Fate of 17α-Ethynylestradiol in the Presence of Vegetable Wastes

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ABSTRACT: Estrone (E1), Estradiol (E2), 17α-Ethynylestradiol (EE2), and Estriol (E3) are estrogens that can result in endocrine-disrupting effects in human and wildlife populations, impacting reproduction and development. Estrogens display characteristics typical of organic compounds and are typically excreted in conjugated forms, with rapid conversion to the unconjugated molecules in the environment. Entry to the environment can occur via multiple pathways which minimize the possibility of source control as a remediation strategy and, as such, responsibility for their removal falls on wastewater treatment plants. As expected, the majority of research on the control of estrogens is reported in the municipal wastewater treatment literature. A recent study assessed the abiotic transformation of estrogens in the presence of model vegetable matter (rabbit food). That study reported the catalytic or enzymatic polymerization of E1, E2, E3 and EE2 in the presence of rabbit food and dissolved oxygen. Greater than 80% reduction in the parent compounds was achieved for each target estrogen after 72 h of exposure. Interestingly, testosterone, androstenedione and progesterone did not undergo any transformation when exposed to the same conditions. It was speculated that the presence of a phenolic group in the estrogen skeleton was responsible for polymer formation. This study was undertaken to demonstrate a treatment process for the removal of estrogens from water matrices using vegetable matter.

Introduction

Endocrine-disrupting compounds (EDCs) comprise a diverse group of heterogeneous contaminants, with the common effect of altering the normal functioning of the endocrine system of living organisms (Birkett and Lester, 2003; Bolong et al., 2009; Al-Khateebet al., 2014; Muz et al., 2013). Recently EDCs have been identified as a major environmental concern due to their adverse effects on human and ecosystem health, generating a vast amount of attention among scientific communities worldwide (Tan et al., 2007).

Estrogens are EDCs that display characteristics typical of organic compounds, including low solubility in water, high affinity to organic matter, and lipophilic traits that lead to ready diffusion through the cell membrane and into the cytoplasm of target cells. Although typically excreted in conjugated forms, quick conversion to unconjugated molecules occurs in the environment (Lai et al., 2000; Hanselman et al., 2003). Estrone (E1), Estradiol (E2), 17 $\propto$-Ethynylestradiol (EE2), and Estriol (E3) can result in endocrine-disrupting effects in human and wildlife populations, impacting reproduction and development (Ogino et al., 2007; Sone et al., 2004; Sumpter and Jobling, 1995). The potency of these compounds can lead to detrimental health consequences at concentrations as low as 1 ng per liter (Irwin et al., 2001; Jobling et al., 1995; Caldwell et al., 2010), however predicting the effects of human exposure to EDCs at varying concentrations is difficult because EDCs often do not follow the typical linear dose-response relationships used in classical toxicology, where greater exposure to a chemical has increased health effects (Vogel, 2004). On the other hand, impact of exposure to EDCs on wildlife is well documented and includes disturbance of the immunological system and fertility, reproductive failure, feminization and masculinization, and altered sexual development (Hester and Harrison, 1999). Fish species are known to be the most affected due to palpable impacts imparted by estrogens (Sumpter and Jobling, 1995; Sumpter and Johnson, 2005). The chemical structure of E1, E2, E3, and EE2 is shown in Figure 1.

