

Scientific paper

Conversion and Estrogenicity of 17 β -estradiol During Photolytic/Photocatalytic Oxidation and Catalytic Wet-air Oxidation

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Received: 05-03-2012

Abstract

Estrogen 17 β -estradiol (E2), produced by human body and excreted into municipal wastewaters, belongs to the group of endocrine disrupting compounds that are resistant to biological degradation. The aim of this study was to assess the efficiency of E2 removal from aqueous solutions by means of catalytic wet-air oxidation (CWAO) and photolytic/photocatalytic oxidation. CWAO experiments were conducted in a trickle-bed reactor at temperatures up to 230 °C and oxygen partial pressure of 10 bar over TiO₂ and Ru/TiO₂ solids. Photolytic/photocatalytic oxidation was carried out in a batch slurry reactor employing a TiO₂ P-25 (Degussa) catalyst under visible or UV light. HPLC analysis and yeast estrogen screen assay were used to evaluate the removal of E2 and estrogenicity of treated samples. The latter was completely removed during photolytic/photocatalytic oxidation under UV (365 nm) light and photocatalytic oxidation under visible light. In CWAO experiments, complete removal of both E2 and estrogenicity from the feed solution were noticed in the presence of TiO₂ and Ru/TiO₂ catalysts.

Keywords: Catalytic wet-air oxidation, endocrine disrupting compounds, 17 β -estradiol, estrogenic activity, photolytic/photocatalytic oxidation, yeast estrogen screen (YES) assay

1. Introduction

17 β -estradiol (E2) is a natural estrogen hormone produced by human body, mostly women (up to 100 μ g per day), but in small amounts also in men (up to 25 μ g per day).¹ It is excreted from human body via urine into sewage systems. Untreated wastewaters and effluents from wastewater treatment plants are the main sources of surface waters pollution. E2 is estrogenically active and belongs to the group of endocrine disrupting compounds (EDCs), which are considered as emerging contaminants of concern.² EDCs have been shown to elicit negative effects on the endocrine systems of humans and wildlife at very low concentrations.^{3–5} The European project COMPREHEND has demonstrated the ubiquitous presence of EDCs in the aquatic environment across the Europe.⁶ Therefore, research efforts have been directed in development of methods for their efficient elimination from the environment.⁷

Although some of the conventional biological treatment systems have been reported to be able to reduce EDCs in wastewaters,^{8,9} a large part of EDCs is in fact removed by adsorption onto activated sludge^{7,8,10} or different colloids.¹¹ Incomplete removal of estrogens by conventional wastewater treatment processes has been demonstrated by Ternes et al.¹² and concentrations causing estrogenic effects have been detected in treated wastewaters as well as in aquatic environment after WWTPs discharges.^{5,12,13} Together with other steroid EDCs, such as estrone (E1), estriol (E3) and 17 α -ethinylestradiol (EE2), E2 has been reported to be responsible for around 90% of the estrogenicity of municipal wastewaters.¹⁴

The application of different advanced oxidation processes (AOPs), such as photocatalysis, ozone based technologies and ultrasound oxidation have been reported to be effective in removal of EDCs from aqueous samples.^{15–19} E1, E2, EE2 and BPA were effectively removed by means of AOPs using ozone, ozone/UV and UV/H₂O₂

techniques.^{20,21} They are easily photocatalytically degraded at the UV-irradiated TiO₂ surface.²² The estrogenicity of remaining parent compound and/or its by-products was not investigated in these studies. Contrary, some studies included also evaluation of estrogenic activity of treated samples.^{20,23,24} The addition of H₂O₂ greatly increases the degradation of E2 and EE2 as well as estrogenic activity during the direct UV photolysis indicating that oxidation of both pollutants and removal of estrogenic activity occurred simultaneously.^{23,24} In addition, products were detected as estrogenic active.

In many cases harmful secondary products can be formed during the treatment of organic pollutants by means of advanced oxidation processes, which are not eliminated by the same process and may be more estrogenically active than the original compound.^{16,25} However, the photolytic/photocatalytic oxidation²⁶ and CWAO processes²⁷ are known to be efficient for the treatment of a variety of organic pollutants. Therefore, our study was focused on the assessment of removal efficiency of E2 from aqueous samples and its estrogenicity by means of these techniques. For this purpose, estrogenic activity of treated E2 aqueous samples was evaluated by means of a yeast estrogen screen assay (YES assay)²⁸ and by HPLC analysis, which was used for quantification of remaining E2 in aqueous solutions after the treatment.

