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Effect of biological and chemical oxidation on the removal of estrogenic compounds (NP and BPA) from wastewater: An integrated assessment procedure

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ABSTRACT

A major source of the wide presence of EDCs (Endocrine Disrupting Compounds) in water bodies is represented by direct/indirect discharge of sewage. Recent scientific literature reports data about their trace concentration in water, sediments and aquatic organisms, as well as removal efficiencies of different wastewater treatment schemes. Despite the availability of a huge amount of data, some doubts still persist due to the difficulty in evaluating synergistic effects of trace pollutants in complex matrices. In this paper, an integrated assessment procedure was used, based on chemical and biological analyses, in order to compare the performance of two full scale biological wastewater treatment plants (either equipped with conventional settling tanks or with an ultrafiltration membrane unit) and tertiary ozonation (pilot scale).

Nonylphenol and bisphenol A were chosen as model EDCs, together with the parent compounds mono- and di-ethoxylated nonylphenol (quantified by means of GC–MS). Water estrogenic activity was evaluated by applying the human breast cancer MCF-7 based reporter gene assay. Process parameters (e.g., sludge age, temperature) and conventional pollutants (e.g., COD, suspended solids) were also measured during monitoring campaigns.

Conventional activated sludge achieved satisfactory removal of both analytes and estrogenicity. A further reduction of biological activity was exerted by MBR (Membrane Biological Reactor) as well as ozonation; the latter contributed also to decrease EDC concentrations.

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1. Introduction

In recent decades, concerns regarding the occurrence of Endocrine Disrupting Compounds (EDCs) in the environment have rapidly increased worldwide. Municipal sewage and Waste Water Treatment Plant (WWTP) effluents are considered to be major sources of pollution due to the documented presence of such compounds at relevant concentrations (see, *inter alia*: Auriol et al., 2006; Ternes and Joss, 2006; González et al., 2007; Stasinakis et al., 2008; Ying et al., 2009; Sanchez-Avila et al., 2009).

EU Directive 2008/105/EC (amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/4/9/EEC, 86/280/EEC and amending Directive 2000/60/EC) sets strict quality standards for water bodies (many EDCs being included among priority substances). Therefore, in the future, efforts to adopt feasible and reliable treatment techniques for wastewater cleaning will be made. Accordingly, two important tasks should be pursued: (1) the assessment of the removal capacity of conventional biological processes and, consequently, (2) the evaluation of possible requirements for additional (tertiary) treatment.

Even though data is available in the literature on both issues, some lack in knowledge still persists: (1) the removal potential of many EDCs by conventional activated sludge plants is well-established (see for instance Farré et al., 2002; Ternes and Joss, 2006; González et al., 2007; Joss et al., 2008; Pothitou and Voutsa, 2008; Press-Kristensen et al., 2008), nevertheless, data are not easily comparable due to different treatment conditions, sampling procedures and analytical methods; (2) tertiary chemical oxidation has been successfully tested (Rosenfeldt and Linden, 2004; Auriol et al., 2006; Zhang et al., 2006; Esplugas et al., 2007; Gultekin and Ince, 2007; Ning et al., 2007; Bolong et al., 2009; Racz and Goel, 2010) but technical-economic feasibility is still to be fully demonstrated (Auriol et al., 2006; Gultekin and Ince, 2007; Koh et al., 2008); (3) chemical analysis alone is not useful to investigate synergistic effects among mixtures of different pollutants and their degradation by-products (a well-known phenomenon in the case of endocrine disruptors: Hjelmborg et al., 2006; Bjorkblom et al., 2008; Mnif et al., 2010). Several authors (Svenson et al., 2003; Hashimoto et al., 2007; Fernandez et al., 2008; Mispagel et al., 2009) have pointed out that water biological activity should also be monitored in order to better evaluate treatment suitability; actually, endocrine activity assays have been proposed in the last few years (Harris et al., 1997; Céspedes et al., 2003; Isobe et al., 2003; Korner et al., 2004; Tan et al., 2007; Fernandez et al., 2009; Jugan et al., 2009; Creusot et al., 2010; Sousa et al., 2010).

In this work, an integrated assessment procedure, based on both chemical and biological analyses, was adopted to evaluate the performance of biological and chemical oxidation in the removal of target EDCs from municipal wastewater. The following estrogen-like substances were considered: 4-nonylphenol (NP), its parent compounds 4-nonylphenol monoethoxylate (NP1EO) and 4-nonylphenol diethoxylate (NP2EO), and bisphenol A (BPA). These substances were chosen as model EDCs since they are diffusely detected in the aquatic environment (Kolpin et al., 2002; Belmont et al., 2006; Gultekin and Ince, 2007; Loos et al., 2007; Sun et al., 2008; Ying et al., 2009) and are included in the EU priority list (EU Directive 2008/105/EC).

Experimental work was conducted at two full scale WWTPs located in Northern Italy equipped with either conventional settling tanks (CAS, Conventional Activated Sludge: Verona municipality) or with an ultrafiltration unit (MBR, Membrane Biological Reactor: Brescia municipality). Tertiary chemical oxidation was tested by means of an ozone pilot plant located at the Verona WWTP.

The duration of the analytical campaigns was extended so as to enable the accurate calculation of mass balances of target compounds. Hormonal activity in water samples was measured by means of human breast cancer MCF-7 based reporter gene assay, using 17β-estradiol (E2) as a standard. This cell line was chosen due to its high concentration of estrogenic receptors and sensitivity (Pons et al., 1990; Urban et al., 2001; Soto et al., 2006; Higashi et al., 2007).

2. Materials and methods

2.1. Treatment plants

2.1.1. *Verona* WWTP

This is a CAS plant (design size 370,000 p.e.) treating mainly domestic wastewater. The process scheme includes primary settling (volume = 10,400 m^3, 3 parallel basins); pre-denitrification (volume = 7200 m^3, 5 parallel basins); oxidation-nitrification (volume = 16,600 m^3, 5 parallel basins); secondary settling (volume = 26,100 m^3, 6 parallel basins).

The sludge treatment line consists of: dynamic thickening, anaerobic digestion and mechanical dewatering.

