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Removal of nalidixic acid and its degradation products by an integrated MBR-ozonation system

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ABSTRACT

Chemical–biological degradation of a widely spread antibacterial (nalidixic acid) was successfully obtained by an integrated membrane bioreactor (MBR)-ozonation process. The composition of the treated solution simulated the wastewater from the production of the target pharmaceutical, featuring high salinity and a relevant concentration of sodium acetate. Aim of treatment integration was to exploit the synergistic effects of chemical oxidation and bioprocesses, by adopting the latter to remove most of the COD and the ozonation biodegradable products. Integration was achieved by placing ozonation in the recirculation stream of the bioreactor effluent. The recirculation flow rate was three-fold the MBR feed, and the performance of the integrated system was compared to the standard polishing configuration (single ozonation step after the MBR). Results showed that the introduction of the ozonation step did not cause relevant drawbacks to both biological and filtration processes. nalidixic acid passed undegraded through the MBR and was completely removed in the ozonation step. Complete degradation of most of the detected ozonation products was better achieved with the integrated MBR-ozonation process than using the sequential treatment configuration, i.e. ozone polishing after MBR, given the same ozone dosage.

1. Introduction

Wastewater from the pharmaceutical industry may contain chemicals that are unbiodegradable and/or potentially harmful for microbial consortia and environmental systems. Although these streams are usually treated separately (in-house), the produced effluents may still contain relatively large amounts of refractory compounds that would affect the composition of municipal wastewater if discharged into the sewer or contaminate directly the aquatic environment if discharged without further treatments. To limit these effects, specific treatment approaches are needed for the removal of pharmaceuticals from industrial wastewater. Conventional biological processes often do not provide satisfactory results for the treatment of wastewater from the pharmaceutical industry, due to the above mentioned scarce biodegradability or toxicity. An alternative treatment approach is the combination of advanced oxidation processes (AOPs) and biological treatments [1]. AOPs are well known for their capacity of partially or completely mineralising organic contaminants, although their practical large scale applications are limited by their high costs, mainly due to energy consumption (radiation, ozone, etc.) and the need of chemical reagents (catalysts and oxidizers) [2]. The combination of chemical and biological oxidation processes can be beneficial if the synergistic effects of these two types of treatment are exploited. Conventional applications of chemical oxidation processes as pre-treatments or final polishing are usually non-optimal from the point of view of process costs. Indeed the achievement of good treatment performances with these treatment trains often requires the adoption of highly intensive chemical oxidation steps [3–8].

A full integration of biological treatment and chemical oxidation may allow for both the limitation of the concentration of organic compounds undergoing chemical oxidation, and the biological removal of the biodegradable oxidation products. Biological and chemical treatment integration can also be considered a safer "multiple barrier approach", where the biological process limits the load of incoming compounds reaching the chemical oxidation process by removing the biodegradable fraction, and eliminates the biodegradable products resulting from this step. This approach had limited applications so far, and its potential is probably not completely exploited yet [9].

In the present study, the integrated approach described above was adopted, and ozonation was placed in the effluent recirculation flow of a lab-scale membrane bioreactor (MBR) treating a solution containing a commercial antibacterial compound (nalidixic acid) and simulating the wastewater produced in the drug's production process. The same target compound was recently adopted

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Table 1
Composition of the synthetic wastewater.

Parameter	Unit	Amount
pH		7.5
Conductivity	$mS\,cm^{-1}$	9.6
DOC	$mg\,L^{-1}$	1300
COD	$mg\,L^{-1}$	2900
TN	$mg_N\,L^{-1}$	78.5
NH_4^+	$mg_N\,L^{-1}$	72.4
PO_4^{3-}	$mg_p\,L^{-1}$	14.6
Sodium acetate	$mg\,L^{-1}$	3800
Nalidixic acid	$mg\,L^{-1}$	48
NaCl	$mg\,L^{-1}$	4200

for testing different removal techniques [10]. In the experimental campaign presented here, MBR was selected among the different available bioprocess configurations in order to exploit its flexibility and selectivity towards specialized biomass. Preliminary experiments performed on a less recalcitrant wastewater stream had shown this approach to be promising [8]. The nalidixic acid (trade names Nevigramon, Neggram, Wintomylon and WIN-18320) is an effective antibacterial used in treating urinary tract infections caused, for example, by *Escherichia coli*, Proteus, Shigella, Enterobacter, and Klebsiella. In lower concentrations it acts as bacteriostatic, inhibiting bacterial growth and reproduction, while in higher concentrations it displays bactericidal effects.

