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Influence of water matrix on copper-catalysed continuous ozonation and related ecotoxicity

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ABSTRACT

The continuous ozonation of a mixture of carboxylic acids (formic, acetic, oxalic and maleic) has been performed under non-catalytic and copper-catalysed ozonation using a synthetic water matrix and a real sewage treatment plant (STP) effluent. The aim was to study the effect of water matrix on catalytic performance, particularly considering the toxicity of treated water to aquatic organisms. The non-catalytic ozonation of carboxylic acids in synthetic water resulted in a low reduction (36%) of the total organic carbon (TOC), the main feature being the accumulation oxalic acid due to the partial oxidation of maleic acid. Catalytic ozonation, adding copper concentration of 20 $\mu g\,L^{-1}$, achieved a TOC reduction of 75%, mainly due to the total depletion of oxalic acid. In wastewater effluent, the same general pattern was found with oxalic acid as the main by-product and its almost complete removal in catalytic ozonation. However, to attain the latter it was necessary to use copper concentrations as high as 100 $\mu g\,L^{-1}$. Copper proved to be a good catalyst for the oxidation of oxalic at near neutral pH, with short reaction times and matrix with high scavenging rate. The aquatic toxicity of treated mixtures was studied by means of five standard species placed on different trophic levels: *Vibrio fischeri*, *Pseudomonas putida*, *Pseudokirchneriella subcapitata*, *Tetrahymena thermophila* and *Daphnia magna*. The results showed that copper in STP effluent was less toxic than in synthetic water, an effect attributed to copper complexation with organic and inorganic compounds present in the matrix. The reduced biological availability could also explain the lower catalytic effect observed in real wastewater.

1. Introduction

Ozone is widely used in drinking water and wastewater reclamation treatments due to its high disinfection power and oxidation potential [1]. The direct ozonation of organic compounds results in many refractory oxidation by-products, particularly carboxylic acids [2]. Different ozone-based processes have been developed to improve ozone oxidation performance in order to increase the degree of mineralization. These technologies include O_3/OH^-, O_3/H_2O_2, and O_3/UV and belong to the group of advanced oxidation process (AOP) based on the generation of hydroxyl radicals ($OH^\bullet$). Contrary to ozone, $OH^\bullet$ reactions are not selective, but their concentration depends on the scavenging rate of the water matrix [3–5].

Catalytic ozonation has also been proposed to increase the degree of mineralization and reduce ozone consumption [6,7]. Different transition metals and oxides have been studied as ozonation catalysts [3]. Among them, copper has shown a significant catalytic effect in the degradation of carboxylic acids [4,8–12]. It has been noted that the performance of catalytic ozonation strongly depends not only on the catalyst itself, but on the composition of water matrix [2,12]. Moreover, most catalytic ozonation studies have been carried out in batch or semi-batch conditions, but more relevant data would be obtained from continuous ozonation devices. Contrary to batch processes in which a well-defined reaction time is established, continuous treatments display a statistical distribution of residence times [13].

Catalytic ozonation is able to remove certain pollutants, but can generate new compounds as oxidation by-products and from the leaching of catalyst active phases, which may be more hazardous

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than the original mixture [6,14]. Treated water is a complex mixture of organic and inorganic compounds, whose ecotoxicological impact cannot be predicted by simple chemical determinations due to the potential interactions among pollutants [14]. The chemical analyses in which regulations are based identify and quantify trace metals in an aquatic environment. However, they do not provide direct indication of the potential effects of the metals on the biota [15]. Thus, ecotoxicological bioassays are required to provide a holistic direct estimation of the environmental hazard of a given mixture. In particular, metal ecotoxicity is directly affected by physico-chemical parameters such as pH, alkalinity, hardness and dissolved organic and suspended matter, which alter its speciation and bioavailability [16,17], and, indirectly, through synergistic or antagonistic effects [18,19]. Therefore, aquatic toxicological assessment should include a battery of different species representative of the different taxa in the trophic chain [20], with emphasis on organisms placed at the bottom, like phytoplankton and zooplankton, where damage caused by metals primarily occur [21]. Many ozonation catalytic studies have been carried out in ultrapure water neglecting the effects on catalyst performance of the organic and inorganic species present in real matrices. Similarly to the influence of water matrix composition over metal ecotoxicity through the bioavailability concept, the same behaviour could be applied to the influence of water matrix on copper catalytic availability.