2. Experimental

2.1. Catalyst Preparation

The catalyst sample containing 3.0 wt. % of Ru was prepared by incipient-wetness impregnation of TiO₂ extrudates (Degussa-Hüls AG, Aerolyst type, d_p: 1.4 mm, S_{BET}: 51 m²/g, V_{porc}: 0.36 cm³/g, d_{porc}: 28 nm) with an aqueous solution of RuCl₃ · xH₂O (Acros Organics), concentration of which was accurately determined by ICP-AES before impregnation. The TiO₂ support was dried at 100 °C in an oven for 2 h and then impregnated at room temperature with an appropriate volume of solution containing the Ru salt to obtain 3.0 wt. % nominal Ru content. After the impregnation step, the catalyst precursor was dried (overnight at room temperature, then at 40 °C for 5 h, and finally at 105 °C for 2 h) and reduced directly in H₂ flow of 250 ml/min at 300 °C for 1 h without previous calcination. The reduced sample was cooled down to room temperature under N₂ flow. Reduction conditions were determined in this work by TPR analysis of dried catalyst precursor. The actual Ru loading in the synthesized Ru/TiO₂ catalyst, determined by using ICP-AES analysis, was found equal to 2.9 wt. %. Before the analysis, the sample was dissolved by sequential treatment in NaOCl and HF/HNO₃ solutions.

2.2. Catalyst Characterization

The specific surface area, total pore volume and average pore width of the supports and catalyst samples were

determined from the adsorption and desorption isotherms of N₂ at –196 °C using a Micromeritics ASAP 2020 MP/C instrument. This characterization was carried out after degassing of samples to 4 μm Hg for 1 h at 90 °C and 2 h at 300 °C. The same apparatus was used to perform static H₂ chemisorption analyses following the procedure described by Shen et al.²⁹ Ru dispersion measurements in the synthesized Ru/TiO₂ catalyst were performed at T = 35 °C. Prior to analysis, the catalyst surface was degassed in Ar atmosphere at 350 °C. The measurements were performed by measuring the amount of chemisorbed H₂ at various hydrogen partial pressures. Since the equilibrium is attained at each measuring data point, slow chemisorption kinetics on ruthenium exhibits no influence on measured data.

XRD patterns of fresh and used catalyst samples were obtained on a PANalytical X'Pert PRO diffractometer using Cu Kα radiation (λ = 0.15406 nm). Data were collected from 20 to 80° 2θ, at 0.034° and 100 seconds per step. Crystalline phases were identified by comparison with PDF standards from the International Centre for Diffraction Data (ICDD).

The high resolution electron micrographs of Ru/TiO₂ catalyst sample were recorded on a FE-SEM SUPRA 35VP (Carl Zeiss) microscope equipped with an EDAX energy dispersive X-ray spectrometer Inca 400 (Oxford Instruments).

Purity of TiO₂ supports used in the present study was determined by means of XRF analysis (Rigaku, model NEX CG). The carbon content of carbonaceous deposits accumulated on the titania and catalyst surface during the reaction course was determined, after washing, and drying the spent support and catalyst samples, using a CHNS elemental analyzer (Perkin Elmer, model 2400 Series II). Eventual leaching of Ru and Ti from the titania support and Ru/TiO₂ catalyst during the CWAO reaction was verified by ICP-AES analysis of collected liquid-phase samples.

2.3. CWAO Experiments

CWAO experiments were carried out in a Microactivity-Reference unit (PID Eng&Tech, Spain), which is an automated and computer-controlled, continuous-flow trickle-bed reactor for catalytic microactivity tests. The properties of the catalyst bed and operating conditions are listed in Table 1. Concentration of E2 (min. 99%, Aldrich) in the feed aqueous solution was 0.272 mg/l (*i.e.* 1.0 × 10⁻⁶ mol/l). Accordingly to literature data, this value is well below the E2 solubility in water (11.4–47.6 × 10⁻⁶ mol/l).³⁰

A fixed-bed tubular reactor (Autoclave Engineers, USA) was made of a 305 mm o.d. × 9 mm i.d. Hastelloy C-276 tube that was heated with a reactor furnace and integrated within the hot box. Liquid and gaseous flows were introduced into the hot box system that includes an

Table 1. Experimental conditions of the CWAO of E2 ($c_{\text{feed}} = 0.272$ mg/l) carried out in a continuous-flow trickle-bed reactor.

Mass of catalyst in bed, g	3.0
Bed density, g/cm ³	0.94
Bed porosity, /	0.41
Equivalent catalyst particle diameter, mm	1.42
Catalyst particle density, g/cm ³	1.59
Reaction temperature, °C	200, 230
Total operating pressure, bar	25.5, ^a 38.0
Oxygen partial pressure, bar	10.0
Gas flow rate, ml/min	60
Superficial gas flow rate (G), kg m ⁻² s ⁻¹	0.357, ^a 0.500
Liquid flow rate, ml/min	0.5
Superficial liquid flow rate (L), kg m ⁻² s ⁻¹	0.134, ^a 0.132
$t_{\text{res.L.}}$, min	0.24, ^a 0.23

