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Are UV photolysis and UV/H_2O_2 process efficient to treat estrogens in waters? Chemical and biological assessment at pilot scale

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ABSTRACT

In this study, UV based treatments were implemented at pilot scale to assess their ability to remove hormones from treated wastewater, especially with the view to equip small and medium size Wastewater Treatment Plants (WTPs). To this end, the degradation of a mixture of estrogenic hormones (Estrone (E1), β-Estradiol (E2), and 17α-Ethinyl Estradiol (EE2)) in waters by UV photolysis and UV/H_2O_2 process was investigated in real conditions. A particular attention was paid at designing a well validated laboratory scale pilot in order to optimise oxidant concentrations and UV fluence. A Low pressure lamp (254 nm) was used in a flow through commercial reactor. The effects of water matrices (drinking water and treated wastewater) and H_2O_2 concentrations (10, 40, and 90 mg/L) on the pilot efficiency were first determined. Only E1 could be partially degraded by UV photolysis whereas hormones were all well removed by UV/H_2O_2 process in both matrices. The second part of the study focused on a chemical and biological assessment of UV photolysis and UV/H_2O_2 process (30 and 50 mg/L). Degradation rate constants of hormones as well as changes in estrogenic activity (YES bioassay) and toxicity (*Vibrio fischeri*) were followed at the same time. UV photolysis could not remove neither estrogens nor estrogenic activity at relevant UV fluence in waters. However 80% of initial estrogenic compounds and estrogenic activity could be removed from treated wastewater by combining UV fluence of 423 and 520 mJ/cm^2 with 50 and 30 mg/L of H_2O_2, respectively. No high estrogenic or toxic by-products were detected by the two bioassays following UV photolysis or UV/H_2O_2 process. Operating costs were estimated for a full scale pilot. H_2O_2 was the major cost. By combining the appropriate concentration of H_2O_2 and UV fluence, it could be possible to design a cost effective treatment for treating estrogens in small and medium size WTPs.

1. Introduction

Recent progress in analytical chemistry allows to identify numerous micropollutants in Wastewater Treatment Plants (WTPs) discharge coming from urban, agricultural or industrial activities. Conventional WTPs are not designed to treat organic micropollutants and can only partially degrade them (Hashimoto et al., 2007). Amongst hundreds of detected molecules, endocrine disrupting compounds (EDCs) are found at very low concentrations (in the ng/L range) (Pereira et al., 2011) but often sufficient to induce biological effects due to their high estrogenic potency (Jurgens et al., 2002). They can disrupt the reproduction and the development of aquatic organisms by interfering with the normal functioning of the endocrine systems (Sumpter and Jobling, 2013). EDCs in the environment may affect not only wildlife but also human fertility (Rozati et al., 2002). Exposure to EDCs have also been associated with disruption of the immune and neurological functions (Colborn et al., 1993). The natural estrogens, estrone (E1), 17β-estradiol (E2) and the synthetic one, 17α-ethinylestradiol (EE2) are amongst the most active and commonly found estrogens in wastewater (Racz and Goel, 2010). Growing concerns about steroid hormones have led European authorities to list the natural steroid hormone E1 and E2 and the synthetic one EE2 as substances to

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watch under the Water Framework Directive. Actions will have to be taken in a near future to reduce or eliminate emission of these substances. Therefore, WTPs upgrade will be needed as they are a major cause of EDCs discharge in the environment. Recently, great attention has been given to Advanced Oxidation Processes (AOPs) due to their ability to remove EDCs from wastewater. Highly reactive hydroxyl radicals generated by AOPs can efficiently react with carbon-carbon double bonds and attack the phenolic ring of refractory organic compounds such as estrogens (Zaviska et al., 2009). Ozone (O_3), O_3/UV, UV/TiO_2 and UV/H_2O_2 are amongst the main studied AOPs for EDCs removal. They have been demonstrated as effective processes for degrading estrogens E1, E2 and EE2 (Esplugas et al., 2007; Yuan et al., 2009). When utilities consider to set up new treatment technologies, energy consumption, running costs and by-products formation are of major importance. Moreover, additional costs particularly impact on small and medium WTPs (<10 000 inhabitant equivalent (eq. inh.)). Since they represent up to 90% of the overall French WTPs it would be of great interest to offer them a cost effective and reliable AOP in order to treat estrogens whom discharge could be soon regulated. The homogeneous advanced oxidation process combining UV and H_2O_2 could be an adequate solution. Previous works (Besnault et al., 2014; De la Cruz et al., 2013) demonstrated the cost-effective efficiency of UV/H_2O_2 process on removal of micropollutants (pharmaceuticals and biocides) at low water flow rate and relevant H_2O_2 concentrations. UV/H_2O_2 process can also decrease estrogenic activity of EDCs mixtures (BPA, nonylphenol, E2 and EE2) at environmentally relevant concentrations (μg/L-ng/L) in lab water and natural water (Chen et al., 2007). Rosenfeldt et al. (2007) achieved 90% of estrogenic activity removal of E2 and EE2 using a combination of 5 mg/L H_2O_2 and a UV fluence of less than 350 mJ/cm^2 in surface water. When adding 10 mg/L H_2O_2 in a collimated beam apparatus, IJpelaar et al. (2010) could degrade between 70% and 90% of E1, E2 and EE2 in surface water (spiked at 40 ng/L) depending on the UV fluence (300 and 600 mJ/cm^2, respectively). Different studies pointed out that UV/H_2O_2 process could be a feasible way to remove estrogens from surface water (Pereira et al., 2012) and wastewater (Hansen and Andersen, 2012) in terms of removal efficiency and energy consumption. Nevertheless, most of the present studies do not consider realistic residence time and flow through conditions in real wastewater. Water quality can highly impact the water treatment efficiency due to the presence of potential radical scavengers (natural organic matter and HCO_3^-/CO_3^{2-}) or radical precursors (Li et al., 2013). By-products formed during the treatments can potentially be more toxic or estrogenic than the original compound (Ioan et al., 2007; Olmez-Hanci et al., 2014). Numerous photodegradation by-products of hormones have been identified (Mazellier et al., 2008) but little is known about their potential (eco)toxicological effects. Therefore, biological and chemical analysis should be combined to follow the removal efficiency of EDCs by AOPs (Miège et al., 2009). In the present study, UV photolysis and UV/H_2O_2 process treatment using a commercial low pressure (λ = 254 nm) UV light reactor coupled with hydrogen peroxide (UV/H_2O_2) is evaluated for the removal of a mixture of three hormones (E1, E2 and EE2) under real conditions. Preliminary experiments using different H_2O_2 concentrations were conducted at pilot scale either in drinking water or treated wastewater spiked with hormones in order to optimise the treatment. Hormones removal rates were followed by HPLC-UV. In a second part, estrogenic activity (*In vitro* YES bioassay) and acute toxicity (*Vibrio fischeri* bioassay) were assessed on two relevant concentrations of H_2O_2 in treated wastewater and compared to chemical analysis. Data were used in order to design a full scale pilot for a small WTP.

