Ultrasound assisted removal of estrogen hormones

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Abstract

Estrogen compounds are being detected in significant concentrations in surface water, wastewater, soil, sediments and groundwater. These estrogenic compounds influence the growth and performance of the reproductive system. Several reports indicate that the major source of these contaminants to the ecosystem is the effluents from wastewater treatment plants. These contaminants are found at significant concentrations in the effluent and the water bodies into which they are discharged. Reports also indicate that these estrogens are found in trace level concentrations in drinking water. Conventional treatment technologies are not designed to completely remove these pharmaceutically active chemicals (PhACs). Therefore there is a need to develop new treatment technologies in addition to the existing technologies. Ultrasound is an advanced oxidation process that effectively destroys many toxic organic chemicals. Ultrasound involves the process of passing high frequency sound waves into the liquid media. These sound waves create acoustic cavitations in the liquid media. The removal of these chemicals occurs through three different mechanisms, which are thermal degradation in the cavitation region, supercritical oxidation and hydroxyl radical oxidation in the interfacial or the bulk region. In the current work, ultrasound technology is employed as a mechanism of removal of several estrogen hormones. The estrogens targeted in this study were 17α-estradiol, 17β-estradiol, estrone, estriol, 17α-dihydroequilin, 17α-ethinyl-estradiol and equilin. Also included in the results are the effect of various process conditions such as variation of pH and presence of oxidizing agents on the removal of estrogens. The degradation rates for each of the estrogen compound have been investigated in clean water. The effect of ionic strength of the system and the system alkalinity are also presented.

Keywords: estrogen hormones, ultrasound, pH, alkalinity, salt, peroxide.

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1 Introduction

Estrogen hormones are an important category of emerging contaminants due to their potential endocrine disrupting effects on wildlife and human health by interfering with the reproductive system. Many reports show the presence of excess estrogen hormones [1, 2].

The major pollution source of estrogen contaminants originates from the wastewater treatment effluents as well as from the animal farms [3]. The conventional wastewater treatment processes are not designed to completely remove compounds as estrogen hormones therefore notable concentrations are often detected in the final effluent and sludge [4, 5]. Treatment techniques as ozonation, chlorination, adsorption and photo-degradation have been studied for removal of estrogen. However, these methods require off-gas treatment, adsorbent disposal/regeneration, or catalyst separation and recovery. In the recent years ultrasound has bee proved to be one of the most advanced treatment technology for destruction of toxic organic chemicals [6].

The application of ultrasound for estrogens destruction in aqueous systems has been studied in our laboratory [7, 9].

System variables such as solution pH, alkalinity, presence of salt and peroxide can influence the degradation of estrogens. In this study, the effect of the above parameters was examined for the destruction of 17α-estradiol, 17β-estradiol, 17α-dihydroequilin, 17α-ethinyl estradiol, estriol, estrone and equilin in aqueous solution.

2 Materials and methods

The estrogen hormones, minimum purities were obtained from Sigma Aldrich. All solvents, HPLC grade, and other chemicals were purchased from Fisher Scientific. Varian Bond Elute solid phase extraction (SPE) cartridges were obtained from Varian Inc. All the glassware was silanized before use [5]. A 2 kW ultrasound system (20 kHz) was used in this study.

Stock solutions of hormones were prepared in methanol using silanized amber glass volumetric flasks that were stored at 4°C. Known concentrations of reaction solutions were prepared in deionized water from the concentrated estrogen stock solutions. A fixed amount of internal standard was added to each sample after SPE extraction to account for any losses that may have occurred during sample processing. 3-O-methyl estrone was used as internal standard. The pH of the solutions was adjusted with HNO_3 and NaOH solutions.

The SPE was performed by passing 400mL sample through Varian Bond Elute C-18 cartridges at a flow rate of 5 ml/min. Prior to loading, the SPE column was activated with 3 ml of methanol, and then rinsed with 3 ml of deionized water. After the SPE extraction the cartridges were washed with 3 ml of deionized water and then eluted with 3 ml of methanol. The methanol eluent was collected into clean, silanized test tubes and dried in a Genevac centrifugal evaporator at 45°C and 12 mbar.