The aim of this study was to explore the effect of the water matrix on the non-catalytic and copper-catalysed continuous ozonation of a mixture of carboxylic acids (formic, acetic, oxalic and maleic acid). These compounds are present in ozonated water as reaction intermediates or final ozone-refractory by-products. We used homogeneous catalyst due to simplicity of application in continuous processes, but in view of the low concentration used, the results could be extrapolated to the effect of active phase leaching in heterogeneous catalysis. The ecotoxicity of ozonated water was tracked using a battery of bioassays composed of five single species tests: *Vibrio fischeri*, *Pseudomonas putida*, *Pseudokirchneriella subcapitata*, *Tetrahymena thermophila* and *Daphnia magna*.

2. Materials and methods

2.1. Materials

Formic, acetic, oxalic and maleic acid and copper ($Cu(NO_3)_2 \cdot 3H_2O$) of analytical degree were purchased from Fluka. The initial carboxylic acid mixtures were prepared with a concentration of 7 mg L^{-1} each. These organic acids and concentrations have been chosen because they have been previously identified and quantified as the main final ozonation by-products in a previous work dealing with the ozonation of pharmaceutical and personal care products in the same STP effluent [22]. These acids were the main responsible of the relative low mineralization degree achieved in direct ozonation runs.

In order to study the effect of the water matrix over the ozonation performance, two different matrices were used: a synthetic matrix and wastewater from the effluent of a sewage treatment plant (STP) located in Alcalá de Henares (Madrid, Spain). Synthetic water was prepared with the required amount of $NaHCO_3$ in ultrapure water to equal the alkalinity and pH values of the STP effluent. Ultrapure water was obtained from a Millipore Milli-Q system with a resistivity of at least 18 MΩ cm at 25 °C. The STP treats a mixture of domestic and industrial wastewater from facilities located near the city and has a nominal capacity of 3000 m^3 h^{-1} of raw wastewater. Details on wastewater characterization are included as supplementary data (Table S1).

2.2. Experimental procedure and analytical methods

The experiments were carried out in continuous mode in a cylindrical reactor made of Pyrex (internal diameter of 6 cm and working height of 51 cm) with a total working volume of 1.44 L operated in co-current mode (Scheme 1). Water flow rate was 142 mL min^{-1} (Gilmont rotameter) and gas flow was 390 mL min^{-1} (Aalborg mass flow controller) with different inlet ozone concentrations (Anseros ozone generator COM-AD-02). Inlet and outlet ozone gas concentration (Anseros ozone GM-PRO analyser), dissolved ozone in the reactor exit (Mettler Toledo-Thomton dissolved ozone sensor), pH and temperature (Easyferm Plus VP 120 Hamilton pH sensor) were constantly monitored and recorded (Keithley 2700 Data Acquisition System). Copper solution was continuously added to the inlet stream at different flows (Harvard 11 plus infusion pump) to achieve the desired final concentration. In order to ensure homogeneity a nine-loop glass coiled pipe was used. The dilution ratio was always lower than 1%.

