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RESEARCH ARTICLE

Comparison of UV photolysis, nanofiltration, and their combination to remove hormones from a drinking water source and reduce endocrine disrupting activity

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Abstract A sequential water treatment combining low pressure ultraviolet direct photolysis with nanofiltration was evaluated to remove hormones from water, reduce endocrine disrupting activity, and overcome the drawbacks associated with the individual processes (production of a nanofiltration-concentrated retentate and formation of toxic by-products). 17β-Estradiol, 17α-ethinylestradiol, estrone, estriol, and progesterone were spiked into a real water sample collected after the sedimentation process of a drinking water treatment plant. Even though the nanofiltration process alone showed similar results to the combined treatment in terms of the water quality produced, the combined treatment offered advantage in terms of the load of the retentate and decrease in the endocrine-disrupting activity of the samples. Moreover, the photolysis by-products produced, with higher endocrine disrupting activity than the parent compounds, were effectively retained by the membrane. The combination of direct LP/UV photolysis with nanofiltration is promising for a drinking water utility that needs to cope with sudden punctual discharges or deterioration of the water quality and wants to decrease the levels of chemicals in the nanofiltration retentate.

Keywords Hormones · Low pressure UV photolysis · Nanofiltration · Water treatment · Yeast estrogen screen assay · Integrated process

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Introduction

The occurrence of endocrine disrupting compounds (EDCs) in drinking water sources has raised public attention. The presence of the hormones 17β-estradiol, 17α-ethinylestradiol, estriol, estrone, and progesterone has been reported in surface waters and groundwaters in USA (Velicu and Suri 2009), Europe (Aydin and Talinli 2013), Asia (Chang et al. 2008), and Australia (Scott et al. 2014), at concentrations up to 180 ng L^{-1}. These hormones highly impact the aquatic ecosystem by inducing severe endocrinologic disorders at very low exposure concentrations (Vajda et al. 2011). Feminization of male fishes (Vajda et al. 2011), abnormal embryogenesis (Saito et al. 2012), and mammary tumors (Turan et al. 2004) are some of the toxicological effects associated with these hormones. The increasing evidence of their impact (Thuy and Nguyen 2013) has led to the recent inclusion of 17β-estradiol and 17α-ethinylestradiol in the EU first watch list (Directive 2013/39/EU). 17β-estradiol, 17α-ethinylestradiol, estrone, and estriol are also included in the United States Environmental Protection Agency (US EPA) Contaminant Candidate List 3, which comprises compounds that are suspected to require regulation under the Safe Drinking Water Act (US EPA 2009). The likelihood of future inclusion

in regulations and their detection in treated waters (e.g. Kuster et al. 2008) therefore demand the development of new treatment technologies that allow utilities to produce treated water with higher quality.

UV photolysis and nanofiltration, as individual processes, have been pointed out as alternative effective treatment processes for the removal of a wide range of organic micropollutants including several EDCs (Narbaitz et al. 2013; Pereira et al. 2012; Sanches et al. 2010, 2012; Wols et al. 2014; Yangali-Quintanilla et al. 2009; Yoon et al. 2007; Zhang et al. 2007). Furthermore, the quest to achieve effective drinking water disinfection led to a shift towards the implementation of UV disinfection, in combination with chlorine, due to its proven high inactivation potential.

Although promising for water treatment applications, UV and nanofiltration processes present specific drawbacks. The formation of by-products, which may be more toxic than the parent compounds, has been previously observed during the photodegradation of carbamazepine (Donner et al. 2013) and sulfamethoxazole (Trovo et al. 2009). For pressure-driven membrane processes, costs associated with the treatment of the retentate generated during membrane filtration of Tagus River water have been estimated to be approximately 17 % of the operating cost if nanofiltration membranes could be used for a production of 10,000 m^3 of water per day (Costa and de Pinho 2006). The reduction of these costs would be of interest for water suppliers employing membrane processes in their treatment plants.

As revised by Pérez-González et al. (2012), research has been developed aiming to reduce the organic load of micropollutants in the retentate of reverse osmosis from desalination and wastewater treatment plants by applying advanced oxidation technologies. The combination of such technologies could also constitute an extra barrier for the removal of the target micropollutants as well as a barrier for by-products generated during the oxidation process. The combination of nanofiltration with oxidation could be more cost-effective since nanofiltration is less energy-consuming than reverse osmosis and it is still expected to be effective to remove micropollutants and UV by-products with low molecular weight (as low as 200 Da) as previously observed for the removal of several pesticides (Sanches et al. 2013). Poor removal of the by-products from the photocatalysis of Gemfibrozil and Tamoxifen was, however, achieved when the NTR 7410 nanofiltration membrane was combined with TiO_2 photocatalysis since by-products were also found in the permeate (Molinari et al. 2006, 2008). The identification of membranes that are able to simultaneously remove specific micropollutants and the respective UV by-products, which may have significantly low molecular weight, is therefore needed.