The following are the main operational data (typical values): influent water flow = 92,000 m^3/d (dry weather); dissolved oxygen concentration in aerated tanks = 2.0–2.2 mg/L; total suspended solids concentration in biological reactors = 4.0–4.5 gTSS/L; influent characteristics (after screens and grit-oil removal): 450 mgCOD/L, 200 mgBOD_5/L, 240 mgTSS/L, 50 mgTKN/L, 5 mgP_{TOT}/L; effluent characteristics: 30 mgCOD/L, 5 mgBOD_5/L, 12 mgTSS/L, 6.5 mgTKN/L; 4 mgNH_4^+-N/L, 4 mgNO_3^--N/L, <0.1 mgNO_2^--N/L, 1.3 mgP_{TOT}/L.

2.1.2. *Brescia* WWTP

This consists of 2 CAS lines and 1 MBR line (design size 380,000 p.e.), treating domestic and industrial wastewater. The process scheme includes equalization/homogenization (volume = 24,000 m^3); pre-denitrification (volume = 11,100 m^3, 3 parallel basins); oxidation-nitrification (volume = 20,600 m^3, 3 parallel basins); secondary settling (for conventional lines, volume = 7800 m^3, 2 parallel basins) and ultrafiltration (for MBR line). This configuration enabled the comparison of the CAS process with the MBR technique.

The sludge treatment line consists of: dynamic thickening, anaerobic digestion and mechanical dewatering.

The following are the main operational data (typical values): influent water flow = 71,500 m^3/d (dry weather); dissolved oxygen concentration in aerated tanks = 1 mg/L; total suspended solids concentration in biological reactors = 2.0 and 5.2 gTSS/L in CAS and MBR lines, respectively; influent

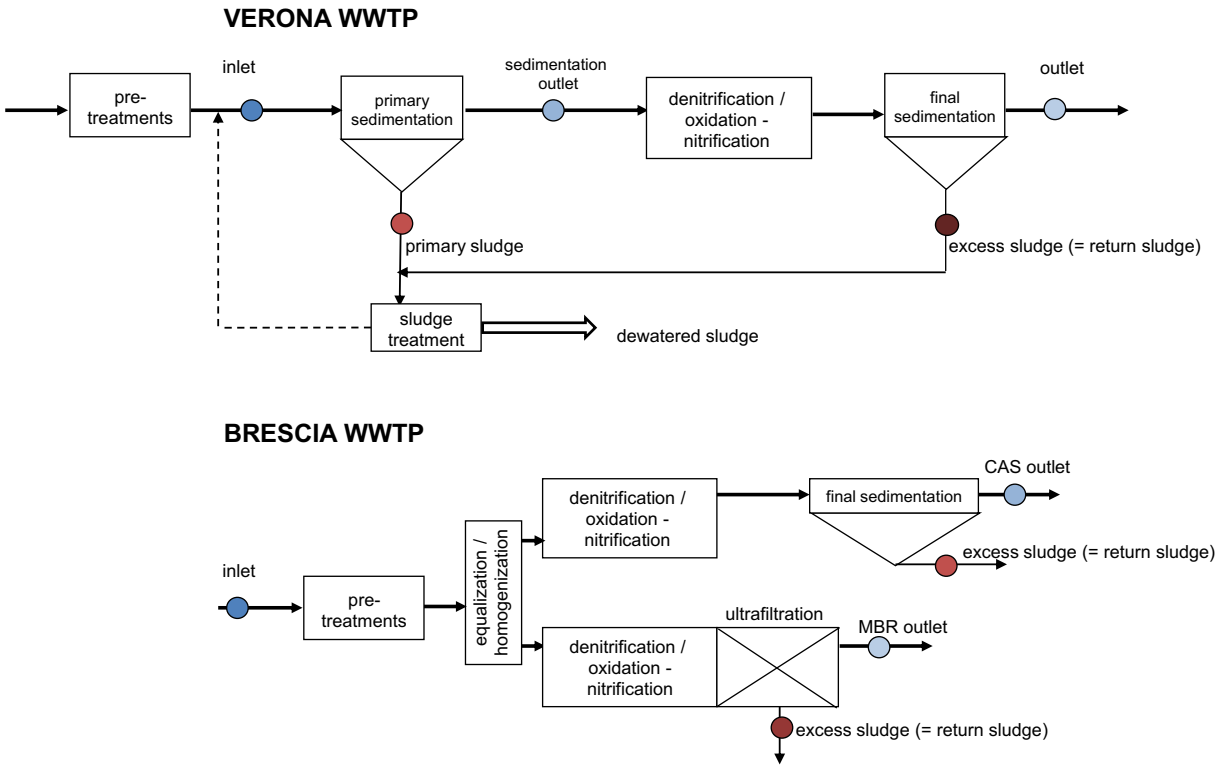

Fig. 1 – Sampling points for the Verona (top) and Brescia (bottom) WWTPs (bold line = wastewater; fine line = sludge; dotted line = supernatant from sludge treatment; double line = dewatered sludge).

characteristics (after screens and grit-oil removal): 310 mgCOD/L, 140 mgBOD_5/L, 140 mgTSS/L, 29 mgTKN/L, 5 mgP_{TOT}/L; effluent characteristics: 15 (CAS line) and 8 (MBR line) mgCOD/L, <5 mgBOD_5/L, <5 mgTSS/L, 2.1 mgTKN/L, 3.1 (CAS line) and 0.5 (MBR line) mgNH_4^+-N/L, 3.5 (CAS line) and 5 (MBR line) mgNO_3^--N/L, <0.2 mgNO_2^--N/L, 0.6 mgP_{TOT}/L.

2.1.3. *Pilot scale ozonation plant*

Supplied by SIAD SpA, Bergamo, Italy, this consists of a stainless steel tubular reactor (volume = 1460 L) and is equipped with a pure oxygen supply system (capacity = 400 gO_3/h). The reactor can be fed with a flow-rate up to 6 m^3/h in a continuous mode of operation.

