Chemical Engineering Journal 283 (2016) 740–749

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Ozonation as pre-treatment of activated sludge process of a wastewater containing benzalkonium chloride and NiO nanoparticles

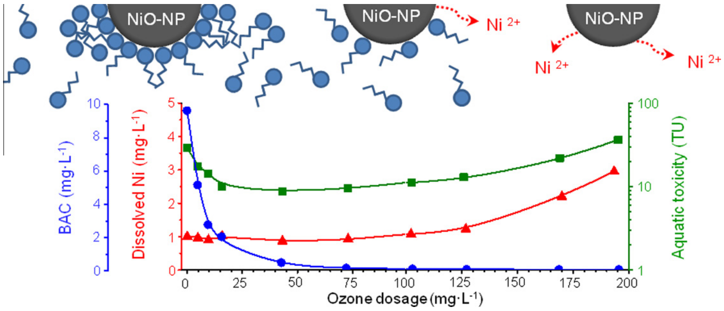
Jose B. Carbajo [a], Alice L. Petre [a,b], Roberto Rosal [a,b], Antonio Berná [b], Pedro Letón [a,b], Eloy García-Calvo [a,b], Jose A. Perdigón-Melón [a,b,*]

[a] *Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcalá, E-28871 Alcalá de Henares, Madrid, Spain*
[b] *Advanced Study Institute of Madrid, IMDEA-Agua, Parque Científico Tecnológico, E-28805 Alcalá de Henares, Madrid, Spain*

HIGHLIGHTS

- BAC was significantly removed by ozonation.
- BAC degradation pathway and ozone dose were influenced by NiO-NPs.
- BAC abatement was influence by water matrix.
- BAC toxicity was completely removed by ozonation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 30 July 2015
Accepted 1 August 2015
Available online 6 August 2015

Keywords:
Continuous ozonation
Quaternary ammonium compounds
Nanoparticles
Transformation products
Water matrix
Aquatic toxicity

ABSTRACT

The continuous ozonation of benzalkonium chloride (BAC) and nickel oxide nanoparticles (NiO-NPs) has been performed in a synthetic water matrix and in a sewage treatment plant influent. This study aims to assess ozonation as pre-treatment of an activated sludge process, with emphasis on the toxicity of treated water. BAC was completely removed in synthetic matrix independently of the presence of NiO-NPs, although the ozone dose was influenced by NPs co-occurrence. The extent of mineralization was limited and a number of intermediate transformation products (TPs) appeared, twelve of which could be identified. The degradation pathway was shown to initiate both on the hydrophobic (alkyl chain) and hydrophilic (benzyl and ammonium moiety) region of the surfactant. The reactions on the hydrophilic region were affected by the presence of NiO-NPs as a consequence of the adsorption of BAC onto NP surface via the aromatic group. Water matrix strongly influenced BAC depletion. The aquatic toxicity of treated mixtures was assessed using a biotest battery composed of single species (the bacteria *Vibrio fischeri* and *Pseudomonas putida* and the protozoan *Tetrahymena thermophila*), as well as a whole biological community assay using activated sludge. Although, BAC showed considerable aquatic toxicity in all bioassays, ozonation decreased the toxic effects of treated water samples at ozone dosages below those required for total BAC depletion. Further treatment would not be justified, neither for a significant increase in BAC abatement nor concerning the toxicity of treated wastewater, which increased as a result of nickel leaching from the NPs.

---

* Corresponding author at: Universidad de Alcalá, Campus Externo, Carretera Madrid Barcelona km 33.600, Edificio Polivalente Despacho 2D23, C.P. 28871, Spain. Tel.: +34 918 856 393; fax: +34 918 855 088.

E-mail address: ja.perdigon@uah.es (J.A. Perdigón-Melón).

http://dx.doi.org/10.1016/j.cej.2015.08.001
1385-8947/

1. Introduction

Quaternary ammonium compounds (QACs) are an important class of industrial chemicals extensively used in domestic and industrial applications such as detergents, emulsifiers, fabric softeners, disinfectants, corrosion inhibitors and processing aids. As a result, about 75% of QAC consumed end up in wastewater treatment systems [1]. In fact, their concentration in hospital wastewater, sewage treatment plant (STP) influents and effluents, and sewage sludge has been reported as 0.05–6.03 mg L^{-1}, 25–300 μg L^{-1}, 0.3–3.6 μg L^{-1} and 22–103 mg kg^{-1}, respectively [2–5]. Because of their chemical properties, QACs rapidly and strongly sorb on suspended solids [1]. Sorption on (bio)solids combined with the persistence of QAC, result in their accumulation on the biomass and their transfer to anaerobic digesters as part of the primary and waste activated sludge. Among QACs, benzalkonium chloride (BAC) has been shown to pose a potential risk for the activated sludge [6]. It is toxic to aquatic organisms at environmental relevant concentration and is classified as "very toxic to aquatic life" according to Regulation (EC) No. 1272/2008. Furthermore, it has been reported that the widespread use of biocides such as BAC could select for antibiotic-resistant bacteria [7].

On the other hand, the increasing use of engineered nanoparticles (NPs) in industrial and household applications will very likely lead to the release of such materials into sewage collection systems [8,9]. Nickel oxide nanoparticles (NiO-NPs) are utilized in various applications such as solar cells, catalysts, lithium-ion batteries, resistive random access memories, light-emitting diodes, electrochemical sensors and biosensors. Toxic effects of NiO-NPs have been observed for freshwater species [10] however, the impact of this nanomaterial on activated sludge microorganisms is still unknown. Once in the STP, nanoparticles such as TiO_2-NPs are captured through adhesion into the sludge and removed from the water stream [11] meanwhile other nanomaterials as CuO-NPs and ZnO-NPs have been demonstrated to cause a severe inhibition of anaerobic wastewater treatments [12,13].

The presence of surfactants in STP influents, such as QACs, has been shown to hinder the removal of NPs from water as a result of the modification of their surface and interfacial properties [11]. QACs adsorption on NP surfaces reduces the tendency of NPs to agglomerate and stick to the sludge. Consequently, QAC + NPs remain suspended in the water stream through the STP, are able to affect the microorganisms in secondary treatment processes, increase the turbidity, foul membranes and influence the efficiency of tertiary disinfection processes [9]. Besides, QAC + NP eventually may leave STP, with NPs acting as a delivery vehicle for QAC into aquatic environments.

The use of ozonation as chemical pre-treatment followed by a biological process has been shown to be a suitable technology for the removal of pollutants which cause toxic effects on microorganisms [14]. Ozonation has several advantages over conventional chemical oxidation processes using potassium permanganate or chloride, including higher oxidation potential, absence of potentially carcinogenic chlorinated by-products, and short life time of the oxidant, which would be toxic to microorganisms in subsequent biological treatments [15]. BAC degradation has not been thoroughly studied, with most studies performed in semi-batch conditions and synthetic water matrix and focused on the degradation and/or mineralization of the target pollutant [16], and not on the biological effects of oxidation by-products. Ozonation as chemical pre-treatment should remove toxic pollutants without producing transformation products (TPs) which could cause an adverse effect on the microorganisms present in the subsequent biological treatment [15]. Continuous treatment is a closer approximation to full-scale systems than batch experiments and allows a better understanding of the fate of pollutants under oxidizing conditions, the influence of water matrix and the toxicity of treated effluents.

The study aims to assess the continuous ozonation of a wastewater contaminated with BAC and NiO-NPs intended as pre-treatment of an activated sludge process. The influence of water matrix was explored using real STP influent. The toxicity of ozone treated wastewater was monitored using standardized single-species tests (the bacteria *Vibrio fischeri* and *Pseudomonas putida* and the protozoan *Tetrahymena thermophila*) and an activated sludge resazurin-based assay.