Given the potential of nanofiltration and UV photolysis to remove several micropollutants from water sources, their individual efficiency towards hormones should be addressed. In this study, nanofiltration and low pressure (LP)/UV direct photolysis were first individually evaluated and then combined as a two-stage treatment. The potential of the multi-barrier approach was assessed in terms of its efficiency to remove five EDCs (17β-estradiol, 17α-ethinylestradiol, estrone, estriol, and progesterone) from surface water collected after sedimentation at a drinking water treatment plant and reduce endocrine disrupting activity—measured using the yeast estrogen screen (YES) assay—while overcoming the aforementioned drawbacks associated with the individual processes, assuring the retention of UV by-products and the production of a concentrated retentate with a lower load.

Experimental section

Chemical reagents

The selected hormones (17β-estradiol, 17α-ethinylestradiol, estrone, estriol, and progesterone) as well as amino acids and salts used in the YES assay were purchased as solids of the highest grade commercially available, higher than 98.9 % (Sigma-Aldrich, Germany).

Sabouraud dextrose agar (Oxoid, UK) supplemented with chloramphenicol (Oxoid, UK) was employed as the culture media for the human estrogen receptor (hER) transfected *Saccharomyces cerevisiae* used in the YES assay.

High-performance liquid chromatography (HPLC) grade acetonitrile (Fisher, UK) was employed in the chromatographic analysis of the EDCs as well as in the preparation of stock solutions. A Milli-Q water system (Millipore, CA, USA) was used to produce water for the chromatographic analysis.

Natural water matrix

The water matrix used was supplied by the water utility Empresa Portuguesa das Águas Livres, SA, that treats and supplies drinking water to approximately 2.8 million people in the region of Lisbon, Portugal. Surface water from the abstraction zone of Tagus River was collected after the sedimentation process in a water treatment utility using 10-L bottles and stored at 5 ± 3 °C until use (pH 7.8). The hormones targeted in this study were not detected in the water collected.

Experimental setup

The experiments were performed in the laboratory scale unit detailed in a previous study (Sanches et al. 2013). This unit is equipped with a feed vessel, a recirculation magnetic drive pump (model MD-15RV, IWAKI, MA, USA), a Puro-Tap UV reactor with an LP mercury lamp that emits primarily monochromatic light at 254 nm (PURO, Italy), a high pressure

diaphragm pump (Hydra-Cell model D/G-03-X, Wanner Engineering, USA), and a membrane unit equipped with a spiral-wound Desal-5 DK membrane (model DK2540F1073, GE Water & Process Technologies, USA). Desal-5 DK is a thin film composite membrane with a polysulfone support layer; it presents a molecular weight cutoff of 150–300 Da (Boussahel et al. 2002) and is negatively charged at neutral pH (GE Osmonics, USA). The nanofiltration filter element presented 1.27 mm spacers, an active area of 1.6 m^2, a water permeability of 5.0 ± 0.2 L h^{-1} m^{-2} bar^{-1}, and a $MgSO_4$ average rejection of 98 %. A transmitter (8864, Trafag, Switzerland) was placed upstream the membrane unit to measure the pressure and temperature of the circulating water. Transmembrane pressure was adjusted on the retentate side to 10 bars with a brass ball valve. The temperature of the feed water was controlled at 25 ± 2 °C using a temperature control unit equipped with a stainless steel heat exchanger coil that was submerged into the feed reservoir. Stirring was provided to the feed solution during the experiments to ensure homogeneity. The tubing of the system was made of polyvinylidene difluoride to minimize the adsorption of the micropollutants.

The laboratory scale unit was tested using different configurations to carry out different three independent studies: degradation of the selected compounds by LP/UV photolysis (using only the UV reactor; detailed in "UV photolysis" section; Fig. 1a), removal by nanofiltration (using only the membrane unit; detailed in "Nanofiltration" section; Fig. 1b), and integration of both processes (using the UV reactor followed by nanofiltration; detailed in "Integration of LP/UV photolysis and nanofiltration" section; Fig. 1c).

