TREATMENT OF ENDOCRINE DISRUPTING COMPOUNDS IN WATER

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Abstract

Historically, compounds such as substituted phenols, non-biodegradable chlorinated solvents, pesticides and surfactants, are recognized as examples of substances that are difficult to remove from water. Recently, pharmaceuticals and personal care products (PPCPs) and especially endocrine disrupting compounds (EDCs) are considered as emerging contaminants, which means that they are still unregulated or in the process of formulating regulations.

There is growing evidence of the impact of these emerging contaminants in the environment. Studies have shown that male fish in detergent-contaminated water express female characteristics, turtles are sex-reversed by polychlorinated biphenyls (PCBs), male frogs exposed to a common herbicide form multiple ovaries, pseudohermaphroditic offspring are produced by polar bears, and seals in contaminated water have an excess of uterine fibroids. Recent work shows that human development can also be feminized by exposure to estrogenic chemicals.

As an example, passive sampling is highly complimentary to spot sampling in environmental analysis. A polar organic chemical integrative sampler (POCIS) was extensively tested to optimize its performance under both controlled and field conditions. Under laboratory conditions, the kinetics of compound uptake by POCIS were linear during 10-day of exposure. POCIS sampling rates of the target compounds were significantly greater using polyethersulfone instead of polysulfone membrane, and was enhanced with increasing sorbent exposure area. Both spot and passive sampling demonstrated that most of the target chemicals were frequently detected in sewage effluent and river waters, and that the daily changes in the pollutant concentrations were greater for pharmaceuticals than for EDCs. The aqueous concentrations of all compounds were elevated at a sewage outfall, which has been confirmed to be an important source of the target compounds in rivers. The validated POCIS was successfully used to estimate the concentrations of the target compounds in effluent and river water, which were in good agreement with those from spot sampling for pharmaceuticals.

Advanced oxidation processes (AOPs) constitute a promising technology for the treatment of wastewaters containing pharmaceuticals and personal care products (PPCPs) and especially EDC's. AOPs are characterized by the generation of hydroxyl radicals. Besides fluorine, the hydroxyl radical is the strongest known oxidant. Therefore, it is possible for the hydroxyl radical to oxidize and mineralize almost every organic molecule into carbon dioxide (CO_2) and inorganic ions. The most common advanced oxidation technique used at the industrial level is the Fenton's reagent process, which uses an iron salt and hydrogen peroxide.

The challenge of EDC's is that some exist in a steroid ring that is very difficult to degrade by standard methods used in waste water treatment systems to remove pathogens. 17α-ethinylestradiol (EE2), the synthetic estrogen commonly found in birth control pills, is one such compound. Using AOP's (Fenton's reagent, hydrogen peroxide and ozone) in combination with other prospective treatment methods (sonication and UV), a solution of EE2 was subjected to treatments of varying methods, durations and intensities. These treated solutions were analyzed

using high performance liquid chromatography (HPLC) in order to evaluate their effectiveness in degrading the endocrine disrupting compound EE2 in wastewater.

Introduction

In recent years, it has been determined that various synthetic and natural compounds can mimic or interfere with the action of natural hormones and disrupt the endocrine systems of humans and wildlife [Colburn *et al*., 1996]. These substances, collectively referred to as endocrine-disrupting compounds (EDCs), have been linked to a variety of adverse effects in both humans and wildlife [Tyler *et al*., 1998; Jobling *et al*., 1998; McLachlan and Arnold, 1996; Sumpter, 2005]. Numerous EDCs, most of which act as estrogens, have been detected in various surface waters and ground waters [Kolpin *et al*., 2002].

A major source of estrogens in rivers is treated wastewater effluent. Investigations worldwide have detected bioactive estrogens in waters receiving treated wastewater [Snyder *et al*., 2001; Murk *et al*., 2002; Sheehan *et al*., 2002; Tilton *et al*., 2002; Gomes *et al*., 2003; Kolodziej *et al*., 2003; Cargouet *et al*., 2004; Hemming *et al*., 2004; Sarmah *et al*., 2006; Lishman *et al*., 2006]. Municipal wastewater is a complex mixture of natural and synthetic organic chemicals. The most powerful EDCs commonly detected in treated wastewater include the natural hormones 17β-estradiol (E2) and estrone (E1), and 17α-ethinylestradiol (EE2), a synthetic estrogen used in birth control pills. Other non-steroidal organic chemicals have been shown to possess estrogenic activity, but are much weaker than the steroid hormones. These include the degradation products of nonionic surfactants, such as alkylphenol-polyethoxylates, and plasti-cizers, such as bisphenol A [Barber *et al*., 2000].