Aim of this investigation is to evaluate the advantages of an integrated approach for removal of the target compound and minimization of the oxidation products in the effluent, by means of their partial chemical oxidation and improved biodegradation in the MBR. MBR/ozonation was chosen as a testing technology for the potential synergies existing between chemical and biological processes. The experimental work was focused on the evaluation of the different contributions to the removal of pollutants, on the investigation of ozonation products formation and their biodegradation during the MBR treatment, and on the possible effects of chemical oxidation products on the biological system. In order to monitor these processes more accurately, a synthetic solution simulating a real wastewater from industrial production was adopted. In particular, the large amount of sodium acetate resulting from the antibiotic's production process and the high salinity due to neutralization of the acidic wastewater were reproduced.

2. Materials and methods

2.1. Chemicals

Nalidixic acid analytical standard was obtained from Aldrich while the technical grade powder of nalidixic acid was supplied from Austep s.r.l. (Italy). Solvents used for high pressure liquid chromatography (HPLC) were HPLC–MS grade and purchased from Sigma–Aldrich. Benzotriazole (purity >99%) was purchased from Aldrich. All other reagents were analytical grade and purchased from VWR or Carlo Erba (Italy).

The composition of the simulated wastewater is reported in Table 1. Nitrogen and phosphorus were dosed according to the expected requirement for biomass growth under the adopted conditions. All reagents were diluted in tap water, which was the only source of micro-nutrients.

2.2. MBR, ozonation and integrated MBR-ozonation systems

Except for the operating volume of the membrane bioreactor, the bench scale plant was similar to the one described in a previous article [8]. In the present experiment, a membrane bioreactor having 6 L operating volume was set-up and fed at a rate of $1.6\,L\,d^{-1}$. A permeate recirculation rate of 3:1 (recirculation flowrate of $4.8\,L\,d^{-1}$) was maintained since the start-up of the plant in order to obtain similar operating conditions for the MBR-alone configuration and for the integrated system. The resulting hydraulic retention time (HRT) of the system was 3.75 days, and the average retention time in the bioreactor also depended on the recirculation rate. The MBR was equipped with a hollow fibre lab-scale membrane module (ZeeWeed 1, GE Water), having a filtration surface of $0.047\,m^2$ and operated out-in, with a membrane flux of $5.7\,L\,m^{-2}\,h^{-1}$. Intermittent cycles of suction and relaxation were alternated (suction: 165 min; relaxation: 15 min) to limit clogging and biofouling of the membrane surface. Moreover, the transmembrane pressure (TMP) was continuously measured in order to monitor the filtration performance, and the membrane module was rinsed with pressurized tap water when the TMP tended to approach a limit value of 500 mbar, as described elsewhere [11]. Start-up of the biological reactor was shortened by using activated sludge from a local wastewater treatment plant as inoculum. The system was operated adopting continuous aeration aimed at the development of aerobic microbial consortia, and a sludge retention time of 30 d. The latter was maintained by regularly withdrawing the appropriate volume of sludge through a sampling port (e.g. daily withdrawal of 1/30 of the mixed liquor volume results in 30 day SRT).

A 7 L ozonation reactor was used and fed with ozonated air (ozone concentration, $11.0\,g\,m^{-3}$; flow, $1.0\,L\,min^{-1}$) produced with a Fisher 502 ozonator (Germany). Ozone concentration was measured in water as well as in both the inlet- and off-gas by an amperometric ozone probe (Orbisphere). The ozone demand was calculated from the ozone off-gas concentration profile by integrating the obtained curve and subtracting the residual ozone in water. Samples were withdrawn from the reactor at scheduled intervals and the residual ozone was stripped by purging with air.

As drafted in Fig. 1a and b, the integrated MBR-ozonation system was operated according to the operational procedure validated in a previous experiment [8]. The synthetic solution was initially fed to the MBR-alone until the steady state was reached and maintained for more than 1 SRT (Fig. 1a). During this period the effluent was collected and used for testing the standard

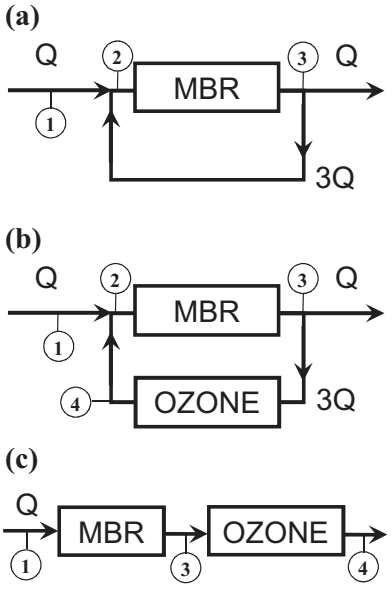

Fig. 1. Schematic of the wastewater treatment performed by (a) MBR-only (first phase), (b) MBR-ozonation (second phase) and (c) standard polishing configuration (reference for the integrated system). Selected sampling points: 1 = raw wastewater; 2 = MBR influent; 3 = MBR effluent = ozonation influent; 4 = ozonation effluent.