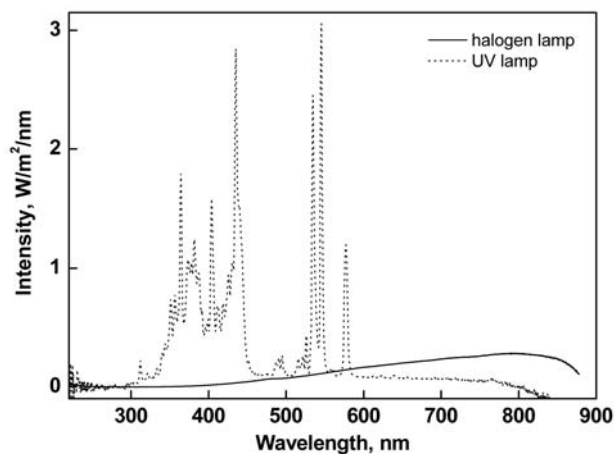
^aT = 230 °C.

electric forced convection heater which permits the process route to be preheated and kept at temperatures up to 190 °C. The liquid reactant was introduced into the unit using a HPLC positive alternative displacement pump (Gilson, model 307). The oxygen source in these experiments was pure O₂ (purity 5.0, Messer), which was fed to the system through an electronic HI-TEC mass-flow controller (Bronkhorst, model EL-FLOW). The preheated gas and liquid streams merge in a T-joint and are then introduced to the top of the reactor through a 10 μm sintered stainless-steel (316) filter (another is located at the outlet of the reactor, which protected the arrangement from possible finely separated catalyst fines). A porous (2 μm) plate made of Hastelloy C-276, supported on a 316 stainless-steel pipe, was placed inside near the middle of the reactor tube to support the fixed bed composed of 3.0 g of either TiO₂ extrudates or Ru/TiO₂ catalyst. The reaction temperature was measured by a K-type thermocouple, which was inserted through the upper end of reactor and was in contact with the catalyst bed, and regulated within ±1.0 °C from the pre-set temperature by a PID temperature controller (TOHO, model TTM-005). The gas and liquid phase, which passed the catalytic bed in a co-current downflow mode and flowed out at the bottom of the reactor, were separated in a high-pressure liquid-gas (L/G) separator cooled with a Peltier cell. The L/G separator equipped with a micrometric servo-controlled valve and capacitive level sensor provides an efficient liquid discharge from the unit. Representative liquid-phase samples were collected at the reactor outlet in intervals of at least 8 hours, which was required in order to obtain a sufficient volume of samples subjected to further preconcentration. The gas stream from the L/G separator was discharged through a second micrometric servo-controlled regulating valve, which was employed to provide continuous and constant flow of gases at the outlet (*i.e.*, pressure control). In the off-gas stream, production of CO₂ and eventual formation of CO were monitored by a non-dispersive IR de-

tor (Rosemount, model BINOS 1001). To avoid eventual water condensation, the stainless-steel pipelines as well as the IR detector were heated to about 60 °C.

2. 4. Photolytic/Photocatalytic Oxidation Experiments

Photolytic/photocatalytic oxidation of E2 ($c_0 = 0.272$ mg/l in ultrapure water (resistance 18.2 MΩ-cm)) was tested at atmospheric pressure in a batch slurry reactor ($V = 250$ ml) thermostated at $T = 20$ °C (Julabo, model FP 25), magnetically stirred (360 rpm) and continuously sparged with purified air (45 l/h). The catalyst concentration (TiO₂ P-25 from Degussa; $S_{\text{BET}}: 52$ m²/g, $V_{\text{pore}}: 0.17$ cm³/g, $d_{\text{pore}}: 13$ nm) was in the range of 0–0.5 g/l. After 30 min “dark” period (for the establishing of equilibrium of the sorption process), the reactor content was illuminated by either: (i) a UV high-pressure mercury lamp (150 W, emission in the 300–400 nm region with a maximum at ~365 nm) laid in a water-cooling jacket immersed vertically in the slurry, or (ii) a halogen lamp (150 W; the irradiation is labeled in the text as visible light). The spectra of lights used in this investigation are illustrated in Figure 1.

**Figure 1.** Spectra of UV and halogen lamp used in the present study.

Representative aqueous-phase samples were withdrawn from the reaction suspension after 6 h of operation and filtered through the 0.22 μm membrane filter (Sartorius) in order to remove catalyst particles.

2. 5. Analysis of End-product Solutions (HPLC)

All samples were extracted and concentrated using solid-phase extraction (SPE) technique by being passed through the Oasis® HLB 6cc (500 mg) SPE cartridges (Milford, Massachusetts, USA). Conditioning of cartridges

was performed with 4 ml of methanol, followed by 4 ml of distilled water. After loading samples, the cartridges were washed with 4 ml methanol (5 v/v %), dried under a gentle stream of nitrogen (N_2) and eluted with 4 ml of methanol. Eluted samples were collected in test tubes and concentrated under a gentle stream of N_2 to the volume of 0.5 ml.