2. Materials and methods

2.1. Materials

Benzalkonium chloride (BAC; ⩾95%, consisting of homologues of different alkyl chain lengths, mainly, ~60% C12 and ~40% C14) and nickel (II) oxide nanopowder (NiO-NPs; ⩽50 nm particle size, 99.8% trace metals basis) were purchased from Fluka and Sigma–Aldrich, respectively. Raw water was prepared with an initial BAC concentration of 10 mg L^{-1}, with and without the addition of NiO-NPs (20 mg L^{-1}). A 1000 mg L^{-1} of NiO-NPs suspension in water was prepared by sonication for 15 min at 20 kHz and 200 W L^{-1} (BioBlock Scientific, France). 500 mL of the concentrated suspension was added to 25 L of the water to be treated in order to achieve 20 mg L^{-1}. Water was then kept under agitation at 400 rpm with a two-arm propeller 3 h prior to the ozonation with the aim of reaching the adsorption equilibrium of BAC on NiO-NPs.

Two different matrices were used: a synthetic water matrix and a municipal STP influent. The synthetic matrix was prepared in ultrapure water (resistivity ⩾18 MΩ cm at 25 °C) with the required amount of sodium bicarbonate to equal the alkalinity and pH values of the STP influent. Raw wastewater was collected from the outlet of mechanical preliminary treatment and before biological treatment in the Carrión de los Céspedes Experimental Plant in Seville (Spain), which treats domestic wastewater and has a capacity of 2500 population equivalent. Wastewater was autoclaved at 121 °C during 20 min before use. Details on wastewater characterization are included as Supplementary data (Table S1).

2.2. Experimental procedure

The experiments were carried out in a cylindrical reactor with a total working volume of 1.44 L, which operated in continuous co-current mode. The retention time distribution curve yielded an average retention time of 10.3 min. The reactor modeling using the continuous stirred tank reactor (CSTR) in series model determined an equivalent value of 1.13 tanks, indicating that the bubble column can reasonably approach a perfect CSTR.

Water and gas flow rates were 142 and 390 mL min^{-1}, respectively, with different inlet ozone concentrations. During the runs, the inlet ozone dosage was stepwise increased from 5 to 300 milligrams of ozone per liter of wastewater (mg L^{-1}). For the different ozone dosages, samples were withdrawn for analysis at the column outlet once the stationary state was reached. This was ensured by circulating four times the hydraulic retention volume after constant ozone concentration was obtained both in the liquid and gas phases at the column outlet. Assuming CSTR behavior, the amount of ozone consumption at the stationary state can be obtained from the following mass balance:

$$\text{Consumed } O_3 = F_{O_3}^{\text{gas,in}} - F_{O_3}^{\text{gas,out}} - F_{O_3}^{\text{liq,out}} \tag{1}$$

in which F_{O_3} is the rate of ozone entering the system in the gas phase (gas, in) or leaving it either in the exhaust gases (gas, out) or dissolved in water (liq, out). Details are given elsewhere [17].

2.3. Analytical methods

BAC concentrations and high accuracy mass analyses of TPs were performed using a time-of-flight mass spectrometer (TOF/MS, Agilent Technologies) with an ESI (electrospray ionization) source coupled to an Agilent 1100 Series HPLC system (LC–TOF/MS). Cationic surfactant concentrations were measured using bromophenol blue method (Hach-Lange LCK 331). Dissolved organic carbon (DOC) was determined by a TOC-V_{CSH} Shimadzu TOC analyzer. Carboxylic acids were measured by a Dionex DX120 IC and inorganic ions were determined by means of a Metrohm 861 Advance Compact IC. Raman spectra of BAC adsorbed onto NiO-NPs were recorded using a Thermo Scientific DXRxi Raman imaging microscope (Waltham, MA, USA). Water samples were analyzed for their nickel oxide and dissolved nickel content by an Agilent 7700× ICP-MS. The size distribution of nanoparticles was obtained using dynamic light scattering (DLS, Malvern Zetasizer Nano ZS). ζ-Potential was measured via electrophoretic light scattering combined with phase analysis light scattering in the same instrument equipped with a Malvern autotitrator MPT-2. Analytical methods are detailed in Supplementary data.

2.4. Aquatic toxicity tests

The aquatic toxicity of water samples was determined using a battery of bioassays composed of single-species tests of two bacteria (*V. fischeri* and *P. putida*) and one protozoan (*T. thermophila*), which represent the microorganisms present in activated sludge, and a whole biological community assay using activated sludge. Single-species tests were conducted according to standard operational guidelines. Meanwhile, the activated sludge assay was carried out by evaluating the effect of water samples on metabolic activity of activated sludge microorganisms (Table S2, Supplementary data) using the resazurin method under the experimental conditions described in OECD Method 209 [6]. Supplementary data show more details about the aquatic toxicity test procedures.

The toxic-effects obtained were transformed into toxic units (TUs) following the procedure described by Persoone et al. [18]. TU is defined as the reciprocal of the wastewater dilution (expressed in percentage) need to achieve 50% inhibition (EC_{50}):

$$TU = \frac{100}{EC_{50}} \tag{2}$$

TUs of non-diluted samples whose effect percentage observed was higher than controls but below 50% (<1 TU) were estimated using the approach proposed in the literature [18] (i.e. TU = *inh*/50, in which *inh* is the percentage of inhibition). On the basis of TU values, water samples were classified into *non-toxic* (<0.4 TU), *slightly toxic* (0.4–1 TU), *toxic* (1–10 TU) and *highly toxic* (>10 TU).

3. Results

3.1. Synthetic water matrix

The evolution of BAC, DOC, consumed ozone and dissolved ozone as a function of the amount of ozone supplied is represented in Fig. 1A. Fig. 1B shows the concentration of cationic surfactants and carboxylic acids (sum of oxalic, acetic and formic acids) during the ozonation process. Based on the evolution of consumed and dissolved ozone profiles, three zones can be observed throughout ozone dosages. In all of them, dissolved ozone was detected ($\geqslant 0.01$ mg L^{-1}) due to the fact that ozone mass transfer rate was greater than that of ozone consumption. This suggests that BAC ozonation reactions are relatively slow [17]. In zone 1, up to 54 mg L^{-1}, consumed and dissolved ozone linearly increased with ozone dosage. In it, BAC decreased steeply with ozone exposure reaching a value as low as 0.4 mg L^{-1}. Cationic surfactants declined in parallel to BAC. However, although the concentration of surfactants was significantly reduced, a large organic load remained as shown by DOC values, which slightly decreased. A remarkable increase was observed for carboxylic acids, whose concentration increased steadily with ozone exposure, which suggests a partial oxidation of BAC molecules. In zone 2, ozone consumption steadily increased up to a dosage of 168 mg L^{-1}. This additional ozone input (from 54 to 168 mg L^{-1}) was necessary to attain a final BAC concentration below 0.1 mg L^{-1}. In this zone, the depletion of cationic surfactants continues while DOC stabilized at 6.5 mg L^{-1}, even though the concentration of organic acids rose slightly. The increasing consumption of ozone indicated the presence of organic matter oxidized but not mineralized. Mineralization is not a single chemical process and represents a series of reactions that are slow for highly oxidized molecules such as carboxylic acids [19]. The contribution of low molecular weight acids to total mineralization is an insignificant fraction of the overall rate, which is mainly produced in the ozonation of the high molecular weight compounds as in zone 1 [20]. For dosages above 168 mg L^{-1} (zone 3), ozone consumption remained constant and ozone concentration at the reactor outlet (gas and liquid) increased proportionally to ozone input. Under these conditions, the upper operational limit of the system, the consumed ozone and the ozone dose were 32 mg L^{-1} and 3.17 mg O_3 (mg BAC)$^{-1}$, respectively.