2. Material and methods

2.1. Chemicals

E1, E2, and EE2 were purchased from Sigma–Aldrich (France) and used as received. Stock solutions of a mixture of the three hormones (1.5 mM) were made in acetonitrile (Sigma, France). Hydrogen peroxide (H_2O_2; 30% w/w) was provided by Carl Roth (Germany). All other chemicals required for analytical and experimental procedures were at least of analytical grade.

2.2. Experimental procedure

2.2.1. Water matrices and H_2O_2 concentrations effect

Treated wastewater quality can be very different from one WTP to another and can also vary on the same place depending on the season or the weather. Therefore, water matrices effect was investigated in order to assess the impact of water quality on the pilot efficiency. Drinking water was used as a matrix with a low organic matter content and a high UV transmittance (97%) and results were compared to treated wastewater with higher organic matter content (DOC = 11 mg/L) and low UV transmittance (70%). Preliminary experiments were conducted in order to determine the role of the water matrix, the oxidant concentrations and the UV fluence in the pilot running in real conditions. Experiments were conducted at 20 °C with a commercial UVC reactor (COMAP WT) and a 55 W (17.6 W germicide) low pressure lamp (Phillips) emitting at 254 nm. The reactor is 81.5 cm long with a diameter of 5.4 cm and the distance between the lamp sleeve and the inner side of the chamber is 1 cm. The total volume of water in the reactor is 1.12 L. Experimental set up is shown in Fig. 1. The system includes a 50 L glass tank where water was continuously thermoregulated and mixed by a stirrer. Data were integrated with a program developed under LabVIEW software. Local drinking water (DW) was directly used (City of Villeurbanne, France). Treated wastewater (TW) was obtained from a local wastewater treatment plant (Feyssine, Lyon) and collected in 35 L plastic containers. Water was used within 24 h after the sampling. The chemical characterization of the different matrices is shown in Table 1. A mixture of E1, E2, and EE2 was added at a final concentration of 5 μM for each compounds in the agitated tank. Final concentration of each hormone was approximately 1.3 mg/L for E1 and E2 and 1.5 mg/L for EE2. Initial concentrations of hormones were higher than commonly found in treated wastewater in order to easily follow the degradation by chemical analysis (HPLC-UV). Water was circulated through the closed system and samples where regularly taken in order to determine the degradation kinetic after UV photolysis or UV/H_2O_2 process. Hormone concentrations were measured by HPLC-UV. The UV fluence delivered by the system was measured by biodosimetry at full lamp power and at water flow rates of 20, 30 and 40 L/min according to ÖNORM protocol (ÖNORM, 2001). Results were compared with UV fluence obtained by using the UVCalc®2A software (Bolton Photosciences Inc., Canada) and a correction factor was determined. The software gave UV fluence slightly over-estimated compare to biodosimetry. All the other UV fluences were calculated by this software based on the time of treatment, the water flow rate (generally 40 L/min), the UV-254 Transmission (UVT) at 10 mm and reactor dimensions. A correction factor of 0.88 was systematically applied. Different H_2O_2 concentrations were investigated: 10, 40 and 90 mg/L. When hydrogen peroxide was used, residual H_2O_2 was scavenged by adding Na_2SO_3 (≥98%, Sigma-Aldrich) at 1 g/L before further analysis. Initial and residual H_2O_2 concentrations were monitored using Merck peroxide test Spectroquant® in the range of 2.0–20.0 mg/L H_2O_2. The yellow color produced in the reaction is due to the formation of pertitanic acid. Absorbance were measured

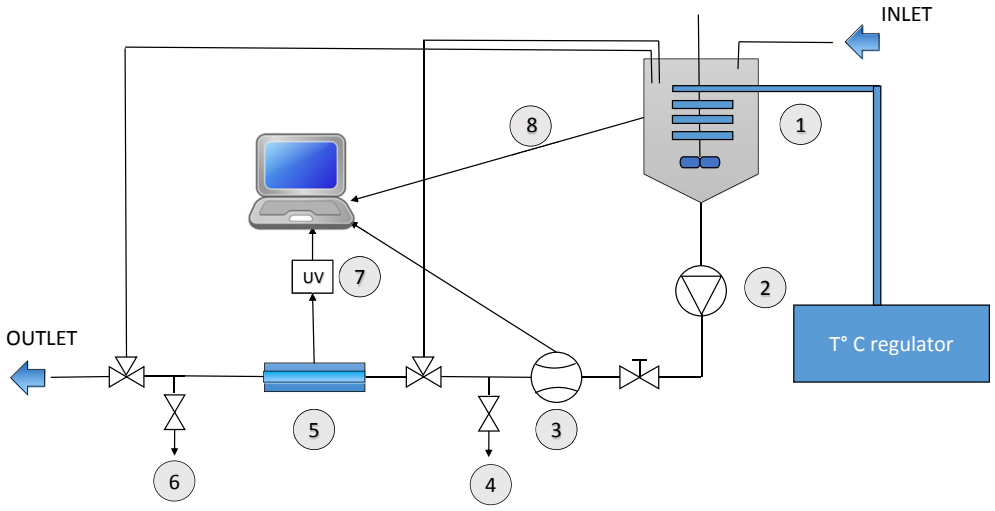

Fig. 1. The schematic diagram of the pilot used in this study. 1) Glass tank; 2) pump, 3) flow meter, 4) and 6) sampling points, 5) UVC reactor, 7) UV intensity control, 8) temperature control.

Table 1
Environmental characterization of the drinking water (A) and the treated wastewater samples (B). TW: Treated Wastewater; COD: chemical oxygen demand; DOC: Dissolved Organic Carbon; BOD5: Biochemical oxygen demand; SS: suspended solids; TUV: UV Transmittance at 254 nm, 10 mm pathway, NK: Nitrogen Kjedalh.

