



## Photocatalytic degradation of estradiol under simulated solar light and assessment of estrogenic activity



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### ABSTRACT

The ability of nanostructured titanium materials developed in the FP7/EU collaborative Clean Water project to photocatalytically degrade pollutants was tested, using 17 $\beta$ -estradiol (E2) as the model compound. The photocatalytic degradation of E2 was carried out under simulated solar light (both the UV part (280–400 nm) and full spectrum (200 nm–30  $\mu$ m)). The efficiency of the process was assessed using several indicators including the conversion yield, the mineralization yield, the formation of by-products and their endocrine disrupting effects. The newly synthesized catalysts significantly degraded E2 and their efficiency was found to depend on the irradiation wavelength range. Some of the intermediates formed during the photocatalytic treatment with ECT-1023t and Evonik P25 were identified and their estrogenic effect was evaluated *in vivo* using the ChgH-GFP transgenic medaka line. This analysis confirmed that in the structure of the identified by-products, the phenol group is not destroyed and that the estrogenic effect is still present in the corresponding solution. The persistence of the estrogenic effect after the photocatalytic treatment is hypothesized to be due to the presence of the phenol group in the by-products.

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### 1. Introduction

Numerous studies reveal the presence of micropollutants in surface and ground water which can interfere with the endocrine system of humans and animals [1–4]. These endocrine disrupting compounds (EDCs) have been shown to cause a number of reproductive and sexual abnormalities in wildlife as well as a decline in sperm count. They are also suspected of being involved in numerous types of cancer in human beings [5–7].

EDCs, of natural and/or anthropogenic sources have been shown to disrupt the estrogen axis. 17 $\beta$ -Estradiol (E2) is the most potent natural estrogen [8], presenting an estrogenic effect even at low concentrations (ng/L level) [5,9]. Around 90% of E2 excreted in

human and live-stock urine is endogenously produced but an additional <10% results from pharmaceuticals used in hormone replacement therapy.

The European commission has established water policies in order to ensure a sufficiently good water quality and environmental protection throughout the EU. The main objective of the Water Framework Directive of 2000 is to achieve a healthy state of water by 2015. In Brussels, in January 31st 2012, the Commission proposed the addition of 15 chemicals to the list of 33 pollutants that are monitored and controlled in EU surface waters. Because of its adverse effects, 17 $\beta$ -estradiol is found in the proposed list of additional priority substances.

Conventional waste water treatments are not able to completely remove this compound. Therefore, it is released into the environment *via* the effluent of conventional waste water treatments plants [10,11]. Thus, in order to achieve the requirements stated in the Water Framework Directive, there is a need for sustainable

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treatment technologies capable of removing estradiol from surface water.

Advanced oxidation processes (AOPs) and technologies (AOTs) have proved to be a promising alternative for the remediation of organic pollutants. The most popular AOPs studied are heterogeneous photocatalysis with semiconductors, ozonation and the photo-Fenton process [12,13]. Heterogeneous photocatalysis and the photo-Fenton process are of special interest since sunlight can be used as the irradiation source [14]. Among the catalysts used in heterogeneous photocatalysis, TiO<sub>2</sub> has been gaining attention due to its strong photoinduced oxidation power [15].

Most photocatalytic treatments applied to remove E2 have used UV light [16–19], but this is costly and potentially dangerous for human health. On the contrary, the use of solar light as an irradiation source is more appropriate since it is freely available and environmentally friendly.

Nowadays, the research for extremely active TiO<sub>2</sub> catalysts under near UV–vis irradiation is a hot topic. The strategies aiming at exploiting solar light efficiently focus on titanium nanostructured materials with high photocatalytic activity as well as doping of TiO<sub>2</sub> with transition metals or with non-metallic elements [20].

In this context, the collaborative project Clean Water (FP7 Grant Agreement number 227017) aimed to synthesize innovative nanostructured photocatalysts with photocatalytic activity within the UV–vis range of solar light [21–23]. The catalysts manufactured within this project were tested with high success for the degradation of several micropollutants [21–25].

Results are presented here for the photocatalytic degradation of E2 under different experimental conditions: UV spectrum (280–400 nm) or the full UV–vis spectrum simulating solar light (200 nm–30 μm).

Special attention was paid to the reaction intermediates as a function of the type of catalyst as well as to the estrogenic effect of the intermediates.

In a number of studies dealing with the photocatalytic degradation of E2, the evaluation of the estrogenic effect of the treated solution was performed using the Yeast Estrogen Screen [16,19]. This test is carried out *in vitro* and can detect estrogen receptor agonists and antagonists. However, estrogen axis disrupting compounds do not only act as directly on the estrogen receptor, they can also inhibit enzymatic catalysis reactions, the transport of hormones in the blood or the production of hormone. In this case, only *in vivo* analysis can identify the full spectrum of possible mechanisms of disruption caused by these compounds in a whole organism. Thus, to identify a broad range of endocrine disrupting compounds, it is more rigorous to carry out *in vivo* estrogenic tests which enable the detection of several mechanisms of endocrine disruption.

In the present study, the estrogenic effect of E2 intermediates was assessed using a ChgH-GFP transgenic medaka line, enabling the detection of multiple types of estrogen axis disruption (estrogen agonists and antagonists, inhibitors and activators of enzymatic catalysis reactions, aromatizable androgens).