2.2. *Monitoring campaign and treatment tests*

2.2.1. *Full scale CAS and MBR WWTPs*

The Verona WWTP monitoring campaign was conducted in winter (dry weather) from 5 to 20 February 2008: sampling points were located as shown in Fig. 1 (top). It is important to

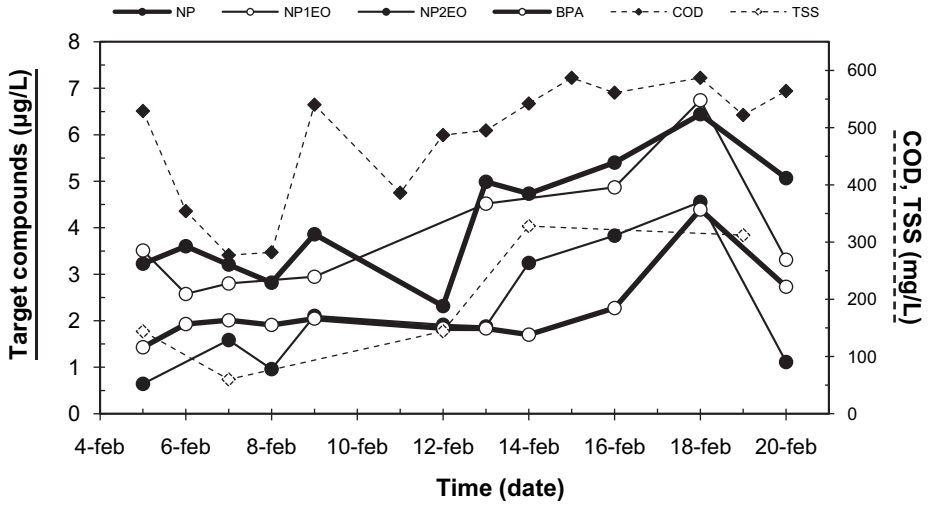

Fig. 2 – Verona WWTP: daily average concentration of pollutants in influent wastewater.

Table 1 – Verona WWTP: average concentrations of target EDCs and percentage attached to 1.6 μm particulate fraction.

	Influent		Primary settling tank effluent		Final effluent
	Total (μg/L)	Particulate (%)	Total (μg/L)	Particulate (%)	Total (μg/L)
NP	4.15	47	3.65	41	0.85
NP1EO	3.90	49	3.96	33	0.52
NP2EO	2.18	38	2.15	39	0.70
BPA	2.19	41	2.43	30	0.31

Table 2 – Brescia WWTP: average concentrations of target EDCs and percentage attached to 1.6 μm particulate fraction.

	Influent		Final effluent (CAS)	Final effluent (MBR)
	Total (μg/L)	Particulate (%)	Total (μg/L)	Total (μg/L)
NP	4.70	64	0.74	0.79
NP1EO	7.89	51	0.29	0.30
NP2EO	5.01	45	0.64	0.96
BPA	1.94	63	0.47	0.50

note that sewage entering the primary settling tanks includes supernatants from the sludge treatment line.

The Brescia WWTP was monitored during a dry weather summer period (23 June – 11 July 2008). Sampling points are shown in Fig. 1 (bottom); unlike the Verona plant, influent samples were not affected by supernatants from the sludge line.

For both plants, wastewater was collected daily, over 24 h, by automatic refrigerated auto-samplers equipped with Teflon pipes and dark glass containers (pre-washed with hydrochloric acid and acetone); sludge was sampled instantaneously and submitted immediately to analysis. The following parameters were measured on collected samples: NP (mixture of 4-nonylphenol isomers), NP1EO (mixture of 4-nonylphenol monoethoxylates isomers), NP2EO (mixture of 4-nonylphenol diethoxylates isomers), BPA, COD, total suspended solids (TSS). Estrogenic activity was measured only at the Brescia WWTP, on three 24-h samples collected during the monitoring campaign.

2.2.2. *Ozonation plant*

Two series of tests were conducted in order to assess the effect of ozone dosage (12 and 20 mgO_3/L) and, for each ozone concentration, three runs were performed at increasing contact times (15, 22 and 30 min, respectively). During each test, at 1, 2 and 3 HRT (Hydraulic Retention Time) time intervals, grab samples of influent and effluent wastewater were taken and immediately submitted to chemical (NP, NP1EO, NP2EO, BPA), microbiological (total coliforms and *Escherichia coli*) and biological (estrogenic activity) analyses. Based on instrumentally detected data (ozone production and residue in offgas), the actual ozone dissolution percentage was calculated.

2.3. *Chemical analyses*

The method of Gatidou et al. (2007) was successfully adopted for the extraction of analytes from liquid phase.

The following chemicals were purchased from Sigma Aldrich (Taufkirchen, Germany): (a) standard reagents: bisphenol A, NP1EO, NP2EO, 4-NP technical mixture of isomers, as proposed by ISO 18857-1 (2005); (b) derivatization reagents: MSTFA and pyridine; (c) internal standard: bisphenol A-d_{16}.