Representative and preconcentrated liquid-phase samples, which were continuously collected from the L/G condenser/separator during the CWAO experiments or from the batch slurry reactor during photolytic/photocatalytic oxidation runs, were analyzed for residual E2 content by using HPLC analysis. Determinations were performed in the isocratic analytical mode using a 250 mm \times 4.6 mm Phenomenex Luna C18 5 μ column thermostated at 30 °C (UV detection at $\lambda = 210$ nm with a mobile phase of methanol (75%) and ultrapure water (25%) at a flow rate of 0.8 ml/min). The reliability of employed analytical protocol was investigated by determining the recovery factor, which was found to be in the range of 95 to 102% that is within an experimental error.

2. 6. YES Assay

Estrogenic activity of initial and treated samples was evaluated by “Yeast Estrogen Screen Assay” (YES assay) using recombinant yeast strain *Saccharomyces cerevisiae* BJ1991, developed in the Genetics Department at Glaxo Corporation under the guidance of Professor John P. Sumpter. Yeast hosts an integrated gene coding for human estrogen receptor (hER) in its genome and expression plasmids carrying the reporter gene *lac-Z*, which encodes the enzyme β -galactosidase. Following the activation of *lac-Z* gene in the presence of estrogenic active compounds, β -galactosidase degrades substrate β -D-galactopyranoside (CPRG), which changes its color from yellow to red. The YES assay was performed according to Routledge and Sumpter.²⁸ On each microtiter plate positive, negative and blank controls were used. 17 β -estradiol (E2) was used as a positive control (in concentration 27.2 μ g/l) and progesterone (P) as natural human androgen hormone, without the ability of binding to the human estrogen receptor, was used as a negative control (in concentration 31.4 μ g/l). As a blank control (B), yeasts exposed to the growth medium with CPRG were used in order to detect whether yeasts themselves, without exposing to estrogen active compound, could degrade CPRG. For determining estrogenic activity (EA), the absorbance measurements at 575 and 620 nm were carried out on the microtiter plate reader PowerWave XS (BioTek, USA). The values of EA were expressed as the activity of enzyme β -galactosidase.³¹ The relative estrogenic activity (REA, %) was calculated regarding estrogenic activity of initial samples:

$$REA(\%) = \frac{EA_{\text{treated sample}} - EA_{\text{blank}}}{EA_{\text{initial sample}} - EA_{\text{blank}}} \times 100$$

3. Results and Discussion

3. 1. Catalyst Characterization

Specific surface area, total pore volume and average pore width of TiO_2 in the form of extrudates and fresh Ru(3.0 wt. %)/ TiO_2 catalyst (S_{BET} : 50 m^2/g , V_{pore} : 0.34 cm^3/g , d_{pore} : 27 nm) show that the deposition of Ru did not significantly modify the textural properties and the corresponding XRD powder patterns (not shown). The XRD pattern of titania supports, which contain more than 99.5% TiO_2 as determined by XRF analysis (the rest was found in the form of Zr, Al, Si and Ca oxides), revealed the presence of anatase (Titanium oxide, PDF 03-065-5714) and rutile (Titanium oxide, PDF 00-004-0551) phases, and that anatase is the prevailing crystallographic form. The XRD pattern is not affected by the deposition of ruthenium, as the diffraction peaks of metallic Ru (Ruthenium, syn, PDF 06-0663: $2\theta = 38.39^\circ$, 42.15° and 44.01°) were not observed. It is believed that the Ru phase was not detected by XRD due to low Ru loading. It was further found out that the accessibility of ruthenium is poor on Ru(3.0 wt. %)/ TiO_2 catalyst. Low dispersion (5.4%) determined on the catalyst after reduction at 300 °C can be, at least partially, explained by an inhibiting effect of residual chloride on the fraction of Ru surface available for H_2 chemisorption³²⁻³⁴ and the use of large-sized TiO_2 extrudates.³⁵ The presence of rather large and discrete Ru particles on the surface of synthesized catalyst, as determined by H_2 chemisorption measurements (25 nm) is supported by SEM examination (Figure 2), which reveals the presence of Ru clusters in the range of 14–34 nm. The identification of Ru particles on the catalyst surface was performed by means of linescan EDX analysis.

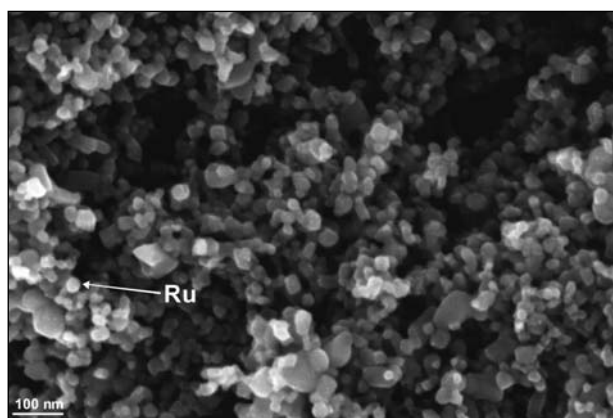


Figure 2. SEM micrograph of fresh Ru(3.0 wt. %)/ TiO_2 catalyst sample.

