ELSEVIER

Selective photocatalytic oxidation of steroid estrogens in water treatment: urea as co-pollutant

T. Karpova *, S. Preis, J. Kallas

*Department of Chemical Technology, Lappeenranta University of Technology,
Skinnarilankatu 34, 53850 Lappeenranta, Finland*

Abstract

The objective of the present research concerns the competitive photocatalytic oxidation (PCO) and adsorption of steroid estrogens (SEs) on titanium dioxide in presence of urea. The results showed the indifference of SEs towards the presence of urea in concentrations characteristic for the domestic sewage. The selective PCO of SEs appeared to be feasible in alkaline media, in which the PCO exhibited the highest efficiency. Ethanol used for hydrophobic SEs dissolution in water appeared to be interfering with the PCO.
© 2007 Elsevier B.V. All rights reserved.

Keywords: 17α-Ethynylestradiol (EE2); β-Estradiol (E2); Aqueous pollution; Endocrine disrupting compounds (EDCs); Urea

1. Introduction

The public concern over the possible consequences of exposure to endocrine-disrupting compounds (EDCs) has drawn the attention of national and international organizations due to their extreme hazard [1]. Among the other EDCs, steroid estrogens (SEs) were identified to have the highest endocrine-disrupting activity, being potent already at nanogram concentrations. The primary sources of these micropollutants are municipal wastewaters and farming. The extent of pollution with SEs is increasing due to the growing population, more intensive farming and the development of synthetic hormones (contraceptive pills, hormone replacement therapy, cattle hormonal growth promotion, etc.).

Natural and synthetic SEs, present in the sanitary fraction of wastewaters, mainly enter the wastewater treatment plants (WWTPs) in a form that is largely not estrogenic. They are excreted primarily as a variety of inactive glucoronide or sulfonide conjugates. However, during the sewage process these inactive compounds are readily transformed into ones that are potentially more harmful than original [2]. This happens presumably due to the large amounts of enzyme produced by the faecal bacteria *Escherichia coli* [3]. The inadequate removal of SEs in WWTPs results in their release to the environment. Substances that enter the water cycle via discharged wastewaters will ultimately find the way into the potable water.

Advanced oxidation processes (AOPs) are promising in the ultimate removal of SEs, although a certain risk of residual estrogenic activity at doses of ozone commonly applied in potable water treatment has been pointed out [4]. Photocatalytic oxidation (PCO) was confirmed to effectively eliminate the SEs and is considered as a potential alternative. Several publications revealed the following observations: alkaline medium was found to be beneficial for PCO [5,6], in which adsorption behaviour of the target compounds was found to play a major role [6]. The effect of initial SEs concentration and light intensity have been reported: within the solubility limits the higher the initial concentration the higher the PCO rate, which increases with increasing UV irradiance [7]. The predominant mechanism of PCO starting from the phenolic moiety of SE's molecule has been suggested [8,9]. Even several reactor designs have been suggested and tested [10–12]. The study on the estrogenicity of PCO by-products showed no formation of estrogenic intermediates [8,12]. However, the influence of other constituents of municipal wastewaters on the PCO of SEs and the selectivity of the PCO towards SEs were so far not reported. Being present in much higher concentrations, these co-pollutant substances may interfere with the PCO of SEs and presumably hinder the oxidation efficiency due to competition for the active adsorption sites.

The sanitary fraction of the municipal wastewaters is a combination of compounds present in urine and faeces. Normal urine

---

is actually a highly complex aqueous solution of organic and inorganic substances. The most important organic substances are urea, uric acid and creatinine. Urea is a product formed by the liver from ammonia and carbon dioxide. Ninety-five percent of the nitrogen content of urine is in the form of this substance, the median daily load of excreted urea in urine is 17.3 g person^{-1} day^{-1} [13].

The objective of the present research was to study the selectivity of PCO of SEs in the presence of interfering compounds: the PCO of natural and synthetic SEs with and without urea as a competing substance was studied in a wide range of pH.

2. Materials and methods

The natural SE β-estradiol (E2) and synthetic 17α-ethynylestradiol (EE2) (Sigma-Aldrich) were used as target compounds (Fig. 1). These compounds are of the greatest environmental concern: E2 and EE2 are found to be the most potent among other SEs [2,14]. The molecules of these SEs differ only in the ethynyl group in EE2 connected to the tertiary carbon atom with the alcohol moiety. Due to their hydrophobic character they were first dissolved using 99.5%-ethanol (Altia Oy, Finland) as a solvent and then gradually diluted with Milli-Q water to prepare the stock aqueous solution of 10 mg L^{-1}. This solution was constantly stirred at room temperature in a hermetically sealed flask to avoid recrystallisation of the SEs. The solutions with required concentrations for the experiments were prepared by dilution of the stock solution with Milli-Q water under stirring for 30 min. The initial concentration of the solutions to be treated was 500 μg L^{-1} if not otherwise specified. These solutions contained about 4 g L^{-1} of ethanol. All PCO experiments were conducted at room temperature of $23 \pm 2\,^\circ\text{C}$. The pH value was adjusted with sulphuric acid or sodium hydroxide. The dissolution of SEs in a stock solution without ethanol was conducted in similar manner, in which ethanol was replaced with water with strongly alkaline reaction (pH 12.4).