UV photolysis

The degradation of the selected hormones was assessed over time by continuously recirculating the water solutions through the UV lamp in the setup illustrated in Fig. 1a. Surface water collected after sedimentation (2 L) was therefore spiked with a mixture of the target hormones to achieve a concentration of 500 μg L^{-1} of each hormone. This concentration was set to carry out all the experiments described in this paper to ensure that the concentration of the target micropollutants would be well above the detection limits of direct injection HPLC as well as to attempt the identification of UV by-products formed. These compounds were spiked as a mixture of hormones since, in the environment, these micropollutants are also present in the water as mixtures and concurrent effects may influence their degradation and removal rates.

Based on the actinometry principle and using the degradation of atrazine as a reference, 1 h of circulation in the LP/UV system corresponds to a UV fluence of approximately 2200 mJ cm^{-2}.

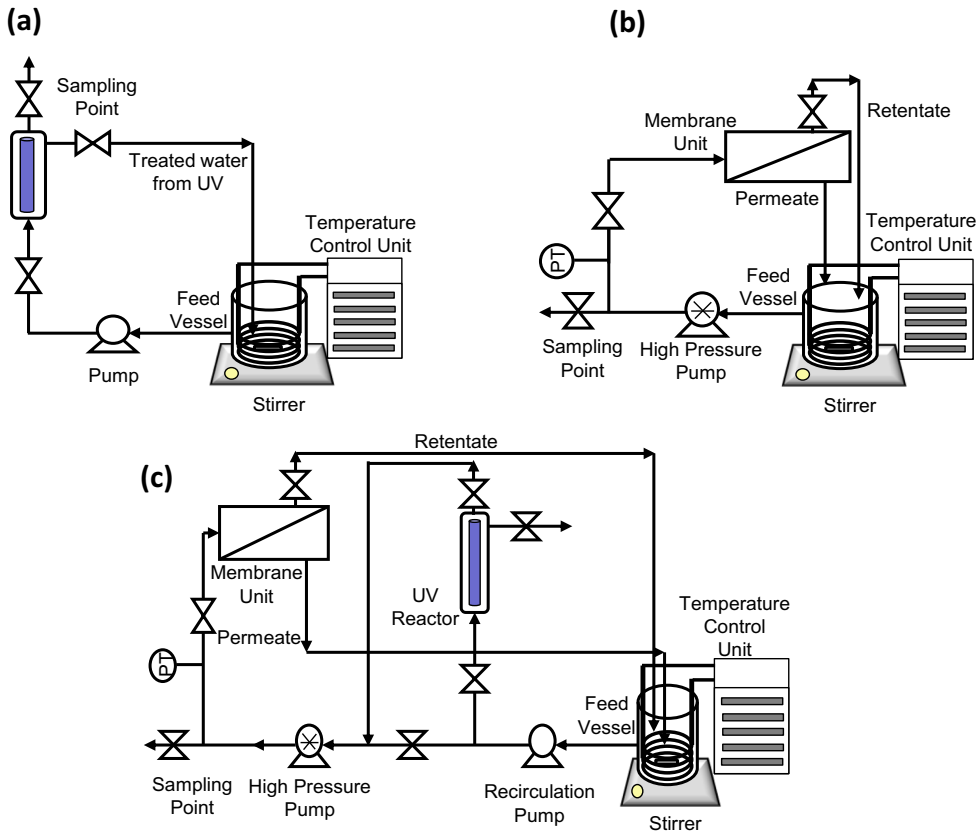


Fig. 1 Laboratory scale unit used to carry out **a** UV photolysis, **b** nanofiltration, and **c** the integrated treatment

Samples (2 mL) were taken throughout the assays at different exposure times, filtered with 0.2 μm filters made of regenerated cellulose (Whatman, USA), and analyzed by direct HPLC to determine the percent degradation of the compounds. The filters were tested in terms of their ability to adsorb the selected compounds; adsorptions lower than 12.1 % were obtained for all the target hormones (6.5 % for 17β-estradiol, 12.1 % for 17α-ethinylestradiol, 6.3 % for estrone, 4.9 % for estriol, and 8.1 % for progesterone).

Control experiments were performed to assess the loss of target hormones due to adsorption on the system. These experiments were carried out under the same conditions as photolysis assays except for the absence of irradiation. The results obtained for the removal of the compounds from the control solution due to adsorption after 6 h were the following: 9 % 17β-estradiol, 22 % 17α-ethinylestradiol, 21 % estrone, 2 % estriol, and 59 % progesterone. The higher adsorption of progesterone is probably related with its high hydrophobicity. The adsorption values obtained over time for all the target hormones were therefore used to correct the degradations obtained during photolysis experiments.