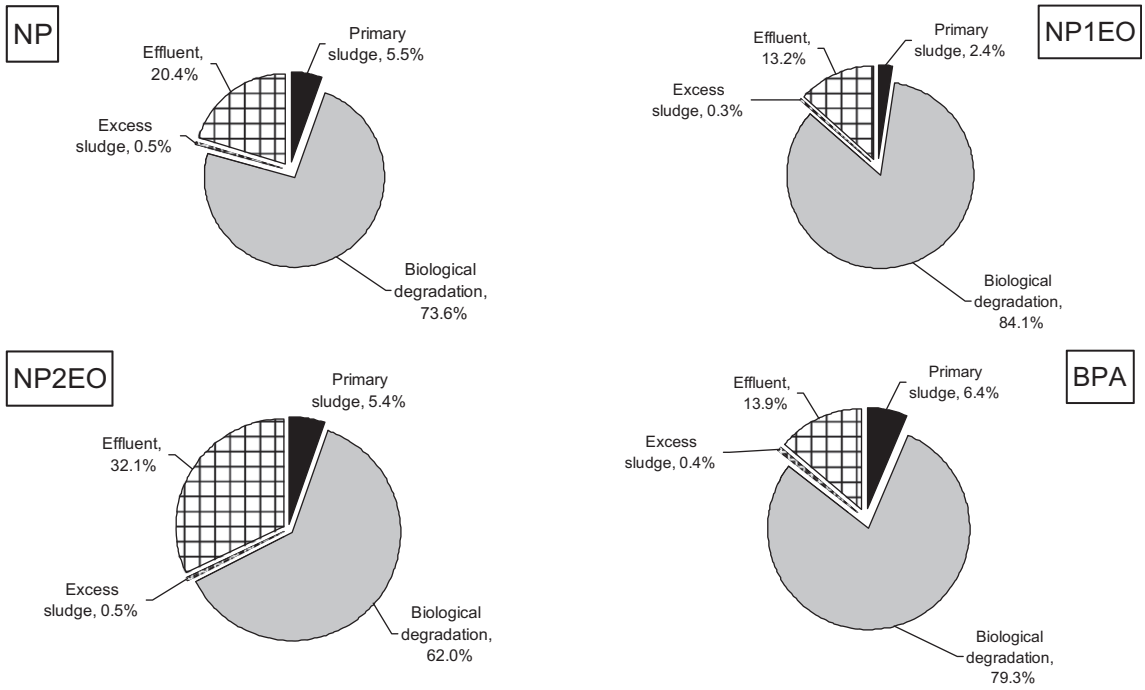

Fig. 3 – Verona WWTP: mass balance of trace pollutants. "*Degraded*" mass obtained by subtracting the sum of each effluent mass flow (final effluent, primary and excess sludge) from influent load.

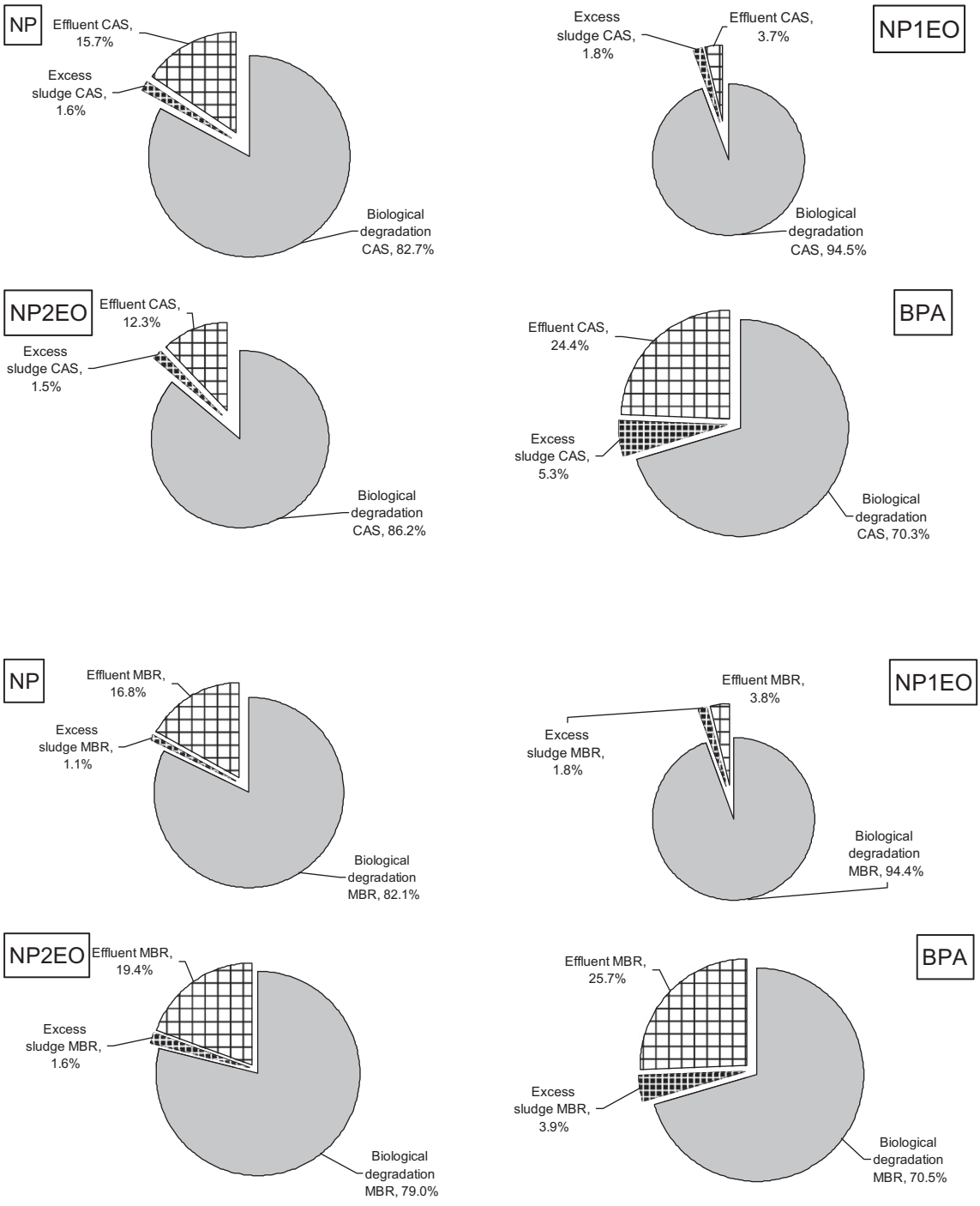

Fig. 4 – Brescia WWTP: mass balance of trace pollutants: CAS (top) and MBR (bottom) line, respectively. "*Degraded*" mass obtained by subtracting the sum of each effluent mass flow (final effluent and excess sludge) from influent load.

Influent samples were filtered on glass fiber filters (Whatman GF/A, $\varphi = 1.6$ μm particle retention) in order to separate particulate matter from the liquid phase. Liquid samples were submitted to enrichment on SPE C18 (Supelco, Bellefonte, USA) and consequent elution. Filters were weighed prior to filtration; solids retained by the filter were weighed by using a thermobalance set at 60 °C. Afterward, filters were placed into 50 mL vials, and 9 mL dichloromethane-hexane 4:1, 1 mL BPA-d_{16} (500 ppb) and 100 μL HCl 6 N were added. Vials were submitted to sonication for 30 min at 50 °C.