Urea (Merck), dried at 105 °C, was added to the solutions to be treated simultaneously with the SEs. The medium concentration of urea of 75 mg L^{-1} attributable to the municipal wastewater [15] was used in the experiments.

Titanium dioxide (TiO$_2$, Degussa P25) under near-UV irradiation (365 nm) was used as photocatalyst. Its concentrations in suspensions were 10 mg L^{-1} as was thoroughly selected in the previous study [6]. Centrifugation at 10,000 rpm was applied to separate TiO$_2$ from the samples before the analysis.

The PCO experiments were performed in 0.25-L capacity thermostatted batch glass reactors with an inner diameter of 100 mm, supplied with a magnetic stirrer. The agitation intensity

was maintained with a standard 25-mm Teflon stirrer at 300 rpm. A 365 nm UV-lamp was positioned horizontally over the reactor; the irradiance was about 1.1 mW cm^{-2} measured by UVX Radiometer (UVX, USA). All PCO experiments were compared with reference samples, treated under identical conditions except UV-radiation.

The adsorption experiments were performed in 0.50-L capacity flasks, where the solutions were mixed with TiO$_2$ at a stirring frequency of 900 rpm for 24 h at 25 °C. Each flask was handled separately as a single-point sample, i.e. without intermediate sampling, and each experiment was repeated three times. The equilibrium concentration of compounds adsorbed on the surface of the catalyst q, μg g^{-1} TiO$_2$, was calculated from the mass balance of the sample.

The concentrations of SEs were determined by means of HPLC with 150-mm ZORBAX Eclipse XDB-C18 column (Agilent) supplied with UV-detector at the wavelength 278 nm, mobile phase acetonitrile/water 1:1 at a flow rate of 1.0 mL min^{-1}. The concentrations of urea were measured according to the method of Mulvenna and Savidge [16] with modifications of Goeyens et al. [17] and the authors of the present paper. The 10-mL test tubes were filled with 3.5 mL of the 35-fold diluted sample, 0.25 mL of the reagent A and stirred. The 0.8 mL of reagent B was then immediately added with subsequent stirring. The test tubes were stored at room temperature for 72 h in the dark. The light absorbance was measured after that at 520 nm. Reagent A was made of 8.5 g diacetylmonoxime in 250 mL of water together with 10 mL of solution, containing 0.95 g of thiosemicarbazide in 100 mL of water (Sigma-Aldrich). Reagent B was prepared by dissolving 300 ml of concentrated sulphuric acid in 535 mL of water with addition of 0.5 mL of the solution, containing 0.15 g of ferric chloride in 10 mL of water.

3. Results and discussion

3.1. The PCO of SEs without urea in presence of ethanol

The results of the PCO of E2 and EE2 at different pH with the initial concentrations of 500 μg L^{-1} can be seen in Fig. 2 (the reaction rate) and Fig. 3 (the PCO efficiency).

The PCO efficiency relative to the catalyst concentration was calculated as the decrease in the concentration of the target compound divided by the amount of energy reaching the surface of the sample and by the concentration of the catalyst used [6,18]:

$$E = \frac{\Delta c \times V \times 1000}{I \times s \times t \times m} \tag{1}$$

where E is the PCO process efficiency, μg W^{-1} h^{-1} (g L^{-1} TiO$_2$)$^{-1}$; Δc, the decrease in the compound concentration, μg L^{-1}; V, the volume of treated sample, L; I, the irradiation intensity, mW cm^{-2}; s, the solution irradiated surface area, cm^2; t, the treatment time, h; and m is the concentration of TiO$_2$, g L^{-1}.

One can see that the efficiency of PCO of both natural and synthetic SEs increases with the increasing pH. The strongly alkaline medium was observed to be the most efficient for the PCO. In our previous research [6], the PCO efficiency was found
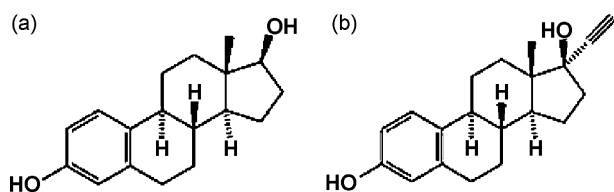