Nanofiltration

Nanofiltration experiments were conducted in cross-flow mode, with total recirculation of permeate and retentate to simulate an extended exposure of the membrane to a water stream containing the target micropollutants. Conducting the assays without recirculation would not be feasible since (i) much larger volumes of feed solutions would be needed to follow rejection over time in the absence of total recirculation because the dead volume of the system is approximately 800 mL and the linear velocity must be sufficiently high to ensure adequate mass transfer conditions; and (ii) a considerable quantity of micropollutants would be needed.

Surface water collected after the sedimentation process (5 L) spiked with a mixture of the hormones (500 μg L^{-1} each) was fed to the nanofiltration system depicted in Fig. 1b at a flow rate of 0.2 m^3 h^{-1} and a transmembrane pressure of 10 bars during 6 h. Samples of feed, permeate, and retentate were taken throughout the experiments, acidified to prevent degradation of the compounds, and kept refrigerated until analysis (the analytical methods used are detailed in "Analytical methods" section).

The apparent rejection of the selected compounds over time ($R_{app,t}$) was determined using Eq. (1).

$$R_{app,t}(\%) = 100 \times \left(1 - \frac{C_{p_t}}{C_{f0}}\right) \quad (1)$$

where C_{Pt} and C_{f0} are the concentrations of a given compound in the permeate over time (t) and in the feed solution, respectively.

Integration of LP/UV photolysis and nanofiltration

Surface water collected after sedimentation (5 L) was spiked with a mixture of the selected EDCs (500 μg L^{-1} each) and fed to the combined system. LP/UV photolysis and nanofiltration were combined as shown in the setup illustrated in Fig. 1c, where UV photolysis took place at atmospheric pressure, followed by nanofiltration at a transmembrane pressure of 10 bar. Operation was carried out in sequential steps (reaction, filtration, and recirculation) during 6 h, with total recirculation of permeate and retentate, as detailed in "Nanofiltration" section. Although UV disinfection is often placed after filtration in water utilities, the UV photolysis unit was placed upstream the nanofiltration unit to attain an integrated treatment able to treat the retentate generated during nanofiltration.

Samples of permeate and retentate were taken at regular intervals to follow the removal of the selected compounds, identify UV by-products, and evaluate the variation of the estrogenic activity of the water using the YES assay.

Analytical methods

Liquid chromatography analysis

Different analytical methods were used according with the different micropollutants and their concentrations in the samples.

Feed and retentate samples were analyzed by direct HPLC injection using a Waters system (Alliance e2695 Separations Module) equipped with a photodiode array detector (2998, Waters Chromatography, Milford, MA, USA), a Multi λ Fluorescence Detector (2475, Waters Chromatography, Milford, MA, USA), and a Luna 5 μ C18(2) 100 A (150 × 3.0 mm) column (Phenomenex Inc, Torrance, CA, USA). The chromatographic conditions to analyze samples containing the mixture of the five EDCs are presented in Table 1.

Since concentrations below the detection limits attained by direct injection were expected in nanofiltration permeate samples, a higher volume of sample was collected and a concentration step—solid-phase extraction using Oasis HLB cartridges (Waters)—was employed prior to quantification by ultra performance liquid chromatography with mass spectrometry detection. The solid-phase extraction procedure was conducted using an AutoTrace Workstation system and Oasis HLB cartridges (6 mL, 200 mg). A conditioning step was performed with 6 mL of a mixture of methanol and acetone (3:2), followed by 3 mL of methanol and 3 mL of ultra-pure water at a flow rate of 10 mL min^{-1}. Then, 500-mL samples were loaded at a flow rate of 30 mL min^{-1}. In the elution step, 6 mL of the same mixture of solvents used in the activation step was used. The final extract was concentrated to a volume of

Table 1 HPLC methods used for the quantification of the EDCs in the direct HPLC analysis

Compound	Mobile phase composition (%)	Temperature (°C)	Flow rate (mL min^{-1})	HPLC/UV λ_{UV} (nm)	HPLC/FL		Detection limit (μg L^{-1})
					λ_{em} (nm)	λ_{exc} (nm)	
17β-Estradiol	0–6 min: 40ACN/60H_2O	35	1.0	220	–	–	50
17α-Ethinylestradiol	6.5–9.5 min: 65ACN/35H_2O			220	–	–	100
Estriol	10.5–15 min: 40ACN/60H_2O				300	217	5
Estrone				220	–	–	25
Progesterone				220	–	–	25

Volume of injection, 50 μL

ACN acetonitrile, *H_2O* Milli-Q water, *UV* ultraviolet, *FL* fluorescence, *λ* wavelength

