Scientific paper

Feasibility of Fenton's Oxidation for Removal of Estrogens from Aqueous Solutions

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Received: 14-09-2009

Dedicated to the memory of the late Prof. Dr. Valentin Koloini

Abstract

Presented research has been focused on the effectiveness of degradation of natural (17β-estradiol – E2) and synthetic (17α-ethinylestradiol – EE2) hormones with Fenton's oxidation. Numerous studies have demonstrated, that the impact of hormones on organisms was shown already at environmental concentrations close to 1 pg L^{-1}, so their effective removal from wastewaters is important. Fenton's oxidation is very efficient in terms of oxidation of organics, while its main withdrawal is the formation of waste sludge, leading to adsorption of organics, which sometimes makes it impossible to reliably value the extent of actual degradation. The ratio among degradation and adsorption of estrogens on sludge during oxidative treatment was determined as a part of our study. It depends upon the molar ratio of reagents Fe^{2+} and H_2O_2 (1:0.5, 1:10; 1:20; 1:33), reaction time and initial concentrations of the investigated estrogens E2 (0.279/27.9 mg L^{-1}) and EE2 (0.296/29.6 mg L^{-1}). The most efficient removal of E2 was achieved at 1:33 molar ratio of reagents (100%, 26–50% adsorption) while EE2 removed efficiently at 1:10 and 1:33 ratios (99–100%, 20–76% adsorption). It was confirmed, that adsorption plays an important role in Fenton's oxidative treatment of estrogens. It is prevailing process at higher pHs, while at lower ones (pH = 3.0–5.0) oxidation is dominant, because it is not limited by mass transport.

Keywords: Adsorption; Degradation; E2 (17β-estradiol); EE2 (17α-ethinylestradiol); Endocrine Disrupting Chemicals (EDCs), Fenton's Oxidation.

1. Introduction

Endocrine disrupting chemicals (EDCs) have the capacity to modulate the endocrine system and could affect reproductive health of wildlife and even humans.[1] EDCs are widespread, and even some remote areas have been contaminated.[2, 3] Main sources of EDCs in natural environment are conventional wastewater treatment plants, unable to effectively remove EDCs from municipal and industrial wastewaters. The effects, associated with the presence of EDCs in environment are: 1) increase in the breakage of eggs of birds, fishes and turtles; 2) feminization of male fish; 3) different malfunctions in the reproductive system in fishes, reptiles, birds and mammals; and 4) changes in the immunological system of marine mammals. In some cases, these effects can lead to decline in population. The effects of EDCs in human beings reported so far have been: 1) reduction of the amount of sperm; 2) increase of the incidence of breast, testicle and prostate cancers; and 3) the endometriosis.[4] In surface waters, different types of estrogens are present at a level from pictograms per litre to nanograms per liter.[5, 6] 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 enter in the aquatic environment through conventional wastewater treatment, because they are not completely removed there.

E2 (17β-estradiol) is by far the most endocrine disrupting chemical. The concentration of E2 detected in surface water is usually near or below 2 ng L^{-1}, while in effluents concentration of E2[7] ranged from below detec-

tion limit to 64 ng L^{-1}. It has been reported[8] to exhibit some estrogenic activity for human estrogen receptors at 41 ng L^{-1} and even to cause vitellogenin production in male fish at environmental concentration of 1 ng L^{-1}. EE2 (17α-ethinylestradiol) is also one of more potent estrogens present and has been shown to induce the synthesis of the yolk-precursor protein vitellogenin in male fish at a concentration[9] as low as 0.1 ng L^{-1}. E2 (17β-estradiol) and EE2 (17α-ethinylestradiol) are difficult to remove from wastewaters by conventional treatment that is why new methods are needed for removing them from wastewaters prior to their release into receiving waters. Three possible approaches could be 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 actual destruction of pollutants to less hazardous ones, not only their transfer from one medium to another.[10]

All advanced oxidation processes (AOPs) are characterized by a common chemical feature: the capability of exploiting the high reactivity of $OH^{\cdot}$ radicals. The oxidation potential of oxidizers is $FeO_4^{2-} > O_3 > S_2O_4^{2-} > H_2O_2 > Cl_2 > ClO_2$ thus selection of Fenton's oxidation with application of Fe^{2+} and H_2O_2 is evident.[11] These processes are capable of converting the organic contaminant completely to carbon dioxide, water and inorganic salts if desired and the chemicals used in the process decompose to the harmless byproducts.[12] 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.[12] The hydroxyl radical generating reaction of Fenton's reagent is written as follows /1/[12]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \qquad /1/$$

The products of the Fenton's reaction are ferric ion, hydroxyl radical ($OH^{\cdot}$) and hydroxide ion (OH^-). Hydrogen peroxide and ferrous ions are usually more stable in a strong acid. But if there is aqueous system containing an organic substrate (RH) and excess of ferrous ions (Fe^{2+}) in strong acidic conditions, different complex reaction could occur. Formed Fe^{3+} can react with H_2O_2 in the so-called Fenton-like reactions (Reactions /2/-/4/) regenerating Fe^{2+} and thus supporting the Fenton process[12]:

$$HO^{\cdot} + Fe^{2+} \rightarrow Fe^{3+} + OH^- \qquad /2/$$

$$HO^{\cdot} + RH \rightarrow R^{\cdot} + H_2O \qquad /3/$$

$$Fe^{3+} + R^{\cdot} \rightarrow Fe^{2+} + R^+ \qquad /4/$$

Theoretically, all organic compounds, containing hydrogen (RH) could be oxidised in Fenton's reaction, but the process is efficient only in the pH range of 2.0–4.0 and it is usually the most efficient at around 2.5–3.0 but rather inefficient in the pH range of where most natural waters belong to (pH 5.0–9.0).[12,13] Required reagents for Fenton's process are Fe^{2+} salts, activated by the addition of H_2O_2. The reagents are inexpensive, readily available, easy to store, save and easy to handle, and process can be performed at ambient temperature. Hydrogen peroxide brings no lasting environmental threat since it readily decomposes to water and oxygen. Iron is highly abundant and may be naturally occurring in the system being treated and is non-toxic whereas many of other potentially suitable metallic catalysis are toxic to varying degrees.[14] Weakness of the Fenton's process is formation of waste sludge, containing various iron salts and hydroxyls. Mass of the sludge is dependent upon the ratio and the volume of added reagents.

Adsorption is also suitable method for removal of estrogens, but they are not destroyed, only transported into another phase.[10] It generally expresses high removal efficiency that enables removal of toxic organic compounds. It has been observed to play important role during biological removal of estrogens, where significant amount of EDCs could be adsorbed to bio solids (up to 75% for steroid estrogens) and possible biotransformations could occur.[10,15] The same problem of adsorption, which has not been investigated by now, could probably occur in the case of waste sludge, formed during Fenton's oxidation. It results in competition of adsorption and oxidation processes leading to less effective destruction of pollution.

The adsorption isotherm is the most extensively used method for representing the equilibrium states of an adsorption system. It can give useful information regarding the adsorbate, the adsorbent, and the adsorption process. It helps in the determination of the surface area of the adsorbent, the volume of the pores, their size distribution, and the heat of adsorption.[16] The Langmuir isotherm equation is the first theoretically developed adsorption isotherm. Many of the equations proposed later fitting the experimental data over a wide range are either based on this equation, or these equations have been developed using the Langmuir concept. Langmuir assumed that adsorption surface is saturated when a monolayer has been adsorbed.[16] Langmuir equation under constant temperature is presented[17] in equations (1) and (2):

$$q_e = \frac{q_e K_L C_e}{1 + K_L C_e} \qquad (1)$$

or

$$\frac{C_e}{q_e} = \frac{1}{q_e K_L} + \frac{C_e}{K_L} \qquad (2)$$

where q_e quantity of adsorbate per unit of adsorbent [mg g^{-1}]
C_e equilibrium concentration of adsorbate in solution [mg L^{-1}]
K_L Langmuir equilibrium constant of adsorption [L g^{-1}]

The Freundlich isotherm is a limiting form of the Langmuir isotherm and is an empirical equation which gives an accurate description of adsorption of organics from aquatic medium.[16] Freundlich equation under constant temperature is written[17] as equations (3) and (4):

$$q_e = KC_e^{\frac{1}{n}} \quad (3)$$

$$logq_e = logK_F + (\tfrac{1}{n})\, logC_e \quad (4)$$

where K_F Freundlich adsorption constant [mg g^{-1}]
n empirical constant
q_e quantity of adsorbate per unit of adsorbent [mg g^{-1}]
C_e equilibrium concentration of adsorbate in solution [mg L^{-1}]

A plot of *log* q_e against *log* gives a straight line with an intercept on the ordinate axis. The values of n and K_F can be obtained from the slope and the intercept of the linear plot. High concentration of adsorbent lead to surface saturation of the adsorbate, while Freundlich equation declares unlimited rising amount of substance with growth of concentration.[16]

The aim of our work was to assess the feasibility of Fenton's oxidation for removal of E2 (17β-estradiol) and EE2 (17α-ethinylestradiol) from aquatic solutions at different concentrations and to determine the relative importance of adsorption in comparison to degradation processes.

2. Materials and Methods

Oxidation experiments were conducted with natural and synthetic estrogens E2 (17β-estradiol) and EE2 (17α-ethinylestradiol), respectively. The effectiveness of oxidative removal was studied at different ratios of reagents (Fe^{2+}:H_2O_2) as well as adsorption capacity of sludge, formed during oxidative treatment has been investigated.

The chemicals used in experiments were $FeSO_4 \cdot 7H_2O$ (Fluka Analytical, p.a.) as catalyst, 30% w/v H_2O_2 (Belinka) as oxidant, natural hormone 17β-estradiol (E2, Sigma, p.a.), synthetic hormone 17α-ethinylestradiol (EE2, Sigma, p.a.) and 1M HCl (Merck) or 5M NaOH (Merck) for pH value adjustments. Stock solutions of E2 (0.272 and 27.2 mg L^{-1}) and EE2 (0.296 and 29.6 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 and adsorption experiments were high in comparison to average expected environmental concentrations.[8,9] Due to the complexity of Fenton's reaction higher concentrations were applied in this particular study to assure more reliable data and to lessen the impact of the analytical preparation of the samples.

2. 1. Fenton's Oxidation Experiments

Fenton's oxidation experiments were carried out at ambient temperature (22 ± 2 °C) according to the following sequential steps:

1) Solution of the test substance E2 – 17β-estradiol (0.272/27.2 mg L^{-1}) or EE2 – 17α-ethinylestradiol (0.296/ 29.6 mg L^{-1}) was put in a baker and stirred (N = 200 rpm).
2) pH was adjusted to attain requested pH (3.0/ 5.0/8.0).
3) The calculated Fe^{2+} dosage was achieved by adding the necessary amount of solid $FeSO_4 \cdot 7H_2O$ into a beaker and waiting for a few minutes to be dissolved.
4) A known volume of 30% w/v H_2O_2 solution was added in a single step.
5) 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. Finally samples were cooled down and filtered through black ribbon.
6) In filtered samples GC-MS analyses were performed to determine the concentration of E2 or EE2 after Fenton's oxidation.