## 2. Materials and methods

### 2.1. Materials

Estradiol was purchased from Sigma Aldrich. Titanium dioxide (AEROXIDE® TiO<sub>2</sub> P25,  $S_{BET} = 50 \text{ m}^2/\text{g}$ ) was obtained from Evonik Degussa GmbH (Frankfurt, Germany) and used as reference. Analytical reagents were obtained from Merck. Three catalysts in powder form were received from project partners: sol–gel synthesized, sieved (where aggregates were selected before

thermal treatment and calcined at 1023 K) nanocrystalline TiO<sub>2</sub> (ECT-1023t,  $S_{BET} = 18.3 \text{ m}^2/\text{g}$ ) [21]; nitrogen-modified TiO<sub>2</sub> (N-TiO<sub>2</sub>,  $S_{BET} = 141 \text{ m}^2/\text{g}$ ); and reduced graphene oxide-TiO<sub>2</sub> composite (GO-TiO<sub>2</sub>,  $S_{BET} = 110 \text{ m}^2/\text{g}$ ). GO-TiO<sub>2</sub> (graphene oxide content of 4.0 wt.%) were prepared by liquid phase deposition followed by post-thermal reduction at 200 °C [22], whereas N-TiO<sub>2</sub> was synthesized as the hydrolysis condensation product of tetrabutyl titanate reaction with urea [23].

### 2.2. Photocatalytic experiments

Photocatalytic experiments were carried out in a cylindrical reactor irradiated from above with a solar simulator (Newport, USA), equipped with a 450 W Xenon arc lamp. A quartz cover was placed on the top of the glass reactor to minimize water loss due to evaporation. An AM1.5 filter was placed at the beam output to obtain a solar-like spectrum and, by using dichroic mirrors, a correct working wavelength range was selected. To conduct experiments, two wavelength ranges were chosen: 280–400 nm and 200 nm–30 μm. The volume of the reactor was 1 L. Catalyst load (P25, ECT-1023t, N-TiO<sub>2</sub> and GO-TiO<sub>2</sub>) was 20 mg/L and the initial E2 concentration was 1 mg/L in ultra pure water (measured pH between 6 and 7). The solution of E2 and catalyst was allowed to equilibrate for at least 2 h before beginning each experiment. No significant adsorption phenomenon was observed. During irradiation, the solution was shaken and continuously bubbled with atmospheric oxygen. Aliquots were taken at various time intervals to determine the E2 residual concentration, the dissolved organic carbon concentration and the endocrine disruption effect. Prior to analysis, the solution was filtered through glass micro filters (GF/B,  $D = 1 \mu\text{m}$ , Whatman).

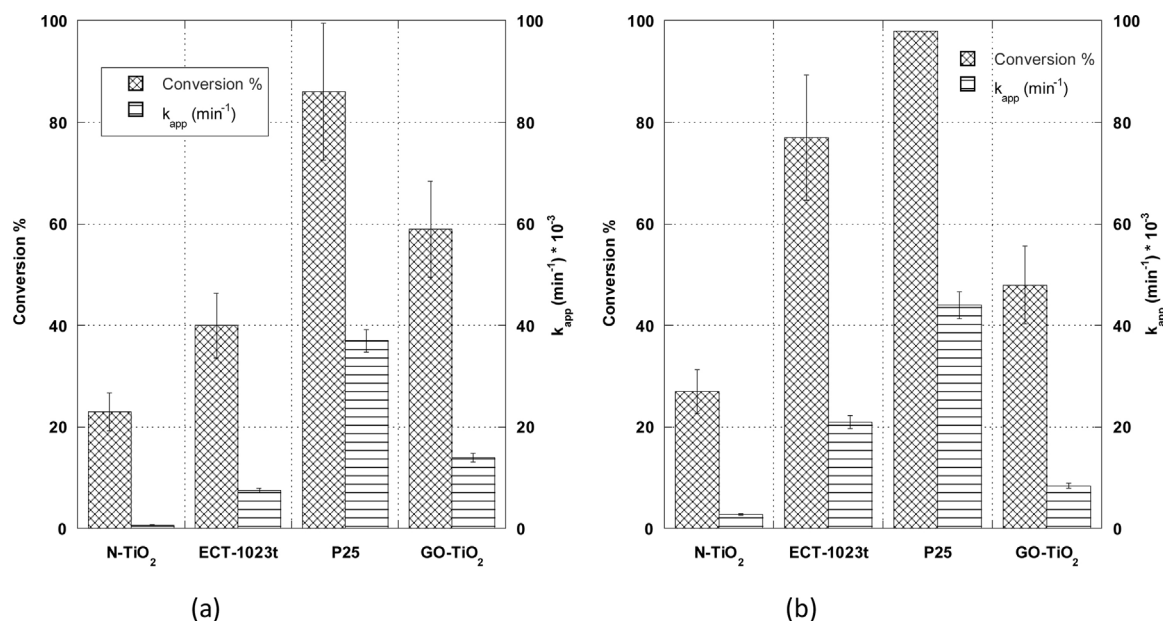
### 2.3. Analytical methods

The analysis of E2 was performed by HPLC (Model 600E, Waters) using a Nova Pack C18 reverse phase column (150 mm × 3.9 mm, I.D. 4 μm, Waters). A mobile phase isocratic elution program was applied with two solvents; Milli-Q water and acetonitrile ( $V_{\text{water}}/V_{\text{acetonitrile}} = 55/45$ ) at a flow rate of 1 mL/min. The detection was performed with a UV detector (Model 486, Waters) at 197 nm. Dissolved Organic Carbon (DOC) was monitored with a Shimadzu 5000 TOC analyzer. The detection and quantification limits were 12 μg/L and 40 μg/L for the HPLC/UV and 16 μg/L and 32 μg/L for the TOC analyzer.