It is interesting to emphasize the steep BAC depletion at low ozone dosages because ozone reacts slowly with aromatic compounds with electron-withdrawing substituent, quaternary amines

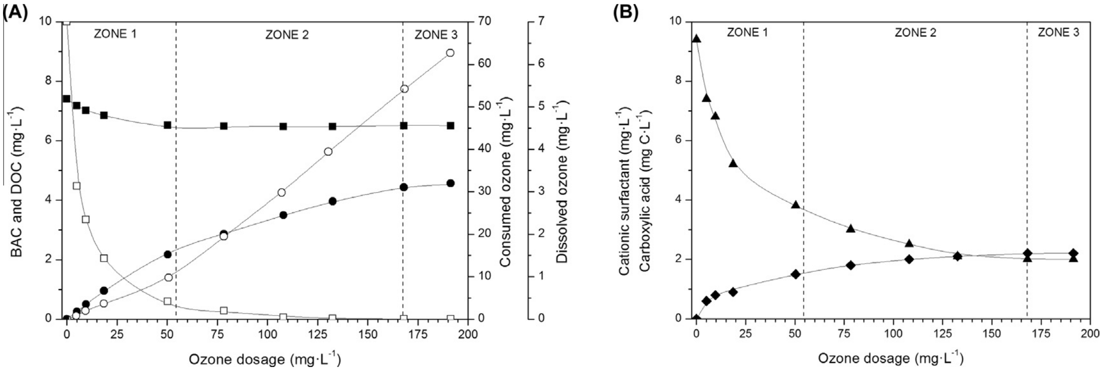


Fig. 1. Evolution of BAC (□), DOC (■), consumed (●) and dissolved ozone (○) cationic surfactants (▲) and carboxylic acids (sum of oxalic, acetic and formic acids, ◆) at different ozone dosages in synthetic water matrix.

and aliphatic chains [21]. Hence, the degradation of BAC under the working conditions used in this work (pH 8.5), seems to be predominantly driven by the attack of hydroxyl radicals, whose rate constants are in the range of 10^9 to 10^{10} M^{-1} s^{-1} for the moieties present in the BAC molecule [21]. The prevalence of indirect mechanism was confirmed by the strong inhibition of BAC abatement occurring in the run carried out using *t*-butanol (30 mM) as radical scavenger. Nonetheless, although the radical chain mechanism is predominant, the maximum mineralization was low, 12%. A third of the remaining DOC is explained by measured carboxylic acids: 1.7 mg L^{-1} of oxalic and 4.4 mg L^{-1} of acetic. These results are consistent with previous studies of the degradation of other surfactants by means of ozonation processes [14].

3.2. NPs co-occurrence effect

The influence of NiO-NPs on BAC ozonation was studied using a synthetic water matrix. The detailed characterization of BAC and NiO-NPs co-occurrence was performed before ozonation runs (Fig. 2). Adsorption isotherm (carried out by measuring BAC concentration without previous extraction) showed that the initial working conditions (BAC: 10 mg L^{-1}; NiO-NPs: 20 mg L^{-1}) correspond to a concentration of BAC in equilibrium of 7.5 mg L^{-1} and consequently, 2.5 mg L^{-1} were adsorbed onto 20 mg L^{-1} of NiO-NPs. The high adsorption of BAC is most probably a consequence of the high surface area-to-volume ratio (BET specific surface area: 86 m^2 g^{-1}) and surface charge (ζ-potential: −15 mV) of the nanomaterial. The potentiometric titration displayed that increasing BAC concentration caused a stepwise rise in the ζ-potential of NiO-NPs from −15 to +15 mV as a consequence of potential surface charge neutralization by positively charged BAC ions and admicelles [22]. The hydrodynamic diameter of NiO-NPs reflected considerable particle aggregation, but the size of the nanomaterial was influenced by BAC concentration. BAC as surfactant has the ability to enhance the dispersion of NPs as well as reduce the charge repulsion between NPs in suspension [11]. NiO-NPs in the presence and absence of BAC were also examined by Raman spectroscopy (Fig. 2C), which is a sensitive technique able to provide direct evidence of molecular conformation or interactions of adsorbed surfactants [23]. For the BAC + NiO-NPs mixture, signals with characteristic Raman bands such as those related to the alkyl chain ($\nu(CH_2)$ at 3000–2800 cm^{-1} and the $\delta(CH_2)$ at 1449 cm^{-1}), to the aromatic group (ν(CH) at 3100–3000 cm^{-1} and ν(phenyl ring) at ~1600, 1448, 1002 cm^{-1}) and to quaternary amine ($\nu(CH_3)$ at 2852 cm^{-1}) were observed. From the comparison between the Raman spectra for pure BAC and the mixture of BAC + NiO-NPs, some conclusions about the adsorption configuration can be extracted. The change in relative intensity between bands in BAC + NiO-NPs mixture (2852 cm^{-1}/1002 cm^{-1} ~5/5) compared to pure BAC (2852 cm^{-1}/1002 cm^{-1} ~6/5), indicated that there is a preferential orientation of BAC on the surface of the NiO-NPs with respect to the random configuration in solution (pure BAC spectra). In the absence of specific interaction between BAC and NiO-NPs, the relative intensity of BAC bands in both spectra should remain equal. These observations suggest that molecules of BAC adsorb on NiO-NP surfaces via head group, aided by favorable electrostatic attraction. These data are in line with the conclusions found for the adsorption of cationic surfactants onto NPs by other authors [23].

Fig. 3A represents the evolution of BAC, DOC and the profiles for consumed and dissolved ozone during the ozonation of BAC + NiO-NPs in synthetic water matrix. A similar behavior to ozonation of BAC alone was observed. For lower ozone dosages (zone 1), BAC was significantly abated with ozone exposure up to 0.4 mg L^{-1}, while for input levels above 176 mg O_3 L^{-1} (zone 3), almost total

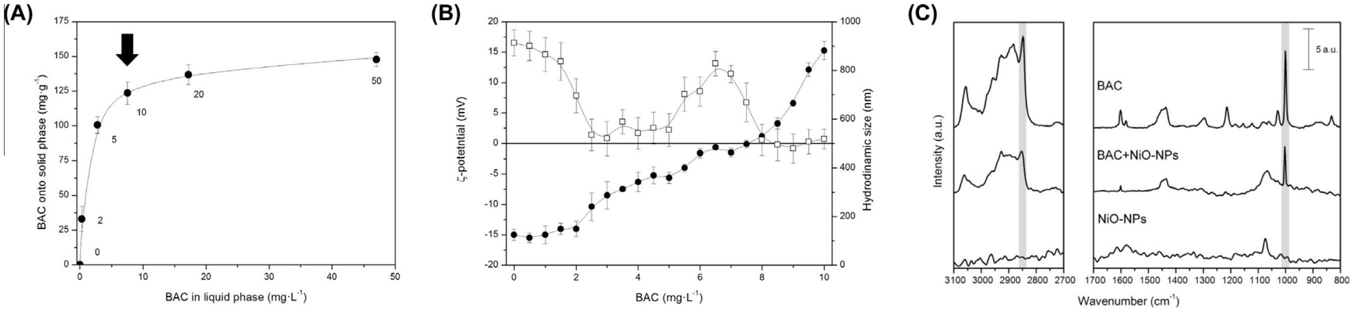


Fig. 2. (A) Characterization of the co-occurrence of BAC and NiO-NPs: Adsorption isotherm (the numbers represent initial BAC concentration and the arrow indicates initial ozonation working conditions: BAC: 10 mg L^{-1}, NiO-NPs: 20 mg L^{-1}). (B) Potentiometric titration ζ-potential (●) and hydrodynamic diameter of NiO-NPs (□). (C) Raman spectra (gray lines represent wavenumbers 1002 and 2852 cm^{-1}).