A	Total chlorine	Colour	Conductivity	Turbidity	$N-NH_4^+$	TUV (254 nm)	pH
Measure unit	mg/l	Pt	µS/cm	NFU	mg/l N	%	
DW	0.05	<5 mg/L	332	0.16	<0.05	97	7.5

B	COD	DOC	BOD5	SS	NK	$N-NH_4^+$	$N-NO_2^-$	$N-NO_3^-$	Pt	TUV (254 nm)	pH
Measure unit	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l N	mg/l N	mg/l N	mg/l P	%	
TW A	21.7	11	5.8	4.7	2.4	0.6	0.1	3.8	2.2	70	7.8
TW B	18.5	6.1	3	2	5.04	4.24	0.09	4.54	1.28	60	7.5
TW C	19	10.6	3	3	2.36	1.31	0.54	3.68	0.9	55	7.8

at 410 nm with a spectrophotometer Jasco V-630-bio. DOC analysis were performed using a Shimadzu TOC-VCPH total organic carbon analyzer.

2.2.2. Estrogenic activity removal

Similar procedure was conducted in order to assess estrogenic activity removal following UV photolysis or UV/H_2O_2 process in treated wastewater. The mixture of the three hormones was added at 5 µM. 30 and 50 mg/L of H_2O_2 were tested in the pilot in TW. Wastewater chemical parameters are shown in Table 1. TW B was used to assess UV + 30 mg/L of H_2O_2 treatment and TW C to assess UV + 50 mg/L of H_2O_2 treatment. Each sample was analyzed by HPLC-UV and submitted to YES bioassay in order to follow degradation of hormones and estrogenic activity removal at the same time. The *V. fischeri* test was also conducted on the highest studied concentration (50 mg/L) in order to identify potential high toxic by-products formation.

2.2.3. HPLC analysis

All samples were analysed in triplicate using a UHPLC Ultimate 3000+ (Thermo Fisher) equipped with an Extend-C18 reversed-phase column (X-Bridge BEH 2.5 µm, 4.6 × 75 µM) and a Diode-Array Detector set at 200 nm. The mobile phase consisted of 50% water and 50% acetonitrile acidified at 0.1% HCOOH at a flow rate of 1.5 mL/min. The instrument detection and quantification limit of all hormones for an injection volume of 80 µL was calculated as 10 µg/L and 25 µg/L, respectively. Based on this conditions, elution occurred at Retention Time (RT) = 1.51 min for E2, RT = 1.79 min for EE2 and RT = 2 min for E1.

2.2.4. Yeast estrogen screen assay

The YES assay is based on a genetically modified *Saccharomyces cerevisiae* strain which hosts a gene coding for a human estrogen receptor in its genome (hERα), thus constitutively expressing this receptor, and a plasmid carrying the reporter gene lac-Z, under the control of the Estrogen Response Element (ERE). When an estrogenic active compound bonds to the ERE, it activates the lac-Z gene transcription. Then, the synthesized β-galactosidase degrades substrate Chlorophenol red-ß-D-galactopyranoside (CPRG) turning culture medium yellow color to red-purple color. All chemicals used for this test were purchased from Carl roth (Germany). YES assay was adapted from Routledge and Sumpter (1996). Growth medium (15 mL) was inoculated with 130 µL of 10 times concentrated yeast stock solution and incubated at 28 °C for approximately 48 h on an orbital shaker (150 rpm) until Optical Density (OD) at 640 nm reached ≈1.0. Each sample to be tested has been diluted ten times in growth medium when needed and transferred to 96-well microplates in sterile conditions. Serial dilutions were made in growth medium and 10 µL aliquots of each well were transferred in triplicate to new 96-well microplates. Growth medium (200 µL) containing yeast and CPRG was then added in each well. Controls were used on each plate: E2 (4.9 pM–10 nM) as positive control and complete culture medium (with yeasts and CPRG but no other chemicals) as negative controls. All plates were incubated at 29 °C for approximately 96 h. The absorbance of each sample was measured at 540 nm (specific red color) and 620 nm

(assessment of the cell density) on the microtiter plate reader TECAN Safire. The estrogenic activity of a sample expressed in E2 equivalents (EEQs) was calculated as the ratio of the effective concentrations to the half maximum response (EC50) for E2 to the EC50 for the sample.

2.2.5. V. fischeri assay

The acute toxicity of the different treatments was tested using the commercial assay kit BioTox™ (Aboatox Oy, Finland) according to the test protocol ISO 11348-3 (2007). This test is based on the inhibition of the enzyme luciferase, which oxidises luciferin to generate light. When toxic substances are present in samples, light emission decreases. Following UV photolysis or UV/H_2O_2 process (50 mg/L of H_2O_2), pH of the samples was adjusted to ~7 when needed (using NaOH or HCl) and 2% NaCl (w/v) was added. Bacterial suspension (200 µL) were added to 200 µL of all samples (in triplicates) in 96-well microplates (Thermo Scientific Nunc®). The maximal light emission of each sample was measured during the first 5 s of contact using a Luminoscan Ascent (Thermo scientific). Light emission decrease was assessed after t = 30 min. All experiments were carried out at 15 °C. A 2% NaCl solution was used as toxicant free control. Untreated wastewater was used as negative control and potassium dichromate (18.75 mg Cr^{6+}/L) as positive control. Percentage of inhibition of *V. fischeri* was calculated on the basis of initial luminescence of toxicant free control and test samples as well as luminescence intensity of samples after 30 min contact time. Toxicity is expressed as EC50, which is the effective concentration of a toxicant causing 50% inhibition of luminescence.

3. Results and discussion

3.1. Preliminary experiments: water matrices and H_2O_2 concentrations effect

Preliminary experiments were conducted in order to i) assess and validate the pilot on different water matrices ii) assess UV photolysis and UV/H_2O_2 process treatment efficiency on a mixture of three hormones (E1, E2 and EE2) in real conditions. Two water matrices (drinking water and treated wastewater) and three different concentrations of H_2O_2 (10, 40 and 90 mg/L) were tested. Pseudo-first-order degradation rate constants were determined for all hormones from the linear relationships between ln([C]/[C_0]) and the UV fluences. [C_0] represents the initial concentration of each hormone spiked in solution and [C] is the concentration of each hormone at a given UV fluence. The UV fluence was used instead of time because experiments were conducted in semi-batch reactor. Furthermore, it enables to take into account the difference of transmittance between drinking water and treated wastewater. The degradation rate constants (k) were calculated for each hormones in drinking water and TW A in the case of UV photolysis and UV/H_2O_2 process.