In order to elucidate an influence of reaction medium and reaction conditions on eventual structural and textural modifications of catalysts (*i.e.*, poisoning, sintering), XRD, N_2 adsorption-desorption isotherms (at -196 °C) and ICP-AES analyses were performed on catalysts (*i.e.* bare TiO_2

extrudates and Ru-containing solid) before and after the CWAO runs (used catalyst samples were thoroughly washed with distilled water and dried prior to analysis). A comparison of X-ray powder diffraction pattern of spent Ru(3.0 wt. %)/TiO₂ catalyst to that of the fresh one reveals that no peak characteristic of metallic Ru, ruthenium oxide phases or other phases (*i.e.* Fe, Fe oxides, carbon) was identified; the reflections correspond to those of the TiO₂ support (not shown). Furthermore, the measurements of textural properties of used catalysts revealed no differences in total pore volume and average pore width, while a small drop of specific surface area at an extent of about 5% was observed in comparison to fresh solids. In the light of these findings, Ru dispersion in spent Ru(3.0 wt. %)/TiO₂ catalyst was found to be equal to 5.3%. This is very similar to the value reported above, which confirms that in the applied range of CWAO operating conditions sintering of Ru particles doesn't occur to a measurable extent.

3. 2. E2 Catalytic Wet-air Oxidation

Liquid hold-up, residence time of the liquid phase in the catalytic bed, and catalyst external wetting efficiency during CWAO runs were determined using a procedure described in detail in our previous study.³⁵ Given the experimental conditions listed in Table 1, all CWAO runs performed in this study were conducted in a low-interaction (LIR) trickle-flow regime (L was in the range of 0.132–0.134 kg m⁻² s⁻¹), which means that the liquid trickles down the packing in the form of droplets, films and rivulets, while the continuous gas phase occupies the remaining porous space and flows separately.^{36,37} It can be further seen in Table 1 that the residence time of the liquid phase in the catalytic bed was in the range from 0.23 to 0.24 min. Analysis of the wetting efficiency has shown that at the used operating conditions this parameter was equal to 0.75, which implies that the external surface of catalyst particles was only partially wetted and thus directly exposed to the gas-phase stream.

Figure 3 shows E2 conversion as a function of time on stream in consecutive CWAO runs performed at different reaction temperatures (200 and 230 °C, respectively) in the presence of inert SiC (average particle diameter of approx. 0.5 mm, BET specific surface area below 0.1 m²/g), bare TiO₂ support or Ru(3.0 wt. %)/TiO₂ catalyst. The effect of temperature was investigated in this range, because it was observed in our previous studies^{38,39} that CWAO of phenol as an aromatic pollutant, carried out over titania- or CNF-supported catalysts at temperatures below 200 °C resulted in catalyst deactivation due to strong adsorption of partially oxidized C-6 intermediates (such as benzoquinones and hydroquinones) on the catalyst surface. Under He, there was no removal of organic carbon from the liquid phase; thus, the E2 conversion is only a result of oxidation pathways. In any of runs, no carbon monoxide was detected in the off – gas stream.

Elevated E2 conversions were already obtained when the reactor was filled with inert SiC, even at short residence time of the liquid phase calculated to be about 0.23–0.24 min (Table 1). Since it was verified that the measured E2 conversions are not influenced by adsorption effects, these observations confirm that in the given range of operating conditions E2 oxidation undergoes