Current technologies for treating municipal wastewater are only partially successful at removing EDCs [Gomes *et al*., 2003; Svenson *et al*., 2003; Clara *et al*., 2005; Falconer *et al*., 2006; Escher *et al*., 2006].. Steroid hormones are especially difficult to remove completely. They contain steroid rings that are resistant to degradation by the microorganisms used in wastewater treatment plants [Johnson and Sumpter, 2001]. Not only are steroids not efficiently removed from the waste during treatment but, those not removed are actually activated by the treatment. Steroids are excreted in human waste as biologically inactive glucuronide or sulfate conjugates, but are hydrolyzed back to the active native molecule by microbial activity in the treatment plant [Johnson and Sumpter, 2001]. As a result, both natural and synthetic estrogens and their degradation products tend to pass through wastewater treatment systems in bioactive forms and can reach concen-trations in receiving waters sufficient to produce deleterious biological effects on organisms living in the waters [Metcalf *et al*., 2001; Jobling *et al*., 2002]..

Reports of EDCs in water have raised substantial concern among regulatory agencies as well as operators of wastewater treatment facilities and water purification plants. It is clear that current methods often fail to remove steroids efficiently. It would be of great benefit to the health and well being of aquatic organ-isms, as well as humans living downstream from wastewater treatment plants (WWTPs), if practical methods could be developed to increase the efficiency of removal of EDCs during the treatment of municipal wastewater. Treatment technologies, such as activated carbon and reverse osmosis appear to be capable of removal of many trace contaminants. However, they are costly to install and maintain. Research is needed to better understand the fate of these compounds during wastewater treatment, removal kinetics, and to develop less expensive treatment alternatives.

The research had two major objectives: (1) to investigate the effectiveness of various advanced oxidation process (AOP) techniques (used singly or in combination) in degrading the potent steroid EE2 in aqueous solution, and (2) to analyze water and sediments in rivers in the Birmingham area receiving treated wastewater to determine whether the estrogens are present in concentrations sufficient to disrupt endocrine systems of aquatic organisms in the receiving waters.

Methods

Advanced Oxidation Processes

Experiments were conducted using 1.0-L volumes of deionized water spiked with 1.0 μM 17α-ethinylestradiol (EE2, Steraloids, Inc., Newport, RI, USA). After treatment, 100 mL of the water was passed through a Varian Speck C18 10 mL column (35 mg sorbent). The columns were eluted with 5 mL HPLC grade methanol, dried under nitrogen gas, and reconstituted to a volume of 100 μL with HPLC grade methanol and subjected to HPLC analysis as described below.

Treatments were done for 5, 10, 15, and 20 minutes and consisted of: Sonication (1.4 kHz), H_2O_2 (1,000 ppm), UV (16 lamps, 5 W each), O_3 (100 ppm) and the following combinations: Sonication + H_2O_2, Sonication + O_3, Sonication + UV, UV + O_3, UV + H_2O_2, UV + O_3 + H_2O_2, and Sonication + UV + O_3 + H_2O_2.

Analysis of River Water Samples

Samples were taken from local rivers (Cahaba River and Buck Creek) up- and downstream from wastewater treatment plants. Samples were collected in 2 L acid-washed glass jars with 15 mL of methanol added. They were immediately placed on ice and transported to the lab. In the lab, 1.0 L of each sample was passed through a series of filters (Whatman 52 [7 μm], followed by Whatman GF/A [1.6 μm], followed by Millipore RW0304700 [0.45 μm]) to remove particulates. The filtered water samples were then each passed through a Varian Bond Elut C18 solid phase extraction column (500 mg sorbent). The columns were eluted with 5 mL HPLC grade methanol, dried under nitrogen gas, and reconstituted to a volume of 100 μL with HPLC grade methanol and subjected to HPLC analysis as described below.

HPLC Analysis

For HPLC analysis, 10.0 μL of the reconstituted sample was injected into a Perkin-Elmer HPLC system. The column used was a Varian CP30705 Microsorb 100 C18, particle size 3 μm, 100 × 46 mm. Organic constituents of the sample were separated using a water:acetonitrile solvent gradient. For spiked samples, the gradient started with a 1:1 water:acetonitrile mixture. The proportion of acetonitrile increased up to 70% in a linear fashion for a period of 10 min and then was increased to 100% acetonitrile and held for a further 10 min. For environmental samples, the gradient started with a 1:1 mixture, increased to 80% acetonitrile over 20 min and then increased to 100% acetonitrile for another 10 min. For both gradients, EE2 elutes from the column at about 6 min. Organic compounds eluting from the column were detected by UV absorbance at 225 nm.