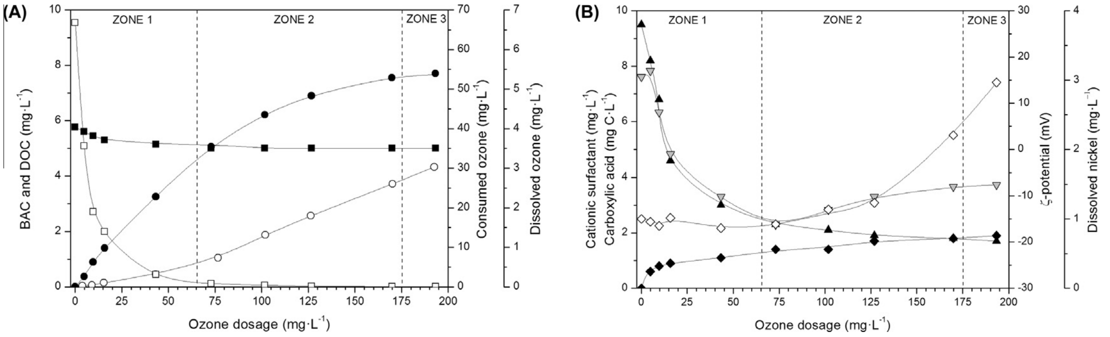


Fig. 3. Evolution of BAC (□), DOC (■), consumed (●) and dissolved ozone (○), cationic surfactants (▲), carboxylic acids (sum of oxalic, acetic and formic acids, ◆), ζ-potential (▽) and dissolved nickel (◇) at different ozone dosages in synthetic water matrix (BAC + NiO-NPs).

BAC depletion was reached and ozone consumption remained constant. As shown in Fig. 3A and B, the profile for DOC, cationic surfactants and carboxylic acids concentration as a function of ozone dosage was also similar to that found for BAC alone. The co-occurrence of NiO-NPs and BAC caused in general a higher ozone dosage and ozone consumption values than BAC ozonation in the absence of NiO-NPs, which suggest that NiO-NPs contribute to ozone consumption. The ozonation of NiO-NPs suspensions resulted in an ozone consumption of 27 mg L^{-1} at the upper operational limit of the system (Fig. S1, Supplementary data). Nevertheless, ozone decomposition by NiO-NPs does not seem to accelerate the production of hydroxyl radicals because BAC profile did not display significant differences with respect to BAC ozonation without NiO-NPs.

The ζ-potential of NiO-NPs sharply declined in parallel with cationic surfactant concentration from +17 mV at ozone dosage of 4.9 mg L^{-1} to −17 mV at 106 mg O$_3$ L^{-1} (Fig. 3B). These values were similar to those obtained for the potentiometric titration of NiO-NPs with BAC (Fig. 2B), which is consistent with a total depletion of adsorbed BAC. ζ-Potential increased slightly for higher ozone dosages throughout zone 2 reaching about −8.0 mV in zone 3. A significant increment in the amount of dissolved nickel from 1 to 3 mg L^{-1} was also observed with the increase of ozone dosage despite pH value of treated waters remained constant (Fig. S2A, Supplementary data). The final nickel concentration was similar to that found in the ozonation of NiO-NPs alone (see Supplementary data Fig. S1). These facts suggest that adsorbed BAC acted as a coating agent, increasing the stability of NiO-NPs dispersion and preventing nickel ions from passing to the solution [10]. Under these conditions, the amount of ozone consumed and the ozone dose were 54 mg L^{-1} and 5.35 mg O$_3$ (mg BAC)$^{-1}$, respectively. The high values for both parameters with respect to the ozonation of BAC could be explained by the ozone consumption driven by NiO-NPs. The amount of ozone consumed by BAC + NiO-NPs at the operational limit was close to the sum of the ozone consumed by water matrix, 7 mg L^{-1} (Fig. S3, Supplementary data), NiO-NPs, 20 mg L^{-1} (Fig. S1), and BAC abatement, 25 mg L^{-1} (Fig. 1).

3.3. Elucidation of transformation products and degradation pathway

Twelve compounds were elucidated as TPs formed during the ozonation of BAC (Supplementary data Table S3). All of them with retention time lower than BAC, which indicates that transformation reactions lead to more polar molecules. The profile of TPs as a function of ozone dosage is shown in Fig. 4. The amounts of TPs corresponded to intermediate products in series reactions, with their counts initially increasing to reach a maximum and then decreasing as a result of their further degradation. The generation pathway of these TPs is expected to include multiple routes due to the presence of different reactive sites. Despite this complexity, and in view of the information obtained from the literature [24], the results could be interpreted to propose the degradation pathway shown in Scheme 1. The degradation of BAC occurred on both its hydrophobic (alkyl chain) and hydrophilic region (benzyl and ammonium moiety), which explains the occurrence of transformation products TP$_{1-6}$ (full symbols in Fig. 4) and TP$_{7-12}$ (empty symbols in Fig. 4), respectively.

On the hydrophobic region, the initiation step was hydrogen abstraction from alkyl chain by means of a hydroxyl radical leading to a carbon centered radical, its reaction with dissolved oxygen to yield a peroxyl radical and the subsequent decomposition to