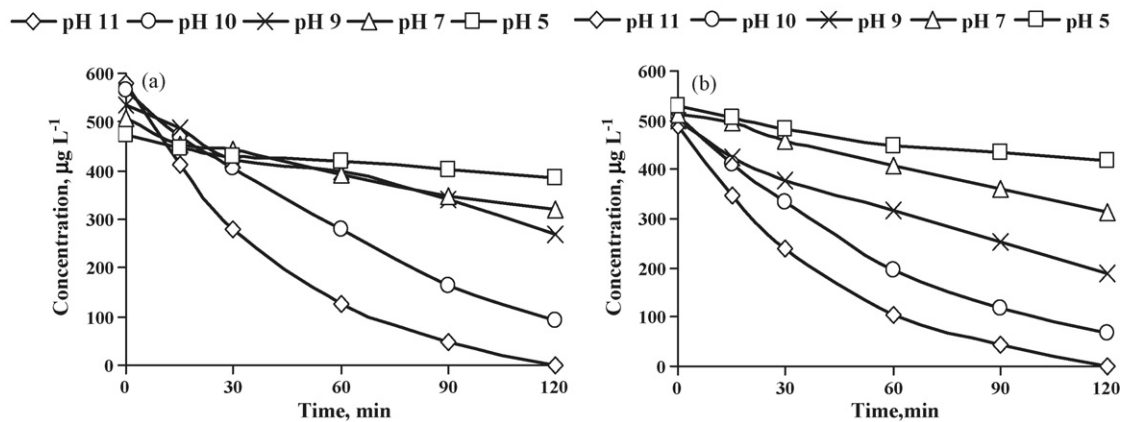Fig. 1. Molecular structures of E2 (a) and EE2 (b).

Fig. 2. The rate of PCO of E2 (a) and EE2 (b) at different pH in presence of ethanol ($4\,g\,L^{-1}$).

to be determined by the adsorption properties of SEs on the TiO_2 surface: the SEs were observed to adsorb to a greater extent under alkaline media conditions. The adsorption increased practically linearly with increasing concentration of OH-ions similar to the PCO efficiency of SEs. This could be attributed to the direct oxidation with the positively charged holes or oxidation with OH-radicals in vicinity of the semiconductor surface.

One can see on Fig. 3 that, at pH 11, the PCO of E2 proceeds with slightly higher efficiency than of EE2. This was also due to the better adsorption properties of E2 on the TiO_2 surface established in [6]: the nonpolar ethynyl group present in EE2 molecular structure possibly hinders the adsorption of molecule with the alcohol moiety. The efficiency is decreasing in treatment time, the reason of which can be the decreasing concentration of the SEs and, thus, the decreasing adsorption and the reaction rate.

3.2. The PCO in presence of urea and ethanol

Urea did not exhibit a substantial yield to PCO under the described experimental conditions: no detectable change in urea concentration was observed as a result of its PCO at the TiO_2 concentration of $10\,mg\,L^{-1}$ at any of the pH values tested within 2 h. The photocatalyst also was tested for the PCO of urea at TiO_2 concentration as big as $1\,g\,L^{-1}$. The adsorption experiments with urea were carried out with its concentration and the concentration of TiO_2 of the same value. Regardless 100-fold increase in TiO_2 concentration, no PCO progress of urea was observed within 2 h. This may be explained by poor urea adsorption on the photocatalyst surface: the series of adsorption experiments with urea on the TiO_2 surface showed no adsorption of this substance within the limits of precision of measurements in the range of pH under consideration.

In the presence of urea, the behaviour of SEs was studied for both PCO and adsorption. The results are shown in Fig. 4 (the reaction rate) and Fig. 5 (the PCO efficiency). One can see that no particular difference in both PCO rate and the efficiency were observed when compared to the PCO of the SEs without urea. This may be explained by the weak adsorption properties of urea and thus its minor interference in PCO.

Figs. 6 and 7 also show that the adsorption of SEs is not influenced by the presence of urea in the solution when ethanol
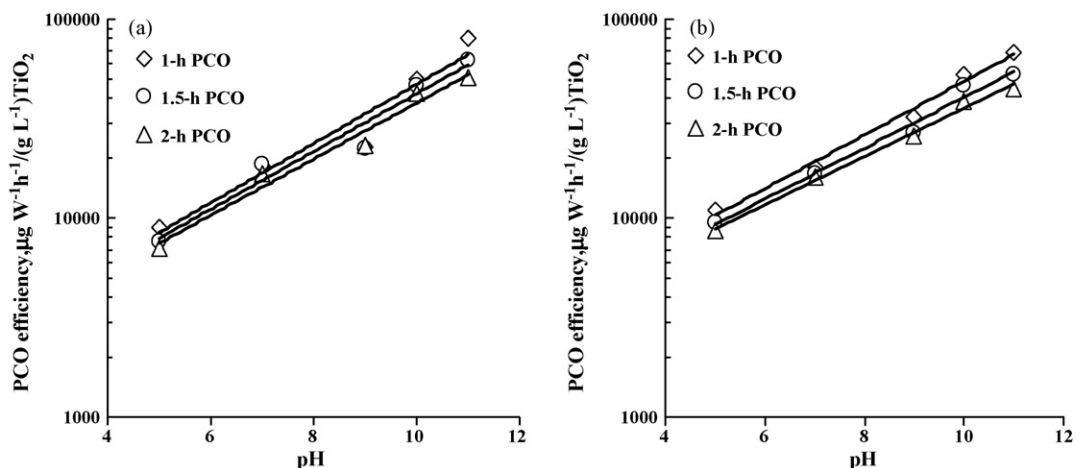