Derivatization was performed with 900 μL MSTFA (5% in isooctane) and pyridine (100 μL).

Instrumental analysis was conducted using a gas-chromatograph 5975B inert XL EI/CI MSD equipped with a split/splitless injector and autosampler (Agilent Technologies, Palo Alto, USA).

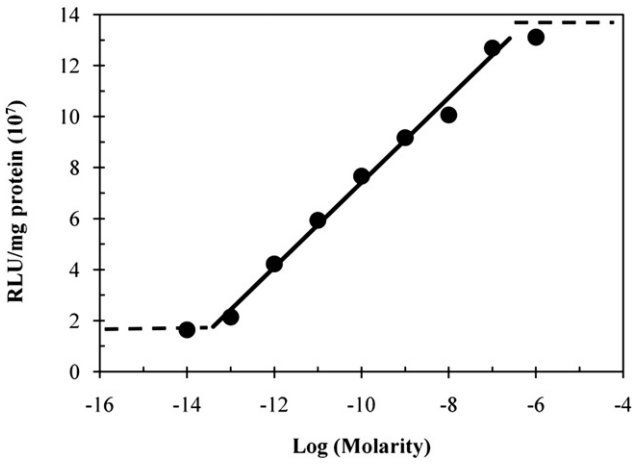

Fig. 5 – Biological assay: calibration curve with the reference estrogen E2.

The RDS% (Recovery Determination Standard) varied from 7.3 to 13.7, depending on the target molecule; mean recovery percentage referring to internal standard of BPA-d_{16} was more than 80% (water samples) and about 60% (sludge samples); the lowest concentration of the calibration curve was equal to 100 ppb for each pollutant (for further details about the analytical procedure, see Pedrazzani et al., in preparation).

COD and TSS were measured as prescribed by the Italian Standard Methods (APAT IRSA CNR No. 5130 and 2090, 2003), the former after $K_2Cr_2O_7$ oxidation, and the latter after 0.45 μm filtration and 105 °C drying process, respectively.

2.4. *Microbiological analyses*

Raw samples of ozonation plant influent and effluent wastewater were diluted in sterile NaCl 0.1% and submitted to total coliforms and *E. coli* determination, accordingly with the MPN (Most Probable Number) technique (Italian Standard Methods: APAT IRSA CNR No. 7010B and 7030B, 2003). DST Colilert® (IDEXX Laboratories, Westbrook, USA) was employed, based on specific enzymatic reactions with ONPG (o-nitrophenyl β-D-galactopyranoside) and MUG (4-methyl-umbelliferyl β-D-glucuronide). Multiplates trays were placed in an incubator at 36 ± 1 °C for 24 h and positive results were read and interpreted as prescribed.

2.5. *Biological analyses*

The pollutant extraction and clean-up procedure was the same as reported for the chemical analyses; extracts were resuspended in 1 mL DMSO (dimethyl-sulfoxide). Human breast cancer cell line MCF-7 stably transfected with the ERE-tK-LUC construct was maintained in DMEM (Modified Dulbecco's Medium, Euroclone, Milan), supplemented with 5% calf serum, at 37 °C and 5% CO_2. 24 h before treatment with pollutants, cells were plated at a density of $6.0 \cdot 10^5$ cells/well in six-well plates containing phenol red free DMEM and 5% charcoal-stripped fetal calf serum.

Cells were treated with either reference estrogen (E2) or pollutants culture medium solutions; dishes were kept at 37 °C for 24 h (Chau et al., 1998; Spink et al., 2003). Cells were then harvested in TEN buffer (10 mM Tris, 10 mM EDTA, 150 mM NaCl, pH 8.0) and pellets were lysed in luciferase assay buffer (25 mM Tris, 150 mM NaCl, 10 mM EDTA, 1 mM dithiothreitol, 5% glycerol, 0.5% Triton X-100, pH 8.0). Lysate was spun for 20 s at 13,000 *g* and supernatant submitted to luciferase activity quantification, which was performed in triplicate by means of a luminometer (Centro 960, Berthold Tech., Germany) over 10 s (De Wet et al., 1987), expressed as RLU (Relative Light Units) and normalized toward protein concentration. Reference estrogen E2 (dissolved in absolute ethanol) was employed for calibration curve definition, at concentrations corresponding to physiological/sub-physiological doses, i.e., from 10^{-13} to 10^{-7} M (the lower approaching LOD – Limit of Detection).

3. Results and discussion

3.1. *Verona WWTP: CAS process*

The mass balance of target compounds was calculated based on measured concentrations and recorded flow-rates of different streams (wastewater and sludge). It should be highlighted that the daily flow-rate was quite stable during the entire period (average value: 82.500 $m^3/d \pm 5\%$), thus yielding reliable calculations, despite an expected slight variability of influent concentrations (similar patterns were observed for EDCs and conventional pollutants COD and TSS: Fig. 2).

Average weighted concentrations of pollutants in different plant sections as well as solid-liquid phase partition percentages are detailed in Table 1; the complete mass balance is shown in Fig. 3.

As far as influent wastewater is concerned, the results confirm the data from the literature, even though NP1EO and NP2EO concentrations are close to the lowest values found by several authors (Di Corcia et al., 1994; Solé et al., 2000; Körber et al., 2000; Fuerhacker et al., 2001; Farré et al., 2002; Planas et al., 2002; Fauser et al., 2003; Laganà et al., 2004; Vethaak et al., 2005; Mart'ianov et al., 2005; Fountoulakis et al., 2005; Lee et al., 2005; Jiang et al., 2005; Clara et al., 2005a, 2007; Shen et al., 2005; Komori et al., 2006; Cantero et al., 2006; Vogelsang et al., 2006; Nakada et al., 2006; Belmont et al., 2006; Levine et al., 2006; González et al., 2007; Loyo-Rosales et al., 2007; Stasinakis et al., 2008).