Investigated ratios of reagents (Fe^{2+}:H_2O_2) were 0.0003M:0.01M (1:33), 0.0005M:0.01M (1:20), 0.0005M:0.005M (1:10), 0.001M:0.01M (1:10) and 0.001M:0.0005M (1:0.5) for lower concentrations of E2 and EE2 but 0.03M:1M (1:33), 0.05M:1M (1:20), 0.1M:1M (1:10), 0.2M:2M (1:10) and 1M:0.5M (1:0.5) for higher concentrations of estrogens.

2. 2. Adsorption Experiments

For adsorption experiments waste sludge was prepared separately under uniform conditions. 55.6 g of $FeSO_4 \cdot 7H_2O$ and 204 mL of 30% w/v H_2O_2 were added in 0.5 L of distilled water. Formed ferrous sludge was neutralized with 5M NaOH, boiled and settled down. After 30 minutes liquid was poured away while remained sludge was dried in the dryer at 105 ± 1 °C for 2 days till the constant mass was obtained. Dry sludge was homogenized and sieved (0.2 mm) to assure comparable size of sludge particles.

Adsorption experiments were carried out with both substances, E2 (17β-estradiol) and EE2 (17α-ethinylestradiol). E2 or EE2 solutions were prepared by dissolving test substance in 1 mL ethanol and 500 mL distilled water and stirred (N = 200 rpm). Initial concentrations of E2 were varied from 0.272 to 27.2 mg L^{-1} and EE2 from 0.296 mg L^{-1} to 29.6 mg L^{-}1. Prepared solution was divided in up to twelve beakers where it was mixed under the same regime. The influence of contact time, pH, the initial concentration of adsorbent (waste sludge) and the concentration of absorbate (E2/EE2) were investigated. Selected pH was adjusted with 1M HCl or 5M NaOH and measured by a digital pH-meter (Hanna). All of the samples

from adsorption experiments were filtered through a black ribbon before GC-MS analyses.

2. 2. 1. Effect of Contact Time

The effect of contact time to adsorption process was studied in 2000 mL beakers at ambient temperature (22 ± 2 °C). A stock solution of E2/EE2 was diluted with 1 mL ethanol in distilled water to obtain 1000 mL of E2 (0.272 mg L^{-1}) or EE2 (0.296 mg L^{-1}) solution and prepared waste sludge (adsorbent) with concentration of 0.1 g L^{-1} was added afterwards. The selection of concentrations of waste sludge was based on average concentration of sludge, formed during Fenton's oxidation at low concentration of E2 or EE2 (0.272 or 0.296 mg L^{-1}, Chapter 3.1.1). Reaction was carried out without any pH adjustment. During 2 hours of mixing, samples were redrawn at the time of 0, 5, 10, 30, 60, 90 and 120 minutes and E2/EE2 concentrations determined by GC-MS analysis.

2. 2. 2. Effect of Adsorbent Dose

The study on the effect of adsorbent dose (waste sludge) to adsorption efficiency was performed at ambient temperature (22 ± 2 °C) without any pH adjustment. Experiments were performed using two concentrations of E2 (17β-estradiol: 0.272/27.2 mg L^{-1}) and EE2 (17α-ethinylestradiol: 0.296/29.6 mg L^{-1}), respectively. The sample volume was 50 mL. Adsorbent doses were varied from 0.0 g L^{-1} to 0.5 g L^{-1} for lower concentration of E2 and EE2, and from 0.0 g L^{-1} to 10.0 g L^{-1} for higher concentrations of E2 and EE2. The systems were mixed 45 minutes prior to analytical determination.

2. 2. 3. Effect of Initial pH

To study the effect of initial pH on the process of adsorption, several experiments (50 mL of the sample) at different initial pHs, ranged from 2.0 to 12.0 with adsorbent dose of 6.0 g L^{-1} at higher concentration of E2/EE2 and 0.1 g L^{-1} at lower concentration of E2/EE2 were carried out. Experiments were accomplished at the ambient temperature (22 ± 2 °C) for about 45 minutes. Initial concentrations of E2 (17β-estradiol) were 0.272 mg $L^{-1}$and 27.2 mg L^{-1} but 0.296 mg L^{-1} and 29.6 mg L^{-1} for EE2 (17α-ethinylestradiol). Appropriate initial pH was attained with 1M HCl and 5M NaOH.

2. 3. Analytical determinations of E2 and EE2

18 mL of each filtered subsample 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 first exposed to micro extraction procedure (SPME) using SPME fibre coated with polyacrylate (PA) 85 μm.[18,19] 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.[18] 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-5MS (5% phenyl- methylpolysiloxane, 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.

3. Results and Discussion

3. 1. Fenton's Oxidation of Estrogens

We have studied the efficiency of E2 (17β-estradiol) and EE2 (17α-ethinylestradiol) removal by Fenton's oxidation at different molar ratios of Fenton's reagents Fe^{2+} and H_2O_2 and at different initial pH values. In spite of the fact, that Fenton's oxidation is exothermic process, the amounts of added reagents for lower concentrations of E2 and EE2 were low enough not to increase the temperature of the experiment (22 ± 2 °C). Possible oxidation by-products were not identified, but some low, non-determined peaks appeared in some cases in GC-MS spectrogram.