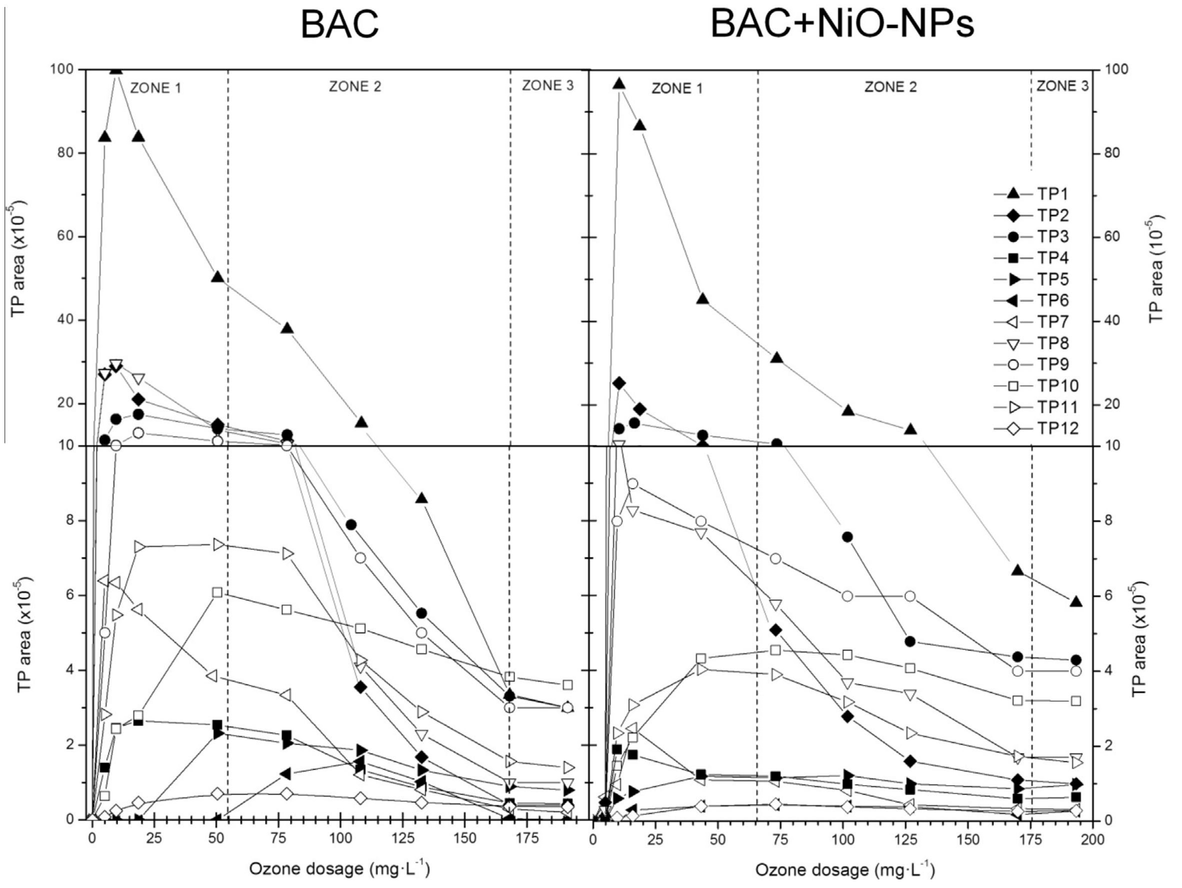
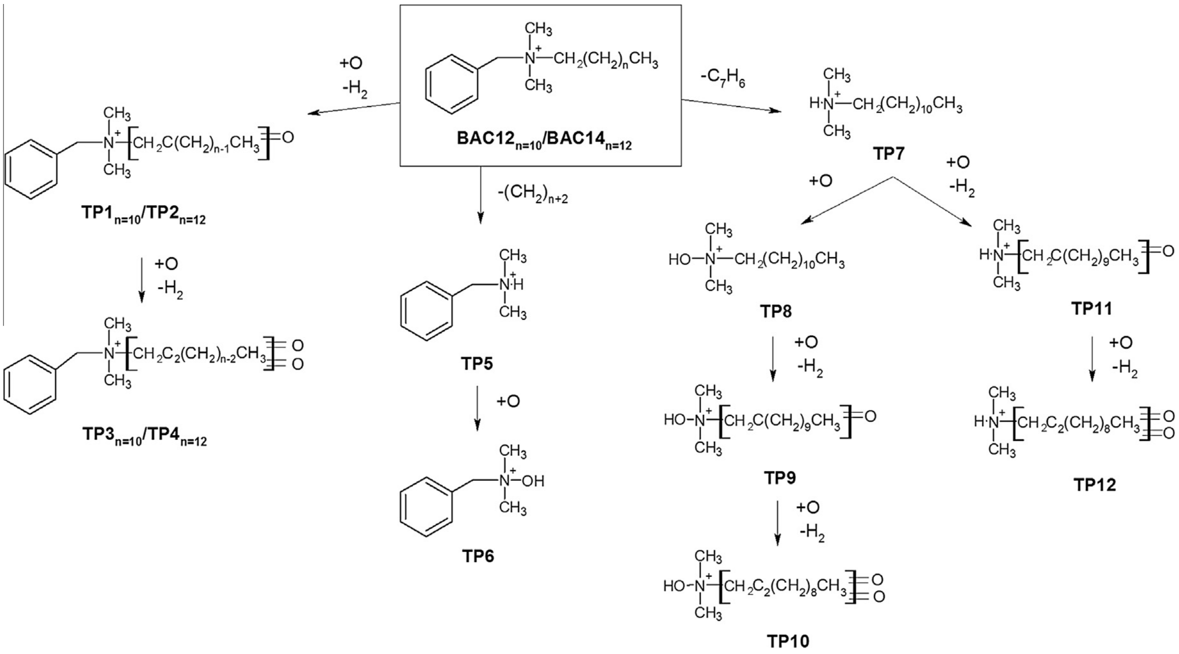


Fig. 4. Evolution of TPs of BAC at different ozone dosages in synthetic water matrix.



Scheme 1. Proposed degradation pathway during the ozonation of BAC.

carbonyl compounds TP_1 and TP_2 [20]. Carbonyl moiety may occur at different positions along the alkyl chain resulting in a series of isomers with similar counts: nine for TP_1 (m/z 318.2791, $C_{21}H_{36}NO^+$) and eight for TP_2 (m/z 346.3104, $C_{23}H_{40}NO^+$). In the same way, the alkyl chains of TP_1 and TP_2 may suffer hydrogen abstraction to yield TP_3 (m/z 332.2584, $C_{21}H_{34}NO_2^+$) and TP_4 (m/z 360.2897, $C_{23}H_{38}NO_2^+$), respectively. The occurrence of benzyldimethylamine (TP_5, m/z 136.1121, $C_9H_{14}N^+$) suggests an α-hydroxylation of the alkyl moiety followed by a central fission of the C_{alkyl}–N bond (dealkylation). Then, benzyldimethylamine can be hydroxylated to TP_6 (m/z 152.1070, $C_9H_{14}NO^+$). On the hydrophilic region, the initiation step would be the degradation of benzyl group to yield carboxylic acids [21]. These reactions lead to dodecyltrimethylamine (TP_7, m/z 214.2534, $C_{14}H_{32}N^+$). The degradation of TP_7 may give rise to TP_8 (m/z 230.2478, $C_{14}H_{32}NO^+$) through the addition of a hydroxyl radical. The aliphatic tertiary amine may undergo hydrogen abstraction along the aliphatic chain to yield a group of eight positional isomers (TP_9, m/z 244.2271, $C_{14}H_{30}NO_2^+$). Further hydrogen abstraction reactions on TP_9 could give rise to TP_{10} (m/z 258.2064, $C_{14}H_{28}NO_3^+$). TP_6 could also be oxidized to TP_{11}, a group of seven isomers (m/z 228.2322, $C_{14}H_{30}NO^+$), which would be further transformed to TP_{12} (m/z 242.2115, $C_{14}H_{28}NO_2^+$).

BAC degradation pathways on both the hydrophobic and hydrophilic region justified the large amount of carboxylic acids detected at the upper operational limit. Acetic acid (4.4 mg L^{-1}) was a clear outcome of the aliphatic chain oxidation, whereas oxalic acid (1.7 mg L^{-1}) seems to be the final product of ring-opening reactions [21]. Nitrate reached the maximum concentration of 0.06 mg L^{-1} at zone 3 (3% of the total nitrogen content in BAC molecules), indicating negligible nitrogen mineralization. These facts suggest that the remaining organic carbon contains a high amount of nitrogen in compounds such as amines, whose protonated species react slowly with ozone ($k < 0.1\ M^{-1}\,s^{-1}$, von Sonntag and von Gunten [21]). The accumulation of transformation products such as TP_1, TP_3, TP_9 and TP_{10} could explain the incomplete depletion of cationic surfactants (Figs. 1B and 3B).

All TPs mentioned above were also detected in the presence of NiO-NPs, which suggest that the same degradation pathway took place. However, significantly lower area counts of TPs from reactions on the hydrophilic part of BAC (TP_7–TP_{12}), especially in zone 1, were found. This is consistent with the already explained adsorption of BAC on NiO-NPs, which should proceed through the benzyl group and prevent it from oxidation.

3.4. Water matrix effect

The influence of water matrix on the ozonation of BAC + NiO-NPs was also studied in a real STP influent. Non-spiked raw wastewater required an instantaneous ozone demand of 77 mg L^{-1} (Supplementary data Fig. S4A). High values of consumed ozone were also observed at the upper operational limit, 87 mg L^{-1}. Both facts were mainly a result of the oxidation of wastewater organic matter, the concentration of which was elevated (DOC_0 = 61 mg L^{-1}). A disintegration of suspended solids also took place during ozonation process, leading to an increase of inorganic anions such as nitrate, phosphate and sulfate, as well as DOC (Supplementary data Fig. S4B). The evolution of dissolved organic matter nature with ozonation has been followed using UV–Vis absorption (Supplementary data Fig. S5). Specific UV absorbance (SUVA) is defined as the UV absorbance of a water sample at a given wavelength normalized for dissolved organic carbon (DOC) concentration. SUVA determined at 254 nm, is strongly correlated with percent aromaticity and conjugated double bonds. A strong reduction of $SUVA_{254}$ took place with increasing ozone dosage (Fig. S4), indicating that the proportion of aromatic compounds in organic matter decrease. This fact is commonly expected because ozone attacks preferentially electron reach moieties which are mainly responsible of ultraviolet absorption at 254 nm. The reaction of ozone with unsaturated bonds or aromatic rings leads to oxygenated saturated functional groups, such as aldehydes, ketones and especially carboxylic groups (Fig. S4B) [20].