For the identification of estradiol intermediates, samples taken during the photocatalytic experiments were analyzed in infusion by tandem mass spectrometry (MS/MS) (Thermo Fischer) with an electrospray ionization (ESI) source, a triple quadrupole analyzer and a photomultiplier electron detector (TSQ Quantum Discovery). Mass spectra were obtained as an average of 50 scans, each requiring 0.02 s. ESI source conditions were as follows: negative mode, heated capillary temperature 350 °C; sheath gas (N<sub>2</sub>) 40 psi, auxiliary gas (N<sub>2</sub>) 15 psi, spray voltage 3500 V, tube lens offset voltage 100 V.

### 2.4. Evaluation of estrogenic effect

The estrogenic test was performed by WatchFrog using a ChgH-GFP transgenic medaka line. The ChgH-GFP transgenic medaka line harbors the coding sequence of the green fluorescence protein (GFP) gene driven by the regulatory sequence of the estrogen responsive choriogenin H (ChgH) gene. In the WatchFrog test, the estrogenic activity is indicated by the fluorescence of transgenic medaka eleutheroembryos when they are exposed to estrogenic compounds.



**Fig. 1.** Percentage of E2 conversion and apparent kinetic constants during photocatalytic treatment under simulated solar light (a) full spectrum (200 nm–30 μm) and (b) UV part (280–400 nm).

In a typical experiment, the estrogenic test is conducted in parallel in 6-well plates with four replicate wells per test condition; the entire experiment is repeated two times. The exposure time in each well is 48 h at 26 °C. After 24 h, the medium is renewed and finally, after 48 h, the fluorescence of the elutheroembryo is observed with a MZ10F stereomicroscope (Leica), photographed with an Infinity 1–3C camera (Lumenera Corporation) and quantified with Image J Software (U.S. National Institute of Health).

Each test group has a specific objective (Table 2). There are control groups, raw sample groups to detect activators of the estrogen axis and groups exposed to sample spiked with testosterone to detect anti-estrogens and aromatase disruptors [25]. The results obtained on the fluorescence of ChgH-GFP fry are analyzed according to the Organization for Economic Co-operation and Development (OECD) guidelines for the statistical analysis of ecotoxicology experiments (Document on the Statistical Analysis of Ecotoxicity Data, OECD 2003) and are then classified into three levels of risk: 0, 1 and 2 [25].

For level 0, the fluorescence of fry exposed to sample is not significantly different from that of negative control 1 (pure water). Similarly there is no significant difference between fry treated with positive control 2 (testosterone) and the sample spiked with testosterone; compounds in this sample are considered to be inert. For level 1, the fluorescence of fry exposed to treated sample differs significantly from that obtained in the negative control 1, therefore the compounds are suspected of having estrogenic effects. Alternatively the fluorescence of the fry exposed to sample spiked with testosterone differs significantly from that obtained in the positive control 2 (testosterone) so the compound is suspected of having effects on aromatase enzyme activity. In level 2, the fluorescence of fry exposed to treated sample differs significantly from that obtained in the negative control 1 and is higher than that of positive control 1 (ethinylestradiol, EE2). Alternatively the fluorescence of fry exposed to sample with testosterone differs significantly from that obtained in the positive control 2 (testosterone) and is above the level of positive control 3 (testosterone plus EE2), or is below the level of positive control 4 (testosterone plus prochloraz). Therefore the sample has sufficiently strong activity to provoke physiologically relevant effects on the estrogen axis.

### 3. Results and discussion

#### 3.1. Photocatalytic experiment

Prior to conducting photocatalytic degradation tests, adsorption of E2 on the catalysts to be used was evaluated. To do this, an aqueous solution of E2 (1 mg/L) containing catalyst was placed in the dark and magnetically stirred. After 3 h of agitation, no significant variation in E2 concentration was observed. Therefore the adsorption of E2 onto the catalysts is considered as negligible.

The results obtained after 60 min of E2 photocatalytic treatment under simulated solar light (UV–vis light (200 nm–30 μm) and UV light (280–400 nm)) are presented in Fig. 1. It can be observed that the photocatalytic degradation of E2 depends on the nature of the catalyst and on the irradiation wavelength range (UV–vis or UV). Among the catalysts developed by the Clean Water Project partners, GO-TiO<sub>2</sub> is the most efficient catalyst under UV–vis irradiation and ECT-1023t is the most efficient catalyst under UV irradiation. However, irrespective of the irradiation type, P25 is more efficient than the other catalysts. In fact, after 60 min of photocatalytic treatment under UV irradiation, P25, ECT-1023t, GO-TiO<sub>2</sub> and N-TiO<sub>2</sub> degraded E2 by 98%, 77%, 48% and 27%, respectively. Under simulated UV–vis solar light, 86%, 40%, 59% and 23% of E2 is degraded using P25, ECT-1023t, GO-TiO<sub>2</sub> and N-TiO<sub>2</sub>, respectively.