3.1.1. UV photolysis

UV photolysis can better degrade E1 than E2 and EE2 in both matrices. Degradation rate constants are 18 and 10 times lower for E2 and EE2 in drinking water, respectively (Fig. 2). In treated wastewater, differences are in the same range (15 and 9 times lower for E2 and EE2, respectively). The degradation rate constants of estrogenic hormones obtained in drinking water and treated wastewater are in the same order of magnitude, particularly for E2 and EE2 for which degradation constants are very low, and only a slight decrease is observed for E1. Table 2 summarizes estrogens removal rates in different water matrix found in the literature. Only studies presenting similar parameters (initial concentration of hormones and UV fluence) were selected in order to compare the

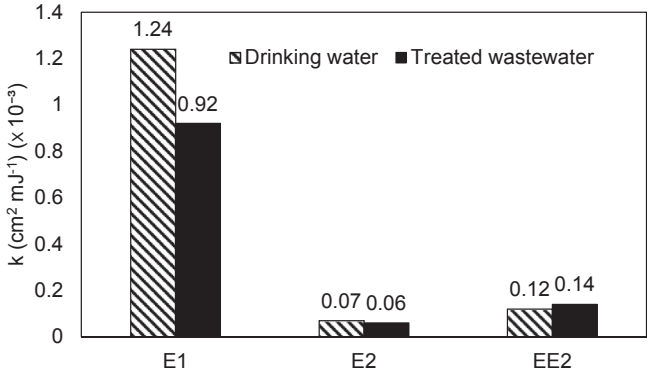

Fig. 2. Effect of UV photolysis on the degradation rate constants of a mix of three hormones (E1, E2 and EE2 at 5 µM each) in drinking water or treated wastewater A.

different degradation rates. E1 is always better degraded than E2 and EE2 following UV photolysis treatment. Moreover, water matrices have a low impact on degradation rates. This was explained by Canonica et al. (2008): reactive oxygen species formed by the action of UV on organic molecules present in complex matrices probably counterbalanced the low transmittance of the treated wastewater. The matrix used for this study (TWA) contains nitrates (Table 1) which are known to act as hydroxyl radicals precursor (Brezonik and Fulkerson-Brekken, 1998). Indirect photolysis could occur and could partly explain relative low difference between DW and TW. Generally, degradation of a compound by UV photolysis is affected by its molar extinction coefficient. High molar extinction coefficient means that the compound can absorb much UV energy which could be used for its degradation. Molar extinction coefficients are very low for the three hormones ranging from 314 M^{-1} cm^{-1} for EE2 to 395 M^{-1} cm^{-1} for E1 (Li Puma et al., 2010). Mazellier et al. (2008) found values slightly higher for E2 (420 M^{-1} cm^{-1}) and EE2 (440 M^{-1} cm^{-1}). Therefore, the three hormones are weak candidates for UV photolysis degradation. The better degradation rate of E1 in comparison with E2 and EE2 is likely due to its ability to absorb more photons at 254 nm than the other hormones. Indeed, E1 exhibits a high quantum yield (5.45 mol $Einstein^{-1}$) compared to E2 (0.06 mol $Einstein^{-1}$) and EE2 (0.09 mol $Einstein^{-1}$) (Pereira et al., 2012). It means that a larger number of moles of E1 is transformed for a given number of photons absorbed compared to E2 and EE2.

In this study, 70% of E1 could be removed from drinking water and 60% from treated wastewater (Fig. 3) at 1000 mJ/cm^2. E2 and EE2 could barely be removed from both matrices (<7% and <13%, respectively). Higher removal rates of these hormones can be found in other studies, arising from different experimental conditions (Table 2). Best degradation rates are observed in mono component solutions with low initial concentrations of hormones (Zhang et al., 2010). When comparing mono- and multi-component solutions, or solutions with different concentrations, at a given UV fluence, an increase in the number of molecules in a given volume lead to a decrease of the “emitted photons/targeted molecules” ratio, hence a lower degradation by UV photolysis. Besides, previous works conducted under experimental conditions similar to the present study, namely in complex matrices and with hormones mixture, reported similar results (Pereira et al., 2012). However, most of the studies selected in Table 2 used collimated beam laboratory scale apparatus and matrices with low organic matter content. Experimental conditions used in the present study are closer to the reality.

At least 80% of the targeted compounds should be removed according to the Swiss new wastewater legislation dealing with micropollutants. Present results showed that UV photolysis is able

Table 2
Comparison of the degradation of estrogens in different matrices following UV photolysis or UV/H_2O_2 process. DW = Drinking Water; TW = Treated Wastewater; DeW = Deionized Water; SW = Surface Water; TOC = Total Organic Carbon, DOC = Dissolved Organic Carbon.

Matrix	Estrogens concentrations	UV treatment (254 nm)	UV photolysis degradation (%)	UV/H_2O_2UV/H_2O_2 degradation (%)	Ref.
DW	E1 = E2 = 1.3 mg/L	55 W		[H_2O_2] = 40 mg/L	This study
	EE2 = 1.5 mg/L	1000 mJ/cm^2	E1 (70%)>EE2 (13%) >E2 (7%)	E1 = E2 = EE2 ≈ 99%	
	in mixture				
DW	E1 = E2 = EE2 = 50 μg/L			[H_2O_2] = 10 mg/L	Ma et al. (2015)
	in mixture	1050 mJ/cm^{2*}	Negligible	EE2 (100%)	
TW	E1 = E2 = 1.3 mg/L	55 W		[H_2O_2] = 40 mg/L	This study
DOC = 8.35 mg/L	EE2 = 1.5 mg/L	1000 mJ/cm^2	E1 (60%)>EE2 (13%) >E2 (6%)	E1 = E2 = EE2 > 90%	
	in mixture				
TW	EE2 = 100 μg/L	11 W		[H_2O_2] = 10 mg/L	Frontistis et al. (2015)
		15min	EE2 (≈20%)	EE2 (100%)	
SW	E1 = E2 = 1 mg/L			[H_2O_2] = 100 mg/L	Pereira et al. (2012)
	in mixture	1500 mJ/cm^2	E1 (82%)>E2 (6%) > EE2 (1%)*	E1 (86%)>E2 (75%) = EE2 (75%)*	
DeW	E1 = E2 = 3 mg/L	30 W		–	Liu and Liu (2004)
	Mono-component	60 min	E1 (93%)>E2 (60%)	–	
DeW	E1 = E2 = 1 mg/L	–		[H_2O_2] = 40 mg/L	Pereira et al. (2012)
	in mixture	1500 mJ/cm^2	E1 (76%) > E2 (7%) > EE2 (4%)*	E1(85%) > E2(14%) > E2(10%)*	
DeW	E1 = E2 = EE2 = 50 μg/L	–	–	[H_2O_2] = 10 mg/L	Ma et al. (2015)
	in mixture	1050 mJ/cm^{2*}		EE2 (100%)	
UW	EE2 = 2 mg/L	30 W		[H_2O_2] = 5 mg/L	Zhang et al. (2010)
		960 mJ/cm^{2*}	EE2 ≈90%*	EE2 ≈99%	
UW	E1 = E2 = EE2 = 50 μg/L			[H_2O_2] = 15 mg/L	Ma et al. (2015)
	in mixture	1050 mJ/cm^{2*}	E1 (97%) > E2 (32%) ≈ EE2 (28.2%)	E1(99.7%) > EE2(78%) > E2(76%)	

* Calculated from the available data in the study.