3. 1. 1. Effect of Fe^{2+} to H_2O_2 Molar Ratio

Four different molar Fe^{2+} : H_2O_2 ratios (1:0.5, 1:10, 1:20 and 1:33) were investigated to find optimal one for

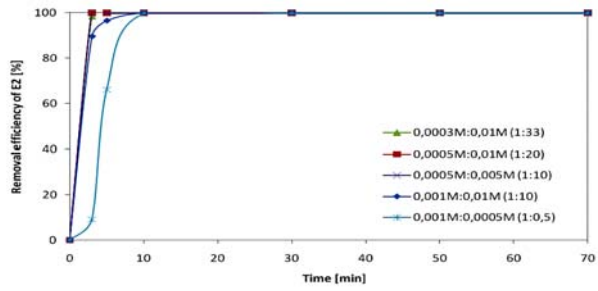

Fig. 1: Removal efficiency of E2 (0.272 mg L^{-1}) at different molar ratios of Fenton's reagents versus time.

removal of natural (E2: 17β-estradiol) and synthetic (EE2: 17α-ethinylestradiol) estrogens. Two 1:10 molar ratios were investigated, the first one with higher concentrations of reagents and the second one with lower ones, to determine optimal treatment efficiency based on minimal consumption of chemicals as well as minimal waste sludge production.

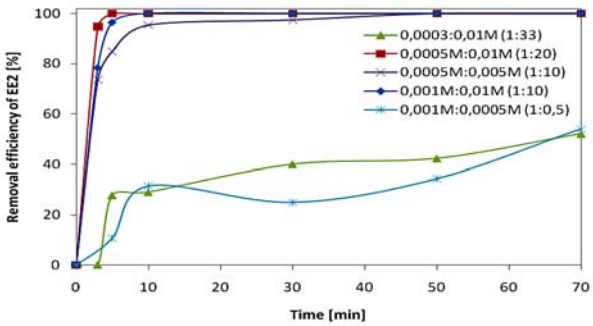

Fig. 2: Removal efficiency of EE2 (0.296 mg L^{-1}) at different molar ratios of Fenton's reagents versus time.

At a low concentration of E2 (0.272 mg L^{-1}, Fig. 1) or EE2 (0.296 mg L^{-1}, Fig. 2) removal efficiency increased rapidly with contact time and with higher molar ratio. In all of the cases investigated natural E2 (17β-estradiol) and synthetic EE2 (17α-ethinylestradiol) were successfully removed in the first ten minutes of reaction (Fig. 1 and Fig. 2), except at Fenton's oxidation of EE2 by higher molar ratio of Fe^{2+} : H_2O_2= 0.0003M:0.01M (1:33) and by lower molar ratio of Fe^{2+} : H_2O_2 = 0.001M : 0.0005M (1:0.5), where removal rate at the end of reaction reached 52% and 54%, respectively. In all of the cases where 17β-estradiol (E2) has been studied, the molar ratio of Fenton's reagent had no significant influence on removal rate, but from the economic perspective it is more convenient to use lower amounts of reagents. The most effective molar ratios of regents at low concentration of E2 were 1:20 and 1:33 and for removal of 17α-ethinylestradiol (EE2) it was 1:20. In both cases the less effective was the lowest molar ratio (1:0.5).

At higher concentration of E2 (27.2 mg L^{-1}, Fig. 3) or EE2 (29.6 mg L^{-1}, Fig. 4) the situation was different, because higher amounts of H_2O_2 and Fe^{2+} were added, but the ratios of the reagents remained the same (Fig. 3 and Fig. 4). At all of the tested molar ratios E2 (17β-estradiol) removal rate had never reached 100%, not even after one hour. The best molar ratio at high concentration for E2 removal was Fe^{2+} : H_2O_2= 0.03M:1M (1:33) and for removal of EE2 it was Fe^{2+} : H_2O_2= 0.2M : 2M (1:10). As for the experiments with low concentration of estrogens, the least effective was the lowest ratio (1:0.5).

Mass of the waste sludge produced was dependent upon the ratio and the volume of reagents added. 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 reliably determine the actual extent of degradation. For this reason a lot of attention has been devoted to adsorption of E2 (17β-estradiol) and EE2 (17α-ethinylestradiol) onto waste sludge, to determine the contribution of adsorption process to removal of investigated substances reliably.

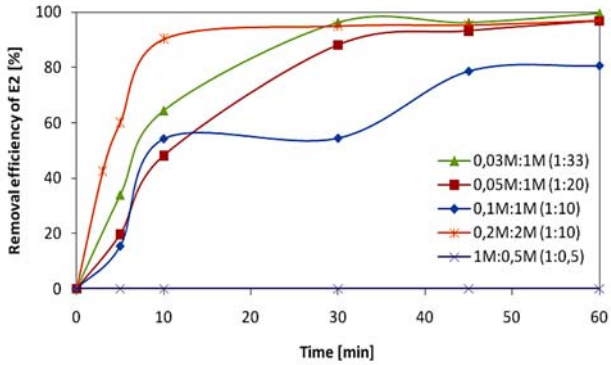

Fig. 3: Removal efficiency of E2 (27.2 mg L^{-1}) versus time at different molar ratios of Fenton's reagents.