0.5 mL using a TurboVap evaporation system (Zymark, USA) with a nitrogen stream at 0.2 bar and at a temperature of approximately 35 °C. The chromatographic analysis of the hormones was conducted using an Ultra Performance Liquid Chromatography Acquity System (Waters) and an Acquity BEH C_{18} (2.1 × 50 mm, 1.7 μm) column from Waters. Gradient conditions were used to analyze the hormones: 30 % methanol/70 % water with 0.05 % ammonia (starting at 0 min); 95 % methanol/5 % water with 0.05 % ammonia (3–4.2 min); and 30 % methanol/70 % water with 0.05 % ammonia (5–6 min). The flow rate of mobile phases was 0.5 mL min^{-1}, the oven temperature was set at 40 °C, and an injection volume of 20 μL was used in the analysis. A mass spectrometer triple quadrupole Acquity TQD, equipped with a Z-spray electrospray ionization source, was used. Electrospray ionization source operating conditions were set using a capillary voltage of 3.0 kV, a source temperature of 150 °C, an extractor voltage of 2.0 V, desolvation temperature and gas flow of 500 °C and 900 L h^{-1}, respectively, a RF lens of 0.1 V, and a cone gas flow of 20 L h^{-1}. Table 2 shows the mass spectrometry conditions used for the detection of the selected hormones.

The detection limit of this method is 0.05 μg L^{-1} for all the selected EDCs, and the uncertainties related with their determination are the following: 23.1 % for 17 β-estradiol, 18.7 % for 17 α-ethinylestradiol, 17.2 % for estrone, 14.7 % for estriol, and 22.5 % for progesterone. This method was also employed using full-scan monitoring analysis in an attempt to identify by-products.

Table 2 Mass spectrometry conditions used for the detection of the target hormones

Hormone	Time (min)	Cone voltage (V)		MRM transition (m/z) precursor ion → product ion	Collision energy (eV)	Dwell time (s)
17β-Estradiol	1.97–2.50	MRM1	65 (ES−)	271.4 → 145.3	40	0.025
		MRM2	65 (ES−)	271.4 → 143.0	65	
17α-Ethinylestradiol	1.97–2.50	MRM1	50 (ES−)	295.4 → 145.0	39	0.025
		MRM2	50 (ES−)	295.4 → 159.0	34	
Estrone	1.97–2.50	MRM1	60 (ES−)	269.3 → 145.2	40	0.025
		MRM2	60 (ES−)	269.3 → 143.0	55	
Estriol	0.00–1.70	MRM1	60 (ES−)	287.4 → 145.4	40	0.078
		MRM2	60 (ES−)	287.4 → 171.3	35	
Progesterone	2.40–2.80	MRM1	50 (ES+)	315.4 → 96.9	20	0.025
		MRM2	50 (ES+)	315.4 → 108.9	25	

YES assay

A YES assay using an hER transfected *Saccharomyces cerevisiae* strain was performed to evaluate the variation of the relative estrogenic activity of the water throughout the integrated process. The hER *Saccharomyces cerevisiae* was used under agreement with Professor J. P. Sumpter at Brunel University, UK. This assay was conducted according to the procedure described by Routledge and Sumpter (1996) with specific modifications introduced by Stanford and Weinberg (2010).

17β-Estradiol was used as reference in terms of estrogenic activity response. The concentrations of 17β-estradiol and samples tested that induce 50 % of the maximum response (EC_{50}) were determined and used to obtain and compare the estradiol equivalents (EEQ) of the samples over time. EEQ are a measure of the relative estrogenic activity and were determined using Eq. (2).

$$\text{EstradiolEquivalents} = \frac{EC_{50,17\beta-\text{Estradiol}}}{EC_{50,\text{Sample}}} \quad (2)$$

A detailed description of this method is provided as Supplementary Material.

Results and discussion

UV photolysis

Table 3 shows the time-based pseudo-first-order rate constants—k'_t—determined from the slope of the linear regression described by Eq. (3).

$$-\frac{d[C]}{dt} = k'_t[C] \Rightarrow \ln\frac{[C]}{[C_0]} = -k'_t \times t \quad (3)$$

where C_0 and C are the initial concentration and the concentration of the selected compounds at a given time (t), respectively.

Even though 17β-estradiol, 17α-ethinylestradiol, and estriol were poorly degraded by direct photolysis (only 41 to 56 % degradation was observed after 6 h of treatment), extremely high degradations of estrone and progesterone (higher than 93 %) were achieved by this process after 1 h of treatment. Figure 2 depicts the degradation of the selected EDCs obtained after 6 h of LP/UV direct photolysis.