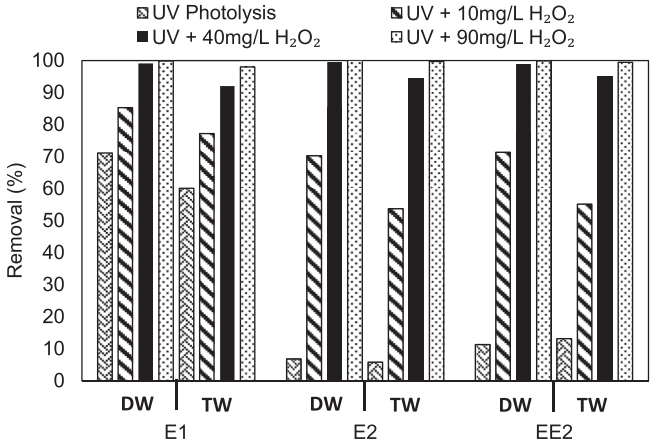

Fig. 3. Removal efficiency of E1, E2 and EE2 in drinking water or treated wastewater A following UV photolysis or UV/H_2O_2 process at 1000 mJ/cm^2.

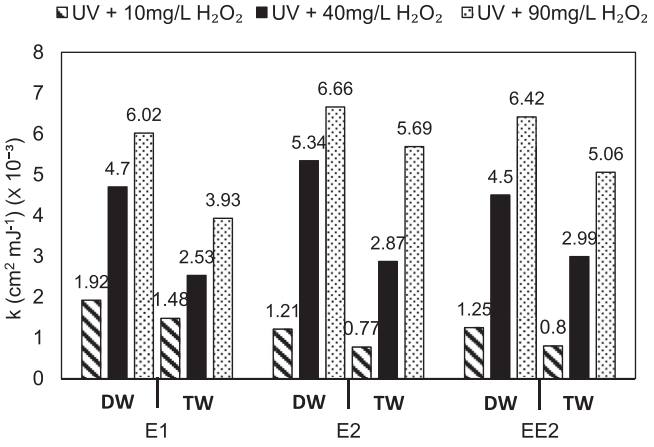

Fig. 4. H_2O_2 concentrations effect on the degradation rate constants of a mix of three hormones (E1, E2 and EE2 at 5 μM each) in DW or TW A.

to degrade E1 but a very high UV fluence is needed: approximately 1300 mJ/cm^2 and 1800 mJ/cm^2 are necessary to remove 80% of E1 in drinking water and TW A, respectively. Moreover, E2 and EE2 removal by UV photolysis is insignificant. It can thus be concluded that UV photolysis is not an efficient process for estrogens removal in treated wastewater at a relevant UV fluence. Subsequently, UV/H_2O_2 process has been investigated.

3.1.2. UV/H_2O_2 process

Results show that UV/H_2O_2 process highly increases all removal of hormones (Fig. 3). A concentration of H_2O_2 above 40 mg/L can remove more than 90% of all compounds at 1000 mJ/cm^2 in both matrices. Similar results are found in different matrices: H_2O_2 concentration ranging from 5 mg/L to 100 mg/L can generally remove more than 80% of estrogens (Table 2). Degradation rate constants are higher in both matrices (Fig. 4) compared to UV photolysis (up to 95 times for E2 at [H_2O_2] = 90 mg/L in DW). Moreover, the removal of the three hormones increases with increasing concentration of hydrogen peroxide. The highest degradation rate constants are observed with a concentration of 90 mg/L of H_2O_2 for the three hormones. Fast degradation of hormones in water was also shown to depend on high H_2O_2 concentrations in other studies (Hansen and Andersen, 2012; Ma et al., 2015). Similar phenomena occurred with pharmaceuticals products (Rosario-Ortiz et al., 2010). H_2O_2 can indeed be easily converted into hydroxyl radicals under UV light, so addition of H_2O_2 enhances the degradation rate constants through increasing the concentration of hydroxyl radicals. Unlike UV photolysis, UV/H_2O_2 process seems to be significantly affected by the water matrix: the UV/H_2O_2 process degradation rate constants obtained in treated wastewater are all lower than in drinking water (Fig. 4). The decrease in treatment efficiency could be explained by the capacity of H_2O_2 to be hindered by the Natural Organic Matter (NOM) competing for UV irradiation (Olmez-Hanci et al., 2014). The competing reaction of hydroxyl radicals by the humic substances and other scavengers in treated wastewater is also thought to be a consequence of this difference. For example, Cl^-, HCO_3^-, SO_4^{2-}, and NO_3^- arealso known to react with hydroxyl radicals (Li et al., 2013).

H_2O_2 in combination with UV considerably reduces UV fluence needed to reach 80% of the removal of hormones in treated wastewater in comparison with UV photolysis (Table 3). A UV fluence close to 400 mJ/cm^2 and 90 mg/L of H_2O_2 would allow to remove 80% of all hormones in treated wastewater. A four times increase of H_2O_2 concentration (10 mg/L to 40 mg/L) approximately reduces the UV fluence needed by 4 for an 80% removal of E2 and EE2. However, increasing H_2O_2 from 40 to 90 mg/L does not improve the treatment as expected (2.25 fold): factors between 1.3 and 2 are observed meaning that a high concentration of H_2O_2 could be counterproductive at a certain point. Indeed, previous works have shown that an excess of hydrogen peroxide could decrease the treatment efficiency (Hansen and Andersen, 2012; Pereira et al., 2012). When H_2O_2 is used in excess, it could act as a scavenger itself and compete for hydroxyl radicals and eventually inhibit oxidation of the targeted organic compounds. As oxidant is a major cost for UV/H_2O_2 process, its concentration has to be adjusted as much as possible. Table 4 presents the consumption of H_2O_2 after the different treatments. Between 30% and 40% of the oxidant is consumed during the UV/H_2O_2 process (at 1000 mJ/cm^2) depending on the water matrix. This is higher than Hansen and Andersen (2012) who conducted experiments in a flow through reactor and activated 21% of H_2O_2 (60 mg/L) in drinking water and 11% in wastewater with medium pressure lamp. Optimisation of the process (lamp efficiency, water residence time and distance between water and UV lamp) must be considered for a full scale process. However, on the one hand, water residence time in a full scale flow through pilot (generally a few seconds) will limit the possibility of H_2O_2 activation. On the other hand, UV fluence should stay under 1000 mJ/cm^2 because of the number of UVC lamps needed to reach high UV fluence. Therefore, the oxidant has to be in excess in order to reach high removal rates. Table 3 reveals that only a low reduction of UV fluence is gained when adding more than ~40 mg/L of H_2O_2. Consequently, it is not worth adding high concentration of oxidant. On the contrary, low concentrations of H_2O_2 require very high UV fluences (>1000 mJ/cm^2) which are not relevant at industrial scale because of associated cost such as electricity. Therefore concentrations of 30 and 50 mg/L were chosen for further chemical and biological investigations.