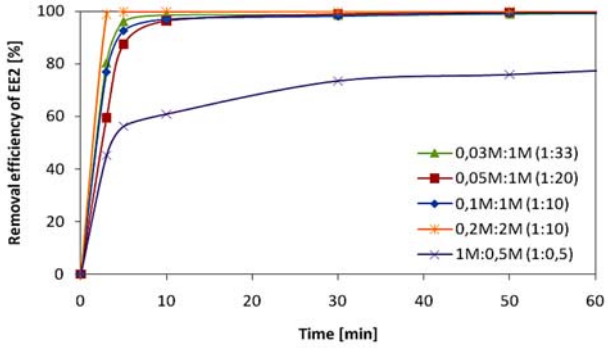

Fig. 4: Removal efficiency of EE2 (29.6 mg L^{-1}) versus time at different molar ratios of Fenton's reagents.

3. 1. 2. Effect of pH

Earlier studies have demonstrated,[14,20] that at low pHs (2.5–3.0) the most efficient oxidation of organics is attained, while oxidation is rather inefficient at the pH range where the most natural waters belong to (pH 5.0–9.0). In order to investigate the effect of initial pH on pathway of

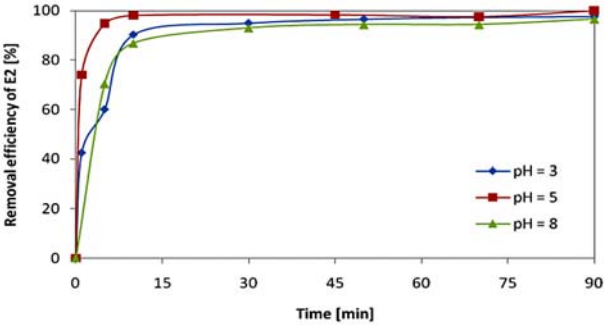

Fig. 5: Removal efficiency of E2 (27.2 mg L^{-1}; 0.2M:2M) at different pHs versus time.

Fenton's oxidation, three different pH values were tested (3.0, 5.0 and 8.0) with Fe^{2+} : H_2O_2= 0.2M:2M (1:10) molar ratio. It was observed, that initial pH did not show high influence on removal efficiency at high concentrations of E2 and EE2 (Fig. 5 and Fig. 6), because at all tested pH values removal reached more than 95% for E2 (17β-estradiol) and 100% for EE2 (17α-ethinylestradiol) in less than 70 minutes.

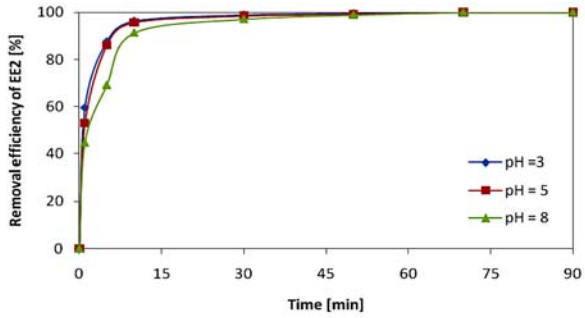

Fig. 6: Removal efficiency of EE2 (29.6 mg L^{-1}; 0.2M:2M) at different pHs versus time.

Removal efficiency of EE2 was 100% at all pHs, but the reaction was the fastest in the case of the lowest pH of 3.0 applied (Fig. 5). In both cases (E2 and EE2) pH value of 8.0 showed the slowest removal rates. Relative unimportance of initial pH of the process leads us to the conclusion, that there should be another removal mechanism responsible for good removal of estrogens, not only oxidation, which expects to be pH dependent.[14, 20]

3. 2. Adsorption of Estrogens on Waste Sludge

Possible adsorption of estrogens on during Fenton's oxidation formed waste sludge has been studied. The impact of contact time, initial pH and initial concentration of the adsorbent (waste sludge) and adsorbate (E2, EE2) have been investigated.

3. 2. 1. Effect of the Contact Time

The experiment was accomplished to determine necessary contact time for achieving adsorption equilibrium. Removal efficiency of E2 (0.272 mg L^{-1}) and EE2 (0.292 mg L^{-1}) versus time is presented in Figure 7. Both curves show fast initial adsorption (10 minutes) followed by soothe adsorption. It means that for adsorption of E2 (17β-estradiol) and EE2 (17α-ethinylestradiol) on sludge with concentration 0.1 g L^{-1}, 30 minutes of reaction is more than enough to reach equilibrium state. From the fast achievement of equilibrium state it can be concluded, that surface diffusion is not rate-limiting mechanism of this adsorption process.[16] On the bases of these results it has been considered, that these 30 minutes were sufficient also for adsorption of higher concentrations of E2 and EE2 and for larger amount of added sludge and was therefore used for all further experiments. The difference between removal efficiency of E2 and EE2 has also been observed. EE2 expressed much lower removal efficiency according to adsorption on waste sludge than E2, probably because of its lower solubility in water.

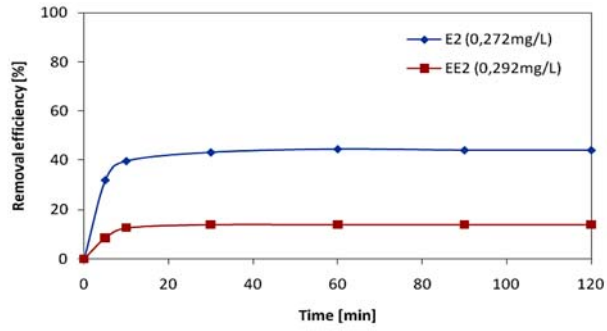

Fig. 7: Effect of contact time on E2 and EE2 adsorption on sludge (0.10 g L^{-1}).