The evolution of BAC, DOC, and the dissolved and consumed ozone during the ozonation in spiked STP influent are represented in Fig. 5A. Important differences were observed with regard to the synthetic water matrix. The amount of consumed ozone increased with ozone input and no dissolved ozone was detected (<0.01 mg L^{-1}). The profile of BAC depletion was also different.

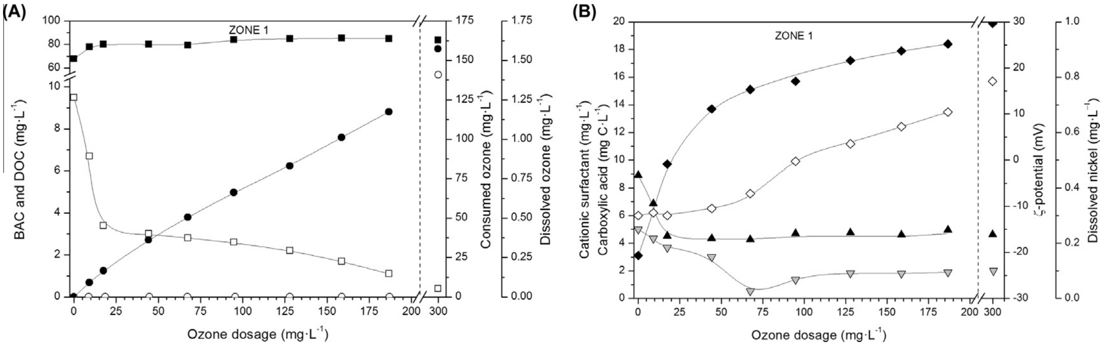


Fig. 5. Evolution of BAC (□), DOC (■), consumed (●) and dissolved ozone (○), cationic surfactants (▲), carboxylic acids (sum of oxalic, acetic and formic acids, ◆), ζ-potential (▽) and dissolved nickel (◇) at different ozone dosages in STP influent (BAC + NiO-NPs).

BAC decay in STP influent could be split in two parts. First, up to ozone exposures of 18 mg L^{-1}, BAC concentration decreased sharply (up to 65% removal). For higher ozone dosages, BAC concentration declined slowly, probably influenced by the elevated amount of DOC in solution. DOC rose from 67 to 80 mg L^{-1} during the first part of the reaction because of the solubilization of suspended solids, which increased ozone demand. Under these conditions, an ozone consumption of 157 mg L^{-1} (dosage of 300 mg L^{-1}) was required to achieve 0.4 mg L^{-1} of BAC, a concentration that required 54 mg L^{-1} of ozone in synthetic water. On the whole, consumed ozone in real wastewater was close to fivefold the corresponding value in synthetic water matrix.

The high ozone demand of wastewater was also related to the lower depletion of cationic surfactants, which followed the same profile as BAC. A significant removal of cationic surfactants was reached for ozone dosage of 18 mg L^{-1} but their concentration stabilized at 4.6 mg L^{-1} without further reduction. The evolution of carboxylic acids followed a similar trend to that observed in synthetic matrix, but displaying higher concentrations as a consequence of the oxidation reactions of dissolved organic matter [20,25]. Specifically, formic, acetic and oxalic acid achieved values of 14, 36 and 6.5 mg L^{-1}, representing altogether close to 40% of the remaining dissolved carbon. Meanwhile, ζ-potential displayed negative values during all the ozonation process, reaching −28 mV for an ozone dosage of 68 mg L^{-1}. A possible cause is that organic matter could adsorb on NP surfaces and confer them a negative charge [25]. The concentration of dissolved nickel at almost neutral pH value (7.52, see Fig. S2 in Supplementary data) reached a value significantly lower than that found in synthetic water (0.8 mg L^{-1}). This fact is most likely due to the barrier caused by adsorbed organic matter on NiO-NPs, which stabilizes NP dispersion and reduces the rate of dissolution [10].

3.5. Aquatic toxicity assessment

The toxicity of BAC and NiO-NPs to single species and activated sludge microorganisms was assessed by determining concentration–response curves as shown in Supplementary data (Fig. S6). All the bioassays were sensitive to BAC with the following EC_{50} values: 0.26 mg L^{-1} for *V. fischeri*, 8.40 mg L^{-1} for *P. putida*, 4.28 mg L^{-1} for *T. thermophila* and 3.43 mg L^{-1} for activated sludge. The EC_{50} values are in good agreement with previously reported data and are consistent with the use of BAC as biocide [6,26,27]. The growth inhibition tests using *P. putida* and *T. thermophila* were also sensitive to NiO-NPs with EC_{50} values of 4.25 and 0.58 mg L^{-1}, respectively, while *V. fischeri* and activated sludge assays displayed EC_{50} values >100 mg L^{-1}. The same sensitivity pattern was observed for nickel ions (nickel as nickel sulfate), which displayed a concentration–response curve parallel to NiO-NPs for each bioassay (Fig. S6). This fact suggests that the concentration of nickel ion released from the NP is the driver for the toxicity of NiO-NPs. The low sensitivity of *V. fischeri* and activated sludge to different NPs has also been reported elsewhere [28,29].

Fig. 6 displays the evolution of the toxicity of untreated and treated water samples at different ozone exposures in synthetic matrix and STP influent for the organisms used in the present study. The aquatic toxicity of untreated synthetic water (BAC = 10 mg L^{-1} and/or NiO-NPs = 20 mg L^{-1}) displayed significant inter-bioassay differences, which essentially corresponded to the already described sensitivity to BAC and NiO-NPs. The growth inhibition of *P. putida* and *T. thermophila* was severely inhibited because BAC and NiO-NPs concentrations were considerably higher than their EC_{50} values. Moreover, untreated synthetic water could be classified as *toxic*, or even *highly toxic*, to subsequent biological treatment according to the scoring system elaborated by Persoone et al. [18]. *V. fischeri* and activated sludge tests were also strongly affected by water spiked with BAC (*toxic* or *highly toxic*), but not with single NiO-NPs, which is consistent with their lower sensitivity to the nanomaterial.

The aquatic toxicity of BAC + NiO-NPs was lower in STP influent than in the synthetic matrix except to *V. fischeri*, which could be explained by the toxicity of the raw wastewater itself. For the rest of biotests, mixture toxicities of BAC + NiO-NPs were notably influenced by matrix (i.e., high concentration of solids, organic and inorganic matter), which reduces the bioavailability of the cationic surfactants and dissolved metals [1,10,26]. Moreover, the high amount of nutrients contained in the STP influent (assimilable organic carbon, nitrogen, phosphate) could partially mask the toxic effects of the studied contaminants.

In the synthetic water matrix, the evolution of the aquatic toxicity throughout ozonation of BAC showed that the increase of ozone dosage up to 54 mg L^{-1} (zone 1) caused a gradual toxicity reduction. By the end of zone 1, the toxicity reached a constant value similar to that of non-spiked synthetic water for all bioassays. TU values remained essentially constant thereafter in zone 2 and 3. Ozone treated water could then be classified as *non-toxic* for *P. putida* and activated sludge tests. Aquatic toxicity and BAC concentration follow a similar profile with increasing ozone dosage. Despite the fact that BAC was not completely depleted at the end of zone 1 (0.4 mg L^{-1}), the reduction of its concentration brought about a considerable reduction of its toxic effects. The degradation reactions caused changes in the molecular structure of BAC, affecting moieties which were directly responsible for its biocide activity [30]. In fact, TOF/MS determinations showed that benzyl and alkyl groups were oxidized in parallel with the depletion of surface activity.