3.2. Estrogenic activity removal

The aim of this study is to assess whether the different treatments can remove the estrogenic activity of the effluent in the same time than the three estrogens without forming other estrogenic or toxic compounds. Identification of these potential toxic or estrogenic by-products or reaction intermediates was not further investigated.

3.2.1. UV photolysis

The estrogenic activity removal of the mixture of the three hormones (5 μM) was studied in the pilot either by UV photolysis or UV/H_2O_2 process ([H_2O_2] = 30 and 50 mg/L (in treated wastewater B and C, respectively)). The chemical characteristics of the treated wastewaters used in these experiments are shown in Table 1. *In vitro* estrogenic activity (measured by YES bioassay) of each sample is assessed as E2 equivalent quotients (EEQs). When expressed as ln(EEQ/EEQ$_0$) estrogenic activity removal follows a pseudo-first-order kinetic. Estrogenic activity removal at 1000 mJ/cm^2 and degradation rate constants are compared to chemical analysis by HPLC-UV in Figs. 5 and 6, respectively. H_2O_2 consumption are shown in Table 4 and are similar to preliminary studies in TW.

Photolysis cannot remove estrogenic activity even if degradation of hormones reached 73% for E1, 20% for E2 and 23% for EE2 at 1000 mJ/cm^2 (Fig. 5). Removal of hormones is in accordance with the preliminary study (Section 3.1.1) where UV photolysis could partially degrade E1 but had almost no effect on E2 and EE2. Knowing that the highest estrogenic potency is due to E2 and EE2 compounds (Thorpe et al., 2003), high estrogenic activity is still expected. Nevertheless it should slightly decrease since hormones are partially degraded. Estrogenic activity stability could be explained by the formation of estrogenic by-products in addition to low degradation of E2 and EE2. Whidbey et al. (2012) showed that E1 direct photolysis (solar simulator) yields lumiestrone, an unnatural steroid hormone product that retains estrogenicity. Photolysis of E1 with high pressure mercury lamp can even form higher estrogenic by-products than parent compounds (Souissi et al., 2014). In the present study, the fact that estrogenic activity remains constant is probably due to estrogenic by-products arising from the degradation of hormones and contributing to the global estrogenic activity. Similar results were obtained in distilled water by Rosenfeldt et al. (2007). They reported that UV photolysis could not remove estrogenic activity at UV fluence as high as 12 000 mJ/cm^2 with ~3 μg/L of EE2 or E2 but they did not look for by-products and did not investigated more complex matrices with low pressure lamps. A recent study from Li et al. (2016), identified a new

Table 3
Calculated UV fluence (mJ/cm^2) needed to remove 80% of E1, E2 and EE2 following UV photolysis or UV/H_2O_2 process treatment at three different H_2O_2 concentrations in DW or TWA. ND: UV fluence not relevant.

	E1		E2		EE2	
	DW	TW	DW	TW	DW	TW
UV photolysis	1289	1773	ND	ND	ND	ND
UV + 10 mg/L H_2O_2	833	1099	1342	2204	1311	2072
UV + 40 mg/L H_2O_2	334	636	301	558	333	535
UV + 90 mg/L H_2O_2	257	382	227	277	236	314

Table 4
Oxidant consumption at 1000 mJ/cm^2 in DW and TW for different initial H_2O_2 concentrations.

	Preliminary studies			Estrogenic activity removal studies	
Initial [H_2O_2] mg/L	10	40	90	30	50
Consumed [H_2O_2] in DW (%)	20	20	16	–	–
Consumed [H_2O_2] in TW (%)	39	30	30	35	30

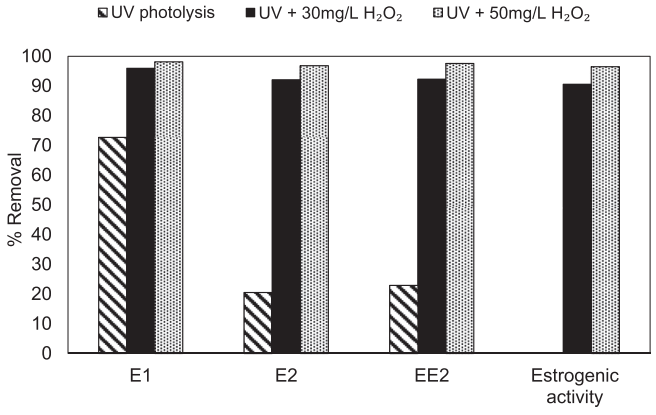

Fig. 5. Comparison of removal efficiency of E1, E2 and EE2 and estrogenic activity in treated wastewater following UV photolysis or UV/H_2O_2 process at 1000 mJ/cm^2.

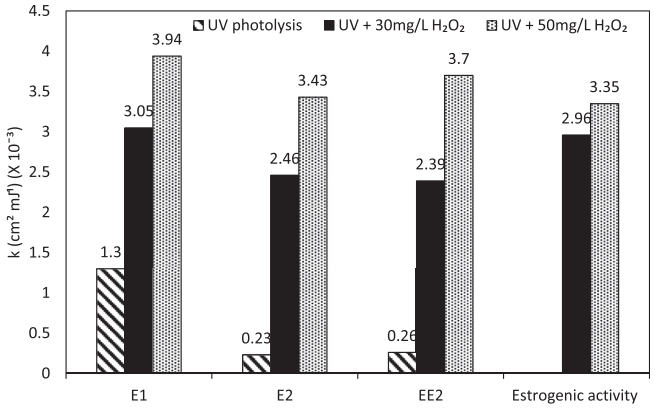

Fig. 6. Comparison of degradation rate constants of hormones in mixture (E1, E2 and EE2 at 5 μM each) and estrogenic activity removal rate constants in treated wastewater.

estrogenic by-product formed after UV photolysis and UV/chlorine treatment in secondary effluent: the dehydro-estradiol. It was shown to derivate from E2 and to present high estrogenic activity (about one fifth that of E2) leading to a weak estrogenic activity removal (<10%). These different studies showed that several estrogenic by-products can be generated after UV photolysis but major challenges remain in identifying other intermediates and their degradation pathways (Sornalingam et al., 2016). The present study clearly shows that UV photolysis can't remove estrogenic activity but further investigations are needed to verify whether by-products detected in previous studies can be responsible of this residual estrogenic activity.