Average concentrations (Table 1) indicate that primary sedimentation exerted negligible removal of trace pollutants, notwithstanding an appreciable abatement of TSS (50%: data not shown) and the relevant percentage of pollutants associated with particulate matter. As a confirmation, mass balance revealed that only 5–6% (Fig. 3) of the influent amount of these contaminants was in primary sludge, detected concentrations being in the range 3–7 mg/kgTSS. This is in agreement with published data (González et al., 2004; Levine et al., 2006), even though removal percentages up to 20–30% are reported as well (in particular for NPnEO, Ahel et al., 1994). However, an exhaustive comparison with the literature is not possible because primary settling performance is likely to be

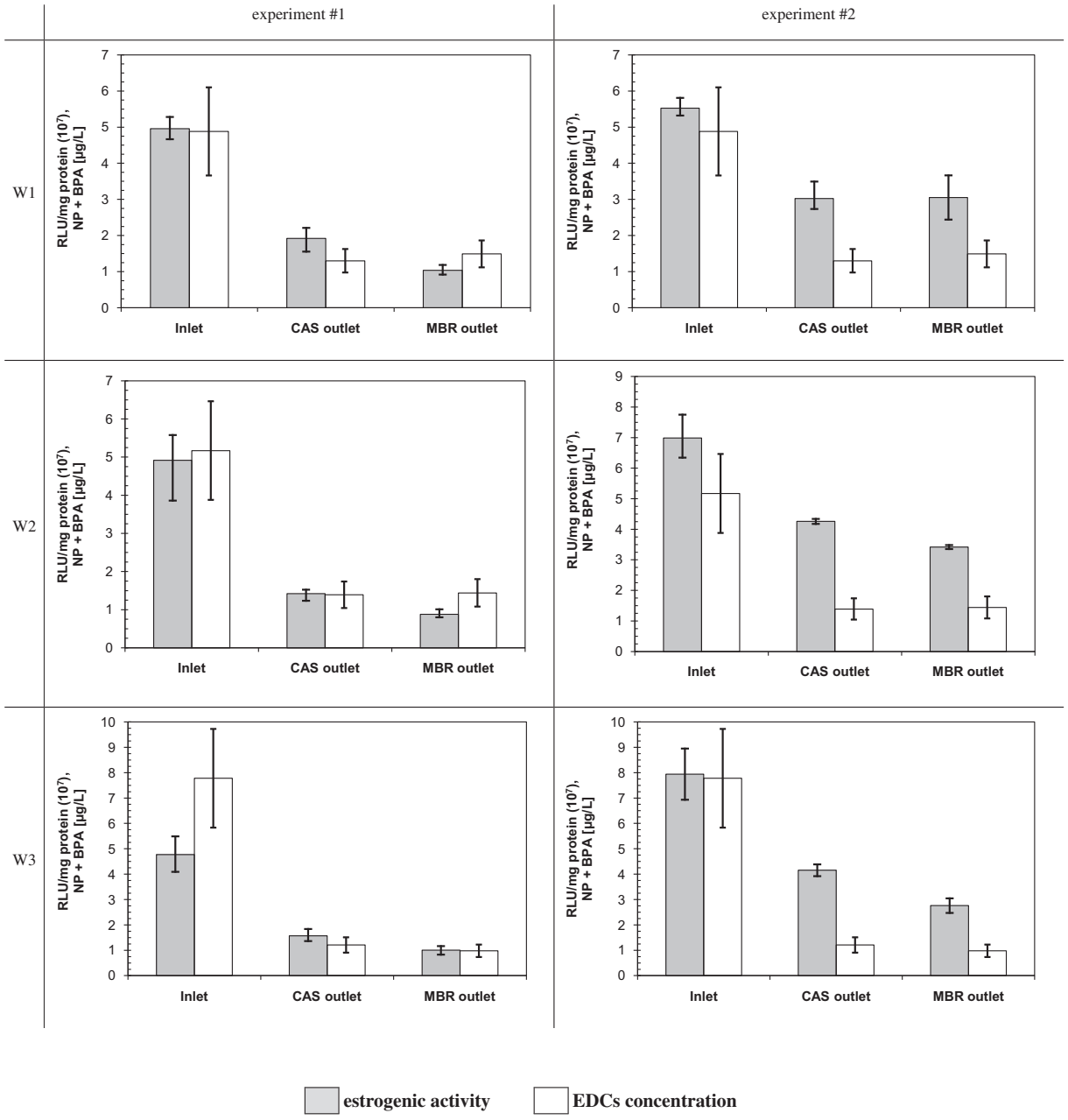

Fig. 6 – Brescia WWTP: comparison between estrogenic activity (measured in two different experiments and in three different days W1, W2, W3) and EDC concentration (NP + BPA). Error bars represent maximum and minimum values measured in 3 replicates in the case of biological data, while they show variation percentage in the case of chemical analyses.

influenced by hydraulic retention time and sewage temperature, and these data are often missing.

Taking into account final effluent, it can be observed that biological process was able to reduce the concentrations of target organics to a significant extent. These results are in accordance with the data from the literature (Koh et al., 2005; Auriol et al., 2006; Huntsman et al., 2006; Levine et al., 2006; Nakada et al., 2006; Vogelsang et al., 2006; Clara et al., 2007; Loos et al., 2007; Loyo-Rosales et al., 2007; Stasinakis et al., 2008). It must be noted that the residual amount of NP, NP1EO and NP2EO in the effluent is the result of both removal (by means of biodegradation/sorption) and generation (as metabolites of parent compounds) processes. Therefore, while in the case of BPA we focus on primary degradation, for NP, NP1EO and NP2EO we refer to an apparent degradation. Trace pollutants were also detected in excess sludge at concentrations ranging from 0.26 mg/kgTSS (BPA) to 4.08 mg/kgTSS (NP1EO); however, mass balance showed that the amount found in excess sludge accounted for less than 0.5% of the mass entering the biological system. As already noted for primary sludge, these pollutants were not removed with solid phase (sludge). Based on the comparison between TSS (data not shown) and trace pollutant

concentrations in final effluent, a clear correlation could not be evidenced, as already stated in the literature (see, among others, Jiang et al., 2005), who observed that tertiary filtration does not improve the removal of EDCs.