Quantification of the amount of EE2 eluting from the HPLC column was done by comparison of the peak volume with that of a known sample containing 10.0 nmoles in 10.0 μL (1 mM solution). By passing a 1mM EE2 standard solution through a C18 column, extracting

and reconstituting, as was done with all samples, it was determined that the process has a 78.5% extraction efficiency.

The rapidity by which the treatments broke down EE2 was quantified by estimating the first order decay equation rate constant.

Results

Advanced Oxidation Processes

Preliminary results (see Figure 1) indicate that the AOPs, performed individually, break down up to ~16% of the EE2 with 20 minutes of treatment. Combinations of treatments appear to be much more effective. Sonication+ozone+peroxide+UV+Fenton's reagent, in combination, broke down >28% of the EE2 in 20 minutes.

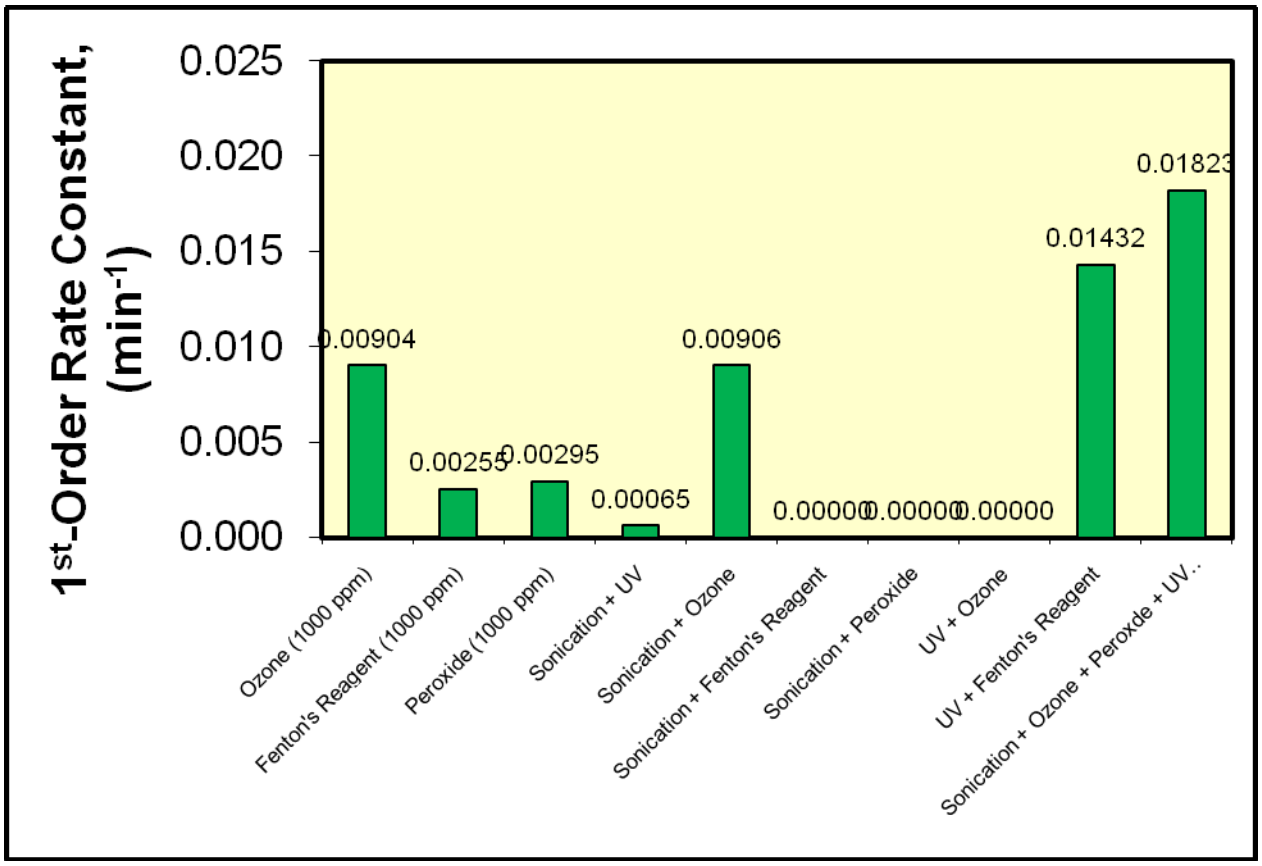

Figure 1. Estimated first order decay rate constants for the breakdown of 17α-ethinylestradiol by different advanced oxidation treatments.