By considering the conversion yield (Fig. 1), it could be observed that there is a decrease in the catalytic efficiency for P25 and ECT-1023t under UV–vis irradiation (Fig. 1a) when compared to the catalytic efficiency under UV irradiation (Fig. 1b). This is not the case for the GO-TiO<sub>2</sub> and N-TiO<sub>2</sub> nonmaterial. For GO-TiO<sub>2</sub>, no decrease in catalytic efficiency for E2 degradation was observed and for N-TiO<sub>2</sub> the efficiency is quite similar under UV and UV–vis.

One of the reasons for the decrease in efficiency could be related to the light intensity. Measurement of the light intensity in the photocatalytic reactor showed a decrease in the UV part of the simulated UV–vis solar irradiation when irradiance was measured at 365 ± 5 nm with a VLX-3W radiometer (Vilber Lourmat). In fact, at 365 ± 5 nm, the measured intensity was 2.35 mW/cm<sup>2</sup> under simulated UV light and 1.85 mW/cm<sup>2</sup> under simulated UV–vis light. This decrease in intensity may be the principal cause of the decrease in photocatalytic efficiency between irradiation under simulated UV

**Table 1**  
Characterization of photocatalytic materials.

Catalysts	Anatase/Rutile	Cristallite size (nm) A	Cristallite size (nm) R	Specific surface (m <sup>2</sup> /g)	Gap (eV)
N-TiO <sub>2</sub>	100/0	7.9	–	140.0	2.3
ECT-1023t	89–94/11–6	57.0	86.3	18.3	2.9
GO-TiO <sub>2</sub>	100/0	4.0–5.0	–	147.0	2.9
P25	80/20	25.0	85.0	50.0	3.1

solar light and simulated UV–vis solar light obtained for the P25, ECT-1023t and N-TiO<sub>2</sub> materials.

Despite the decrease in the UV part of the simulated UV–vis solar irradiation, an increase of GO-TiO<sub>2</sub> efficiency for E2 degradation (40 ± 6%) was obtained under simulated UV–vis in comparison to the E2 degradation under UV irradiation. One possible explanation for this behavior is that the GO-TiO<sub>2</sub> catalyst may exhibit a higher photocatalytic activity in wavelengths closer to visible light (above 400 nm). This hypothesis is supported by the fact that the photocatalytic activity of GO-TiO<sub>2</sub> under visible light irradiation has already been demonstrated for the degradation of the pharmaceutical diphenhydramine [22]. As the determined band gap of GO-TiO<sub>2</sub> is similar to ECT-1023t (Table 1), the performance of GO-TiO<sub>2</sub> under visible light was attributed to the optimal assembly and interfacial coupling between the reduced GO sheets and TiO<sub>2</sub> nanoparticles with the graphene oxide sheets acting as sensitizers in GO-TiO<sub>2</sub>. The scheme that initiates the photocatalytic activity was described as follows: under visible irradiation, electrons from the excited state localized sp<sup>2</sup> are injected into the conduction band of TiO<sub>2</sub> and then, the injected electrons are scavenged by O<sub>2</sub> molecules to produce reactive radicals that can attack estradiol molecules [26].

However, in a previous study, GO-TiO<sub>2</sub> has been also tested for the photocatalytic degradation of bisphenol A (BPA) under similar experimental conditions to the present study and no increase in photocatalytic activity under visible light irradiation was demonstrated [25]. Taken together these results imply that the optimal wavelength of light for a given photocatalyst depends also on the substrate to be degraded.

### 3.2. Estrogenic effect

Estradiol was photocatalytically degraded under UV irradiation (280–400 nm) and under this condition; P25 and ECT-1023t were the most efficient catalysts among those tested. After 80 and 180 min of photocatalytic treatment with P25 and ECT-1023t respectively, E2 concentration in solution was below the detection limit (LOD = 12 µg/L) of the HPLC/UV apparatus (data not shown). However, E2 is a compound with significant estrogenic activity even at low concentrations (ng/L) [5,8,9]. Thus, even if E2 was not detected in the solution, this does not imply that there was no remaining estrogenic effect. Moreover, during the photocatalytic

**Table 2**  
Description of the samples in the test for evaluation of estrogenic effect.

Name of samples	Contents	Objectives
Negative control	Fry, 8 mL of pure water	Gives the level of fluorescence of a non-treated fry
Positive control 1	Fry, 0.488 µg/L of ethinylestradiol in water	Gives the level of fluorescence above which a physiological effect on the reproductive efficiency and the production of vitellogenin is proven in the OECD 21 day test
Positive control 2	Fry, 30 µg/L of testosterone in water	Gives the basal level of aromatase enzyme activity
Positive control 3	Fry, 30 µg/L of testosterone and 0.488 µg/L of ethinylestradiol in water	Gives the level of fluorescence in a spiked sample above which a physiological effect on the reproductive efficiency and the production of vitellogenin is proven in the OECD 21 day test
Positive control 4	Fry, 30 µg/L of testosterone and 100 µg/L of prochloraz in water	Gives the level of fluorescence in a spiked sample below which a physiological effect on the reproductive efficiency and the production of vitellogenin is proven in the OECD 21 day test
Raw sample	Fry, sample taken during the treatment	Indicates the estrogenic effect of the sample
Sample spiked with testosterone	Fry, sample taken during the treatment, testosterone 30 µg/L	Indicates the effect of the sample on aromatase enzyme activity

treatment, intermediates with an higher estrogenic effect than E2 could have been generated, increasing the estrogenicity of the solution. So for these reasons, the estrogenic effect of E2 solution during the photocatalytic treatment was evaluated biologically.