Fig. 3. The dependence of PCO efficiency *E* on pH for E2 (a) and EE2 (b) solutions (initial concentrations $500\,\mu g\,L^{-1}$) at different treatment times in presence of ethanol ($4\,g\,L^{-1}$).
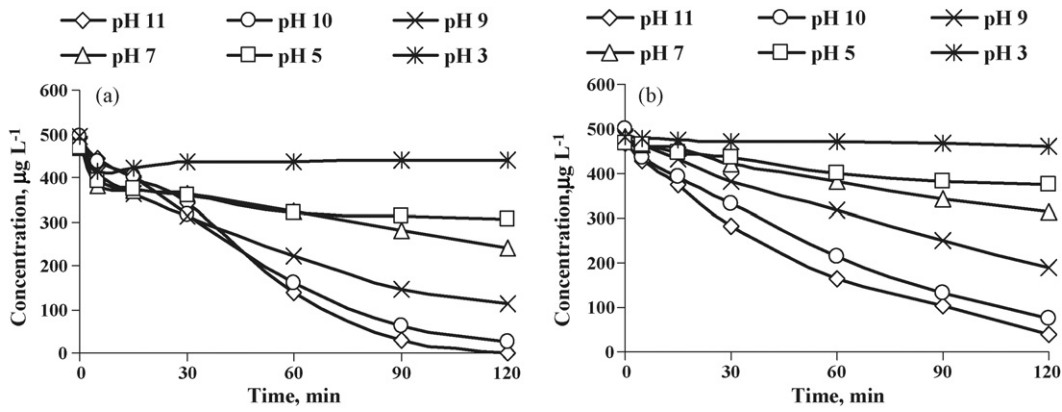
Fig. 4. The rate of PCO of E2 (a) and EE2 (b) at different pH in the presence of urea ($75\,\mathrm{mg\,L^{-1}}$) and ethanol ($4\,\mathrm{g\,L^{-1}}$).
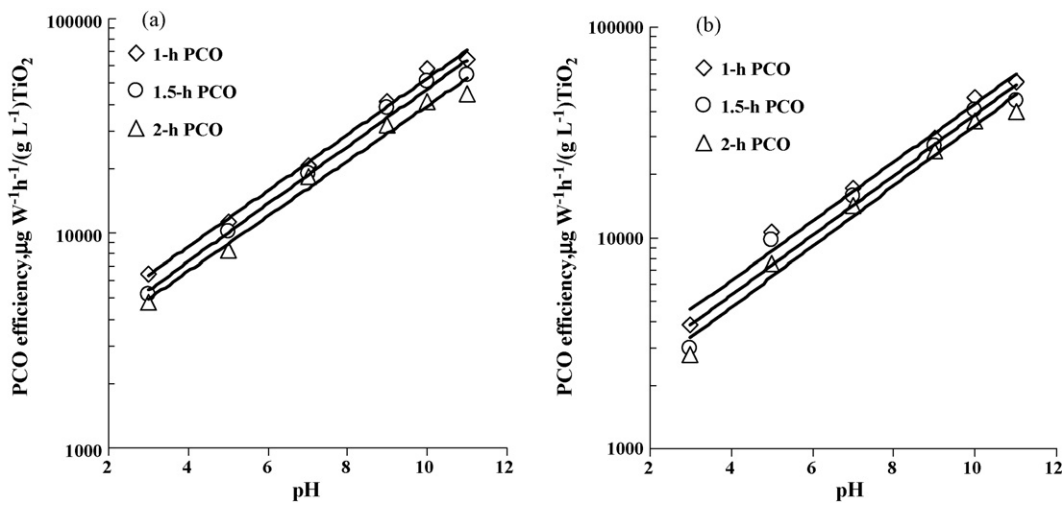

Fig. 5. Dependence of PCO efficiency E on pH of E2 (a) and EE2 (b) solutions (initial concentration $500\,\mathrm{\mu g\,L^{-1}}$) in the presence of urea ($75\,\mathrm{mg\,L^{-1}}$) and ethanol ($4\,\mathrm{g\,L^{-1}}$).

is also present. The character of the dependence of the adsorption of SEs on pH remains similar to the one observed in the absence of urea: the adsorption of SEs follows the same pattern as their PCO. The selective oxidation of SEs thus could be demonstrated at higher pH.