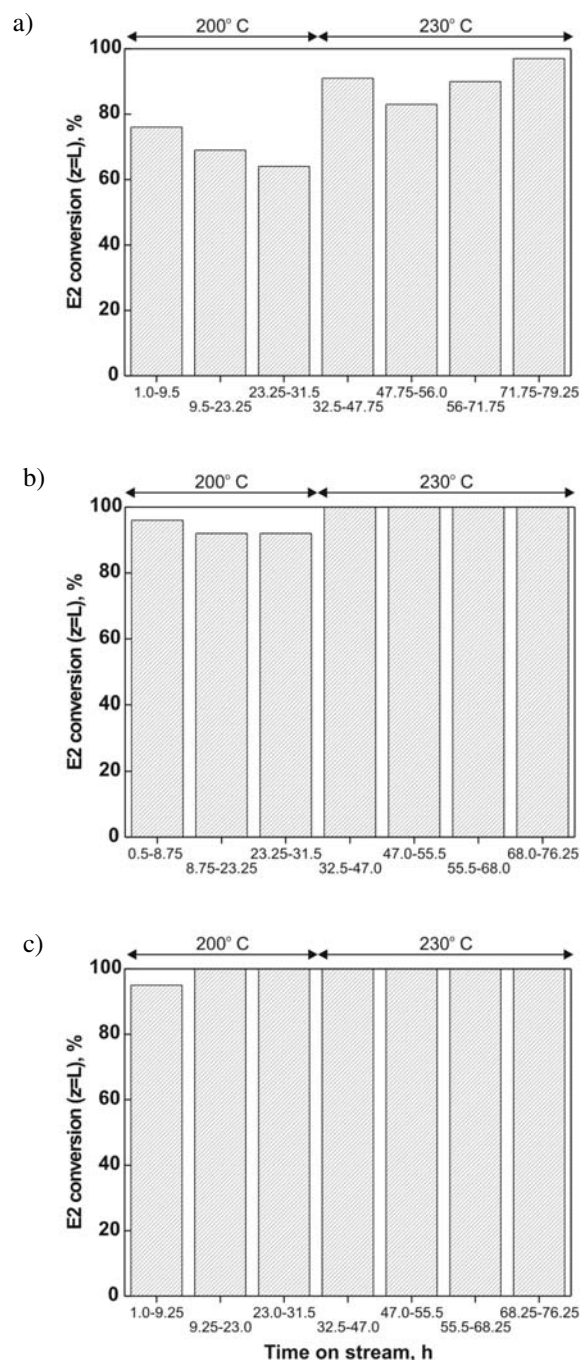


Figure 3. E2 conversion as a function of time on stream obtained over inert SiC (a), TiO₂ extrudates (b) and Ru(3.0 wt. %)/TiO₂ catalyst (c) at 200 and 230 °C. Operating conditions: p(O₂): 10.0 bar, Φ_{vol,i}: 0.5 ml/min, c(E2)_{feed}: 0.272 mg/l.

both noncatalytic as well catalytic oxidative routes. The E2 removal over the bare TiO₂ support was further improved as compared to runs conducted in the presence of SiC (Figure 3). As titania extrudates are more than 99.5% pure (XRF analysis), it is obvious that the observed activity for E2 oxidation is solely caused by TiO₂ itself. This can be attributed to the nonstoichiometric nature of TiO₂.⁴⁰

Furthermore, complete conversion of E2 was attained in the presence of Ru(3.0 wt. %)/TiO₂ catalyst, and no drop of activity was observed in the investigated time period of about 80 hours. This is in agreement with the finding that the TiO₂ support and Ru(3.0 wt. %)/TiO₂ catalyst were found to be very stable in the CWAO conditions used. Indeed, no leaching of either titanium or ruthenium (to the detection limit of 0.01 mg/l) was detected in the experiments performed in the presence of these solids. Finally, examination of SiC, TiO₂ support and Ru(3.0 wt. %)/TiO₂ catalyst after reaction under oxidative conditions by means of CHNS elemental analysis showed that carbonaceous deposits, which could be produced by simultaneous oxidative coupling reactions and result in eventual catalyst deactivation, were not accumulated on the surface of these solids during the CWAO of E2 (Table 2).

Table 2. Carbon content (measured by means of CHNS elemental analysis) on the surface of fresh and spent catalyst samples used in the CWAO of E2 aqueous solution. c(E2)_{feed}: 0.272 mg/l.

Catalyst sample		^a Carbon content, wt. %
SiC	fresh	0.03
	spent ^b	0.02
TiO ₂	fresh	0.13
	spent	0.14
Ru(3.0 wt. %)/TiO ₂	fresh	0.07
	spent	0.05

^a Limit of detection: 0.01 wt. %.

^b After CWAO of E2 carried out at both 200 and 230 °C.

γ-Al₂O₃ and CeO₂ exhibited an accelerating effect in the WAO of a model domestic wastewater,⁴¹ while in the oxidation of acetic acid CeO₂, CeO₂-ZrO₂, ZrO₂ and TiO₂ were identified to possess detectable catalytic properties.^{42,43} The oxidation promoted by these solids can be interpreted by their redox properties via a free-radical mechanism. The TiO₂ surface may first interact with oxygen to produce O₂^{•-} and HO₂[•] radicals. These species are able to initiate a radical mechanism by abstracting H[•] radicals from the adsorbed organic components. The ability of oxides to activate oxygen derives from the ease with which they deviate from the stoichiometric composition resulting in an oxygen deficient surface. Further activity enhancement with ruthenium is likely due to the possibility of this metal to undergo a redox cycle between two oxidation states and thus to activate oxygen.

The results presented in Table 3 list the average values of conversion and estrogenicity (presented as relative estrogenic activity – REA) of E2 aqueous samples treated by means of CWAO process.

Table 3. Conversion (%) and estrogenicity (REA, %) of E2 in end-product solutions obtained during CWAO process.