3. 2. 2. Effect of the Initial Concentrations of E2 and EE2

Initial concentration of E2 (17β-estradiol) was varied from 0.272 mg L^{-1} to 27.2 mg L^{-1} while initial concentration of EE2 (17α-ethinylestradiol) was in the range from 0.296 mg L^{-1} to 29.6 mg L^{-1} at two different adsorbent doses, 6.0 g L^{-1} and 0.1 g $L^{-1,}$ respectively. pH was adjusted to 2.5 and process took place for about 45 minutes. The curves in the Figure 8 show how initial concentrations of E2 and EE2 affect removal efficiency at different doses of adsorbent. E2 and EE2 expressed different behaviour during this adsorption experiment. E2 (17β-estradiol) was removed better at higher concentration of sludge (6.0 g L^{-1}), while EE2 (17α-ethinylestradiol) removal efficiency was higher at lower amount of the sludge (0.1 g L^{-1}).

It has been confirmed, that at lower initial concentrations of estrogens, lower removal efficiency is obtained. The increase of E2 (17β-estradiol) and EE2 (17α-ethinylestradiol) adsorption capacity with higher initial con-

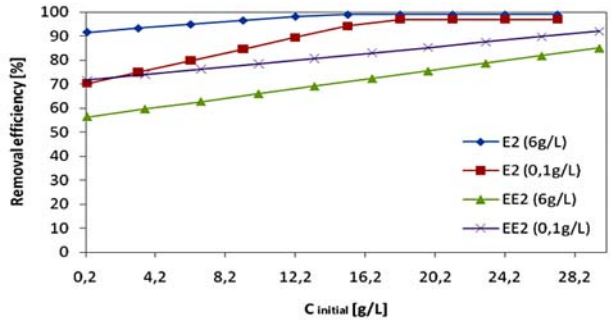

Fig. 8: Effect of initial concentrations of E2 and EE2 to adsorption on sludge (6.0 g L^{-1} and 0.1 g L^{-1}).

centration is probably a consequence of complete utilization of adsorption surface and active sites available which is not possible at low concentration. E2 expressed much higher adsorption tendency than EE2. E2 reached 99% and 97% of removal with 6.0 g L^{-1} and 0.1 g L^{-1} of waste sludge added, while in the case of EE2, only 83% and 93% of the substance was removed. Adsorption of EE2 was also much slower, comparable to findings, presented in Figure 7.

3. 2. 3 Effect of Adsorbent Dose

Effect of adsorbent dose was studied with different amounts of adsorbent (waste Fenton's sludge). Its concentration varied from 0.0 g L^{-1} to 0.50 g L^{-1} for low concentrations of E2 (0.272 mg L^{-1}) and EE2 (0.292 mg L^{-1}) and from 0.0 g L^{-1} to 10.0 g L^{-1} for high concentration of E2 (27.2 mg L^{-1}) and EE2 (29.6 mg L^{-1}). In all of the cases (Fig. 9 – Fig. 12) removal efficiency increased with adsorbent (waste sludge) dose. Higher the concentration of adsorbent was, more E2 (17β-estradiol) or EE2 (17α-ethinylestradiol) was adsorbed. At high concentration of adsorbent, the adsorption efficiency could reach even 100%.

The reduction of sorption capacity (Equation 1, q_e, mg g^{-1}) with increased concentration of adsorbent is related to the fact, that some of the active centres remain unsaturated during adsorption process. But with increase of the dose of the waste sludge, the absolute amount of the active sites increases, resulting in an increase of the overall sorption efficiency of the system.

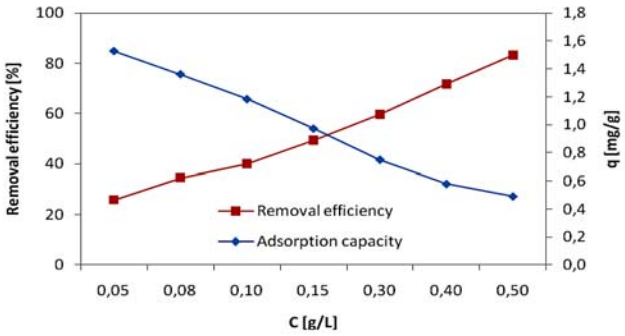

Fig. 9: Effect of sludge dose on E2 (0.272 mg L^{-1}) adsorption.

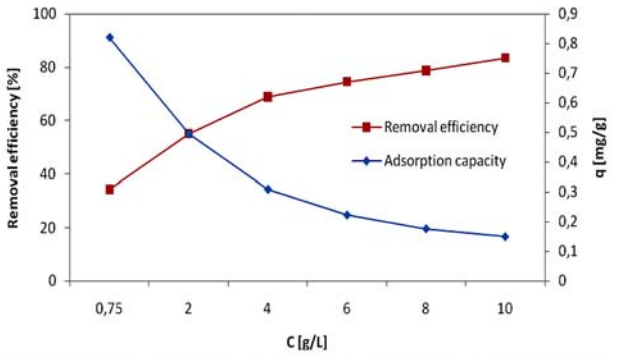

Fig. 10: Effect of sludge dose on E2 (27.2 mg L^{-1}) adsorption.