Derivatization: To the dried sample, 15 µl of pyridine and 65 µl of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane were added. The sample was allowed to react in a capped test tube for 15 min at 26°C. To the derivatized sample, 250 µl of toluene was added, vortexed and placed in an amber glass GC vial containing a 0.25 µl insert. The headspace free vial was placed on the GC-MS for analysis. The GC-MS analysis was performed using an Agilent 6890N GC and a 5973N MS.

3 Results and discussion

3.1 Ultrasound degradation of estrogen hormones

This study shows that ultrasound treatment can be efficient for removal of estrogen hormones as shown in Figure 1. About 60% degradation of all estrogens was observed within 30 min reaction time. 17α-dihydroequilin and equilin showed faster degradation rates compared to the rest of the compounds (data not shown here).

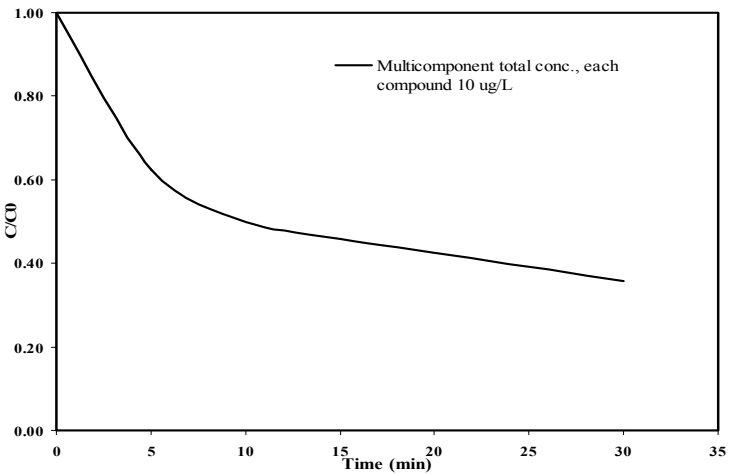

Figure 1: Ultrasound induced destruction of estrogen hormones in a multi-component system, power density 640 W/L, initial pH 7.0, individual initial concentration 10 µg/L.

3.2 Effects of alkalinity

In order to examine the mechanism of hydroxyl radical oxidation, the effects of a radical scavenger was investigated in multiple-component solutions. HCO_3^-, a well-known HO· scavenger was used in this study.

Table 1 lists the first order rate constants of all seven compounds in the presence of different dosages of HCO_3^-. It can be observed that 10 mM HCO_3^- did not have a significant effect on the degradation rate of estrogens except for

17α-dihydroequilin and equilin, which showed slower degradation rate. However further studies are required to determine if there is an optimum dosage of alkalinity that would enhance the removal of estrogens.

Table 1: Alkalinity effects on ultrasound destruction of estrogens; power density 640 W/L, initial pH 7, individual initial concentration 10 μg/L.

Analyte	First order rate constant k (min^{-1})	
	0 mM alkalinity	10 mM alkalinity
17α-estradiol	0.0396	0.0391
17β-estradiol	0.0367	0.0396
17α-dihydroequilin	0.1597	0.0659
17α-ethinyl estradiol	0.0459	0.0465
Estriol	0.0103	0.0365
Estrone	0.0342	0.0432
Equilin	0.0945	0.0537

3.3 Effect of solution pH

The destruction of estrogen hormones was studied at different solution pH. It was observed that pH variation had little or no effect on estrogen concentration. Table 2 shows the variation of degradation rate constants of estrogens with the change of the system pH. It may be observed that the degradation rate constants decrease with pH increase from acidic solution to neutral pH, but then increase with pH increase from neutral to basic pH. Increasing the solution pH to 10 significantly increased the degradation rate. This may be due to the fact that *pKa* of most of these estrogens is around 10, which means more estrogen molecules would be available in ionic form.

Table 2: pH effects on destruction rate constants; power density 640 W/L, individual initial concentration 10 μg/L.