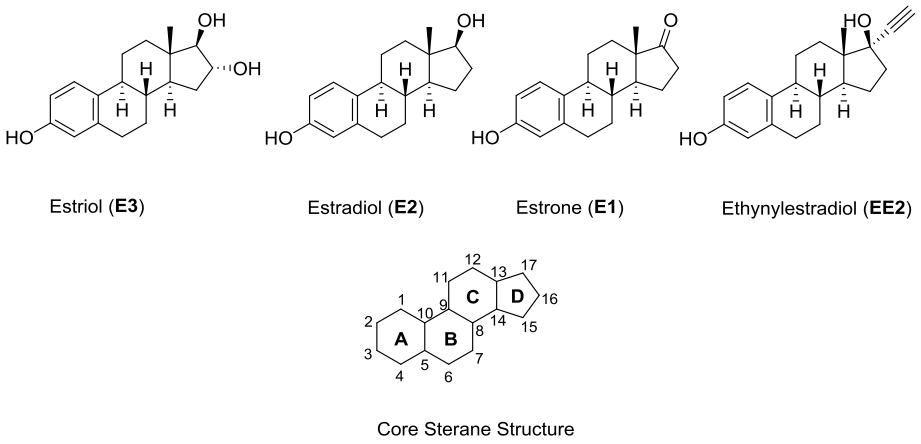

Figure 1 Structures of estrogens and the core sterane structure

Human and animal excretion is cited to be the main source of steroidal hormones in aquatic environments (Mes et al., 2005; Jobling et al., 2006). Johnson et al., (2000) studied the increase in concentration of estrogen secretions during pregnancy, the corresponding excretion levels of E1, E2, and E3 are 600, 259, and 6,000 pg per day, respectively, and among females who are taking birth control pills, the excretion of EE2 is around 35 pg per day. Additionally, the plant-processing industry also significantly contributes toward the presence of phytoestrogens in surface water bodies, this includes biodiesel production facilities (Lundgren and Novak, 2009). After excretion, these compounds enter the environment via multiple pathways including wastewater treatment plant effluents and run-off from manure applied to soils, reaching the receiving waters (Khanal et al., 2006). The majority of research in the field of environmental estrogens is reported in the municipal wastewater treatment literature (Hamid and Eskicioglu 2012). Because conventional WWTPs are considered effective in removing nutrients and solids from WW effluents, estrogens on the other hand follow a different pathway and, consequently, cannot be completely removed (Auriol et al., 2007). Therefore, the residual concentrations of estrogens in treated sewage are still at levels that cause adverse effects on aquatic life and ecosystems (Auriol et al., 2006; Auriol et al., 2006). Figure 2, adopted from Hamid and Eskicioglu (2012), illustrates the pathway of estrogens and steroidal hormones to the environment.

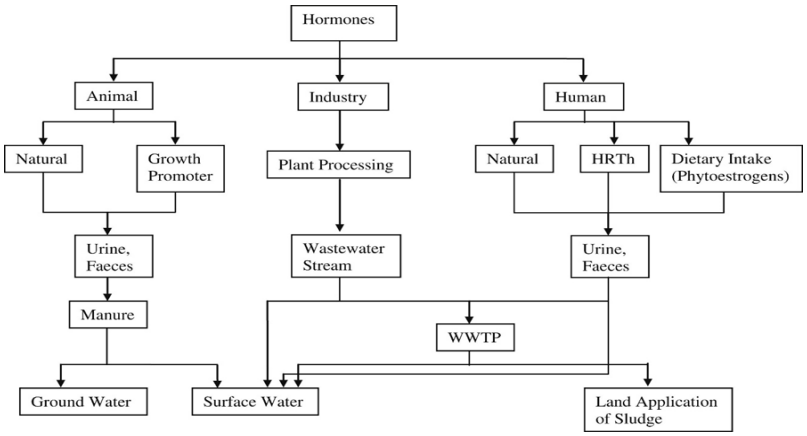

Figure 2 Pathways of estrogens and other steroidal hormones into the environment

The objective of this study is to investigate the fate of EE2 in the presence of rabbit food (RF) by:

1. Characterizing the abiotic conversion of estrogen EE2 upon exposure to RF
2. Assessing the removal of EE2 from water upon exposure to ground RF (breakthrough curve)
3. Determining parameters that promote the removal of EE2 (flow and RF concentrations)

Materials and Methods

In this study, we investigate the fate of EE2 in the presence of rabbit food. Multiple sets of batch experiments were performed over the course of 192 h (8 days), where 50 ng of EE2 was spiked in 100 mL distilled water and placed in serum bottles containing 1.4 g/L of rabbit food. Serum bottles were continuously shaken at 200 rpm until they were sacrificed at preset time internals. Throughout the experiment, liquid and solid phases were analyzed independently at each sampling time using LC-MSMS. Column breakthrough experiments were carried out in columns packed with a mixture of Ottawa sand and ground rabbit food. Each column was packed in three layers. An active middle section was sandwiched between two Ottawa sand layers. The active section contained rabbit food in a matrix of Ottawa sand as demonstrated in Figure 3. Performance was assessed as a function of: flow rate, sorbent to sand ratio, volume of influent treated, and time. LC-MSMS was used to measure the concentration of EE2 in both the aqueous and solid phases. Throughout the experiments sodium azide was added to the feed water to act as an aerobic biological inhibitor.

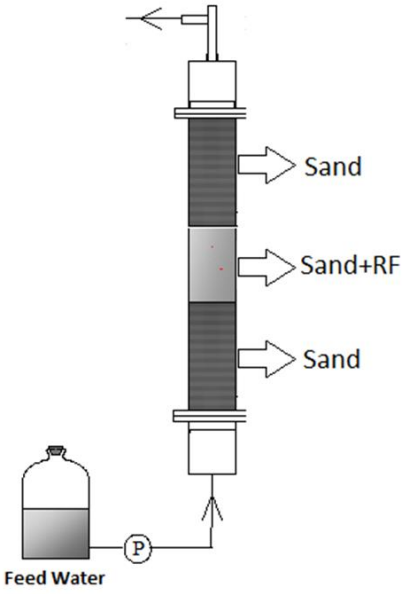

Figure 3 Column setup schematic

After the batch run samples were sacrificed the solid and liquid phases were separated using a 1.2-micron glass microfiber filter. Subsequently, the solid samples of the column and the batch runs were prepared via the following procedure:

- Spiked with surrogate D4EE2
- Frozen at -80 °C and kept for 72 h

- Lyophilized using a freeze dryer for 120 h
- Transferred to a silanized 250 mL flask spiked with the surrogate
- Ultra-sonication extraction
- Alumina cleanup followed by C18 cleanup

Liquid effluent on the other hand was handled as follows:

- Spiked with surrogate
- Solid phase extraction (HLB)
- Alumina cleanup followed by C18 cleanup

All glassware used in this experiment was salinized with 5% dimethyldichlorosilane in toluene. The LC MS-MS condition is shown in

Table **1**, with MRM transition.