Table 2
Main characteristics of the MBR biomass before and after integration with ozonation, and performance comparison of the two systems in terms of COD and NDX removal.

Parameter	Unit	MBR alone	MBR-O_3	Variation
MLSS	g TSS L^{-1}	3.3 ± 0.5	3.2 ± 0.3	n.s.
MLVSS/MLSS	%	80 ± 3	79 ± 2	n.s.
Y_{obs}	g VSS g $COD_{removed}$ $^{-1}$	0.12 ± 0.02	0.11 ± 0.01	n.s.
CST	S	8.0 ± 1.1	6.6 ± 0.7	−18%
COD effluent	mg L^{-1}	89 ± 7	34 ± 11	−62%
COD removal	%	89 ± 4	95 ± 2	+7%
COD after ozonation	mg L^{-1}	–	29 ± 17	–
NDX effluent	mg L^{-1}	48 ± 2	12.5 ± 1.1	−74%
NDX after ozonation	mg L^{-1}	–	0.5 ± 0.3	–

polishing configuration (single ozonation step after the MBR, Fig. 1c). During polishing experiments prolonged ozonation was carried out, and samples were withdrawn at scheduled ozone dosages and analysed for residual concentration of nalidixic acid and ozonation products, in order to plot the formation/degradation profiles of these compounds. Then the integrated configuration was tested by matching the continuous operation of the MBR with a batch, off-line ozonation process. So the MBR effluent was collected daily, ozonated in a batch reactor and then returned to the MBR together with the feed. A daily volume of 4.8 L of MBR effluent was subjected to ozonation using an ozone dosage of 103 mg L^{-1} and then re-circulated. Daily samples of raw wastewater, MBR influent, MBR effluent, and AOP effluent when present were analysed to monitor the system's performance.

2.3. Analytical determinations

Dissolved organic carbon (DOC), total nitrogen (TN), COD and total phosphorus (TP) were measured according to previously described procedures [8,12]. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to standard methods [13]. Dissolved oxygen and conductivity were measured using an Oxi 340 Oximeter (WTW, Germany) and a LF 330 conductivity meter (WTW, D-Weilheim).

Biomass samples from the membrane bioreactor were regularly monitored both in terms of filterability and biological activity. The capillary suction time (CST), measuring the water holding capacity of sludge, was used as an indirect filterability index, as these two parameter were shown to correlate well in membrane systems [11]. A Multi-CST 319 with CST standard papers (Triton Electronics, England) was adopted, and the time required by the water borne by the sludge samples to close a standard electrical circuit was measured. Respirometric tests adopting a discontinuous static gas–static liquid system were regularly done to assess the biomass activity. A small air-tight respiration cell (25 mL) was cyclically filled with activated sludge from a 1 L aerated vessel where the sludge sample had been maintained overnight to obtain endogenous respiration conditions. The oxygen consumption was then measured in the liquid phase of the cell, and in order to evaluate the respiration rates of the different bacterial groups, appropriate substrates were added to the aerated vessel [14].