During the ozonation of NiO-NPs in synthetic water matrix, the toxicity was slightly enhanced at the lowest ozone dosage due to the increment of dissolved nickel that reached 3 mg L^{-1}. For higher ozone exposure, no toxicity changes were observed. The aquatic

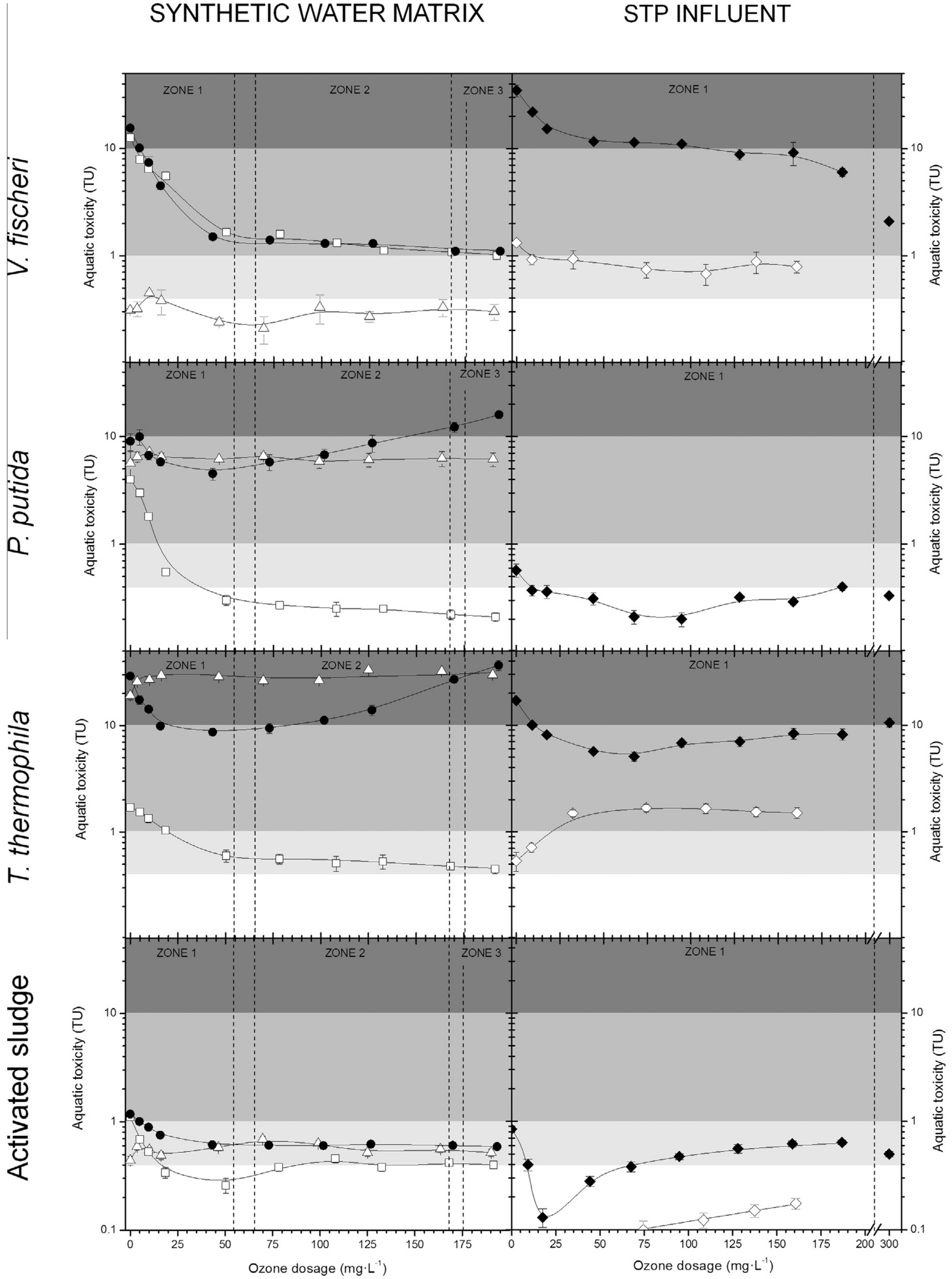


Fig. 6. Evolution of toxic units (TUs) of treated samples at different ozone dosages in synthetic water spiked with BAC (□), NiO-NPs (△), BAC + NiO-NPs (●), STP influent spiked with BAC + NiO-NPs () and non-spiked STP influent (◇). Mean ± 95% confidence interval, ■ *highly toxic*, ▒ *toxic*, ░ *slightly toxic* and ☐ *non-toxic* influent to subsequent biological treatment according to the classification elaborated by Persoone et al. [18].

toxicity pattern of treated BAC + NiO-NPs in synthetic matrix was similar to that of BAC for the bioassays with low sensitivity to NiO-NPs. *V. fischeri* and activated sludge tests reached the same TU values of non-spiked synthetic water at the end of zone 1, with the toxicity remaining constant for further ozone dosages (zones 2 and 3). For *P. putida* and *T. thermophila*, the toxicity declined steadily with ozone exposure up to an ozone dosage of 58 mg L^{-1} to sharply increase thereafter in zones 2 and 3. This increase was parallel with that of dissolved nickel, the concentration of which rose from 1 to 3 mg L^{-1}. As a consequence, the ozonation yielded a *highly toxic* wastewater from a *toxic* influent to *P. putida*. As already described, *P. putida* and *T. thermophila* were highly sensitive to NiO-NPs and dissolved nickel as evidenced by the corresponding EC_{50} values: 0.57 and 0.061 mg L^{-1}, respectively. It is worth mentioning that the toxicity towards *P. putida* at the highest ozone dosage (~190 mg L^{-1}) was significantly higher in BAC + NiO-NPs than in NiO-NPs wastewater, even considering that both had the same amount of dissolved nickel (3 mg L^{-1}). This suggests a synergistic effect between nickel and other mixture components.

The aquatic toxicity of non-spiked STP influent increased steadily with increasing ozone exposure for all biotests except *V. fischeri*, for which it slightly reduced. In spiked STP influent, aquatic toxicity steadily decreased in single-species tests up to 68 mg O_3 L^{-1}, allowing treated wastewater to be classified as *non-toxic* to *P. putida*. For higher ozone exposures, the toxicity to *P. putida* and *T. thermophila* increased progressively in parallel with an increase in the amount of dissolved nickel. For the activated sludge test, ozonation resulted in a sharp toxicity reduction at low ozone dosage (18 mg L^{-1}) allowing ozone treated wastewater to be considered *non-toxic*. For increasing ozone exposure, the toxicity of treated wastewater increased progressively equaling that of non-spiked STP influent. This fact, together with the low sensitivity of activated sludge to nickel (<5% inhibition at 1 mg L^{-1}), suggests that ozonation by-products from STP influent matrix were the main source of toxicity to the activated sludge assay.

4. Conclusions

It was shown that the continuous ozonation with short reaction time and low ozone dosages is a suitable technology for sequential chemical-biological treatment regarding the reduction of toxicity caused by wastewater contaminated with BAC and NiO-NPs.

BAC was significantly removed (>95%) during ozonation independently of NiO-NP co-occurrence or water matrix characteristics. NiO-NPs and wastewater matrix notably increased the ozone dosage required for a given degree of BAC removal. BAC ozonation led to less hydrophobic molecules as a consequence of the reaction on both the hydrophobic (alkyl chain) and hydrophilic regions (benzyl and ammonium moieties) of the parent compound. The presence of NiO-NPs influenced the first steps of the degradation pathway of BAC preventing benzyl group from oxidation.

The aquatic toxicity of raw wastewater for the biotest battery (*V. fischeri*, *P. putida*, *T. thermophila*) and activated sludge assay was considerably reduced for an ozone dosage lower than that required for BAC abatement. Higher ozone dosage BAC + NiO-NPs caused an increase in nickel leaching from the nanomaterial and a toxicity enhancement of treated wastewater. Ozone generation cost is the main drawback for general application of ozonation process and consequently, ozone dosage should be optimized in order to obtain the maximum benefits at the lowest cost. Ozone excess is not only economically detrimental, but also presents a serious drawback due to toxicity increase. Toxicity assessment was shown to be a critical parameter for the optimization of ozonation as pre-treatment of activated sludge process.