3.2.2. UV/H_2O_2 process

UV/H_2O_2 process can remove estrogenic activity in wastewater at both investigated concentrations. At 1000 mJ/cm^2, all individual hormones and estrogenic activity were highly removed (>90%) (Fig. 5). Calculation of the UV fluence needed to remove at least 80% of the initial estrogenic activity gives a value of 520 mJ/cm^2 for UV + 30 mg/L of H_2O_2 (TW B) and 463 mJ/cm^2 for UV + 50 mg/L of H_2O_2 (TW C). This is better than previous work such as Chen et al. (2007) who could remove around 80% of the estrogenic activity of a mixture made of E2 (200 ng/L) and EE2 (1000 ng/L) in surface water (at 1000 mJ/cm^2 and [H_2O_2] = 10 mg/L). In the present study, high initial concentrations of hormones were used (≈1 mg/L) in order to easily follow the degradation of hormones by HPLC-UV. However, these chemicals are commonly found at concentrations below the ng/l range in treated wastewater. Therefore, it is important to wonder whether the degradation rate constants would be faster or slower at environmental concentrations. According to different studies, the same conditions of treatment would lead to a better removal efficiency as hormones act themselves as UV filter, hence decreasing the production of hydroxyl radicals. An increase in removal rate of EE2 with decreasing initial concentration (from 1.98 to 0.4 mg/L) was measured by Zhang et al. (2010). Similar observations were made by Liu and Liu (2004) for E1 and E2 (from 20 mg/L to 3 mg/L). Chen et al. (2013) showed that the photodegradation rate constants increased with decreasing concentrations of E3 (≈from 6 to 1 mg/L) under simulated sunlight and concluded that the removal of E3 would be faster in natural waters where much lower concentrations are detected.

UV in combination with H_2O_2 (30 and 50 mg/L) leads to a fast estrogenic activity removal (Fig. 6). The treatment is slightly faster (~12%) with 50 mg/L of H_2O_2 than with 30 mg/L. For both H_2O_2 concentrations, the estrogenic activity removal rate constants and the degradation rate constants of hormones obtained by chemical analysis are in the same range. It indicates that no high estrogenic by-products seem to be formed during UV/H_2O_2 process in treated wastewater otherwise removal rates would have been delayed (Rosenfeldt et al., 2007). On the contrary, Frontistis et al. (2015) found that 15 min of UV/H_2O_2 treatment (10 mg/L) treatment could chemically degrade EE2 (100%) but estrogenic activity was only partially removed (35%) in TW. Stable estrogenic by-products might be formed during UV/H_2O_2 process but in the present study, the higher concentration of oxidant seems to degrade refractory estrogenic compounds.

Biological and chemical analysis are of high importance in the present work because it clearly demonstrates that estrogenic activity decrease is due to removal of hormones and that no highly estrogenic by-products are formed for these experimental settings. However, other studies showed that a large number of by-products can be produced following AOP treatment of estrogens (Mazellier et al., 2008; Pereira et al., 2011), but their estrogenic activity or toxicity has not been assessed yet. According to Ma et al. (2015), UV/H_2O_2 treatment of E1, E2 and EE2 can also produce unidentified intermediate compounds (named intermediate X in their work).

If by-products are formed during the UV/H_2O_2 process in this study, they don't have high estrogenic activity but other toxicological or ecotoxicological (carcinogenicity, genotoxicity, acute toxicity…) experiments are needed in order to find out whether other toxic by-products are formed or not. To this end, *V. fischeri* assay was conducted in order to have more data about these potential by-products. This test is commonly used for wastewater toxicity assessment but should be associated with other ecotoxicological tests in order to confirm the non-toxicity of the wastewater samples as recommended by Wigh et al. (2016).

3.2.3. Acute toxicity: V. fischeri bioassay

Acute toxicity was monitored with *V. fischeri* bioassay after UV photolysis or UV/H_2O_2 process (50 mg/L) in treated wastewater (Fig. 7). This bioassay can indicate the toxic potency of a broad spectrum of compounds. Treated wastewater (control) causes an inhibitory effect of approximately 35% after 30 min of exposure. The toxicity of the wastewater matrix itself was not investigated because this experiment focused on the detection of high toxic by-products after the different treatments. The same level of inhibitory effect (31%–38%) is measured after UV photolysis or UV/H_2O_2 process at different UV fluence. Regarding that EC50 is > 50%, toxicity to *V. fischeri* is relatively low (Velegraki et al., 2010). From these experiments, it can be concluded that estrogens treatment by UV photolysis or UV/H_2O_2 process doesn't form highly toxic compounds in treated wastewater with operating conditions very close

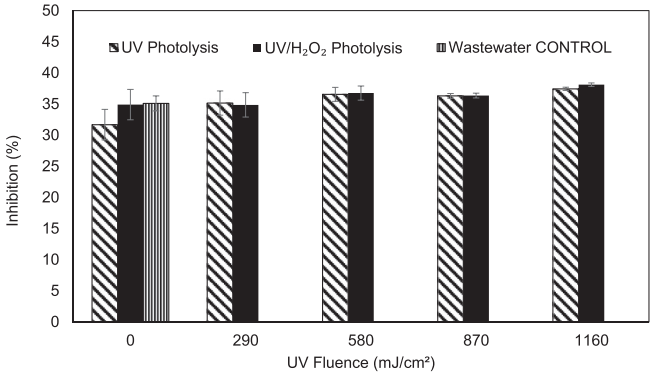

Fig. 7. *Vibrio fischeri* inhibition measured following UV photolysis or UV/H_2O_2 process (50 mg/L) in treated wastewater. Initial hormone concentrations = 5 μM.

to what could be found in large scale WTPs. Other intermediates are probably formed but are not toxic to *V. fischeri* (Frontistis et al., 2011). Therefore, this bioassay can only give a first glimpse on by-products potential acute toxicity but will not be sufficient to assess other kind of toxicities (genotoxicity, neurotoxicity, chronic or sublethal effects …). Interestingly, Jia et al. (2015) run 36 bioassays following different AOP treatments and found that UV/H_2O_2 process (500 mJ/cm^2 and 10 mg/L of H_2O_2) was more efficient than UV photolysis (500 mJ/cm^2) in removing *in vitro* responses. Both treatments could reduce toxicity to *V. fischeri* but were ineffective in removing genotoxicity or mutagenicity or could even lead to higher toxic effects due to the formation of by-products. Using higher UV doses or higher oxidant concentrations might contribute to reduce this toxicity because by-products themselves could be degraded. Bioanalytical tools (Macova et al., 2011) or ecotoxicological studies (Fatta-Kassinos et al., 2011) on relevant models are essential to assure UV/H_2O_2 treatment innocuousness. When considering a full scale pilot, chronic toxicity would also have to be considered. *In vivo* ecotoxicological tests using sensitive aquatic organisms such as fishes (zebra fish) or crustacean (Daphnia, Gammarus) could give a good insight on treated wastewater chronic toxicity (Besse et al., 2013; Rizzo, 2011).