The samples used to evaluate the overall estrogenic effect of the treated solution were taken during the photocatalytic degradation of E2 under UV irradiation with P25 and ECT-1023t. The percentages of conversion and mineralization of E2 in these samples are presented in Table 3 and the fluorescence of fry exposed to these samples is presented in Figs. 2 and 3.

According to the results presented in Figs. 2 and 3, the fluorescence of fry exposed to the E2 solution (1000 µg/L, t = 0 min) is significantly different from the one obtained in the negative control. When the E2 solution is spiked with testosterone, the fluorescence of the fry is higher than the one obtained in the positive control 2 (testosterone alone). The risk level is therefore 2, in concordance with the fact that E2 is as an estrogen disruptor.

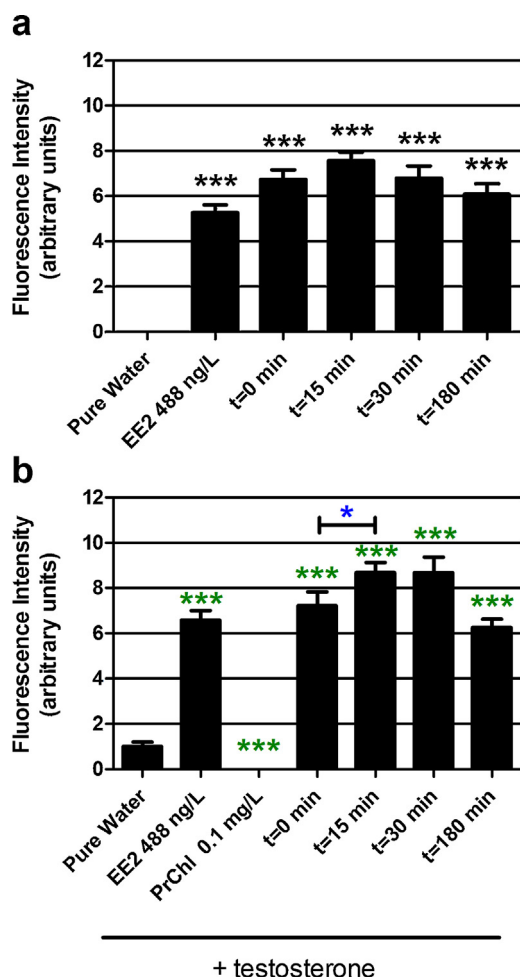
During the photocatalytic treatment with P25, the samples collected at t = 15, 30 and 180 min induce a fluorescence significantly different from the one obtained in the negative control (Fig. 2(a)). When these samples are spiked with testosterone, they also induce a level of fluorescence significantly different from the one obtained in the positive control 2 (testosterone alone). The risk level is therefore 2, the compounds present in these samples are estrogen axis disruptors. Thus, it can be concluded that the photocatalytic treatment did not eliminate the estrogenic effect of the E2 solution over the time scale of the photocatalytic experiments.

The lowest published observable effect concentration (LOEC) for an induction of fluorescence by E2 using ChgH-GFP fry is 0.17 µg/L [27]. For the samples collected at t = 15 min and t = 30 min, E2 concentration in these samples was 500 µg/L and 253 µg/L respectively. So, it can be said that the estrogenic effect observed is due to the residual concentration of E2. However, in comparison to the estrogenic effect induced by the E2 solution at t = 0 min, the sample taken after 15 min of treatment (t = 15 min) caused a statistically significant increase in fluorescence (p < 0.05, blue star, Fig. 2(a) and (b)). This increase in estrogenic effect may be due to the formation of intermediate products with a greater estrogenic activity than E2 or to a combinatorial effect of E2 with its reaction intermediates.

The formation of E2 intermediate products with an estrogenic effect has also been observed during the treatment of E2 using other

**Table 3**Percentage of mineralization and E2 conversion ( $[E2]_0 = 1000 \mu\text{g/L}$ ) and evaluation of the endocrine disruption effect of the photocatalytic products of estradiol.