Table 3 Pseudo-first-order rate constants (k'_t) obtained for the degradation of the studied hormones by LP/UV direct photolysis (standard deviations associated to linear regressions are provided)

Compound	$k'_t \times 10^3$ (min^{-1})	R^2
17β-Estradiol	2.0 ± 0.1	0.94
17α-Ethinylestradiol	1.3 ± 0.1	0.95
Estrone	24.5 ± 2.8	0.91
Estriol	1.9 ± 0.1	0.98
Progesterone	34.7 ± 4.7	0.90

The differences shown in Table 3 and Fig. 2 may be explained by the quantum yield (ratio between the total number of molecules of compound degraded to the total number of photons absorbed by the solution due to the compound's presence) of the selected hormones. Much higher quantum yields have been reported for estrone (5.45 mol $einstein^{-1}$) and progesterone (2.82 mol $einstein^{-1}$) relatively to the other hormones (0.06, 0.09, and 0.40 mol $einstein^{-1}$ for 17β-estradiol, 17α-ethinylestradiol, and estriol, respectively) (Pereira et al. 2012).

Nanofiltration

Table 4 presents the apparent rejection of the selected hormones obtained by nanofiltration.

In the early stage of the nanofiltration process (at 6 min), very similar and extremely high removals (≥99 %) were obtained for all hormones. At this point, the concentrations of the majority of the target compounds in the permeate were below the detection limits of the analytical methods (5 - 100 μg/L) and, therefore, exact removal values were not determined. During the 6 h of treatment, the rejections obtained for the target hormones were often higher than 81 %, with the exception of estrone (with rejections higher than 70 %), showing that nanofiltration is effective to remove these molecules even when they are spiked in surface water at levels higher than their reported occurrence levels. Apparent rejection was generally higher for the molecules having the highest molecular weights (Table 5) throughout the treatment.

Hydrophobic interactions were also likely to play a significant role in the beginning of the assay (at 6 min), contributing to higher rejections by adsorption since membrane saturation has not been accomplished yet, as previously reported by other authors (Kimura et al. 2003; Nghiem et al. 2005). In a previous study, very high removals of these hormones were also attained (often higher than 92 and 72 % in groundwater and surface water, respectively) using the same membrane in a flat-sheet configuration (Sanches et al. 2012). Removals attained by Pereira et al. (2012) for the majority of these hormones using a NF270 membrane are also in line with these findings.

The high removals attained are due to a physical removal process that will lead to high concentrations of these compounds in the membrane filtration retentate. It is thus extremely important to couple this process with other treatment options that will enable the degradation of the highly concentrated retentates produced.

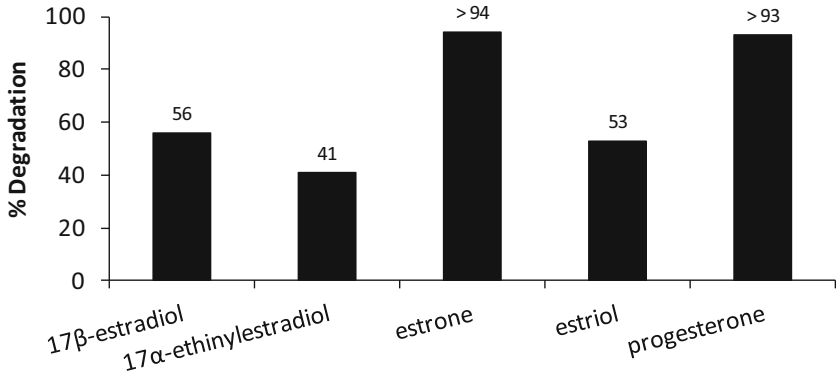


Fig. 2 Comparison of the degradations attained for 17β-estradiol, 17α-ethinylestradiol, estrone, estriol, and progesterone by LP/UV direct photolysis after 6 h of treatment

Integrated process

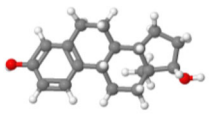
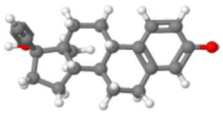
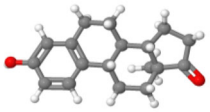
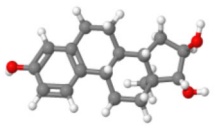
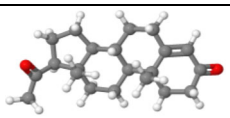
Table 4 shows the global removal of the EDCs throughout the integrated process, reflecting the quality of the drinking water produced (permeate). Extremely high removals were obtained with the combined system throughout the 6 h of treatment. The removals attained are in agreement with those obtained by nanofiltration alone (Table 4). A significant increase from 77 to 95 % was, however, observed for estrone at the 6th hour when the combined treatment was applied relatively to nanofiltration alone. There is therefore advantage in combining nanofiltration with LP/UV photolysis to remove estrone since it is highly degraded by LP/UV radiation. To the best of authors' knowledge, despite the inclusion of the majority of these hormones in the EU first watch list (Directive 2013/39/EU) and the US EPA Contaminant Candidate List 3, there are not regulated values for the occurrence of these hormones. Therefore, concentration values attained in the treated water cannot be taken for comparison with regulations.