Analyte	First order rate constant k (min^{-1})		
	pH 2	pH 7	pH 10
17α-estradiol	0.0787	0.0396	0.3219
17β-estradiol	0.0812	0.0367	0.3706
17α-dihydroequilin	0.8563	0.1597	0.3971
17α-ethinyl estradiol	0.0836	0.0459	0.2816
Estriol	0.0446	0.0103	0.3893
Estrone	0.0596	0.0342	0.3828
Equilin	0.272	0.0945	0.3817

3.4 Effects of dissolved salt

The presence of dissolved salts increases the ionic strength and enhances the diffusion of estrogens to the cavity interface. Enhancement in degradation rate is therefore expected in the presence of dissolved salts. Seymour and Gupta [8] have reported large salt-induced enhancements on ultrasound oxidation of chlorobenzene, *p*-ethylphenol and phenol.

No change in estrogens concentration was observed in a blank control test (no ultrasound) up to 10 g/L of NaCl. Table 2 shows the destruction rate constants of estrogens compounds at pH 7 in the presence of 0.1 g/L and 10 g/L NaCl. A significant increase in degradation rate of most estrogens was observed with increase in salt concentration from 0.1 g/L to 10g/L (data not shown here) of salt. However, the degradation of 17α-dihydroequilin and equilin was not significantly affected by presence of salt.

Table 3: Effects of presence of salt (NaCl) on ultrasound degradation of estrogens at pH 7; power density 640 W/L, individual initial concentration 10 μg/L.

Analyte	First order rate constant *k* (1/min)	
	0 g/L salt	0.1 g/L salt
17α-estradiol	0.0396	0.0581
17β-estradiol	0.0367	0.0566
17α-dihydroequilin	0.1597	0.1228
17α-ethinyl estradiol	0.0459	0.0502
Estriol	0.0103	0.0379
Estrone	0.0342	0.0572
Equilin	0.0945	0.0838

Table 4: Effects of presence of H_2O_2 on ultrasound degradation of estrogens at pH 2; power density 640 W/L, individual initial concentration 10 μg/L.

Analyte	First order rate constant *k* (min^{-1})	
	pH 2, 0 mg/LH_2O_2	pH 2, 100 mg/L H_2O_2
17α-estradiol	0.0787	0.0925
17β-estradiol	0.0812	0.0883
17α-dihydroequilin	0.8563	0.786
17α-ethinyl estradiol	0.0836	0.0907
Estriol	0.0446	0.0595
Estrone	0.0596	0.082
Equilin	0.272	0.4458

3.5 Effect of H_2O_2

The enhancement in the degradation of pollutants in the presence of hydrogen peroxide can be attributed to the increased production of hydroxyl radicals and has been well documented [10].

No effect of H_2O_2 on estrogens concentration was observed in blank control tests in the absence of ultrasound at pH 2. Under ultrasound irradiation, significant enhancement on degradation of estrogen hormones in the presence of 100mg/L of H_2O_2 was observed at pH 2, as shown in Table 3. Further H_2O_2 concentrations need to be considered to optimize the dosage requirement. Inhibition on the sonolytic degradation by addition of H_2O_2 has been reported by Song *et al* [11].

4 Conclusions

This study shows that ultrasound treatment can be effectively used for the degradation of estrogen hormones. 60% degradation of all estrogens was observed within 30 min reaction time. Acidic system pH both showed increase in estrogen degradation rate. At pH 10, the average first order degradation rate constant was 0.36 min^{-1} or 13 min retention time for 99% removal of estrogens.

While 10 mM alkalinity did not have a significant effect on the degradation, higher alkalinity of the solution may inhibit the degradation rate. The presence of salt is also important for the estrogens degradation. A significant increase in degradation rate of most estrogens was observed with increase in salt concentration from 0.1 g/L to 10g/L of salt. Oxidizing agents as hydrogen peroxide can also play a significant role in the treatment process. Our study shows enhancement in the degradation of estrogen hormones in the presence of 100mg/L of H_2O_2 at pH 2.

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