3.3. The influence of ethanol

The observed indifference of the PCO of SEs towards the presence of urea may be explained by the poor adsorption of urea at the TiO$_2$ surface. However, further studies in the influence of
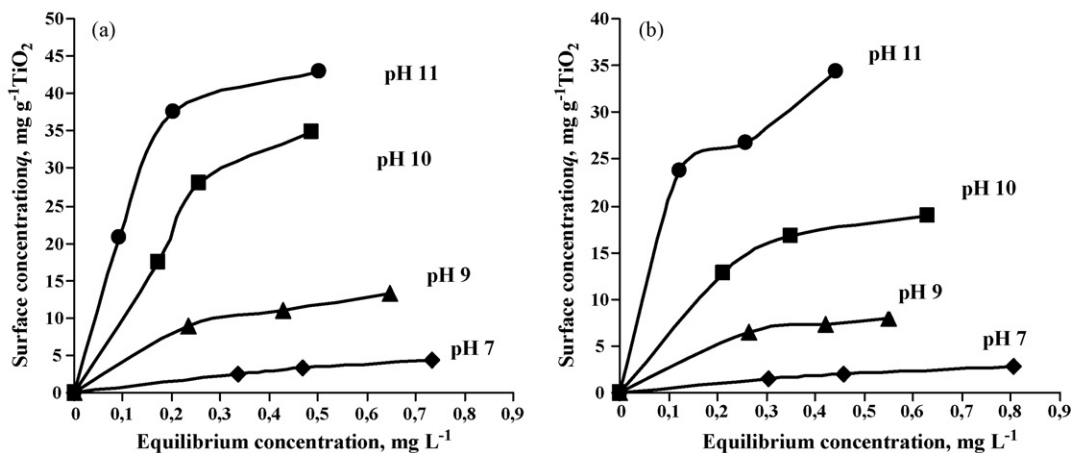

Fig. 6. Adsorption isotherms for E2 (a) and EE2 (b) on TiO$_2$ catalyst surface at different pH in the presence of urea ($75\,\mathrm{mg\,L^{-1}}$) and ethanol ($4\,\mathrm{g\,L^{-1}}$).
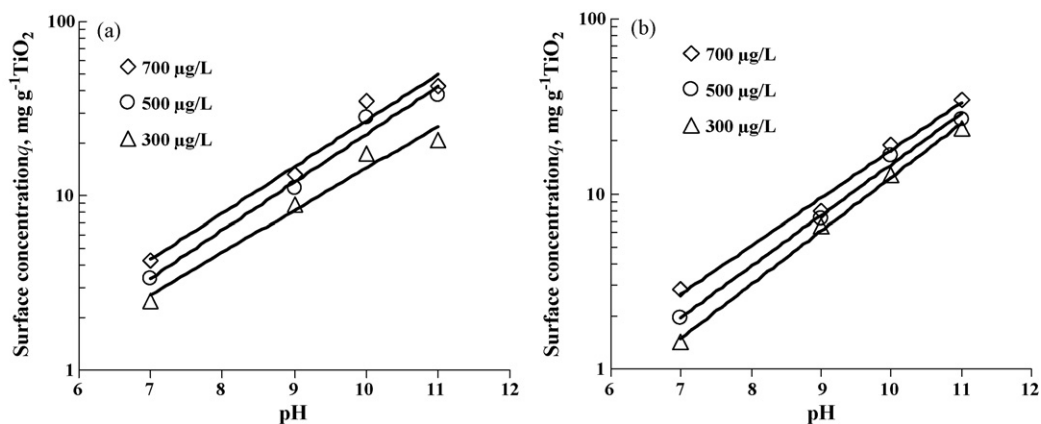
Fig. 7. The E2 (a) and EE2 (b) equilibrium surface concentration on TiO$_2$ vs. pH at different initial concentrations of SEs in the presence of urea (75 mg L^{-1}) and ethanol (4 g L^{-1}).

competing pollutants are needed for the reason of the inevitable presence of the solvent used for the stock solution, ethanol, in concentrations as big as 4 g L^{-1}. Practically all authors use organic solvents for SEs dissolution, making this problem of an interest for other researchers. Ethanol, for example, is the well-known OH-radical scavenger [19] and may compete for the adsorption sites.

Within the present study, the dissolution of E2 and EE2 in strongly alkaline media containing no ethanol was carried out and appeared to be successful: the stock solution of 10 mg L^{-1} was obtained with no visible precipitation. The strategy was used for the presence of phenolic group in the SEs molecules dissociated in alkaline media.

Figs. 8 and 9 represent the adsorption of E2 and EE2 on the surface of the catalyst without and in the presence of urea.

The presence of urea in commonly observed for municipal wastewaters concentration of 75 mg L^{-1} seemed to have negligible effect on the EE2 adsorption on TiO$_2$. The effect of urea on E2 adsorption was more pronounced, the adsorption was deteriorated at all pH values except pH 7, although the adsorption in presence of urea was of the same order of magnitude as without the co-pollutant. The difference in behaviour of the two

substances may be explained by the difference in hydrophilic properties of two molecules: the presence of urea as an additional pollutant may have stronger effect on adsorption of more hydrophilic E2 molecule than of EE2 having more hydrophobic character.

The presence of ethanol seemed to have also negligible effect on the adsorption of SEs (see Figs. 6 and 8).

The results of the PCO experiments in absence of ethanol are shown in Figs. 10 and 11. One can compare these results with the ones containing ethanol (Figs. 2 and 4) and see that the PCO in absence of ethanol proceeds much faster, although the dependence of the PCO efficiency on pH saved the previously observed pattern: the higher the pH, the faster the PCO. The authors would like to point to the circumstance that the PCO of SEs exhibited much stronger sensitivity towards the presence of ethanol than their adsorption on TiO$_2$. This could be explained by the role of OH-radicals in oxidation: radicals may be scavenged by ethanol molecules thus decreasing the PCO rate of SEs.

