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Can zero-valent iron nanoparticles remove waterborne estrogens?

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ABSTRACT

Steroidal estrogens are one of the most challenging classes of hazardous contaminants as they can cause adverse effects to biota in extremely low concentrations. They emerge in both waste waters and surface waters serving as a source of drinking water. Environmental Quality Standards for 17β-estradiol (E2) and 17α-ethinylestradiol (EE2), promulgated within the EU Water Framework Directive, are 0.4 and 0.035 ng L^{-1}, respectively. Because nanoscale zero-valent iron (nZVI) particles have been previously used in numerous remediation technologies and have the advantage of possible magnetic separation, interaction of nZVI with E2 and EE2 in water was investigated to assess the potential role of nZVI in removing steroidal estrogens. A mixture of E2 and EE2 dissolved in water was shaken with varying doses of nZVI for 1–5 h. Concentration-dependent removal of the estrogens was observed but removal did not increase significantly with time. Concentrations of the estrogens were determined by HPLC/MS/MS and a biodetection reporter gene assay. Sorption and nonspecific oxygen-mediated oxidation of estrogens were identified as the most probable removal mechanisms. Two independent experiments confirmed that significant decrease of estrogens concentration is achieved when at least 2 g L^{-1} of nZVI is applied. The presented study provides insights into the mechanisms of nZVI interaction with steroidal estrogens under aerobic conditions prevailing in currently applied water treatment technologies.

1. Introduction

In recent decades, the release of estrogens and other endocrine-disrupting compounds into the environment from anthropogenic sources has become a major environmental problem worldwide particularly affecting rivers and irrigation networks (e.g., Sumpter and Johnson, 2005; Leusch et al., 2010). Estrogens have been detected in wastewater, surface water, groundwater and even drinking water (Leusch et al., 2009). In fish, as well as other aquatic vertebrates, small concentrations (ng L^{-1} range) of estrogens are known to cause adverse effects, including feminization of males, impaired reproduction and abnormal sexual development (Sellin et al., 2009). Disruption of sexual function of wild fish, downstream of municipal wastewater treatment plants, has been observed worldwide and attributed to estrogenic compounds (Jobling and Tyler, 2003). The natural hormone, 17β-estradiol (E2), and synthetic birth control pharmaceutical 17α-ethinylestradiol (EE2) are reported to be the most potent estrogens in complex environmental mixtures, such as contaminated surface waters (Caldwell et al., 2012). Recently, Environmental Quality Standards for E2 and EE2 promulgated as a part of the EU Water Framework Directive have been set at 0.4 and 0.035 ng L^{-1} for E2 and EE2, respectively.

To monitor concentrations of steroidal estrogens in natural water and wastewater, high performance liquid chromatography

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combined with tandem mass spectrometry (HPLC/MS/MS) can be used (Petrovic et al., 2004). However, such analytical method does not provide information on possible interactions among the various endocrine disrupting compounds within the mixture and/or other not-measured or unexpected estrogenic compounds. This information can be complemented by *in vitro* biological assays which evaluate the total estrogenicity of complex mixtures (Leusch et al., 2010).

Improvements and optimization of water treatment technologies and/or development of advanced treatment methods are needed to reduce concentrations of potentially harmful estrogens in effluents and, consequently, surface waters (e.g., Grover et al., 2011) especially when they serve as a source of drinking water. Widely accepted technologies for efficient removal of estrogens are based on advanced oxidation processes, sorption, filtration, and biodegradation (Caliman and Gavrilescu, 2009). Some of these methods are complicated by a decrease in effectiveness of sorbents/filters over time, which therefore need to be replaced (Snyder et al., 2003; Caliman and Gavrilescu, 2009). Ferromagnetic nanoscale zero-valent iron (nZVI) particles are one of the promising advanced nanomaterials suitable for water treatment technologies to remove various inorganic and hazardous organic substances (Li et al., 2006). The high efficiency and versatility of metallic iron in the degradation (through effective reduction and catalysis) and removal of more than 70 different environmental contaminants have been demonstrated in dozens of laboratory and large-scale studies (i.e., pilot and full-scale remediation at polluted sites, Elliott and Zhang, 2001). Moreover, magnetic nanoparticles also allow their simple magnetic separation from different remediation technologies (Yavuz et al., 2006). However, to our knowledge, no published study has focused on the interaction of nZVI with estrogens.

Our previous experiences include synthesis, surface modification, detailed characterization and application of nZVI (Filip et al., 2007; Klimkova et al., 2011; Marsalek et al., 2012; Filip et al., 2014) and also detection of small concentrations of estrogens and evaluation of their activity (Jarosova et al., 2012). The present work examines the interaction of nZVI with estrogens (E2 and EE2). Based on laboratory-scale experiments, including detection of changes in estrogen concentrations and overall estrogenic activity coupled with characterization of the solid materials, we identified possible mechanisms for the interaction between estrogens and commercially available nZVI particles.