Analysis of River Water Samples

Currently, samples have been collected from the Cahaba River and Buck Creek. Some of them appear, based on retention times, to have steroids in concentrations sufficient to affect aquatic organisms living in the rivers (see Figure 2). Further analysis with mass spectrometry will be necessary to confirm the identities of the suspected steroids.

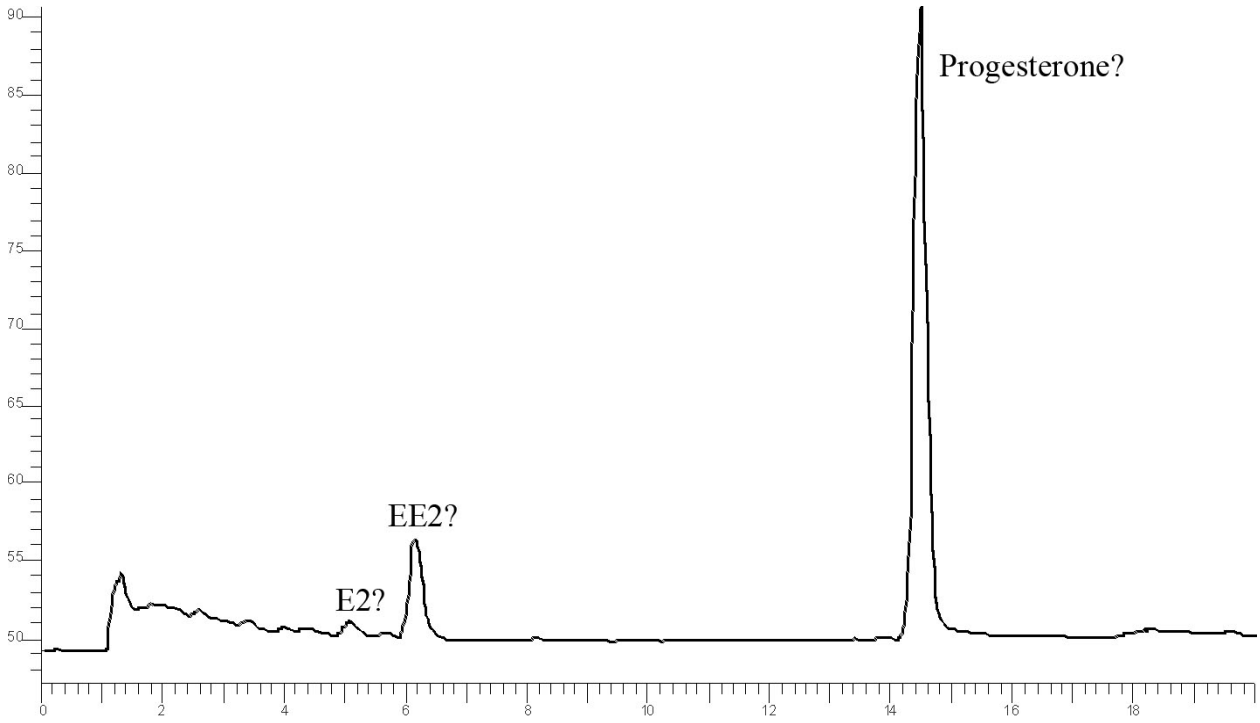

Figure 2. HPLC chromatogram of an extract of water collected from the Cahaba River below a wastewater treatment plant in Hoover. Preliminary identifications are based on similarity of retention time to known standards separated using the same solvent gradient. Definitive identification will require analysis by mass spectroscopy.

Conclusions

Although the project period is over, the project is not complete by any means. Numerous different AOP treatment combinations have been performed and await HPLC analysis. These will be done this semester by a graduate student in biology. Further river water samples will be collected and analyzed this fall as well. Preliminary results (see Figure 1) indicate that the AOPs, performed individually, break down up to ~16% of the EE2 with 20 minutes of treatment. Combinations of treatments appear to be much more effective. Sonication+ozone+peroxide+UV +Fenton's reagent, in combination, broke down >28% of the EE2 in 20 minutes.

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