Most of the benefits observed by integration of both treatment processes are due to the treatment of the retentate stream (Fig. 3) and the decrease in the endocrine disrupting activity of the treated water (Fig. 4). Removals from the retentate higher than 73 % were achieved for all the compounds except estriol in the sample taken at the 1st hour of treatment (Fig. 3). Even though all hormones were analyzed over the 6 h experimental period, estriol was the only compound still detected in the retentate above the respective detection limit at the 6th hour with a removal of 69 %. Conversely, estrone was not detected above the detection limit even at the sample taken at the 1st hour (removal higher than 92 %, which corresponds to the detection limit of estrone). The hormones 17β-estradiol, 17α-ethinylestradiol, and progesterone were not detected after the 1st hour and were also therefore very efficiently removed

Table 4 Degradation and apparent rejection of the selected hormones by LP/UV direct photolysis, nanofiltration, and the integrated process

Compound	Time (h)	Degradation/apparent rejection (%)		
		LP/UV	Nanofiltration	Integrated process
17β-Estradiol	0.1	0	>99	99
	3.0	30	81	86
	6.0	56	84	85
17α-Ethinylestradiol	0.1	5	>99	99
	3.0	21	85	95
	6.0	41	90	94
Estrone	0.1	21	>99	99
	3.0	>95	70	93
	6.0	>95	77	95
Estriol	0.1	6	99	99
	3.0	29	93	89
	6.0	53	94	91
Progesterone	0.1	27	>99	99
	3.0	>95	89	99
	6.0	>95	93	98

Table 5 Molecular structure, molecular formula, molecular weight, and logarithm of octanol-water partition coefficient (log K_{ow})

Compound* [CAS number]	Molecular structure	Molecular formula	MW (Da)	log K_{ow}
17β-Estradiol [50-28-2]		$C_{18}H_{24}O_2$	272.4	4.01[26]
17α-Ethinylestradiol [57-63-6]		$C_{20}H_{24}O_2$	296.4	3.67[26]
Estrone [53-16-7]		$C_{18}H_{22}O_2$	270.4	3.13[26]
Estriol [50-27-1]		$C_{18}H_{24}O_3$	288.4	2.45[26]
Progesterone [57-83-0]		$C_{21}H_{30}O_2$	314.5	3.87[26]

*Molecular structures depicted were obtained from the Jmol software

by the combined treatment. For these compounds, removals higher than 82.5 % were attained (removal determined based on the highest detection limit that corresponds to 17α-ethinylestradiol).

The removal of the target compounds from the retentate was followed by a very high decrease of estrogenic activity (Fig. 4) The integrated treatment was able to effectively treat water containing high levels of endocrine disrupting activity. Very low values of EEQ were attained in the permeate over time (0.04–0.38) relatively to the initial feed (8.3), reflecting removals of estrogenic activity of 95.4–99.6 %.

A noteworthy estrogenic removal of 75 % was attained in the retentate within the 1st hour of treatment (Fig. 4). However, a slight increase was observed between the 3rd and the 4th hours in the retentate. Even though by-products were not identified by the UPLC-MS/MS analysis carried out in this study, this increase in the estrogenic activity may be explained by the formation of estrogenically active transformation compounds that may be more disrupting than the parent compounds at extremely low concentrations that did not enable detection. The efficient removal of the endocrine disrupting activity associated to 17β-estradiol and 17α-ethinylestradiol during photolysis assays using LP and medium pressure lamps was previously reported (Rosenfeldt et al. 2007). The high efficiency of the integrated treatment to remove the target micropollutants and respective endocrine disrupting activity from retentate suggests that further treatment and/or disposal of retentate may not be required, highlighting the advantage of combining membrane processes with UV photolysis to treat the filtration retentate. Furthermore, the fact that the removal of EDCs from the retentate was generally higher than 73 % in the sample taken at the 1st hour of treatment (Fig. 3) and that a considerable decrease of estrogenic activity of 75 % had already been