The determination of residual nalidixic acid concentrations and the detection of ozonation products were performed by HPLC/MS and HPLC/MS–MS using an acquity chromatographic system (Waters) interfaced to an API 5000 mass spectrometer (AB Sciex) by means of a turbo ion spray interface. Samples were properly diluted (1/50 or 1/2 for nalidixic acid and by-product determinations, respectively) and added with benzotriazole (final concentration of 0.2 mg L^{-1}) as an internal standard (IS) before injection in the HPLC/MS system. The adoption of an IS was aimed at compensating any instrumental loss of performance occurring in presence of complex or highly saline solutions, due to ion suppression and/or background increase. Preliminary tests showed similar matrix effect for nalidixic acid and benzotriazole, both in synthetic saline solutions and in MBR effluent, leading to the choice of benzotriazole as IS. Therefore, peak areas measured for the target compound and the ozonation products were normalized to the peak area of the internal standard. Sample volumes of 5 μL were injected by the acquity autosampler equipped with a Rheodyne valve and a 10 μL loop, and eluted at 0.35 mL min^{-1} through a 150 mm × 2.1 mm Ascentis Express C 18 column (Supelco) using an isocratic and a gradient method for residual nalidixic acid and by-product determinations, respectively. The isocratic method employed an eluent containing water + 0.1 formic acid (solvent A) and methanol + 0.1 formic acid (solvent B) at a ratio of 1:1. The mass analysis was performed in positive ion multiple reaction monitoring mode using three characteristic fragment ions of nalidixic acid having m/z 215.1, 204.9, 186.8 while for IS the fragment at m/z 65.1 was monitored. The gradient method was carried out varying the composition of eluent from 95/5 of solvent A/B to 5/95 within 9 min by a concave gradient, then hold for 8 min. Experimental conditions of the MS interface were as follows (positive ion): needle voltage 3000 V; declustering potential 80 V; range of mass-to-charge ratio (m/z) 50-500; scan time 0.6 s; nebulizer gas flow (air) 1.5 L min^{-1}; curtain gas flow (nitrogen) 1 L min^{-1}; auxiliary gas flow (air) delivered by a turbo heated probe 6 L min^{-1} at 450 °C. Ozonation products were first detected using HPLC/MS running in full scan mode. Once the mass spectra data were obtained, in order to obtain an analytical method as specific and sensitive as possible for monitoring the organics during the MBR and MBR-ozonation operation, a HPLC/MS–MS procedure including multiple reaction monitoring was optimized with information obtained from product ion scan experiments.

3. Results and discussion

3.1. Preliminary tests

3.1.1. MBR-alone configuration

COD removal rates in the range of 85–95% were obtained during the test with the MBR-alone configuration, and the influent 2900 mg COD L^{-1} was mostly degraded to 90 mg COD L^{-1} on average (Table 2). However the removal of nalidixic acid was practically absent, confirming its very low biodegradability [12]. Despite the saline conditions and the presence of the antibacterial compound, the load of readily biodegradable substances (0.77 gCOD $L_{reactor}$ $^{-1}$ d^{-1} mostly due to acetate) resulted in a biomass growth of 0.12 gVSS $gCOD_{removed}$ $^{-1}$, comparable to what observed in similar MBR fed on municipal wastewater (0.11–0.15 gVSS $gCOD_{removed}$ $^{-1}$ for SRT of 30 d [15,16]), and normal synthetic feed (0.15–0.18 gVSS $gCOD_{removed}$ $^{-1}$ [17,18]). The biomass concentration reached 3.3 ± 0.5 gTSS $L_{reactor}$ $^{-1}$ (VSS/TSS 80 ± 3%), which is lower than typical values for municipal MBR but consistent with the high HRT adopted for this industrial application.

Respirometric tests were performed for monitoring the endogenous and maximum activities of heterotrophic and

autotrophic biomass. These resulted in average specific activities of 4.3 ± 1.4 mg O_2 mg VSS^{-1} h^{-1} for endogenous respiration, and 43 ± 14 and 6.3 ± 1.7 mg O_2 mg VSS^{-1} h^{-1} for maximum respiration of heterotrophic and autotrophic biomass, respectively. The endogenous respiration was in the same range as for similar MBR fed on municipal wastewater [11], confirming no significant changes in biomass growth and decay rates. In comparison with similar municipal MBR, the maximum respiration rates was found to be higher for heterotrophic biomass, suggesting a good adaptation to the presence of high concentration of easily biodegradable substrate (sodium acetate), despite the negative influence of salinity and the nalidixic acid. On the other hand, lower autotrophic respiration suggests that this biomass was more sensitive to salinity and presence of the nalidixic acid.

The mixed liquor filterability, measured as CST, tended to improve over time during MBR start-up and MBR-alone operation (data not shown). Filterability is negatively affected by the production of membrane fouling compounds (colloids, extracellular polymers, etc.), and these are produced by bacteria as a result of environmental stressing conditions, such as high salinity or presence of toxic or inhibiting compounds such as the nalidixic acid. Therefore, the observed filterability improvement and the similar values of biomass growth and removal performances with those observed with municipal wastewater suggest progressive acclimation of the MBR biomass to these adverse conditions.