Table 1 LC MS-MS condition

LC Conditions			
Columns	Agilent Poroshell 120 Phenyl-Hexyl		
	2.1 x 100 mm, 2.7 um (p/n 695975-321)		
Colum Temperature	20 ^{o}C		
Injection volume	30 uL		
Mobile phase	A: Water + 1 mM Ammonium Fluoride		
	B: 35% Acetonitrile + 65 % Methanol		
Flow Rate	0.3 L/min		
Gradient	Time (min)	% A	% B
	0	100	0
	0.5	90	10
	12.5	0	100
	15	0	100
	22	10	90
	25	100	0
Post time	5 mins		
Total Runtime	30 mins		
MS Conditions			
Ionization Mode	ESI with negative polarity		

Drying gas temperature	350°C
Drying gas Flow	40 psig
Sheath gas temperature	375°C
Sheath gas flow	11 L/min

Results and Discussion

Batch: Our findings demonstrated a viable process for the control of estrogens in wastewater. During the batch experiment, the maximum decrease in the concentration of EE2 as measured by LC-MSMS occurred between 48 and 72 h (2-3 d) into the experiment where 75 percent of starting concentrations was transformed to most likely unknown byproducts formed during the experiment as a consequence of catalytic/enzymatic transformation. On the other hand, the concentration measured by LC-MSMS analysis in the control samples confirmed that the transformation of estrogens was abiotic occurring exclusively in the presence of rabbit food as displayed in Figure 4.

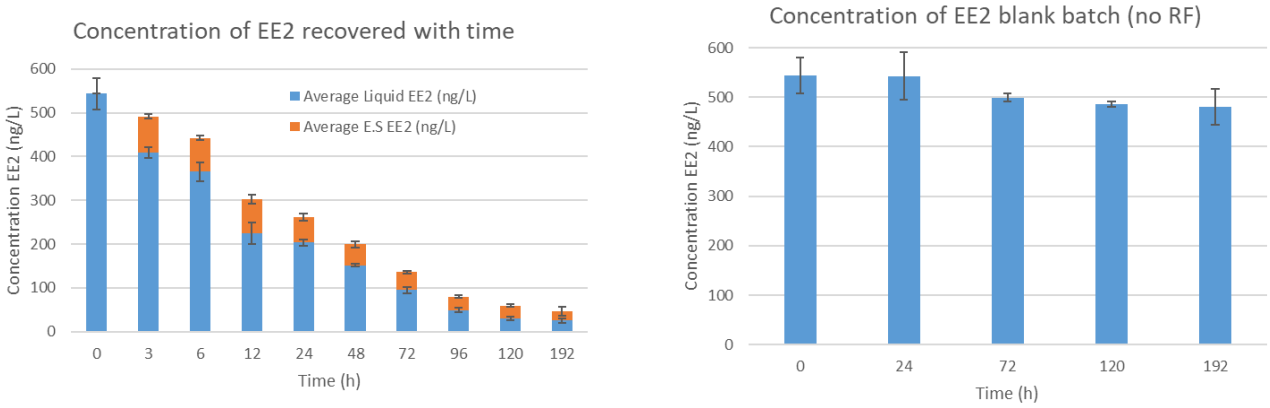

Figure 4 Concentration vs. Time for Batch test results

Column: Starting with an EE2 concentration of 200 ng/L in 5000 mL distilled water, and a flow of 0.35 mL/min, column experiments were carried out, and breakthrough data were collected. Effluent from the column was collected and samples analyzed when the collected volume reached 500 mL. Having 3.2 grams of rabbit food in the column was sufficient to give a removal of 100% of EE2 during the first two sampling events with full breakthrough occurring after Day 9 (4500 mL of spiked feed). Sandwiching a total of 5 grams of rabbit food under the same conditions caused a further delay to breakthrough curve, whereby 100% removal of EE2 occurred during the first three sampling events with full breakthrough occurring on Day 11 (5500 mL of spiked feed). Lastly, sandwiching a total of 7 grams of rabbit food under the same conditions exhibited even better results showing a delay to breakthrough curve, whereby 100% removal of EE2 occurred during the first four sampling events with full breakthrough occurring on Day 24 (6000 mL of spiked feed). A mass balance of the EE2 was performed for the column runs and the findings showed that mass

transformance of EE2 was 32-52%, 18-45%, and 4-19% under influent flow of 0.17,0.35, and 0.5 mL/min respectively with percentages increasing with increasing mass of RF.

Conclusions

In conclusion, understanding the mechanisms involved in estrogen removal from water is needed to help mitigate health and environmental risks resulting from exposure to EDCs. The need to develop a model for removal of estrogens from water matrices is of importance. Most importantly the understanding of abiotic transformation of estrogens as representative compounds of phenolic EDCs can be extrapolated to other micropollutants with similar chemical structure (BPA and alkylphenol surfactants).

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