Acknowledgments

This study has been financed by the Dirección General de Universidades e Investigación de la Comunidad de Madrid (S2013/MAE-2716) and the Spanish MINECO (CTM2008-04239 and CTM2013-45775). JBC thanks the Spanish Ministry of Education for a FPU Grant (AP2008-00572). The authors would like to thank Donna Cassidy Hanley and the Tetrahymena Stock Center at Cornell University, Ithaca, NY for providing us with the *T. thermophila* strain SB210 as well as INQUIFOR group for its support with RAMAN spectroscopy analyses.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2015.08.001.

References

[1] Z.Z. Ismail, U. Tezel, S.G. Pavlostathis, Sorption of quaternary ammonium compounds to municipal sludge, Water Res. 44 (2010) 2303–2313.
[2] K. Kümmerer, A. Eitel, U. Braun, P. Hubner, F. Daschner, G. Mascart, M. Milandri, F. Reinthaler, J. Verhoef, Analysis of benzalkonium chloride in the effluent from European hospitals by solid-phase extraction and high-performance liquid chromatography with post-column ion-pairing and fluorescence detection, J. Chromatogr. A 774 (1997) 281–286.
[3] M. Clara, S. Scharf, C. Scheffknech, O. Gans, Occurrence of selected surfactants in untreated and treated sewage, Water Res. 41 (2007) 4339–4348.
[4] E. Martínez-Carballo, C. González-Berreiro, A. Sitka, N. Kreuzinger, S. Scharf, O. Gans, Determination of selected quaternary ammonium compounds by liquid chromatography with mass spectrometry. Part I. Application to surfaces, waste and indirect discharge water samples in Austria, Environ. Pollut. 145 (2007) 489–496.
[5] E. Martínez-Carballo, C. González-Berreiro, A. Sitka, N. Kreuzinger, S. Scharf, O. Gans, Determination of selected quaternary ammonium compounds by liquid chromatography with mass spectrometry. Part II. Application to sediment and sludge samples in Austria, Environ. Pollut. 146 (2007) 537–547.
[6] J.B. Carbajo, J.A. Perdigón-Melón, A.L. Petre, R. Rosal, P. Letón, E. García-Calvo, Personal care product preservatives: risk assessment and mixture toxicities with an industrial wastewater, Water Res. 72 (2015) 174–185.
[7] K. Kümmerer, Antibiotics in the aquatic environment – A review – Part II, Chemosphere 75 (2009) 435–441.
[8] B. Nowack, T.D. Bucheli, Occurrence, behavior and effects of nanoparticles in the environment, Environ. Pollut. 150 (2007) 5–22.
[9] S.K. Brar, M. Verma, R.D. Tyagi, R.Y. Surampalli, Engineered nanoparticles in wastewater and wastewaters sludge – evidence and impacts, Waste Manage. 30 (2010) 504–520.
[10] K.L. Garner, A.A. Keller, Emerging patterns for engineered nanomaterials in the environment: a review of fate and toxicity studies, J. Nanopart. Res. 16 (2014) 2503.
[11] L.K. Limbach, R. Bereiter, E. Müller, R. Krebs, R. Gälli, W. Stark, Removal of oxide nanoparticles in model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency, Environ. Sci. Technol. 42 (2008) 5828–5833.
[12] L. Otero-González, J.A. Field, R. Sierra-Alvarez, Inhibition of anaerobic wastewater treatment after long-term exposure to low levels of CuO nanoparticles, Water Res. 58 (2014) 160–168.
[13] L. Otero-González, J.A. Field, R. Sierra-Alvarez, Fate and long-term inhibitory impact of ZnO nanoparticles during high-rate anaerobic wastewater treatment, J. Enviorn. Manage. 135 (2014) 110–117.
[14] K. Ikehata, M.G. El-Din, Degradation of recalcitrant surfactants in wastewater by ozonation and advanced oxidation process: a review, Ozone Sci. Eng. 26 (2004) 327–343.
[15] I. Oller, S. Malato, J.A. Sánchez-Pérez, Combination of advanced oxidation processes and biological treatments for wastewater decontamination – a review, Sci. Total Environ. 409 (2011) 4141–4166.
[16] R.F. Dantas, M. Darcissac, C. Lesueur, S. Contreras, C. Sans, M. Fuerhacker, S. Esplugas, Assessment of cationic surfactants mineralization by ozonation and photo-Fenton process, Water Environ. Res. 81 (2009) 201–205.
[17] J.B. Carbajo, A.L. Petre, R. Rosal, S. Herrera, P. Letón, E. García-Calvo, A. Fernández-Alba, J.A. Perdigón-Melón, Continuous ozonation treatment of ofloxacin: transformation products, water matrix effect and aquatic toxicity, J. Hazard. Mater. 292 (2015) 34–43.
[18] G. Persoone, B. Marsalek, I. Blinova, A. Törökne, D. Zarina, L. Manusadzianas, G. Nalecz-Jawecki, L. Tofan, N. Stepanova, L. Tothova, B. Kolar, A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewater, Environ. Toxicol. 18 (2003) 395–402.
[19] A.L. Petre, J.B. Carbajo, R. Rosal, E. García-Calvo, P. Letón, J.A. Perdigón-Melón, Influence of water matrix on copper-catalyzed continuous ozonation and related ecotoxicity, Appl. Catal. B: Environ. 163 (2015) 233–240.
[20] S. van Geluwe, L. Braeken, B. van der Brugge, Ozone oxidation for the alleviation of membrane fouling by natural organic matter: a review, Water Res. 45 (2011) 3551–3570.
[21] C. von Sonntag, U. von Gunten, Chemistry of ozone in water and wastewater treatment: from basic principles to applications, IWA Publishing, London, 2012.
[22] L.K. Koppel, E.M. Lee, M.R. Böhmer, Adsorption of cationic and anionic surfactant on charged metal oxide surfaces, J. Colloid Interface Sci. 170 (1995) 85–97.

[23] W. Wang, B. Gu, L. Liang, Effect of surfactants on the formation, morphology, and surface property of synthesized SiO_2 nanoparticules, J. Dispersion Sci. Technol. 25 (2004) 593–601.

[24] M.A. Patrauchan, P.J. Oriel, Degradation of benzyldimethylalkylammonium chloride by *Aeromonas hydrophila* sp., K. J. Appl. Microbiol. 94 (2003) 266–272.

[25] Y. Zhang, Y. Chen, P. Westerhoff, J. Crittenden, Impact of natural organic matter and divalent cations on the stability of aqueous nanoparticles, Water Res. 43 (2009) 4249–4257.

[26] G. Nalecz-Jawecki, E. Grabinska-Sota, P. Narkiewicz, The toxicity of cationic surfactants in four bioassays, Ecotoxicol. Environ. Saf. 54 (2003) 87–91.

[27] H. Sütterlin, R. Alexy, K. Kümmerer, The toxicity of the quaternary ammonium compound benzalkonium chloride alone and in mixtures with other anionic compounds to bacteria in test systems with *Vibrio fischeri* and *Pseudomonas putida*, Ecotoxicol. Environ. Saf. (2008) 498–505.

[28] A. García, L. Delgado, J.A. Torà, E. Casals, E. González, V. Puntes, X. Font, J. Carrera, A. Sánchez, Effects of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment, J. Hazard. Mater. 199–200 (2012) 64–72.

[29] D. Wang, Z. Lin, Z. Yao, H. Yu, Surfactants present complex joint effects on the toxicities of metal oxide nanoparticles, Chemosphere 108 (2014) 70–75.

[30] A.D. Russell, Similarities and differences in the responses of microorganisms to biocides, J. Antimicrob. Chemother. 52 (2003) 750–763.