Sample	Reaction conditions	Conversion, %	REA, %
	feed E2 solution	/	100.0
	blank	/	0.0
S5a	E2, SiC, T = 200 °C	70.0 ± 6.0	95.2 ± 2.5
S5b	E2, SiC, T = 230 °C	90.0 ± 5.6	90.4 ± 3.5
S6a	E2, TiO ₂ extrudates, T = 200 °C	93.0 ± 2.2	101.5 ± 7.5
S6b	E2, TiO ₂ extrudates, T=230 °C	100.0	0.0
S7a	E2, Ru(3.0 wt. %)/TiO ₂ catalyst, T = 200 °C	100.0	0.0
S7b	E2, Ru(3.0 wt. %)/TiO ₂ catalyst, T = 230 °C	100.0	0.0

As discussed above, high conversion rates of E2 (above 70%) were achieved in all conditions used in the CWAO process. Due to remaining E2 in the samples S5a (81.6 µg/l), S5b (27.2 µg/l) and S6a (19.0 µg/l), and correspondingly high sensitivity of the YES assay, estrogenic activity was still detected in these end-product solutions. In other treated samples (S6b, S7a, S7b), neither E2 nor estrogenic activity were detected, which leads us to the conclusion that E2 was completely removed. This further alludes that reaction intermediates and by-products, if formed, exhibit no estrogenic activity.

3. 3. E2 Photolytic/Photocatalytic Oxidation

The results presented in Table 4 indicate the conversion and estrogenicity (presented as relative estrogenic activity – REA) of photolytically or photocatalytically treated E2 aqueous solution after the completion of experiments.

Table 4. Conversion (%) of E2 in the photolytic/photocatalytic oxidation and remaining estrogenicity (REA, %) after 6 hours of treatment.

Sample	Reaction conditions	Conversion, %	REA, %
	initial E2 solution	/	100.0
S1	E2, visible light	45.4 ± 0.1	100.0
S2	E2, visible light + TiO ₂ P-25	100.0	0.0
S3	E2, UV light (365 nm)	100.0	0.0
S4	E2, UV light (365 nm) + TiO ₂ P-25	100.0	0.0

Temporal course of E2 conversion and corresponding estrogenicity of treated aqueous-phase solutions obtained during photolytic/photocatalytic oxidation runs are presented in Figure 4.

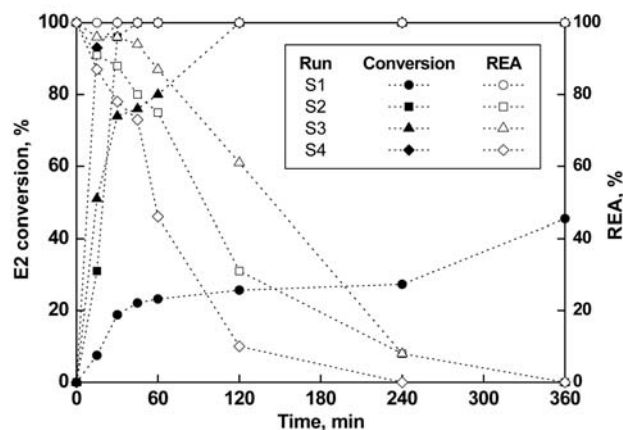


Figure 4. Temporal course of photolytic/photocatalytic oxidation of E2 ($c_0 = 0.272 \text{ mg/l}$) in a batch slurry reactor under various reaction conditions. Lines show trends.

Although 45% of E2 conversion was detected after 6 hours when the aqueous solution was treated by visible light (S1), REA levels remained constant at 100% (Table 4). Complete E2 conversion was detected after 45 min when E2 aqueous solution was treated under visible light in the presence of TiO_2 P-25 (Degussa) catalyst (S2). In this case, REA dropped for 20% after 45 min of exposure and completely disappeared after 6 hours. When E2 aqueous solution was treated by UV (365 nm) light (S3), 100% conversion occurred after 120 min and then REA dropped significantly in the treated sample. Complete conversion of E2 was achieved in 30 min when the solution was treated by TiO_2 P-25 catalyst under UV (365 nm) light (S4). Consequently, estrogenicity decreased for 20% already after 30 min and completely disappeared in the treated samples after 240 min (S4) (see Figure 4). It should be noted that the observed activity of the TiO_2 P-25 photocatalyst under illumination with visible light is attributed to the fact that no UV cut-off filter was used in this work; the employed halogen lamp emitted about 3% of light in the UV range (300–400 nm). In heterogeneously photocatalyzed runs performed in this study, no dissolution of titania in the liquid phase was observed.

Comparing the performed runs, the most effective removal of E2 was obtained during the photocatalytic oxidation under UV 365 nm light in the presence of TiO_2 catalyst, as complete conversion of E2 and removal of estrogenicity were detected after 30 and 240 min, respectively. This was followed by the results obtained by photocatalytic oxidation under visible light and TiO_2 catalyst exhibiting similar efficiency, but longer time was required to

reach both 100% conversion of E2 and complete disappearance of estrogenicity. Similar results were obtained in the case of photolytic oxidation process under UV light. However, photolytic oxidation under visible light was only partially successful, since 45% of E2 was converted after 360 min of exposure. Due to high residual content of E2 (150 $\mu\text{g/l}$) in aqueous solution, estrogenicity remained more or less the same as in the initial sample (Figure 4).