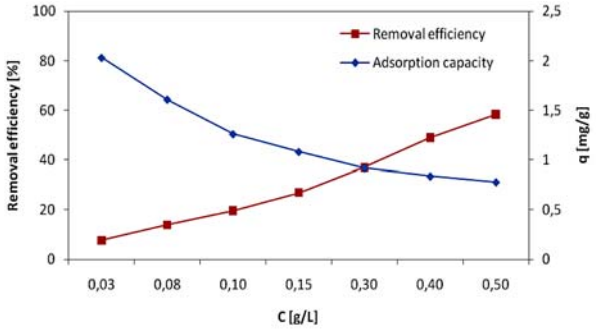

Fig. 11: Effect of sludge dose on EE2 (0.296 mg L^{-1}) adsorption.

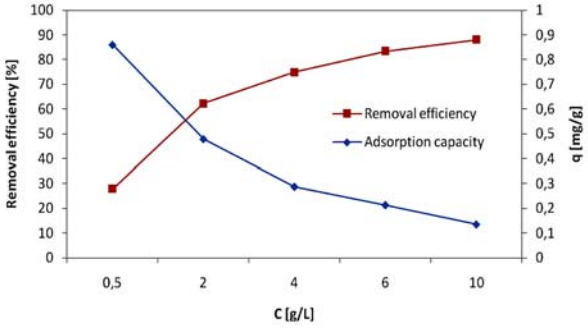

Fig. 12: Effect of sludge dose on EE2 (29.6 mg L^{-1}) adsorption.

3. 2. 4 Effect of pH

The study on the effect of the initial pH on the process of adsorption was carried out by changing initial pH from 2.0 to 12.0 with adsorbent dose 6.0 g L^{-1} for the high concentrations of E2 (27.2 mg L^{-1}) and EE2 (29.6 mg L^{-1}) and 0.1 g L^{-1} of adsorbent for the low concentrations of E2 (0.272 mg L^{-1}) and EE2 (0.296 mg L^{-1}), as shown in Figure 13. Rising pH values increased removal efficiency. The maximum adsorption of E2 (17β-estradiol) and EE2 (17α-ethinylestradiol) at all concentrations was at pH 12.0. Adsorption potential of E2 was higher at higher concentration of the substance (62/48%), while in the case of

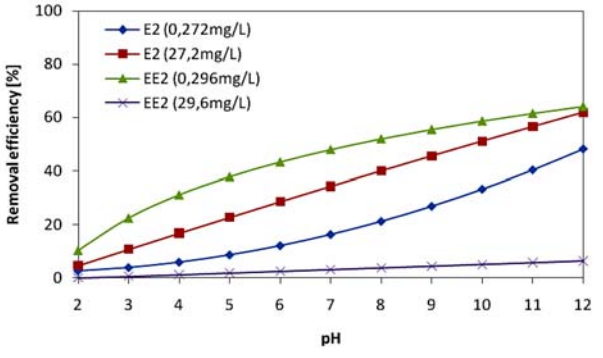

Fig. 13: Effect of initial pH on the removal of E2 and EE2 by adsorption.

EE2, removal was more evident at lower concentration (64/5%, Fig. 13).

The dependency of adsorption on pH could be explained as a consequence of changes at the surface of waste sludge due to the presence of different hydrogen ion concentration.[21]

3. 3. Contribution of Estrogens Adsorption to Fenton's Oxidation Removal Efficiency

The best molar ratio from all perspectives considered is the one producing the least of waste sludge accompanied by minimal amounts of reagents used resulting in complete removal of both investigated substances. At the same time, the removal should be a consequence of oxidation and not adsorption. If all of these facts are considered, the best molar ratios for E2 (17β-estradiol) removal are Fe^{2+} : H_2O_2 = 0.0003M:0.01M (1:33) and Fe^{2+} : H_2O_2 = 0.03M:1M (1:33) for lower and higher concentrations, while in the case of EE2 the most efficient were Fe^{2+} : H_2O_2 = 0.0005M:0.001M (1:20) ratio (low EE2 concentration) and Fe^{2+} : H_2O_2 = 0.03M:1M (1:33) ratio in the case of higher EE2 (17α-ethinylestradiol) concentration. In Table 1 the importance of both processes for removal of estrogens from water solutions is compared. Values of adsorption (%) were defined from diagrams (Fig. 9 – Fig. 12) regarding average concentration of formed sludge and acquired values were then compared to those at pH of 2.7 (Fig. 13), which is favourable for oxidation. The differences between adsorption and removal efficiency, calculated in Fenton's experiments (Fig. 1 – Fig. 4) were then assigned to oxidation. The sum of adsorption (%) and oxidation (%) processes in the last column of Table 1 thus represents the final removal efficiency of E2/EE2 in Fenton's oxidation.

Generally, higher ratios of reagents (1:33) favours oxidation, because there is not a lot of sludge produced, while in the case of lower Fe^{2+} : H_2O_2 ratio, formation of sludge is more evident resulting in higher contribution of adsorption processes to final removal of investigated estrogens. Adsorption has been confirmed in extraction experiments, where waste sludge after Fenton's oxidation experiments (Fe^{2+} : H_2O_2 = 0.2M : 2M (1:10), t = 45 min) with E2 (27.2 mg L^{-1}) and EE2 (29.6 mg L^{-1}) has been dissolved in ethanol solution (2.5 Vol.%) and concentration of estrogen determined after 30 minutes of extraction. Regarding calculations in Table 1, 58% of adsorption of E2 has been expected, while the actual one represented 48%. In the case of EE2, contribution of adsorption was 65% (100% calculated, Table 1). These extraction experiments clearly indicated, that adsorption plays an important role in Fenton's treatment of estrogens.