4. Design of a full scale UV/H_2O_2 process: an economic perspective

More than 90% of the French WTP are designed for <10 000 IE and 80% are under 2000 IE (French ministry for ecology sustainable development and energy). Therefore, an economic study based on De la Cruz et al. (2013) method is briefly proposed for a local WTP of 1200 PE (Reed bed sewage systems, Vercia, France) with a maximal water flow of 10 m^3/h. The UVCalc®2A software (Bolton Photosciences Inc., Canada) was used in order to design a full scale UV/H_2O_2 pilot able to deliver at least 463 mJ/cm^2 with 50 mg/L of H_2O_2 or 520 mJ/cm^2 with 30 mg/L of H_2O_2 (allowing at least 80% of estrogenic activity removal). This would help to determine the optimum wastewater treatment conditions at a low water transmittance (UVT 254 nm) of 50%. H_2O_2 is added by a dosing pump (24 W) and water goes through the reactor by gravity. Designed reactor is approximately 89 L and can receive up to 14 lamps (325 W each). Simulation were conducted with different numbers of lamps in order to obtain a UV fluence close to the targeted ones. Main variable costs are electricity (0.14 €.kWh^{-1}: electricity of France (2015)) and hydrogen peroxide (0.73 €.L^{-1}: Solvay, France). Maintenance cost is estimated at 2000 €/year based on lamp lifetime (12 000 h), quartz replacement and technician cost. The economic study was conducted for two different water flow rates (5 and 10 m^3/h) as the local WTP is designed for a maximal water flow of 10 m^3/h but is more often running between 5 and 7 m^3/h. Costs are presented in Euros (€) per cubic meter of treated wastewater (Table 5). The most economical conditions are always obtained using 30 mg/L of H_2O_2. At a flow rate of 10 m^3/h, operating costs reaches 0.155 €/m^3.

Oxidant is the major operating cost of the treatment (between 43 and 73% depending on the operating conditions). Therefore, it is of high importance to give priority to low H_2O_2 concentrations rather than low UV fluence. Other studies demonstrated that UV/H_2O_2 was a cost/effective treatment process for low water flow treatment. De la Cruz et al. (2013) concluded that a reactor equipped with 5 UVC lamp of 150 W (0.75 kW h) and H_2O_2 concentration of 50 mg/L could remove 95% of 22 emerging micropollutants at a flow rate of 14 m^3/h (residence time: 10 s) in wastewater with operating cost reaching 0.13 €/m^3. Other cost effective processes do exist for estrogens removal such as ozonation (Sarkar et al., 2014). However, this technology requires a permanent trained staff onsite which is not conceivable for small WTPs.

5. Conclusion

The present study aimed at investigating the performance of UV photolysis and UV/H_2O_2 process in removing estrogenic compounds (E1, E2 and EE2 in mixture at 5 μM) and estrogenic activity in treated wastewater. A particular attention was paid to the design of a pilot with a realistic flow through reactor in order to investigate natural matrices and real operating conditions because most of studies mentioned in the literature present results arising either from laboratory scale experiments or from full scale implementation. In the first case, operating conditions are quite far from real conditions, in the second case, operating conditions are not well controlled. The pilot used in the present study can operate with realistic flow rates and residence times, but experimental parameters can be easily controlled. Preliminary experiments focused on matrices (drinking water and treated wastewater) and H_2O_2 concentration effects on the pilot efficiency. Results showed that UV photolysis was not effective in removing estrogens from waters at relevant UV fluence whereas UV/H_2O_2 highly removed estrogenic activity and corresponding estrogens. Estrogens removal from treated wastewater depended on H_2O_2 concentrations with best removal rates obtained at 90 mg/L of H_2O_2 but lower concentrations were tested in a second step for cost effective reasons. UV photolysis and two UV/H_2O_2 process treatments (30 and 50 mg/L of H_2O_2; initial hormone concentration = 5 μM each) were assessed by chemical (HPLC-UV) and biological (YES bioassay, *V.fischeri*) analysis on treated wastewater. Both UV/H_2O_2 process treatments could enhance hormones and estrogenic activity removal at the same time whereas UV photolysis had no ability to remove estrogenic activity. No high estrogenic or high toxic by-products were formed according to the two bioassays used in the study. However, further toxicological or ecotoxicological tests are required in order to confirm that UV/H_2O_2 process is completely safe. A UV fluence of 463 mJ/cm^2 + 50 mg/L of H_2O_2 and 520 mJ/cm^2 + 30 mg/L of H_2O_2 were required to remove at least 80% of the initial estrogenic activity. These data were used to propose a cost effective UV/H_2O_2 reactor able to degrade at least 80% of estrogenic activity in a small WTP. Best economic conditions were obtained with 520 mJ/cm^2 + 30 mg/L of H_2O_2. More realistic hormones initial concentrations should be investigated in order to reduce oxidant use since it is the major operating cost. To this end, a full-scale pilot is

Table 5
Operating costs per cubic meter of UV/H_2O_2 process at different conditions of water flow rate and H_2O_2 concentrations allowing at least 80% of estrogenic activity removal.

Water flow rate	H_2O_2 dose	Targeted UVC fluence	Simulated UVC fluence	H_2O_2	Lamp power needed	Lamps electricity	Replacement	Total cost
m^3/h	mg/L	(mJ/cm^2)	(mJ/cm^2)	€/m^3	kWh	(€/m3)	(€/m3)	(€/m3)
10	30	520	563	0.073	4.225	0.059	0.023	0.155
10	50	463	520	0.121	3.9	0.054	0.023	0.203
5	30	520	614	0.073	2.275	0.032	0.046	0.151
5	50	463	529	0.121	1.95	0.027	0.046	0.194

incidentally being implemented at the outlet of a constructed wetland on a wastewater treatment plant site. Nevertheless, on the basis of the results presented in this paper, UV/H_2O_2 process has shown strong capacity to remove estrogens from waters and can be an economically available technology for small and medium sized WTP.

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