3.1.2. Preliminary ozonation experiments

Preliminary ozonation experiments were performed with both a solution of nalidixic acid in distilled water and the effluent of the MBR-alone configuration. These tests were aimed at investigating the degradation of the target compound and the formation of ozonation products. Additionally, these tests allowed to check for possible effects of the saline matrix in reducing the degradation of nalidixic acid due to scavenging of dissolved ozone and hydroxyl radicals formed by ozone decomposition in water. Results showed that nalidixic acid was completely removed within 30 min of ozonation time, independent of the saline matrix. This corresponds to an ozone dosage of 68.7 mg L^{-1} and a pseudo-first order constant of 0.045 min^{-1}. On the basis of these results and considering the recirculation rate in the integrated process configuration (Fig. 1b), the ozone dosage adopted in the integrated MBR-O_3 was 103 mg $L_{influent}{}^{-1}$, corresponding to 0.5 mg O_3 mg DOC^{-1}, also in order to avoid over-dosing. This value was expected to provide 80% primary degradation of nalidixic acid under steady state conditions, because in this case dilution with the recirculated stream results in a lower concentration of nalidixic acid entering the ozonation step with respect to the one used in the batch tests.

Table S1 reports the molecular weights (MW) and chromatographic retention times of 46 compounds detected in the ozonated MBR effluent. All these compounds were assumed to derive from nalidixic acid degradation because (i) nalidixic acid was the main organic present in the MBR effluent and the remaining DOC was related to residual acetate; (ii) the simulated wastewater was prepared with acetate and nalidixic acid as organic constituents; (iii) the same 46 compounds were identified during ozonation of a solution of nalidixic acid in distilled water. The detected ozonation products were found to have molecular weights ranging between 130 and 316 amu and retention times lower than the parent compound. This suggest that all ozonation products have a higher polarity than the nalidixic acid as a consequence of multiple hydroxylation reactions and molecule breakdown deriving from carbon–carbon oxidation caused by the ozonation treatment.

3.2. Integrated MBR-ozonation system

3.2.1. Performance in terms of process parameters

When the ozone step was placed in the recirculation stream of the MBR, the integrated system was operated adopting an ozone dosage of 103 mg L^{-1} (corresponding to 0.5 mg O_3 mg DOC^{-1}). With this configuration, besides the high COD removal, also the nalidixic acid concentration in the final effluent of the integrated process greatly decreased. As expected, in terms of total COD removal little improvements were achieved by the integrated process with respect to the performance of the MBR-alone (Table 2). This was due to the limited contribution provided by the nalidixic acid to the total COD, mainly composed of biodegradable carbon. The average COD of the MBR effluent decreased from 89 ± 7 mg L^{-1} to 34 ± 11 mg L^{-1} after 10 days from the introduction of the ozonation step. Therefore in addition to the high COD elimination obtained by the MBR treatment, an additional 55 mg COD L^{-1} could be removed due to the integrated configuration, and this included removal of the target contaminant. The contribution of the ozonation step to complete COD removal was very limited, confirming that this process was mostly focused on partial oxidation of the nalidixic acid into other organics.

Considering the theoretical COD of the nalidixic acid (1.6535 mg COD/mg NDX), 79.4 mg L^{-1} of COD can be associated to the influent nalidixic acid concentration (48 mg L^{-1}). After the effluent ozonation most of this COD was available as oxidation products and 75% of it was recirculated back to the MBR, i.e. about 60 mg L^{-1} of COD. The latter value is in the same range of the $\Delta COD_{effluent}$ suggesting that the MBR biomass was able to remove most of the oxidation products produced with the ozonation.

Respirometric tests showed no significant modification of the specific bacterial activities as a result of the introduction of the ozonation step (data not shown). Similarly, the variation of the average mixed liquor filterability in the MBR was not significant (Table 2), although a slight improvement could be associated with the introduction of ozonation in the recirculation stream.

3.2.2. Removal of nalidixic acid and minimization of organic products

The concentration profiles and removal percentages of nalidixic acid during both the MBR-alone and the MBR-ozonation phases are depicted in Fig. 2. As shown in the figure, the nalidixic acid removal efficiency during the first phase (biological treatment alone) was negligible, i.e. the compound was not biodegradable under the tested conditions. In the subsequent second phase, the ozonation step placed in the MBR recirculation stream completely removed the nalidixic acid. The integrated MBR-ozonation treatment was operated for about 70 days. Ozonation aimed at improving biodegradability of the nalidixic acid, and the biomass had already been acclimated to the latter compound during the previous period (MBR-alone). Therefore quick adaptation to the ozonation products was expected, since the antibacterial compound was mostly transformed into simpler compounds. The results have confirmed these expectations, and the system's performance with respect to the target compound was observed to be steady after a few days from the start-up of the integrated configuration (Fig. 2). Moreover, during integrated MBR-ozonation although the nalidixic acid concentration reaching the bioreactor decreased from 48 to 12.5 mg L^{-1} with respect to the MBR-alone configuration due to recirculation of the ozonated stream, the latter concentration remained undegraded after the bioprocess, confirming the persistent nature of the target compound.

