Comparison of Fenton’s oxidation and ozonation for removal of estrogens

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ABSTRACT

This study compares efficiency of Fenton’s oxidation and ozonation of 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) as two possible processes for removal of estrogens from aqueous solutions. The effectiveness of Fenton’s oxidative removal was studied at different ratios of reagents Fe²⁺:H₂O₂ (1:0.5; 1:10; 1:20; 1:33), where with some molar ratios up to 100% removal of E2 and EE2 was achieved in the first few minutes of reaction. The best molar ratio for E2 (17β-estradiol) removal was 1:33, while in the case of EE2 the most efficient one was 1:20 ratio. Ozonation was much faster, because complete removal of estrogens was achieved in 30 seconds (pH ≈ 6), but the time of ozonation was extended up to 60 minutes trying to decompose formed by-products, expressing estrogenic activity, detected by YES (Yeast Estrogen Screening) assay. The obtained results showed that the removal efficiency of estrogens from waters should be assessed by a combination of chemical analyses and bioassay.

Key words | E2 (17β-estradiol), EE2 (17α-ethinylestradiol), Endocrine Disrupting Chemicals (EDCs), Fenton’s Oxidation, ozonation, YES assay

INTRODUCTION

There is a growing concern about release of some micropollutants, known as Endocrine Disrupting Chemicals (EDCs), from municipal and industrial wastewaters to the aquatic environment. Many studies reported their potential harmful impact on human and non-target aquatic organisms. EDCs have the capacity to modulate the endocrine system and could affect reproductive health of wildlife and even humans (Harrison et al. 1997). EDCs are widespread, and even some remote areas have been contaminated (Martin 2002; Priddle 2002). Research published in the literature showed that endocrine disrupters can increase the incidence of different types of cancers as well as they can reduce fertility and spermatozoids number (Harrison et al. 1997; Coleman et al. 2005). The effects of these micropollutants on organisms do not depend only on their concentrations, but also upon others factors, such as lipoflicity, persistence, bioaccumulation potential, exposure time and mechanisms of biotransformation and/or elimination. Some substances in the environment undergo (bio)degradation, resulting in metabolites or by-products more harmful than the original compounds (Esplugas et al. 2007). In surface waters, different types of estrogens are present at a level from picograms per liter to nanograms per liter (Baronti et al. 2000; Kuch & Ballschmitter 2001).

Natural and synthetic EDCs are released into the environment by humans, animals and industry, mainly through sewage treatment systems before reaching the receiving bodies (soil, surface water, sediments and ground water) (Liu et al. 2009). E2 (17β-estradiol) is natural estrogen excreted by humans and animals, while the synthetic estrogen EE2 (17α-ethinylestradiol) is widely used in combination with other steroid hormones in oral contraceptives and in the contraceptive patch. E2 and EE2 are continuously discharged into receiving water bodies, because they are not completely removed in conventional biological wastewater
treatment. Thus new methods are needed to upgrade conventional municipal treatment plant to remove them from wastewaters prior to releasing into watercourses. Three possible approaches are widely considered: 1) physical removal (adsorption, filtration); 2) biodegradation (aerobic and usually less effective anaerobic); and 3) Advanced Oxidation Processes (AOPs). The advantage of AOPs in comparison to other mentioned methods is complete destruction of pollutants to carbon dioxide, water and inorganic salts if desired or they can only be transformed partially to less hazardous ones (Prousèk 1996). Pollutants are not only transported from one medium to another (Liu et al. 2009). AOPs although using different reaction systems, are all characterized by the same chemical feature: production of OH radicals, which have a high electrochemical oxidant potential (Huber et al. 2003; Ternes et al. 2003). Most often used processes are Fenton’s oxidation, wet air oxidation and ozonation.

Fenton’s processes are known to be very effective in the removal of many hazardous organic pollutants from wastewaters. The Fenton's reaction causes the dissociation of the oxidant and the formation of highly reactive hydroxyl radicals that attack and destroy the organic pollutants (Neyens & Baeyens 2003). Fenton’s process is efficient only in the pH range of 2.0 to 4.0 and it is usually the most efficient at around 2.5 to 3.0 but rather inefficient in the pH range of natural waters. Required reagents for Fenton’s process are Fe(II) salts, activated by the addition of H₂O₂ (Simon 2004). Weakness of the Fenton’s process is formation of waste sludge, containing various iron salts and hydroxyls. Amount of the produced sludge is dependent upon the ratio and the volume of added reagents.

