technologies have been used for producing algal biomass for use as fertilizer, a source of products (e.g., paraffin, olefin, glycerol, protein, anti-oxidants, pigment, plastic, etc.), or biofuel, but they also have the advantage of providing a high-quality treated effluent (Craggs et al., 2012). Although the capability of microalgae-based wastewater treatment systems to remove organic matter and nutrients has already been studied, few such studies have focused on the removal of EDCs. Existing laboratory-scale studies dealing with microalgae's capacity to remove EDCs suggest that microalgae treatment systems may remove them by evaporation, photodegradation, biodegradation, and/or microalgae uptake (Ge et al., 2009; Abargues et al., 2013; Ji et al., 2014; Matamoros et al., 2016b). In recent years, with the aim of enhancing pollutant removal and biomass recovery, the use of co-immobilization (also known as joint immobilization) of microalgae and bacteria has increased (de-Bashan and Bashan, 2010). Nevertheless, the deterioration of alginate beads due to different environmental factors such as the pH and water composition is still a matter of great concern (Draget et al., 2005). For instance, some alginate-degrading bacteria have been isolated from wastewater (Cruz et al., 2013). It has been found that immobilizing microalgae in alginate beads increases removal efficiency for heavy metals and some organic pollutants such as organotin and oil spill compounds (de-Bashan and Bashan, 2010). Nevertheless, to date only one study has dealt with the capability of immobilized microalgae to remove EDCs. Gao et al. (2011) found that co-immobilization of microalgae in alginate beads enhances nonylphenol (NP) removal (reactors were spiked at 1 mg L^{-1}) for a short period of time (12–24 h) in a grown medium, but performed worse than free microalgae cells over a longer period of time (96–168 h). Therefore, there is a lack of knowledge regarding the effects of real wastewater and immobilized microalgae on the removal of other EDCs at real environmental concentrations (μg L^{-1}).

This study aimed to quantify the effect of the co-immobilization of microalgae in alginate beads on removal efficiency for 6 EDCs (Table 1-supplementary material, SM) at environmental concentration (10 μg L^{-1}) using secondary-treated wastewater. This is the first time that microalgae technology has been used for attenuating EDCs from secondary-treated wastewater effluents.

2. Material and methods

2.1. Experimental design

In order to study the effect of the presence of microalgae and their co-immobilization with bacteria on EDC removal, different reactor set-ups were assessed. These set-ups included microalgae reactors, control reactors without microalgae, co-immobilized microalgae in alginate beads, and control alginate beads. A total of 12 experimental units were used, consisting of 3 replicates of each of the 4 types of reactors. All reactors were fed with real secondary-treated wastewater from the Montcada i Reixac WWTP. The wastewater used for the experiments was a secondary-treated effluent with the following composition: total suspended solids (TSS), 75.4 mg L^{-1}; chemical oxygen demand (COD), 49 mg L^{-1}; NH_4-N, 36.3 mg L^{-1}; total phosphorous (TP), 0.46 mg L^{-1}; and fecal coliforms, 4.5 log CFU/100 mL. It had a conductivity of 1480 μS cm^{-1}.

All the reactor systems consisted of 2.5 L pre-cleaned glass containers. A standard solution containing the 10 EDCs in methanol solution was added to each reactor (final water volume of 2 L) to obtain a final concentration of 10 μg L^{-1} (200 μL of spiking solution at 100 mg L^{-1} for each compound in methanol). The microalgae reactors were inoculated with a microalgae consortium obtained from an experimental high-rate algal pond treating urban wastewater (Passos et al., 2013). The main microalgae populations were made up of *Chlorella* sp. and *Nitzschia acicularis*. While the inoculum also contained bacteria, the microalgae accounted for over 90% of the biomass, as is usual in microalgae secondary wastewater treatment technologies such as high rate algal ponds (García et al., 2006). The microalgae consortium was pre-acclimatized to the growth conditions (secondary-treated wastewater) for 20 days before the reactors were stocked with microalgae. The free and co-immobilized microalgae were inoculated to a concentration of approximately 80 mg L^{-1} dry weight (dw) biomass per reactor (150 mL of pre-acclimatized microalgae of approximately 800 mg L^{-1} dw biomass). The experiments were run simultaneously for 10 days. The reactors were set up in a temperature-controlled growth room at 23 ± 5 °C and lit by fluorescent tubes at a photon flux density of 150 μmol m^{-2} s^{-1} in a 12 h light/12 h dark cycle (Philips Master TL-D, 36W/840).

2.2. Immobilization of microalgae in alginate beads

Microalgae were immobilized as described previously by de-Bashan and Bashan (2010). Briefly, 150 mL of pre-acclimatized microalgae was mixed with 2% sodium alginate and stirred for 60 min to obtain an alginate solution. The mixture was then dropped into a 2% $CaCl_2$ solidification solution in secondary-treated wastewater using a peristaltic pump (Minipuls 2, Gilson, WI, U.S.A.) with a flow rate of 1.1 mL min^{-1} to produce uniform immobilized microalgal beads (3–5 mm in diameter each). Approximately 4600 beads were automatically produced per reactor. The beads were left for 1 h under a soft stirring for curing and then washed with secondary-treated wastewater. Control beads were prepared in the same way as the microalgal beads, except using secondary-treated wastewater instead of microalgal cells. Finally, 4600 microalgal or control beads were added to each reactor, which was then filled to 2 L with secondary-treated wastewater (approximately 2 beads mL^{-1}).

2.3. Sampling strategy

Aqueous samples of 125 mL were taken regularly during experiments at 0, 0.1, 1, 3, 6, and 10 days. The incubation time was selected on the basis of the hydraulic retention time of microalgae wastewater treatment systems, normally set at 2–4 days (García et al., 2006). No water loss was observed over the experimental period due to evaporation. All water samples were collected in 250 mL amber glass bottles, which were stored at 4 °C until analysis. The sample holding time was less than 12 h.

Water and alginate bead samples were taken during experimentation from reactors and were examined with an optical microscope (Motic BA-310, China) to evaluate the main populations.