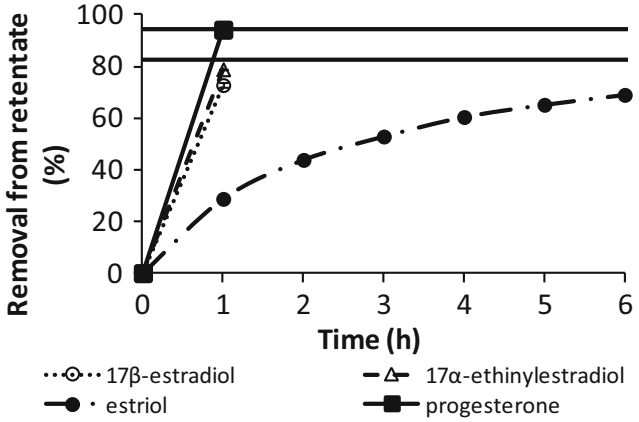


Fig. 3 Removal of the selected hormones from the retentate throughout the integrated treatment over a 6-h experimental period (duplicate injections are provided as *error bars*). *Horizontal lines* represent the percentage of degradations that correspond to the lowest and highest detection limits of the hormones that were not detected after 1 h of operation (25–100 μg L^{-1})

achieved at this point (Fig. 4) suggests that a residence time of 1 h could be sufficient to achieve an effective treatment. Lower retention times would probably be required to attain a satisfactory retentate treatment at the environmental occurrence concentrations. Under such conditions, much lower UV fluences would be needed as well.

The permeate flux measurements throughout experiments indicated that the flux was fairly constant over time since it was determined as 48 ± 4 L h^{-1} m^{-2}. The degradation of natural organic matter present in the water by LP/UV radiation is likely to have contributed to minimize fouling and promote a relatively constant flux. Furthermore, the surface water after sedimentation used presents low concentrations of particulates and colloidal organic matter that usually contribute to membrane fouling and consequently to flux decline.

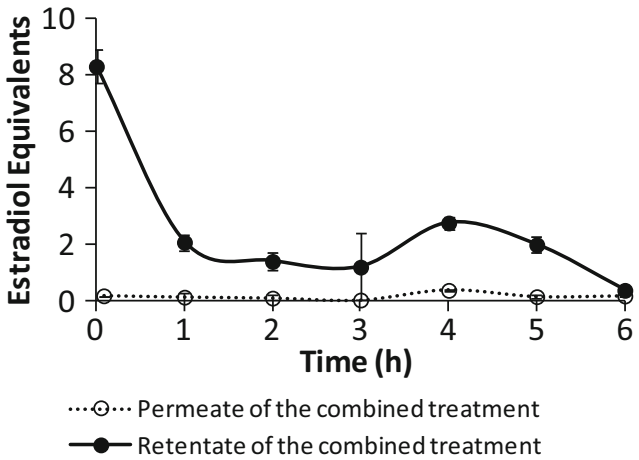


Fig. 4 Variation of the endocrine disrupting activity in the permeate (treated water) and in the retentate throughout the integrated treatment over a 6-h experimental period (duplicate injections are provided as *error bars*). At $t = 0$ h, estradiol equivalents shown in the *plot* for retentate correspond to the feed sample

Conclusions

The integration of the proposed technologies evaluated demonstrated a high treatment potential towards the removal of the spiked target hormones in a real surface water collected after sedimentation. High efficiency is also expected to be advantageous for the removal of other organic compounds with similar structures and properties.

The extremely low estrogenic activity of permeate reported suggests that UV intermediates generated that have higher estrogenic activity than the parent compounds are likely to have been retained by the membrane.

The removal of the target micropollutants from the retentate, as well as the reduction of the estrogenic activity of the retentate to levels comparable to permeate, suggests that additional treatment and/or disposal of retentate will not be necessary. Besides improving the quality of the retentate by reducing its load, the use of UV radiation will also accomplish the inactivation of microorganisms that are resistant to traditional disinfectants, improving the microbial water quality.

The general retention of estrogenically active transformation compounds, as well as the treatment of nanofiltration retentate, constitutes advantages of the integrated process relatively to UV and nanofiltration processes when assessed individually. Furthermore, neither UV photolysis nor nanofiltration are the best treatment technologies for all the compounds addressed: three hormones (17β-estradiol, 17α-ethinylestradiol, and estriol) were better removed by nanofiltration and one by UV photolysis (estrone). A cocktail of numerous micropollutants that are also differently removed by these technologies exists in water treatment utilities, highlighting the benefits of having a multi-barrier approach. Besides the removal of a wide range of organic micropollutants spiked in the surface water collected after sedimentation, the combined process ensured the treatment of retentate, the retention of photolysis by-products, and low fouling development, showing its potential for application in drinking water utilities.

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