Ozone is a strong oxidant and has the standard electrode potential $E_0 = 2.07$ V in acidic medium and 1.27 V in alkaline medium, respectively. Theoretically, it can provide sufficient energy in aqueous solution to degrade the persistent chemical substances (Zhang et al. 2006). Ozone can react directly with a compound or it can produce hydroxyl radicals which then react with a compound (Gottschalk et al. 2000). The main effects of ozonation are decolorization, elimination of taste and odours, degradation of organics and disinfection. Some research also showed that ozonation process could be finally converted to CO₂ and H₂O in special conditions (Eckernfelder et al. 1991). Moreover, excess ozone compared to other oxidants, could be decomposed to oxygen without harmful residue (Konsowa 2003). A major limitation of the ozonation process is the relatively high costs of ozone generation process coupled with very short half-life period of ozone. Thus, ozone needs to be generated always at site (Gogate & Pandit 2004). However, ozone dosages that are economically viable do not always promote the complete mineralization of the micropollutants. Therefore, it is very important to know the by-products formed during oxidation, as well as to evaluate their effects (Blä et al. 2007).

The objective of this work was to study the feasibility of Fenton’s oxidation and ozonation for degradation and removal of E₂ (17β-estradiol) and EE₂ (17α-ethinylestradiol) from aqueous solutions. The YES assay was used to compare the estrogenicity of E₂ and EE₂ before and after Fenton’s oxidation and ozonation, while changes in their concentrations were monitored by SPME/GC-MS, which has also been used for identification of metabolites in certain cases.

**MATERIALS AND METHODS**

Oxidation experiments were conducted with natural E₂ (17β-estradiol) and synthetic estrogens EE₂ (17α-ethinylestradiol), obtained by Sigma. Stock solutions of E₂ (0.272 mg L⁻¹) and EE₂ (0.296 mg L⁻¹) were prepared by dissolving appropriate amount of test substance (E₂/EE₂) in ethanol (96%, 1 mL). The concentrations of E₂ and EE₂ selected for the oxidation experiments were high in comparison to average expected environmental concentrations. Due to the complexity of Fenton’s reaction higher concentrations were applied in presented study to obtain more reliable data and to lessen the impact of the analytical preparation of the samples. For Fenton’s oxidation experiments FeSO₄ · 7H₂O (Fluka Analytical, p.a.) and 30% w/v H₂O₂ (Belinka) were used while 1 M HCl (Merck) and 5 M NaOH (Merck) were prepared for pH value adjustments in all oxidation experiments.

**Fenton’s oxidation experiments**

Fenton’s oxidation experiments were carried out at ambient temperature (22±2°C). Investigated ratios of reagents (Fe²⁺:H₂O₂) were 0.0003 M:0.01 M (1:33), 0.0005 M:0.01 M (1:20), 0.0005 M:0.005 M (1:10), 0.001 M:0.01 M (1:10) and 0.001 M:0.005 M (1:20) for E₂ and EE₂ removal. Solution of the test substance E₂ (0.272 mg L⁻¹) or EE₂ (0.296 mg L⁻¹) was put in a baker and stirred (N = 200 rpm). Then pH was adjusted to attain requested pH. The calculated Fe²⁺ dosage was achieved by adding the necessary amount of solid FeSO₄ · 7H₂O into a beaker and waiting for a few minutes to be dissolved. A known volume of 30% w/v H₂O₂ solution was added in a single step. At different time periods (0, 5, 10, 30, 50, 70 and 90 minutes) samples were redrawn and pH value was adjusted with NaOH above 12.0 to stop the reaction. Sample was boiled afterwards for
about 10 minutes to remove the rest of hydrogen peroxide (San Sebastian Martinez et al. 2003). Finally samples were cooled down and filtered through a black ribbon. After filtering of samples GC-MS analyses were performed to determine the concentration of E2 or EE2 after Fenton’s oxidation. To check the impact of boiling, the solution to determine the concentration of E2 or EE2 after Fenton’s filtering of samples GC-MS analyses were performed at 0.5 L/C1.

External circulation of reaction mixture was maintained 0.2 m high with 0.09 m in diameter, its effective volume was 0.272 mg·L−1 of E2 was boiled and cooled down afterwards. Change in the concentration was 3% thus it was concluded, that performance of Fenton’s procedure is appropriate.