	Irradiation time (min)	$[E2]$ ( $\mu\text{g/L}$ )	E2% conver.	E2% min.	Estrogenic risk level	Conclusion
ECT-1023t	25 min	438	51	14	Level 2	Estrogen axis disruptor
	50 min	255	71	15	Level 2	Estrogen axis disruptor
	240 min	<LOD	99	55	Level 2	Estrogen axis disruptor
P25	15 min	500	54	27	Level 2	Estrogen axis disruptor
	30 min	253	77	29	Level 2	Estrogen axis disruptor
	180 min	<LOD	99	55	Level 2	Estrogen axis disruptor

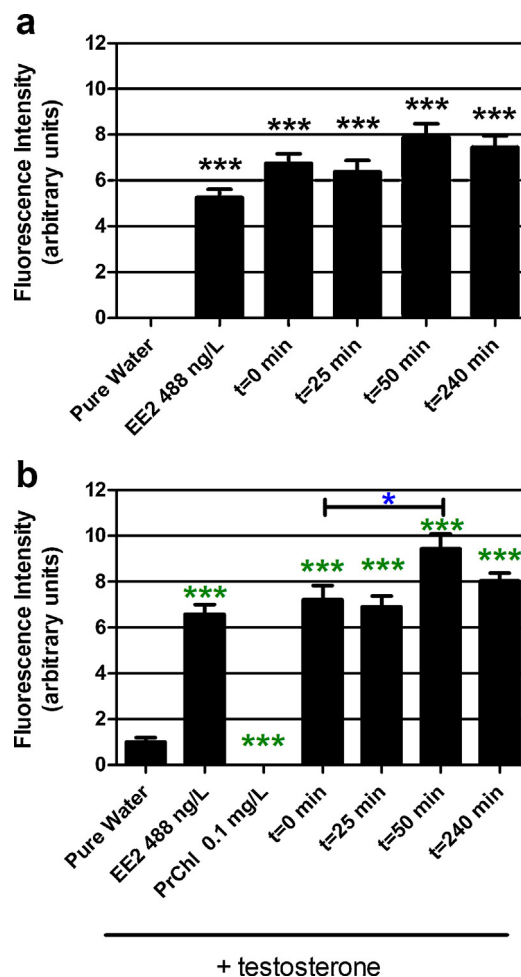


**Fig. 2.** Fluorescence of fry exposed to (a) samples treated with P25 and (b) samples treated with P25 mixed with testosterone. (\*Significant ( $p < 0.05$ ), \*\*\*Extremely significant ( $p < 0.001$ ), EE2: ethinylestradiol and PrChl: prochloraz). Black text: pure water is compared to EE2 sample and treated samples. Green text: testosterone sample compared to EE2 + testosterone sample, PrChl + testosterone sample or treated samples spiked with testosterone. Blue text: sample at  $t = 0$  min is compared to treated samples.

oxidation processes. For example, Bila et al. observed an increase in the estrogenic effect of E2 solution during the treatment by ozonation ( $[E2] = 10\text{--}50 \mu\text{g/L O}_3$  at  $5 \text{g h}^{-1}$ ), using the “Recombinant Yeast Assay” (YES) test [28]. In another study, Maniero et al. [29] using the YES assay also observed an increase of the estrogenic effect in the treatment of E2 by ozonation and also during the treatment of E2 by ozonation in the presence of hydrogen peroxide ( $[E2] = 10 \text{mg/L O}_3$  at  $5 \text{g h}^{-1}$ ). In contrast, Ohko et al. [19] and Rosenfeldt et al. [30] postulated that E2 intermediate products have no estrogenic effect. These authors studied the degradation of E2 by photocatalysis (He-Xe lamp,  $[E2]/P25 = 10^{-9} \text{mol/mg}$ ) and by UV and UV/ $\text{H}_2\text{O}_2$  (medium pressure lamp 1 kW,  $[E2] = 5 \mu\text{M}$ ) respectively. These discrepancies in the literature concerning the

estrogenic effect of E2 intermediate products suggest that the reaction intermediates could be different depending on the treatment process and operational conditions.

For samples collected after 180 min of treatment, a decrease in fluorescence of fry was observed compared to samples collected before initiating the photocatalytic treatment, although this difference did not achieve statistical significance. When these samples were spiked with testosterone, a decrease in fluorescence of fry was also observed (Fig. 2(a) and (b)). These results suggest that the samples collected at 180 min present a lower estrogenic effect than the initial solution of E2 (at  $t = 0$  min) and that the photocatalytic treatment of E2 with P25 has led to a decrease in the estrogenic activity, although complete elimination of the estrogenic activity was not achieved.



**Fig. 3.** Fluorescence of fry exposed to (a) samples treated ECT-1023t and (b) samples treated with ECT mixed with testosterone. (\*Significant ( $p < 0.05$ ), \*\*\*Extremely significant ( $p < 0.001$ ), EE2: ethinylestradiol and PrChl: prochloraz). Black text: pure water is compared to EE2 sample and treated samples. Green text: testosterone compared to EE2 + testosterone, PrChl + testosterone or treated samples spiked with testosterone. Blue text: sample at  $t = 0$  min is compared to treated samples.