One can see that the presence of urea had negligible effect on the PCO of EE2. The effect on the PCO of E2 also was moderate, although the oxidation rate showed the behaviour opposite to the one of adsorption: the PCO in the presence of urea proceeded
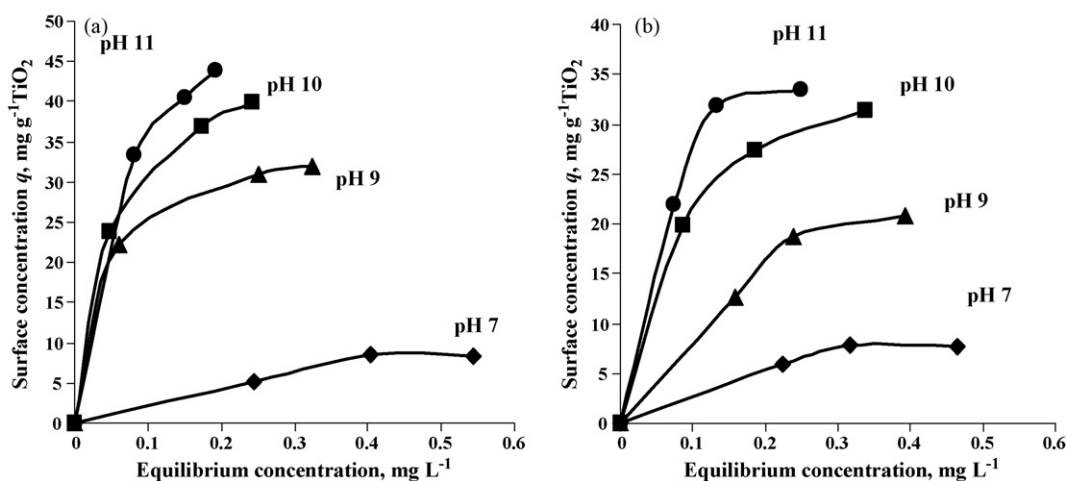

Fig. 8. Adsorption isotherms for E2 (a) and EE2 (b) on TiO$_2$ catalyst surface at different pH without urea (no organic solvent used).
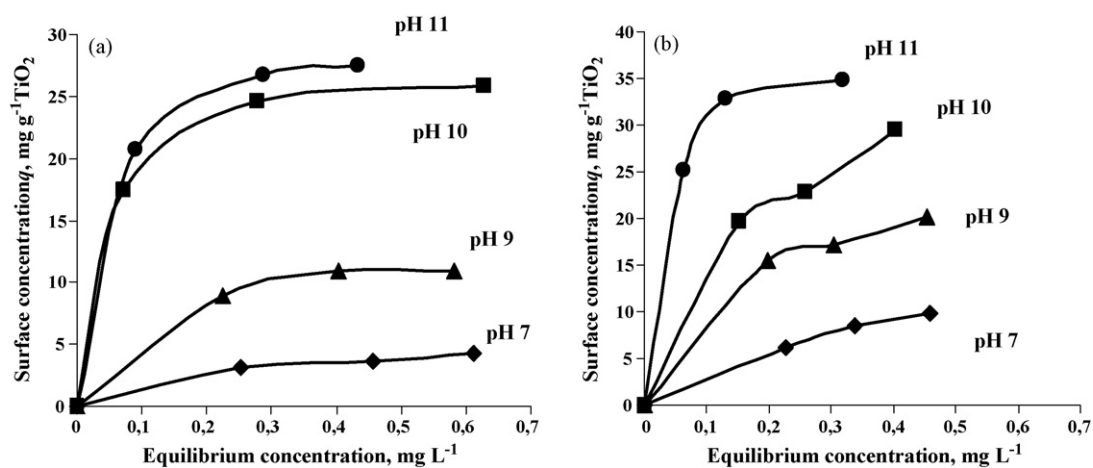
Fig. 9. Adsorption isotherms for E2 (a) and EE2 (b) on TiO$_2$ catalyst surface at different pH in the presence of urea (75 mg L^{-1}) (no organic solvent used).
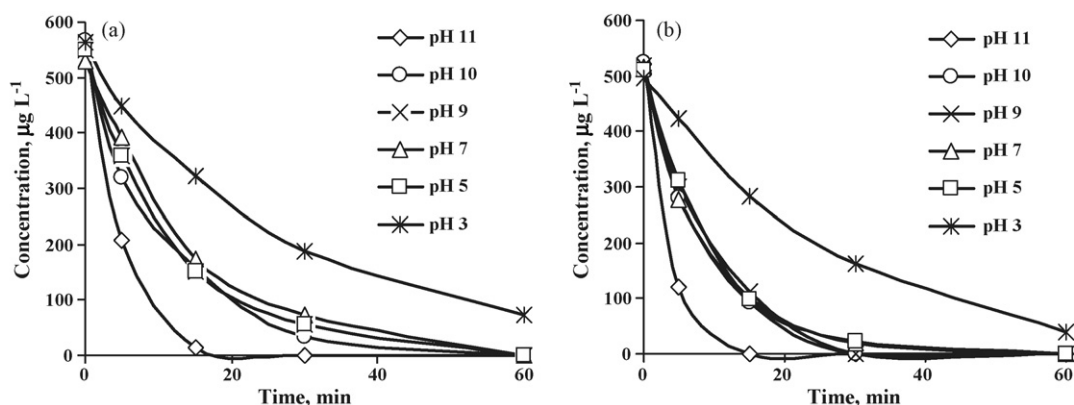