The ozone consumed for oxidation of the MBR effluent was also monitored during each cycle, showing that when the ozonation was placed in the recirculation stream of the MBR the amount of ozone consumed decreased from 31.5 to 25 mg $L^{-1}{}_{ozonation\ influent}$

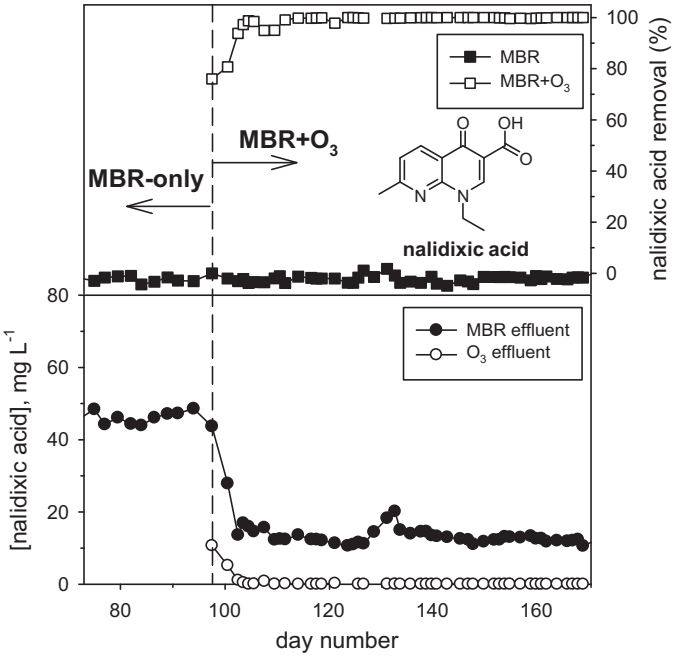

Fig. 2. Nalidixic acid removal during MBR-only and integrated MBR-ozone treatment of the synthetic wastewater, and concentration of the target compound in the effluents.

(Fig. S1). This is consistent with the fact that the concentration of nalidixic acid recirculated through the system tended to decrease as the steady state was approached. Accordingly, the selected ozone dosage allowed 80% oxidation of nalidixic acid at the beginning of the second phase but, after few days, this percentage raised to 100% when steady state conditions were reached (Fig. 2).

The formation/degradation of ozonation products was monitored and compared during standard polishing and integrated MBR-ozonation (see set-ups in Fig. 1b and c). This comparison was aimed at identifying the best configuration in terms of minimization of organic compounds in the effluent under the same ozone dosage of 103 mg $L_{influent}^{-1}$. The oxidation products were identified based on retention time and molecular weight (Table S1), as the chemical structure identification by means of accurate mass measurement was not relevant to the aim of the study. The potential for effluent organics minimization was evaluated in terms of the ratio $R = abundance_{integrate\ system}/abundance_{polishing\ configuration}$, where the abundance is the normalized peak area measured for each ozonation product.

For 3 out of the 46 detected ozonation products (*n*. 14, 21, 39) the integrated system showed a much better removal than the standard polishing configuration (i.e. $R > 3$). Fig. 3a shows the formation/degradation profiles of by-product *n*. 14, and suggests that by-product *n*. 14 is formed during ozonation but effectively removed biologically when the ozonation effluent is recirculated back to the MBR. On the contrary, application of the polishing treatment scheme with the same ozone dosage of 103 mg $L_{influent}^{-1}$ resulted in the detection of ozonation product *n*. 14 in the effluent. Ozonation products *n*. 21 and 39 showed a similar behaviour, and the related results are reported in Supporting Information (Fig. S2).

For 26 out of 46 ozonation products, the integrated system showed better performances than polishing (i.e. $1.5 < R < 3$). As an example, the formation/degradation profiles of ozonation product *n*. 13, representing this group, are reported in Fig. 3b. Results for ozonation products *n*. 6 and 24 from this same group are reported in Supporting Information (Fig. S3). In general, for the ozonation products of this second group a lower percentage of biological removal, when ozonation effluent is recirculated back to the MBR, was also observed. Furthermore, a comparison between the ozonation polishing profiles of products *n*. 14 and 13 (Fig. 3) shows that for the