Ozonation experiments

Ozonation experiments were carried out in lab-scale ozonation system, in bubble column reactor. Initial concentrations of the test substance were for E2 – 17β-estradiol (0.272 mg·L−1) and for EE2 – 17α-ethinylestradiol (0.296 mg·L−1). The system was operated in the batch mode. Ozonation reactor was 0.2 m high with 0.09 m in diameter, its effective volume was 0.25 L. External circulation of reaction mixture was maintained at 0.5 L·min⁻¹ by membrane pump. For ozone production the Lifetech ozone generator was used with the maximum ozone production of 5 L·h⁻¹. Continuous flow of oxygen at 20 L·h⁻¹ was applied for generation of ozone. Ozonation trials were carried out at 50% of the power maximum of ozone generator. For ozonation different time periods (0, 0.5, 1, 5, 10, 20 and 60 minutes) were used. Measurements of input and output ozone concentrations in gas phase were run out. Average ozone inputs were varied from 0 to 100 g O₃·Nm⁻³.

Analytical method (SPME/GC-MS)

18 mL of each filtered sample was transferred directly to the 20 mL glass vial to perform SPME/GC-MS analysis. SPME/GC-MS analysis was performed on GC Agilent Technologies 6890 coupled with MSD 5730 detector. The Gerstel MPS autosampler was used to allow automated SPME analysis. Samples were exposed to micro extraction procedure (SPME) using SPME fibre (Supelco, Sigma-Aldrich) coated with polyacrylate (PA) 85 μm (Eisert & Levens 1996; Brondi & Lancas 2005). The PA fibre was conditioned in the hot injector of the gas chromatograph according to instructions provided by the supplier. The samples were incubated at 55°C for 2 min. Adsorption of compounds from water samples on the PA 85 μm fibre was carried out by stirring the vial on the agitator for 30 min at 55°C and the stirring speed of 250 rpm (Brondi & Lancas 2005). After adsorption the PA 85 μm fibre was transferred to the injector port where the thermal desorption at 250°C occurred. Compounds were separated on the gas chromatograph equipped with a split–splitless injector, operating in the splitless mode and using a 30 m length, 0.25 mm I.D., 0.25 μm film thickness HP-5 MS (5% phenylmethylpolysiloxane, Agilent) capillary column. Helium was used as carrier gas with a flow rate of 1.1 ml min⁻¹. The initial oven temperature was set at 80°C with an initial 4 min hold during the desorption step, followed by a programmed temperature ramp 15°C·min⁻¹ up to the final temperature of 300°C, where it was held for another 10 min. MS monitoring conditions were set as follows: transfer line temperature was 280°C and detector voltage was 350 V. The MS signal was collected over 50–300 m·z⁻¹. Electron impact ionisation in the positive mode and scan acquisition mode were used.

YES assay

The estrogenic activity of E2 and EE2 aqueous samples after Fenton’s oxidation and ozonation was determined by yeast estrogen screen assay (YES assay), using a genetically modified yeast strain Saccharomyces cerevisiae BJ1991, which was developed in the Genetics Department at Glaxo coorporation under the guidance of Prof. Sumpter. When exposed to estrogens or xenoestrogens, genetically modified yeasts produce enzyme β-galactosidase, which is able to convert yellow substrate chlorophenol red-β-D-galactopyranoside (CPRG) into a red product.

YES assay was performed according to the method of Routledge & Sumpter (1996). Appropriate volume of samples after SPE procedure, dissolved in methanol, were transferred in four replicates to 96-well optically flat-bottom microtiter plates (TPP, Switzerland) in the sterile conditions and allowed to dry. After the methanol evaporated, 200 μL of the assay medium was transferred to each well. The assay medium consisted of the yeast cell suspension and substrate CPRG in the growth medium. Natural estrogen hormone 17β-estradiol was used as positive control, as it best binds to estrogen receptor, natural androgen hormone testosterone, which does not bind to estrogen receptor, as negative control and yeast exposed to the growth medium with CPRG as blank control. The microtiter plates with samples and assay medium were shaken vigorously for 3 min on a microtiter plate shaker prior to incubation at 34°C. After 48–52 hours incubation, the optical density at 575 nm, for colour change of degraded substrate CPRG, and optical density at 620 nm for turbidity, as indicator of yeast growth, were measured. The estrogenic activity (EA) was expressed as the activity of enzyme β-galactosidase using equation adopted by Fent et al. (2006). A (575 nm) sample represents the samples absorbance measured at 575 nm, A(620 nm) sample represents the samples
absorbance measured at 620 nm and $A(620\text{nm})_{\text{blank}}$ represents the turbidity of the yeasts in the assay medium with added CPRG.