Fig. 10. The rate of PCO of E2 (a) and EE2 (b) at different pH without urea (no organic solvent used).

even a little faster than in absence of that at pH 7–10. The explanation of this phenomenon may require more knowledge in the PCO of SEs acquired with additional experiments at higher precision level. The dependence of the PCO rate on pH preserved the same pattern: the PCO rate increased with increasing pH.

The characteristics of the reaction kinetics for PCO of E2 and EE2 without and in the presence of urea at different pH were calculated using Langmuir–Hinshelwood equation for the monomolecular reaction:

$$r = \frac{k \times K \times C_e}{1 + K \times C_e} \quad (2)$$

where r is the rate of SE degradation, mg L^{-1} min^{-1}; k, the reaction rate constant, mg L^{-1} min^{-1}; K, the Langmuir equilibrium constant, L mg^{-1}; and C_e is the equilibrium SE concentration, mg L^{-1}.
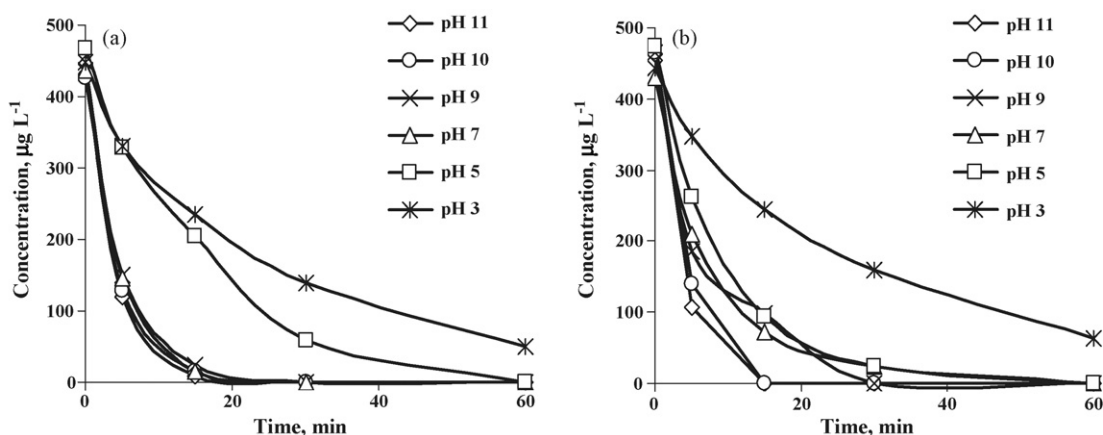

Fig. 11. The rate of PCO of E2 (a) and EE2 (b) at different pH in the presence of urea (75 mg L^{-1}) (no organic solvent used).
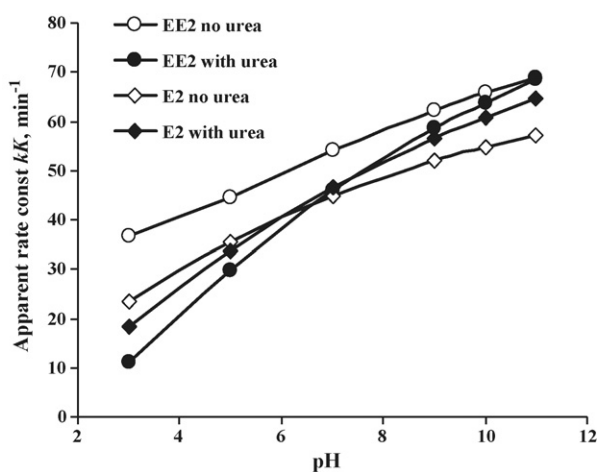
Fig. 12. The dependence of apparent reaction rate constant kK of PCO on pH for E2 and EE2 without and in the presence of urea (75 mg L^{-1}) (no organic solvent used).

The dependence of the apparent reaction rate constant of PCO on pH is given in Fig. 12. One can see the increased sensitivity of the PCO apparent reaction rate constant of both SEs towards pH in presence of urea, the slope of the curves 'pH–kK' increased with the addition of urea. In the pH area of maximum oxidation rate, the alkaline one, the impact of urea seems to be insignificant, which indicates the selective character of PCO of SEs.

4. Conclusions

Steroid estrogens, natural β-estradiol (E2) and synthetic 17α-ethynylestradiol (EE2), are easily photocatalytically degraded on UV-irradiated TiO$_2$ surface at very small (10 mg L^{-1}) concentrations of the photocatalyst. No degradation of urea was observed on the TiO$_2$ surface under the experimental conditions due to its poor adsorption. The presence of urea did not interfere with the PCO of SEs. The alkaline media exhibited the highest efficiency in both PCO and adsorption of SEs.