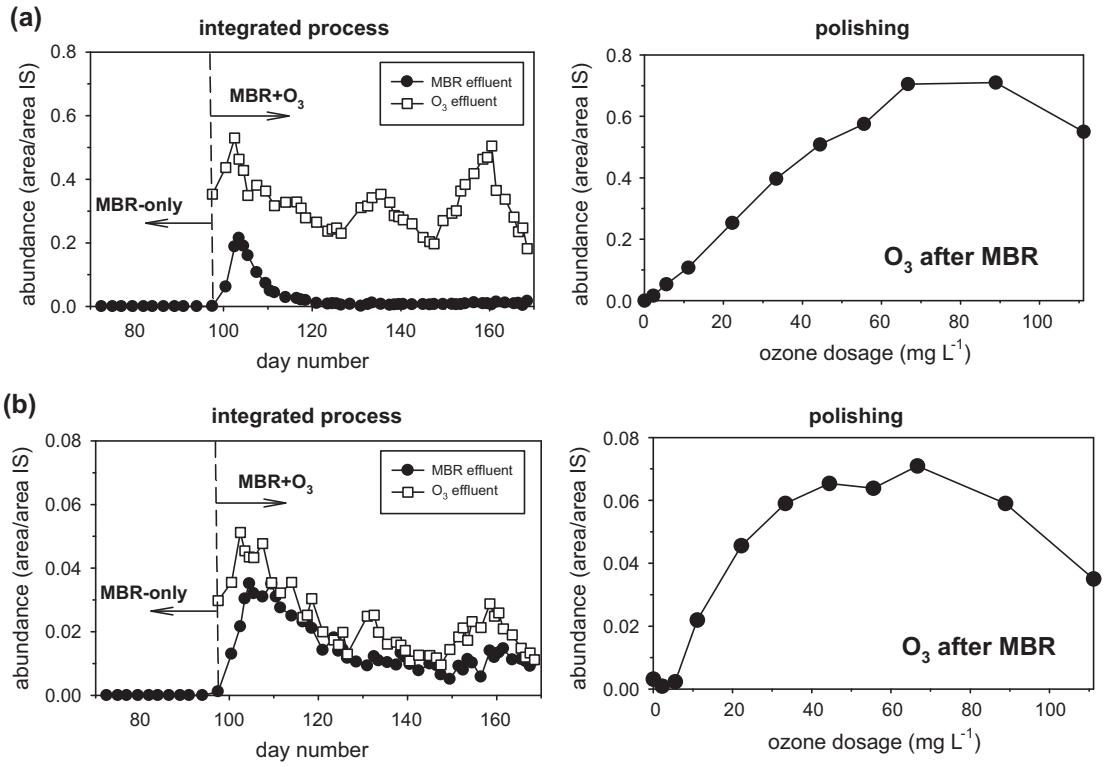

Fig. 3. Normalized abundance (to internal standard, IS) of by-products *n*. 14 (a) and *n*. 13 (b) in the effluents during treatment of wastewater with integrated MBR-ozone treatment (left) and decay profiles of the same compounds during prolonged ozone polishing (right).