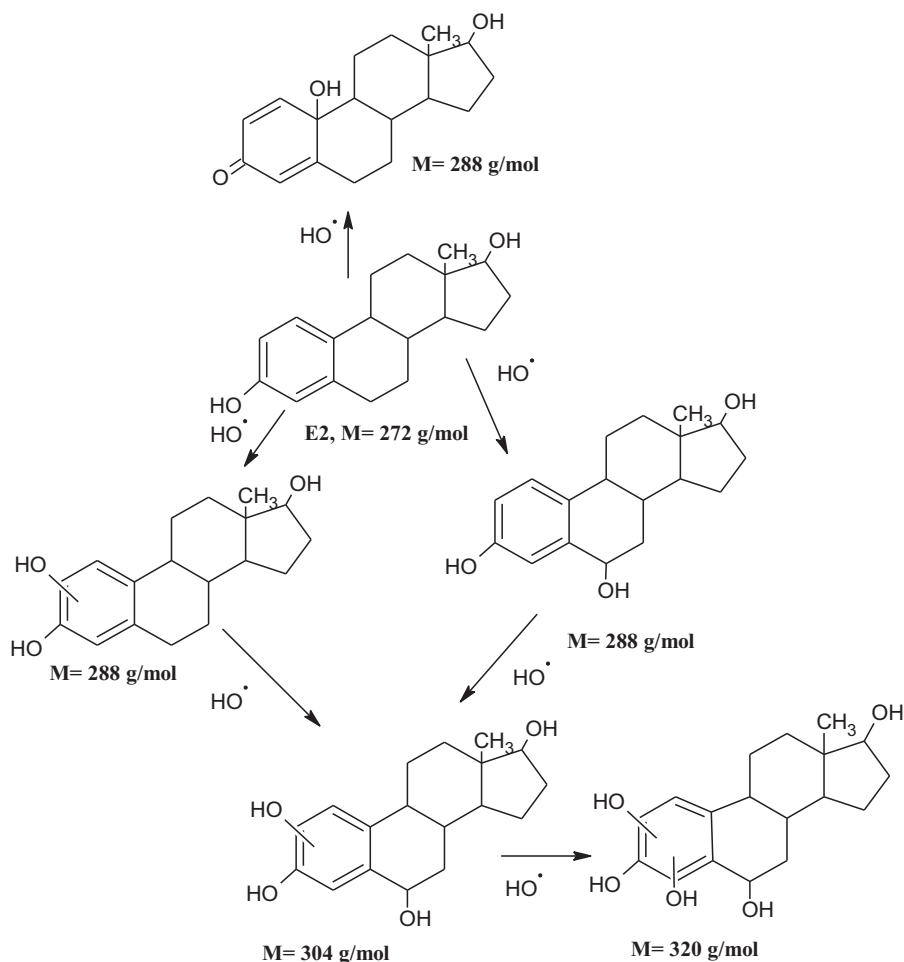


Fig. 4. Proposed reaction pathway for the photocatalytic degradation of 17 $\beta$ -estradiol.

Concerning the E2 solution treated with the ECT-1023t catalyst (Fig. 3), samples collected at  $t = 25, 50$  and  $240$  min, showed statistically significant increases in the fluorescence of fry when compared to the negative control. Likewise co-incubation of fry with testosterone caused a statistically significant increase in fluorescence compared to positive control 2. At  $t = 50$  min the compounds which are present in these samples also showed an increased estrogenic effect compared to  $t = 0$ . In the samples taken at 25 and 50 min, the E2 concentration was  $438 \mu\text{g/L}$  and  $255 \mu\text{g/L}$  respectively. Fluorescence of fry induced by these samples could therefore be attributed to the remaining E2 in the solution. For the sample collected at 240 min, it was observed that this sample induced a higher level of fluorescence than that induced by the initial solution ( $t = 0$  min). Thus, the estrogenic effect after 240 min of treatment with ECT-1023t is greater than the estrogenic effect caused by E2 solution before the photocatalytic treatment.

A summary of the results obtained with the estrogenic test is given in Table 3. As it can be observed, although estradiol was degraded during the photocatalytic treatment under UV irradiation with ECT-1023t and P25, the estrogenic effect of the solution was not removed. Therefore, it is suggested that with both catalysts (ECT-1023t and P25), a number of pro-estrogenic reaction intermediates were formed.

### 3.3. Identification of reaction intermediates

The results obtained using the estrogenic test showed that the endocrine disruption effect persists in the solution during the

photocatalytic treatment. To better understand the persistence of the estrogenic effect of the intermediates in the resulting mixture, it was necessary to extend the investigation to the identification of the major by-products of E2 formed during the photocatalytic degradation.

Aliquots taken during the photocatalytic treatment of E2 with either P25 or ECT-1023t, at identical time points and carried out under identical experimental conditions as previously, were analyzed in infusion by MS/MS. The main parent ions ( $m/z$ ) corresponding to by-products and obtained in a full Q1 MS analysis as well as their respective main fragment ions are presented in Table 4. As observed in Table 4, six main compounds with parent ions at  $m/z$  of 287, 303, 319, 285, 301 and 317 have been detected as E2 intermediates formed during the photocatalytic treatment. Some parent ions at  $m/z$  of 285, 287 and 303 have already been observed during estradiol degradation using oxidation processes [18,19,31–33].

Between the parent ion of E2 with an  $m/z$  of 271 and the ion with an  $m/z$  of 287, there is a mass difference of 16. This could come from the addition of a hydroxyl group. This E2 intermediate with a molecular mass of 288 g/mol, could be 2-hydroxyestradiol or its resonance structure 10 $\epsilon$ -17 $\beta$ -dihydroxy-1,4-estradien-3-one [18,19,31–33]. In fragmentation, the parent ion with an  $m/z$  of 287 gives fragment ions with  $m/z$  ratios of 259 and of 269. The fragment ions with a  $m/z$  of 259  $[\text{M}-\text{H}-28]^-$  and  $m/z$  of 269  $[\text{M}-\text{H}-18]^-$  may come from the loss of carbonyl group and  $\text{H}_2\text{O}$  respectively from the  $m/z$  287 parent ion. Thus, both 2-hydroxyestradiol and 10 $\epsilon$ -17 $\beta$ -dihydroxy-1,4-estradien-3-one could be the intermediate compounds detected in this MS molecular spectral range.