\[
\text{Activity of } \beta - \text{galactosidase}^* (EA) = A (575\text{nm})_{\text{sample}} - \left[A (620\text{nm})_{\text{sample}} - A (620\text{nm})_{\text{blank}}\right]
\]  

Estrogenic activity of treated samples after Fenton’s oxidation and ozonation was compared to initial sample before treatment. % of estrogenic activity remained in treated samples was calculated in comparison to the interval of estrogenic activity determined by the average value of blank control and the maximal value of estrogen activity, which is the average value of the initial samples.

**RESULTS AND DISCUSSION**

**Fenton’s oxidation of E2 and EE2**

We have studied the removal efficiency of E2 – 17β-estradiol (0.272 mg · L⁻¹) and EE2 – 17α-ethinylestradiol (0.296 mg · L⁻¹) by Fenton’s oxidation at four different molar ratios of Fenton’s reagents Fe²⁺ and H₂O₂ (1:0.5, 1:10, 1:20 and 1:33). Two 1:10 molar ratios were investigated, the first one with higher concentrations of reagents and the second one with lower ones, to find the optimal ratio with minimal consumption of chemicals and minimal waste sludge production.

Removal efficiency of Fenton’s oxidation E2 (0.272 mg · L⁻¹, Figure 1) or EE2 (0.296 mg · L⁻¹, Figure 2) increased rapidly with contact time and with higher molar ratio. In all of the cases investigated natural E2 and synthetic EE2 were successfully removed in the first ten minutes of reaction (Figures 1 and 2), except at Fenton’s oxidation of EE2 at higher molar ratio of Fe²⁺:H₂O₂ = 0.0003 M:0.01 M (1:33) and at lower molar ratio of Fe²⁺:H₂O₂ = 0.001 M:0.0005 M (1:20), where at the end of reaction 48% and 46% of estrogens remained, respectively. 1:0.5 molar ratio could be un-efficient due to the lack of peroxide and thus lower formation of active hydroxyl radicals (Gogate & Pandit 2004).

The important withdrawal of Fenton’s oxidation is the formation of waste sludge, containing various iron salts and hydroxyls. It constitutes a serious problem regarding further disposal (hazardous waste). Mass of the sludge formed is dependent upon the ratio and the volume of added reagents, Fe²⁺ and H₂O₂. Lower amounts of added reagent or higher molar ratios resulted in lower concentration – amount of formed waste sludge (Table 1). If above mentioned factors are considered also from the economic perspective, it is more convenient to use lower amounts of reagents which also bring less residual sludge. So the best molar ratio for E2 (17β-estradiol) removal was Fe²⁺:H₂O₂ = 0.0005 M:0.01 M (1:33), while in the case of EE2 the most efficient one was Fe²⁺:H₂O₂ = 0.0005 M:0.001 M (1:20) ratio. The 1:33 ratio was selected as the optimal one for E2 because of 99% removal obtained in a few minutes as in the case of 1:20 ratio, but 1:33 ratio assured the lowest consumption of chemicals (hydrogen peroxide) and the lowest production of waste sludge (0.06 g · L⁻¹ in comparison to 0.12 g · L⁻¹ at 1:20 ratio, Table 1). The selection was later confirmed by YES assay.

Due to the presence of the waste ferrous sludge it could come to adsorption of investigated natural or synthetic hormones on sludge, which sometimes makes it impossible to determine the actual extent of degradation reliably (Nakrst et al. 2010).

**Ozonation of E2 and EE2**

Ozonation appeared to be very efficient for removal of E2 (0.272 mg · L⁻¹, Figure 3) and EE2 (0.296 mg · L⁻¹, Figure 4)
from aqueous solutions. Complete removal of estrogens was fast achieved for both cases just in 1 minute. In spite of fast degradation of estrogens, the time of ozonation was extended because formation of by-products was expected. Longer contact time should allow complete degradation of estrogens.