From the obtained results we can also see that the level of estrogenicity detected by YES assay dropped slower in comparison to the measured concentrations of E2 in the liquid phase determined by means of HPLC analysis. The reason for this obvious discrepancy was probably higher sensitivity of yeast to the remaining E2 (some ng/l) in tested samples in comparison to LoD (40 ng/l) and LoQ (145 ng/l) detection limits of the chemical examination using HPLC analysis.

Nevertheless, the obtained results of photolytic/photocatalytic oxidation of E2 are comparable to the reported results,^{23,24} where it was noticed that the addition of H_2O_2 to the reaction mixture increased the degradation process and decreased estrogenicity of E2. Similarly, our results show that the non-catalytic UV photolysis treatment was efficient regarding the removal of E2 and decrease of estrogenicity; however, both processes were significantly promoted by means of TiO_2 P-25 catalyst. We also confirmed the results obtained by Karpova et al.,²² that E2 is easily degraded in the presence of a heterogeneous TiO_2 catalyst, and upgraded them by confirming the removal of estrogenicity of E2 and its degradation products.

To summarize, high conversions of E2 during both oxidation processes were detected by the HPLC analysis and the obtained results were similar to the reported results.^{20,22–24} Comparing the results of CWAO experiments (Figure 3) with the results of photolytic/photocatalytic oxidation runs (Figure 4), we can conclude that both oxidation processes, when carried out in the presence of TiO_2 and Ru/TiO_2 catalysts were effective in obtaining oxidative destruction of E2 as well as in decreasing the estrogenicity of the initial E2 solution. The products formed during both oxidation processes, which may be those demonstrated by Huber et al.,⁴⁴ seem to possess no ability to cause estrogenic activity, since the latter was not detected in treated samples. Finally, E2 is moderately toxic to aquatic organisms after acute exposure; e.g., the reported 48h EC50 value for *Daphnia magna* is 2.97 mg/l.⁴⁵ In the light of this fact, no toxicity of feed solution and treated samples was determined towards test organisms from various taxonomic groups (luminescence marine bacteria *Vibrio fischeri*, water fleas *Daphnia magna*, unicellular green algae *Desmodesmus subspicatus*, fish embryos *Danio rerio*), which is due to very low E2 concentration of feed solution used. The results of these measurements also confirm that no intermediate or side products were formed at concentration levels that might exert toxic effects.

4. Conclusions

Natural estrogen hormone E2, which is mainly present in municipal wastewaters, was investigated for degradation and estrogenic potential during photolytic/photocatalytic oxidation and CWAO experiments. Comparing the obtained results, it was found that both oxidation processes were highly effective regarding both the removal of E2 and estrogenicity from the initial solution. Catalysts used in both processes clearly enhanced the oxidation of E2, especially Ru/TiO₂ catalyst used in the CWAO process was found to be very effective. Furthermore, the concomitant decrease in estrogenicity and concentrations of E2 in treated samples showed that no estrogenically active intermediates are formed during both processes. From the obtained results we can conclude that E2 can be partially degraded also under sunlight when discharged from WWTPs into aquatic environment, but for the complete removal and estrogenicity elimination of the E2 at the WWTPs additional treatment including AOP process is obviously needed.

5. Acknowledgements

The authors gratefully acknowledge the financial support of the Ministry of Education, Science, Culture and Sport of the Republic of Slovenia through Research program No. P2-0150.

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Povzetek

Estrogen 17β -estradiol (E2), ki ga proizvaja človeško telo, z izločanjem iz telesa prehaja v komunalne odpadne vode. Uvrščamo ga v skupino motilcev endokrinega sistema, ki so pogosto biološko slabo razgradljivi. Cilj raziskave je bil ugotoviti učinkovitost odstranjevanja E2 iz vodnih vzorcev s heterogenima procesoma katalitske mokre oksidacije in fotolitske/fotokatalitske oksidacije. Katalitsko mokro oksidacijo smo izvedli v kontinuirnem, trifaznem kapalnem reaktorju pri temperaturah do 230 °C in parcialnem tlaku kisika 10 bar z uporabo TiO_2 in Ru/TiO_2 katalizatorjev, fotolitsko/fotokatalitsko oksidacijo pa v reaktorju z goščo v prisotnosti TiO_2 P-25 (Degussa) katalizatorja, obsevanega bodisi z vidno bodisi UV svetlobo. Odstranjevanje E2 in estrogensko aktivnost smo ugotavljali s HPLC analizami in s testom YES. Estrogenske aktivnosti nismo več zaznali v vzorcih, obdelanih s fotolitsko/fotokatalitsko oksidacijo z UV svetlobo (365 nm) in fotokatalitsko oksidacijo z vidno svetlobo. V vzorcih, obdelanih s katalitsko mokro oksidacijo, je bila razgradnja E2 v prisotnosti TiO_2 in Ru/TiO_2 katalizatorjev popolna, estrogenske aktivnosti v obdelanih vzorcih pa nismo več detektirali.