4. Conclusions

Fenton's oxidation experiments were conducted by natural and synthetic estrogens E2 (17β-estradiol) and EE2 (17α-ethinylestradiol), respectively. The effectiveness of oxidative removal of estrogens was studied at different ratios of reagents (Fe^{2+} : H_2O_2) as well as adsorption capacity of sludge, formed during oxidative treatment has been investigated. Fenton's oxidation was confirmed as very effective for the removal of natural E2 and synthe-

Table 1. Adsorption and oxidation of E2 and EE2 at different molar ratios of regents in Fenton's oxidation (FO).

Substance	C (mg L^{-1})	Ratio of reagents used in FO	pH	C of sludge formed (g L^{-1})	Adsorption/Oxidation (%/%)
E2	0.272	0.0003M:0.01M (1:33)	2.7	0.06	26/74
		0.0005M:0.01M (1:20)	2.7	0.12	38/62
		0.0005M:0.005M (1:10)	2.7	0.11	37/63
		0.001M:0.01M (1:10)	2.7	0.16	44/56
		0.001M:0.0005M (1:0.5)	2.7	0.37	60/40
	27.2	0.03M:1M (1:33)	2.7	3.25	50/50
		0.05M:1M (1:20)	2.7	6.00	57/40
		0.1M:1M (1:10)	2.7	10.22	64/17
		0.2M:2M (1:10)	2.7	6.55	58/39
		1M:0.5M (1:0.5)	2.7	90.01	99/1
EE2	0.296	0.0003M:0.01M (1:33)	2.7	0.15	19/28
		0.0005M:0.01M (1:20)	2.7	0.24	24/76
		0.0005M:0.005M (1:10)	2.7	0.16	20/80
		0.001M:0.01M (1:10)	2.7	0.30	27/73
		0.001M:0.0005M (1:0.5)	2.7	0.24	24/20
	29.6	0.03M:1M (1:33)	2.7	4.61	76/23
		0.05M:1M (1:20)	2.7	6.03	81/19
		0.1M:1M (1:10)	2.7	13.29	96/4
		0.2M:2M (1:10)	2.7	24.22	100/0
		1M:0.5M (1:0.5)	2.7	126.65	77/0

tic EE2 hormones, reaching up to 100% with some molar ratios of Fe^{2+} and H_2O_2 reagents. The only weakness of this process is the formation of the waste sludge, where amount of sludge formed is strongly dependent upon molar ratios and concentration of reagents applied. The problem of waste sludge is almost always overlooked in treatment processes. It leads to competitive adsorption of estrogens during oxidative treatment, resulting in lower destruction of harmful 17β-estradiol and 17α-ethinylestradiol due to their transport into another phase, adsorption. Intensity of adsorption process depends strongly upon contact time (longer time increased adsorption), initial concentration of E2 and EE2 (the higher is concentration, more evident adsorption is), and adsorbent dose (more sludge causes more adsorption). The effect of pH is also important, because Fenton's oxidation is most efficient at low pHs (3.0–5.0) where the impact of adsorption is less evident, due to fast and prevailing oxidation, which is not limited by mass transport. For the selection of conditions in Fenton's oxidation process it is important to assure the lowest adsorption to attain maximal degradation of estrogens. On the other hand our experiments indicated, that even in the case of minimal sludge production and well regulated pH, adsorption of estrogens reach at least 20%. That means that oxidation of E2 (17β-estradiol) and EE2 (17α-ethinylestradiol) reached only up to 80% and that remained amounts could still pose significant environmental impacts.

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Povzetek

Raziskovalno delo je bilo usmerjeno v študij učinkovitosti razgradnje naravnega (17β-estradiol – E2) in sintetičnega (17α-ethinilestradiola – EE2) hormona s Fentonovo oksidacijo. Številne raziskave so potrdile, da se vpliv estrogenov na organizme kaže že pri koncentracijah blizu 1 pg L^{-1}, zato jih je potrebno v celoti odstraniti iz odpadne vode. Fentonova oksidacija je izredno učinkovit napredni oksidacijski postopek za oksidacijo organskih snovi, slaba stran postopka pa je tvorba odpadnega blata. Zaradi blata tako lahko pride do adsorpcije organskih snovi na oborino, kar onemogoča zanesljivo oceno dejanskega obsega razgradnje. Preučili smo razmerje med dejansko oksidacijo estrogenov in njihovo adsorpcijo na nastalo blato glede na molsko razmerja reagentov Fe^{2+} in H_2O_2 (1:0.5, 1:10; 1:20; 1:33), čas poteka reakcije, pH in glede na začetne koncentracije E2 (0,279/27,9 mg·L^{-1}) in EE2 (0,296/29,6 mg L^{-1}). Kot najbolj primerno molsko razmerje za odstranitev E2 se je izkazalo razmerje 1:33 (100 % odstranitev, 26–50 % adsorpcija), v primeru EE2 sta bili najbolj učinkoviti razmerji 1:10 in 1:33 (99–100 % odstranitev, 20–76 % adsorpcija). Potrdili smo, da ima adsorpcija veliko vlogo pri Fentonovi oksidaciji estrogenov, še posebno pri visokih pH-jih, kjer je prevladujoč proces, medtem ko je oksidacija bistveno bolj učinkovita pri nižjih pH vrednostih (3.0–5.0), predvsem zato, ker je reakcija homogena in masni transport procesa oksidacije ne omejuje.