3.2. Brescia WWTP: CAS and MBR processes

3.2.1. Chemical analyses

Weighted mean concentrations of trace pollutants are reported in Table 2 while mass balance is shown in Fig. 4 for CAS and MBR processes, respectively. Also in this case, no appreciable scattering (±5%) with respect to average value was evidenced for sewage flow-rate during the monitoring campaign.

Considering influent wastewater characteristics, while NP and BPA were detected in similar concentrations as in the Verona WWTP, NP1EO and NP2EO values were higher. This may be due to several factors:

- the origin of influent wastewater (Brescia is located in a heavily industrialized area);
- influent wastewater temperature (higher during the Brescia monitoring campaign), which influences NPnEO degradation pathways, hence metabolite generation by biodegradation processes;
- sewer pipeline features (length, hydraulic retention time, etc.).

Both CAS and MBR lines yielded a noticeable reduction of trace pollutants and, like in Verona the plant, amounts detected in excess sludge were very low: from 1.1% to 5.3% of total influent mass (concentrations ranging from 0.38 mg/kgTSS for NP to 1.51 mg/kgTSS for NP1EO).

3.2.2. Biological analyses

Water samples (influent and both CAS and MBR effluents), taken on three different days of consecutive weeks (W1, W2 and W3) during the monitoring period, were submitted to biological assays, which were repeated twice (experiment #1 and #2). Prior to each experiment, cell responsivity to E2 was checked and a calibration curve was plotted (an example is presented in Fig. 5).

Fig. 6 shows the results of the biological analyses. It is clear that estrogenic activity was significantly reduced by both treatments, and, in five of six cases, with greater efficiency by the MBR system. This is a relevant outcome which emphasizes the importance of biological analyses: actually, while EDC (NP + BPA) concentrations were similar in outlet samples taken from both lines (Fig. 6), estrogenic activity exerted by CAS effluent was almost always higher.

3.3. Overall comparison between CAS and MBR processes performance

Removal efficiency and residual effluent concentration of target compounds for all studied plants and processes are compared in Fig. 7.

The experimental results show that, while the Brescia CAS and MBR lines, where different sludge ages were kept (9 d for CAS and 15 d for MBR, respectively), yielded similar performances, the Verona CAS plant, having the same sludge age as the Brescia MBR line, yielded on the contrary to slightly lower removal efficiencies (apart from BPA). This phenomenon might be due to different sewage temperature (16 °C and 23 °C for the Verona and Brescia WWTPs, respectively).

Actually, it is well known, that sludge age and temperature are crucial parameters: Clara et al. (2005b) argue that the minimum required sludge age is 10 d at 10 °C, and further increases do not lead to noticeable improvements. Moreover, several authors (e.g. Auriol et al., 2006; Koh et al., 2008, 2009) conclude that EDC removal occurs only in plants equipped with nitrification stages (as in the Brescia and Verona WWTPs). In addition, Clara et al. (2004) report that possible MBR efficiency improvements might be ascribed to an increase in sludge age, rather than to filtration.

Nevertheless, biological measurements carried out in this work showed that estrogenic activity was reduced to a greater extent by a MBR process with respect to CAS treatment, even if analytes were removed at a comparable level. While the reason is still under investigation; it might be attributed to metabolic pathways exhibited by different microbial consortia growing in MBR plants (Cicek et al., 1999; Clouzot et al., 2010).

3.4. Tertiary ozonation

3.4.1. Chemical and microbiological analyses

Actual ozone dosages (calculated based on dissolution efficiency) were 8 and 11 mg/L, respectively, during the two series of tests.

Disinfection performance was very high: total coliforms and *E. coli* were abated from 3.2 log efficiency (8 mg/L actual ozone dosage, 15 min contact time) up to 4.2 (11 mg/L actual

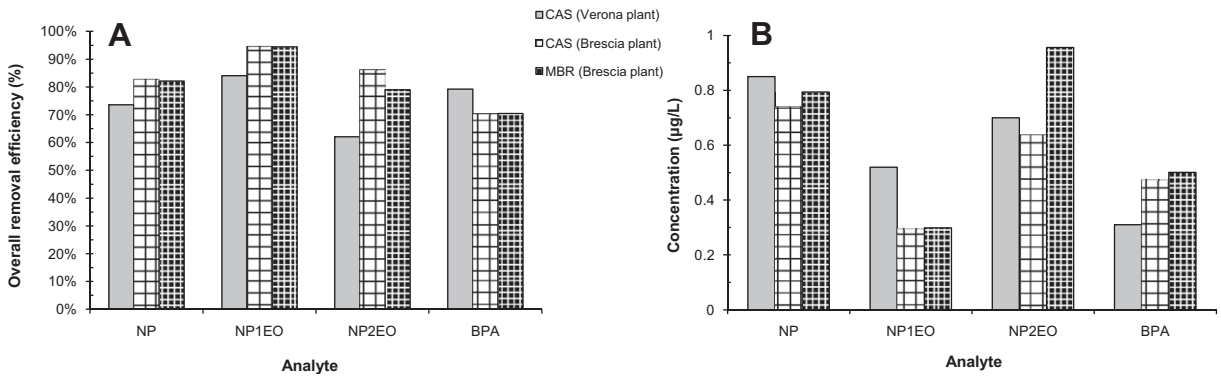

Fig. 7 – Comparison among studied processes: treatment efficiency (A) and effluent residual concentrations (B).