Ozonation was carried out at pH ≈ 6. At lower pHs oxidation occurs via molecular O₃, which is selective and reacts rapidly with specific functional groups. An increase in pH favours the formation of OH radicals which are less selective oxidant regarding organic compounds (Bila et al. 2007).

### Estrogenic activity

The best results of Fenton’s oxidation of E2 and EE2 were achieved when molar ratio Fe²⁺ : H₂O₂ = 0.0003 M:0.01 M (1:33) and 0.0005 M:0.01 M (1:20) were used, respectively. Estrogenic activity decreased in both cases with reaction time, but it was not lost completely during Fenton’s oxidation (Figure 5 and 6). Estrogenic activity of E2 decreased more rapidly than in a case of EE2. A decrease of E2 was almost immediate (70% of removal) and estrogenic activity did not change significantly during next 60 minutes of oxidation (Figure 5). The estrogenic activity of EE2 decreased continuously and reached the final value (57% of removal) in 10 minutes (Figure 6). Remained estrogenic activity could be due to formation of by-products, which were not identified, but some low, non-determined peaks appeared in GC-MS spectrogram.

Oxidation of organic micropollutant by ozone is an efficient process for compounds with functional groups.

### Table 1

<table>
<thead>
<tr>
<th>Substance</th>
<th>Ratio of Fenton’s Reagents</th>
<th>C of waste sludge (g L⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>E₂ (0.272 mg L⁻¹)</td>
<td>0.0003 M:0.01 M (1:33)</td>
<td>0.06</td>
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<td>0.0005 M:0.01 M (1:20)</td>
<td>0.12</td>
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<td></td>
<td>0.0005 M:0.005 M (1:10)</td>
<td>0.11</td>
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<td></td>
<td>0.001 M:0.01 M (1:10)</td>
<td>0.16</td>
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<tr>
<td></td>
<td>0.001 M:0.0005 M (1:0.5)</td>
<td>0.37</td>
</tr>
<tr>
<td>EE₂ (0.296 mg L⁻¹)</td>
<td>0.0005 M:0.01 M (1:33)</td>
<td>0.15</td>
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<td></td>
<td>0.0005 M:0.01 M (1:20)</td>
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<td>0.001 M:0.01 M (1:10)</td>
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<td>0.001 M:0.0005 M (1:0.5)</td>
<td>0.24</td>
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such as amino groups, activated aromatic systems (i.e., phenolics) or double bonds (von Gunten 2003). For that reason ozonation can be efficient for removal of estrogenic activity related to E2 and EE2. In spite of complete degradation of E2 (0.272 mg L⁻¹) and EE2 (0.296 mg L⁻¹) during ozonation, confirmed by GC-MS measurements, estrogenic activity was detected even after one hour of ozonation (Figure 7 and 8); only 41% and 48% of removal of estrogenic activity were detected for E2 and EE2, respectively. Remaining estrogenic activity could be a consequence of formation of by-products during the ozonation process by oxidation via OH radical. Some authors have proposed and identified by-products formed during the ozonation of E2 and EE2 (Huber et al. 2004; Bila et al. 2007). GC-MS system was used to determine benzofenon and 7.9-Di-tet-butyl-1-oksaspiro(4.5)deka-6,9-dien-2.8-dion as possible by-products of 17β-estradiol oxidation, where similarities obtained were 88.6% and 97.0%, respectively. In the case of 17α-ethinylestradiol, dibutyl italate with 93.0% similarities was identified.

**CONCLUSIONS**

Fenton’s oxidation and ozonation were found to be very effective for removal of E2 and EE2 from aqueous samples, which was confirmed by the GC-MS analyses. In Fenton’s oxidation completely removal of E2 and EE2 was achieved at particular molar ratios of Fe²⁺ and H₂O₂ reagents in the first ten minutes of reaction. Ozonation also successfully removed estrogens (E2 and EE2) from aqueous solutions in 30 seconds. However, the estrogenic activity of the treated samples, measured by the YES bioassay, was still present in the samples at the end of experiments. During Fenton’s oxidation and ozonation by-products are formed and they are probably responsible for the remaining estrogenic activity. Experiments confirmed, that only combination of chemical analyses and bioassays assure relevant determination of oxidation efficiency. In spite of slower degradation of estrogens with Fenton’s oxidation, this method is still more appropriate than ozonation, because of better removal of estrogenic activity.

**REFERENCES**