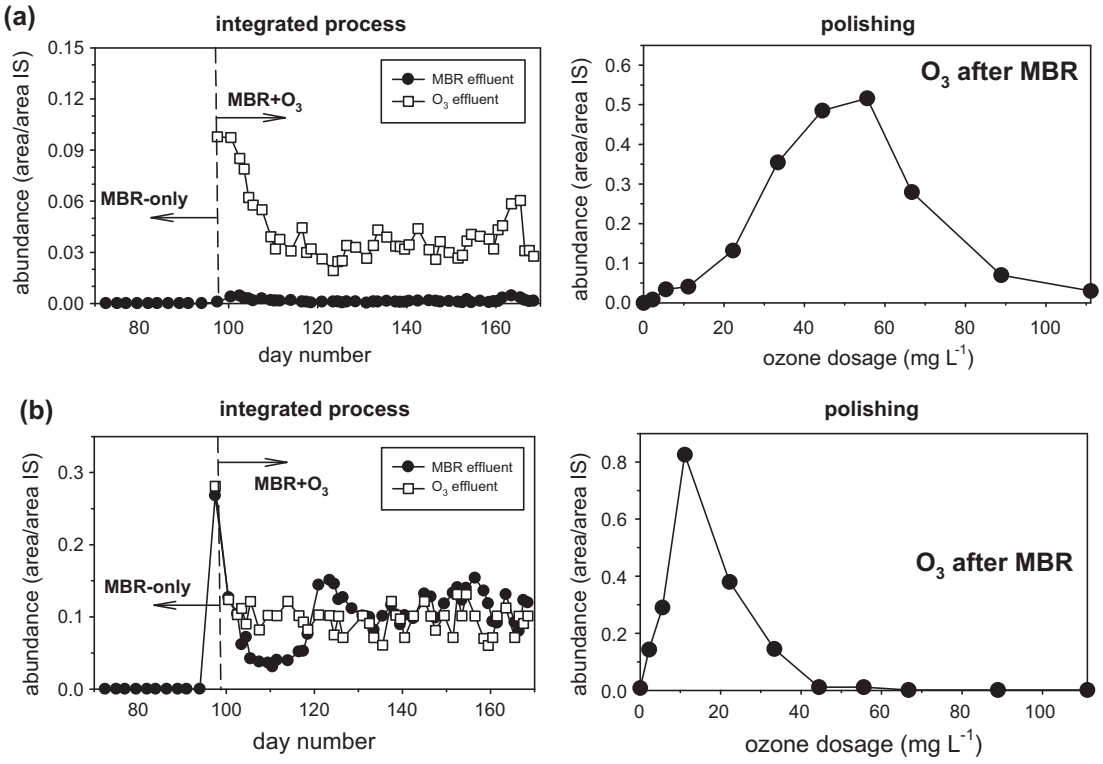

Fig. 4. Normalized abundance (to internal standard, IS) of by-products *n.* 2 (a) and *n.* 44 (b) in the effluents during treatment of wastewater with integrated MBR-ozone treatment (left) and decay profiles of the same compounds during prolonged ozone polishing (right).

latter the maximum formation is reached at a lower ozone dosage than for the former.

For 15 ozonation products the integrated system and standard polishing configuration lead to similar results in term of compounds minimization (i.e. $0.5 < R < 1.5$). As an example for this third group, the formation/degradation profiles of compound *n.* 2 are reported in Fig. 4a. The profiles of compound *n.* 2 are similar to those of other groups, however, the polishing profile shows faster formation/degradation kinetics. This leads to shift the maximum of formation at a lower ozone dosage. Fig. S4 in the Supporting Information reports results of compounds *n.* 23 and 26 from the same group.

Finally, for only 2 compounds (*n.* 44, 46) the integrated system showed a worse removal than the standard polishing configuration ($R < 0.5$). The formation/degradation profiles of compound *n.* 44 are displayed in Fig. 4b, showing that the worse performance of the integrated MBR-ozonation system is due to the much faster kinetics of degradation than formation. Therefore, with an ozone dosage of 103 mg $L_{influent}^{-1}$ an almost complete removal of the compound was obtained in the polishing configuration. The results related to the other compound of this group are reported in Supporting Information (Fig. S5).

Overall, the results showed that for 29 out of 46 ozonation products the performance of the integrated MBR-ozonation system was superior to the standard polishing configuration in terms of their minimization. This suggest that the integrated system allows to better minimize the presence of organic oxidation products in the final effluent, independent of their structure and chemical/biological characteristics.

4. Conclusions

A synthetic saline solution simulating the wastewater resulting from the production of a commercial antibacterial compound was used to test the suitability and effectiveness of an integrated treatment configuration including a membrane bioreactor whose effluent was ozonated and recirculated back to the inlet. Main challenges were related to the intrinsic nature of the antibiotic towards the bacterial consortia, the high salinity of the adopted solution, the comparison of the integrated process with the ozone post-treatment in terms of main compound and ozonation products removal.

Extensive long term monitoring of the proposed system included the determination of the main process parameters, the biomass characterization through different approaches, and the evaluation of the fate of a number of degradation products.

The main results of the experimental activity can be summarized as follows:

- Despite the inherent characteristics of the synthetic solution, the MBR biomass was acclimated and its growth, activity and fouling potential appeared to be scarcely affected by the presence of the antibacterial compound and the extremely high salinity
- The introduction of the ozonation step did not show relevant drawbacks on the biological and filtration processes and resulted in improved effluent quality, with COD reduction possibly related to the biological degradation of the ozonation products of the nalidixic acid
- Comparison between the compounds formed/degraded during the integrated MBR-ozonation process and those resulting from the conventional ozone post-treatment (polishing) showed that in general the former is to be preferred, especially in terms of ozonation products removal effectiveness and required ozone doses.

In the present investigation, TMP monitoring did not show relevant changes in the mixed liquor's fouling propensity across the

two experimental periods (before and during ozonation). Moreover the sludge filterability index CST tended to improve moderately with ozonation. However, further developments of the proposed approach should investigate possible changes in the structure and composition of the membrane fouling agents caused by process modifications occurring with the introduction of ozonation in the recirculation stream of the MBR. Deeper evaluation and specific analyses of potential foulants (e.g. EPS) and sludge physical properties (e.g. viscosity) could provide interesting insights and complement the results of the present campaign, mostly aimed at testing the integrated system towards the removal of the target compound and its by-products.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.11.072.

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