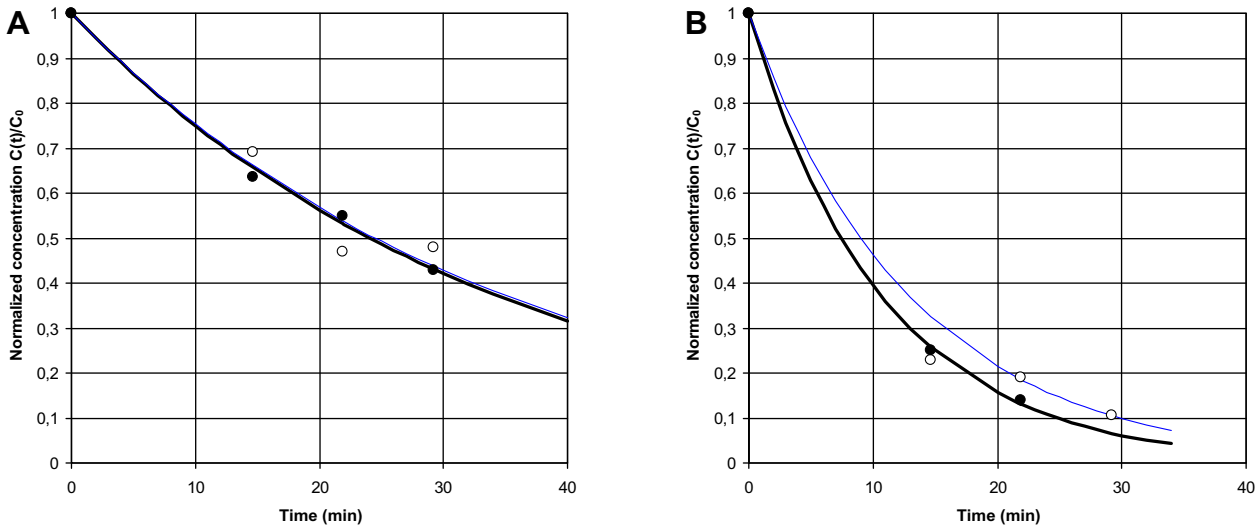

Fig. 8 – Ozonation: target pollutants normalized concentration *vs.* reaction time. White marks and fine line: BPA; black marks and bold line: NP. Actual ozone dosages: (A) = 8 mg/L; (B) = 11 mg/L. C_0 = influent concentration.

ozone dosage, 30 min contact time), notwithstanding the initial concentration ($2.4\cdot10^5$–$1.0\cdot10^6$ MPN/100 mL total coliforms, $4.3\cdot10^4$–$1.9\cdot10^5$ MPN/100 mL *E. coli*).

Influent trace pollutants concentrations were in the range 0.14–0.30 μg/L and 0.20–0.43 μg/L for NP and BPA, respectively, while both NP1EO and NP2EO were below 0.20 μg/L. Time profiles of NP and BPA normalized concentration are shown in Fig. 8; NP1EO and NP2EO are omitted since they were below detection limits. Assuming first order kinetics (and under the hypothesis of plug-flow reactor), it was possible to estimate reaction rate constants, which resulted, for both pollutants, in the range 0.028–0.093 min^{-1} depending on ozone dosage.

3.4.2. *Biological analyses*

The influence of O_3 dosage on estrogenic activity abatement is shown in Fig. 9 (average values). Error bars indicate results obtained during different experiments (i.e., reaction time conditions).

Chemical oxidation was able to reduce estrogenicity of wastewater remarkably. Nevertheless, while a higher O_3 dosage led to an appreciable improvement of EDC (NP + BPA) removal,

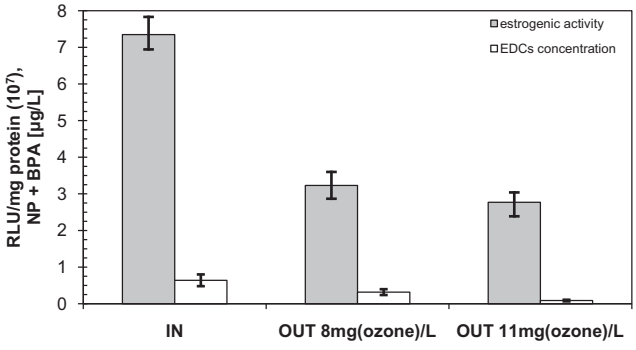

Fig. 9 – Ozonation: comparison between the estrogenic activity and EDC concentration (NP + BPA) as a function of O_3 dosage. Error bars indicate results obtained during different experiments (i.e., reaction time conditions).

only a slight additional reduction of hormonal activity was achieved. This may be due to the persistence of endocrine disruptors (e.g., including natural hormones) or the formation of active by-products, as recently found by other authors (Huber et al., 2004; Bila et al., 2007).

4. Conclusions

In this work, the fate of selected trace pollutants (NP, NP1EO, NP2EO and BPA) in two full scale WWTPs was investigated.

Monitoring campaigns showed that the contribution of primary settling in the removal of studied pollutants was negligible, their content in primary sludge being quite low (<10 mg/kgTSS). Biodegraded fractions ranged from 62.0% (NP2EO, Verona plant) to 94.5% (NP1EO, Brescia plant); final effluent concentrations were always <1 μg/L and excess sludge concentrations ≤5 mg/kgTSS for all analytes. Although the WWTPs considered have different process schemes (CAS and MBR, respectively) similar performances were observed. In fact this finding was expected based on the literature, since the most influential process parameters (sludge age and temperature) were always within the optimal range for EDC biodegradation.

On the contrary, biological assays showed that MBR was more efficient in estrogenicity reduction: this is a very important finding of this research, which would not have been highlighted if only chemical analysis had been performed.

As far as tertiary ozonation is concerned, chemical oxidation of trace pollutants was described by first order kinetics, rate constants being dependent on reagent dosage: for instance, a 90% removal of BPA and NP could be achieved either after 80 min at 8 mgO_3/L, or 27 min at 11 mgO_3/L.

Biological analyses confirmed the beneficial effect of ozonation on the reduction of estrogenicity of CAS effluent. However, unlike analytes, estrogenic activity abatement was not significantly affected by ozone dosage.

In summary, CAS treatment enabled a satisfactory reduction of EDCs and estrogenicity, thanks to adequate process

conditions; a further decrease of biological activity was achieved by means of MBR and ozonation, but the latter, at the same time, yielded an additional reduction in pollutants.

Finally, the efficacy of an integrated (chemical + biological) approach in evaluating performances of wastewater treatment processes was demonstrated: bioassays account for synergistic effects of dozens of pollutants, the simultaneous determination of which might be actually unfeasible.

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