2. Materials and methods

2.1. Materials and reaction mixtures

E2 and EE2 estrogens were purchased from Sigma–Aldrich (Czech Republic). All reaction mixtures were prepared with Milli-Q ultra-pure deionized water (18 MΩ cm^{-1}, Millipore). All chemicals were of analytical reagent grade and used without further purification.

The nZVI particles (commercially available as NANOFER 25N with a specific surface area $a_s \approx 20\ m^2\ g^{-1}$ and being manufactured by NANO IRON company), were synthesized by thermal reduction of iron oxide powder (Zboril et al., 2012; Filip et al., 2014). The as-prepared dry nZVI particles were stored in hermetically enclosed stainless steel containers under gaseous nitrogen at room temperature and subsequently rapidly transferred (under an inert gas but without vigorous mechanical stirring) into deionized water (sample labeled as nZVI-A). The same type of nZVI particles were transferred into deionized water under vigorous mechanical stirring immediately prior to experiments (sample labeled as nZVI-B).

2.2. Experimental design

Mixtures of estrogens were prepared in Milli-Q water at nominal concentrations of 60 μg L^{-1} for E2 and 120 μg L^{-1} for EE2. These initial concentrations are greater than those observed in the environment, but were selected so that 98% removal of the compounds would still be greater than the detection limit of HPLC/MS/MS. Experiments were conducted in 1 L glass bottles, each containing 0.5 L of estrogen solution and various concentrations of nZVI-A particles (0, 2, 4 or 6 g L^{-1}). Bottles were tightly closed, kept at 21 °C and agitated at 180 rpm. Liquid samples were collected prior to nZVI addition and after 1, 3, and 5 h of shaking. Glass vials containing the liquid samples were placed on a magnetic plate for 8 min to separate out the nZVI particles. Aliquots of solution (0.5 mL) were stored at 4 °C until chemical analysis, which was completed within 2 d of sampling. Verification of the effective concentration of nZVI, testing of low concentrations of nZVI particles, and elucidation of the possible mechanisms of E2 and EE2 interaction with nZVI were all carried out with nZVI-B particles (at concentrations of 0, 0.04, 0.4, 2, and 6 g L^{-1}). In these experiments, liquid samples were collected prior to nZVI addition and after 1 and 5 h of shaking. Mixtures containing nZVI-B particles at concentrations of 0.04 and 6 g L^{-1} in Milli-Q water were also prepared to act as negative controls, i.e., without estrogens. Separate samples of E2 and EE2 at maximum solubility in water (i.e., about 1 mg L^{-1}) with 6 g of nZVI-B particles (shaken for 1 h) were also prepared in order to examine the mechanism of nZVI interaction with estrogens in further details.

Concentrations of E2 and EE2 in all the aqueous samples were measured with HPLC/MS/MS. Simultaneously, changes in the estrogenic potencies of samples treated with 0, 2, 4, and 6 g L^{-1} of nZVI-A particles were investigated using an *in vitro* bioassay. To elucidate the mechanism of estrogen interaction with nZVI, X-ray powder diffraction, ^{57}Fe Mössbauer and X-ray photoelectron spectroscopy, Transmission Electron Microscopy (TEM) and SQUID magnetometry were used to analyze the nZVI particles prior to and after interaction with estrogens (i.e., 0.04 g L^{-1} or 6 g L^{-1} nZVI-B interacting with 60 μg L^{-1} of E2 and 120 μg L^{-1} of EE2, and 6 g L^{-1} nZVI-B interacting with 1 mg L^{-1} of E2 or EE2), as well as in the negative controls (for experimental details, see Supplementary material). Particles of nZVI were obtained by magnetic separation from the particular reaction dispersions.

2.3. Instrumentation employed for the analysis of liquid samples (HPLC/MS/MS)

An Agilent 1200 series HPLC system coupled to a 6410 Triple-Quad MS (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray interface was used for HPLC/MS/MS analysis. Separation of E2 and EE2 was performed on a C18 column ACE 3 (250 × 2.1 mm I.D., 3 μm, ACE, Aberdeen, Scotland, UK). Isocratic elution (acetonitrile/1 mM L^{-1} of ammonium acetate at pH = 7, 65/35 v/v) was carried out at 25 °C with a flow rate of 0.1 mL min^{-1}. The mass spectrometer was operated in the multiple reaction monitoring mode with a negative polarity and a collision energy of 46 eV. The capillary voltage and fragmentation energy were 5000 and 220 V, respectively. Transition ions of E2 from *m*/*z* of 271.2 to 145.0, EE2 from *m*/*z* of 295.2 to 145.0 and estrone (E1) from *m*/*z* of 269.3 to 145.0 were monitored with a 250 ms dwell time. Quantification of E2, EE2, and E1 was achieved using external calibration in the range from 5 to 160 μg L^{-1}. The detection limit of HPLC/MS/MS, defined as amount of sample which produces a signal three times higher than the noise level, was less than 2 μg L^{-1} of injected sample based on a 10 μL injection volume (i.e., 20 pg per injection). The experimental error expressed as a coefficient of variation was determined to be 11%.

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