**Table 4**  
Compounds detected during the photocatalytic treatment of E2 and their respective fragment ions (collision energy: 15 eV).

Compounds (molecular mass g/mol)	Parent ions [M–H] <sup>–</sup> (m/z)	Main fragment ions (m/z)
17β-Estradiol (272)	271	183
		145
		143
		269
		269
Product 1 (288)	287	259
		243
		225
		285
		285
Product 2 (304)	303	269
		259
		241
		301
		283
Product 3 (320)	319	275
		247
		267
		259
		241
Product 4 (286)	285	223
		283
		257
		259
		145
Product 5 (302)	301	299
		281
		273
		255
		255
Product 6 (318)	317	273
		255
		281
		273
		255

The compound with the parent ion with an *m/z* of 303 could come from the addition of the hydroxyl radical to the compound with the parent ion at *m/z* ratio of 287. In fact, between the molecular mass of these compounds, there is a mass difference of 16 units. As it can be seen in Table 4, during the fragmentation, this compound loses a H<sub>2</sub>O molecule ([M–H–18]<sup>–</sup>) and forms a fragment ion with an *m/z* of 285. As there is no loss of carbonyl group observed ([M–H–28]<sup>–</sup>), the compound with the molecular mass of 304 g/mol does not contain a carbonyl group in its chemical structure. Moreover, the compounds with molecular masses of 288 and 304 g/mol have common fragment ions *m/z* 259 and *m/z* 269, suggesting that these intermediates have a similar structural base.

The hydroxylation of the product with the molecular mass of 304 g/mol gives a compound with a molecular mass of 320 g/mol. In fragmentation, this compound successfully loses two H<sub>2</sub>O molecules to form fragment ions with *m/z* ratios of 301 and 283. For the compounds with molecular masses of 286, 302 and 318 g/mol, there is a difference of two mass units between the compounds with molecular masses of 288, 304 and 320 g/mol, respectively. This difference in mass units could be due to a dehydrogenation of the compounds with molecular masses of 288, 304 and 320 g/mol. The difference in mass units is also present in fragment ions (Table 4). It could be the alcohol functions of the compounds with molecular masses of 288, 304 and 320 g/mol which are dehydrogenated to form carbonyl groups. However, as no loss of carbonyl groups ([M–H–28]<sup>–</sup>) is observed for the parent ions with *m/z* ratios of 285, 301 and 317 during the fragmentation, it is difficult to propose a chemical structure for the compounds with molecular masses of 286, 302 and 318 g/mol.

According to the fragmentation pattern described for the main intermediates found during the photocatalytic treatment of E2, some chemical structures have been proposed for these intermediates and a photocatalytic degradation pathway is also proposed (Fig. 4). As it can be observed, the phenol group is still present in

most of the intermediates. In addition, these intermediates have OH groups that can form hydrogen bonds with the estrogen receptor. This could explain the persistence of the estrogenic effect of the resulting mixture of intermediates, in agreement with the literature attributing the estrogenic effect of E2 to the interaction of the phenolic group with estrogen receptors [34,35].

#### 4. Conclusions

In this study, the efficiency of the photocatalysts ECT-1023t, N-TiO<sub>2</sub> and GO-TiO<sub>2</sub> was evaluated for the degradation of 17β-estradiol under simulated solar light (both UV and UV-vis). These catalysts significantly degraded E2 and, depending on the irradiation type; ECT-1023t and GO-TiO<sub>2</sub> were the most efficient in terms of E2 conversion. ECT-1023t was the most efficient under UV light and GO-TiO<sub>2</sub> was the most efficient under UV-vis irradiation. The higher efficiency of GO-TiO<sub>2</sub> under UV-vis, in comparison to ECT-1023t under the same irradiation, may be attributed to the photocatalytic activity of GO-TiO<sub>2</sub> under near-visible light. However, some caution must be taken as when GO-TiO<sub>2</sub> was used to degrade BPA under UV-vis light, as described in a previous paper, no significant increase of the photocatalytic activity was observed [25]. This also implies that the efficiency of the catalyst depends on the substrates to be degraded.

Under the present experimental conditions the E2 pollutant was fully degraded to below detection limits, however it is possible that a biologically active concentration of E2 remained. In fact, even if 17β-estradiol is below detection limit in the solution, the estrogenic effect persists during the photocatalytic treatment. Another possibility which explain the persistence of the estrogenic effect is the formation of estrogenic intermediates. The identification of the main reaction intermediates with the reference catalyst P25 (AEROXIDE® TiO<sub>2</sub>) and with the catalyst ECT-1023t showed that the phenol group is still present in these compounds. This could explain the estrogenic effect of the treated solution.

This work outlines the challenge of developing an efficient photocatalyst able to degrade a broad range of pollutants and also to remove the adverse acute toxic effects which appear even at low level pollutant concentration (μg/L to ng/L).

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