The inhibiting effect of ethanol on the PCO rate of SEs occurred to be strong. The organic solvent-free dissolution techniques showed its feasibility for studies of the competitive adsorption on TiO$_2$ surface and PCO of SEs with other co-pollutants; the presence of organic solvents usually used for preparing stock solutions should be avoided.

References

[1] A. Mendes, The endocrine disrupters: a major medical challenge, Food Chem. Toxicol. 40 (2002) 781–788.
[2] A. Johnson, J. Sumpter, Removal of endocrine-disrupting chemicals in activated sludge treatment works, Environ. Sci. Technol. 35 (2001) 4697–4703.
[3] G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, R. Samperi, Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities, Sci. Total Environ. 302 (2003) 199–209.
[4] M. Huber, T. Ternes, U. von Gunten, Removal of estrogenic activity and formation of oxidation products during ozonation of 17α-ethinylestradiol, Environ. Sci. Technol. 38 (2004) 5177–5186.
[5] H. Coleman, B. Eggins, J. Byrne, F. Palmer, E. King, Photocatalytic degradation of 17-β-oestradiol on immobilised TiO$_2$, Appl. Catal., B: Environ. 24 (2000) L1–L5.
[6] T. Malygina, S. Preis, J. Kallas, The role of pH in aqueous photocatalytic oxidation of β-estradiol, Int. J. Photoenergy 7 (2005) 187–191.
[7] H. Coleman, M. Abdullah, B. Eggins, F. Palmer, Photocatalytic degradation of 17β-oestradiol, oestriol and 17α-ethynylestradiol in water monitored using fluorescence spectroscopy, Appl. Catal., B: Environ. 55 (2005) 23–30.
[8] Y. Ohko, K.-I. Iuchi, C. Niwa, T. Tatsuma, T. Nakashima, T. Iguchi, Y. Kubota, A. Fujishima, 17β-estradiol degradation by TiO2 photocatalysis as a means of reducing estrogenic activity, Environ. Sci. Technol. 36 (2002) 4175–4181.
[9] K. Mitamura, H. Narukawa, T. Mizuguchi, K. Shimada, Degradation of estrogen conjugates using titanium dioxide as a photocatalyst, Anal. Sci. 20 (2004) 3–4.
[10] T. Nakashima, Y. Ohko, D. Tryk, A. Fujishima, Decomposition of endocrine-disrupting chemicals in water by use of TiO$_2$ photocatalysts immobilized on polytetrafluoroethylene mesh sheets, J. Photochem. Photobiol., A: Chem. 151 (2002) 207–212.
[11] T. Nakashima, Y. Ohko, Y. Kubota, A. Fujishima, Photocatalytic decomposition of estrogens in aquatic environment by reciprocating immersion of TiO$_2$-modified polytetrafluoroethylene mesh sheets, J. Photochem. Photobiol., A: Chem. 160 (2003) 115–120.
[12] H. Coleman, E. Routledge, J. Sumpter, B. Eggins, J. Byrne, Rapid loss of estrogenicity of steroid estrogens by UVA photolysis and photocatalysis over an immobilised titanium dioxide catalyst, Water Res. 38 (2004) 3233–3240.
[13] I. Fittschen, H. Hahn, Characterization of the municipal wastewaterpart human urine and a preliminary comparison with liquid cattle excretion, Water Sci. Technol. 38 (1998) 9–16.
[14] K. Thorpe, R. Cummings, T. Hutchinson, M. Scholze, G. Brighty, J. Sumpter, C. Tyler, Relative potencies and combination effects of steroidal estrogens in fish, Environ. Sci. Technol. 37 (2003) 1142–1149.
[15] G. Boeije, R. Corstanje, A. Rottiers, D. Schowanek, Adaptation of the CAS test system and synthetic sewage for biological nutrient removal. Part I: Development of a new synthetic sewage, Chemosphere 38 (1998) 699–709.
[16] P. Mulvenna, G. Savidge, A modified manual method for the determination of urea in seawater using diacetylmonoxime reagent, Estuar. Coast. Shelf Sci. 34 (1992) 429–438.
[17] L. Goeyens, N. Kindermans, M. Yusuf, M. Elskens, A room temperature procedure for the manual determination of urea in seawater, Estuar. Coast. Shelf Sci. 47 (1998) 415–418.
[18] S. Preis, M. Krichevskaya, A. Kharchenko, Photocatalytic oxidation of aromatic aminocompounds in aqueous solutions and groundwater from abandoned military bases, Water Sci. Technol. 35 (1997) 265–272.
[19] D. Rassokhin, M. Gokzhaev, L. Bugaenko, G. Kovalev, Sonolysis of aqueous solution under argon: dependence of the rate of hydrogen peroxide formation on hydroxyl radical scavenger concentration, Mendeleev Commun. 4 (1994) 25–27.