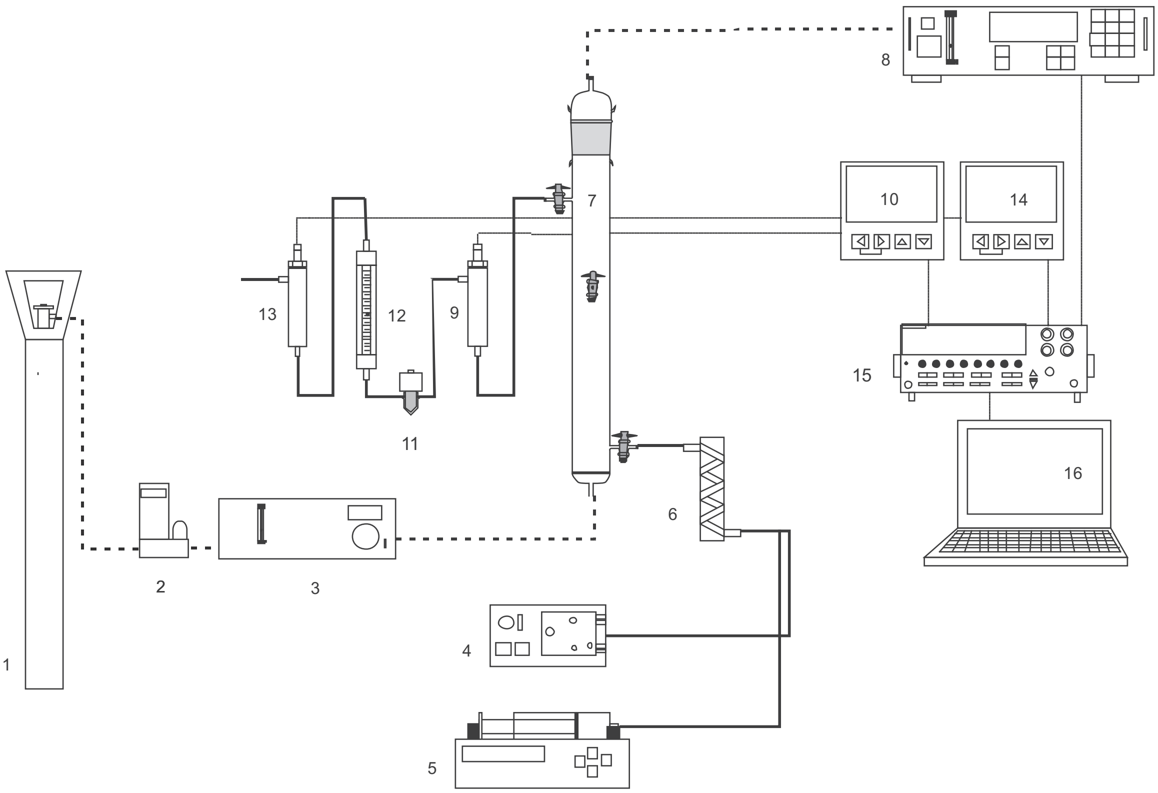
For every set of working conditions, samples were withdrawn for analysis at the column outlet once the stationary state was reached. This was accomplished after circulating four times the hydraulic retention time after a constant ozone value was obtained both in liquid and gas phases at the column outlet. The retention time distribution curve yielded an average retention time of 10.3 min and was analysed using the continuous stirred tank reactor CSTR in series model according to the procedure described in the literature [23]. The equivalent value of 1.13 tanks obtained indicated that the column can be approached to a perfect CSTR. It is generally accepted that short columns with intense gas phase hydrodynamics can be assimilated to a CSTR due to the bubble back mixing [24]. Assuming CSTR behaviour, the amount of ozone consumption at the stationary state $dC_{O_3}^{liq}/dt = 0$ can be obtained from the following mass balance (Eq. (1)) in which F_{O_3} is the rate of ozone entering the system in the gas phase (gas, in) or existing either in the exhaust gases (gas, out) or dissolved in water (liquid, out):

$$\text{Consumed O}_3 = F_{O_3}^{gas,in} - F_{O_3}^{gas,out} - F_{O_3}^{liq,in} \quad (1)$$

The concentration of organic acids was measured using a Dionex DX120 Ion Chromatograph (IC) with conductivity detector. Oxalic and maleic acid concentrations were determined using an IonPac AS9-HC analytical column (4 × 250 mm) with ASRS-Ultra suppressor, whereas acetic, glyoxalic and formic acids were measured with an IonPac ICE-AS6 analytical column (9 × 250 mm) with AMMS ICE II suppressor. Total organic carbon (TOC) analyses were performed on a Shimadzu TOC-V$_{CSH}$ total carbon organic analyser equipped with an ASI-V autosampler. The concentration of copper was determined by Agilent 7700× ICP-MS operating at 3 MHz in helium cell gas mode.

2.3. Aquatic toxicity bioassays

The ecotoxicity of water samples was assessed by means of five bioassays using *V. fischeri*, *P. putida*, *P. subcapitata*, *T. thermophila* and *D. magna*. The battery of tests allowed the combination of acute and chronic assays and the combined use of prokaryotes and eukaryotes at several trophic levels. All these bioassays were conducted according to standard operational guidelines [25–29]. More details about the aquatic toxicity tests procedure are presented in supplementary data.



Scheme 1. Experimental set-up. 1 Oxygen cylinder, 2 mass flow controller, 3 ozone generator, 4 peristaltic pump, 5 syringe pump, 6 nine-loop coil, 7 bubble column, 8 ozone gas analyser, 9 dissolved ozone sensor, 10 dissolved ozone transmitter, 11 needle valve, 12 rotameter, 13 pH sensor, 14 pH transmitter, 15 data acquisition system, 16 computer. Water line is represented as solid line, gas line as dotted line and electrical wiring as dashed line.

3. Results and discussion

3.1. Synthetic water

3.1.1. Non-catalytic ozonation

The non-catalytic ozonation of carboxylic acids in the synthetic matrix was studied by keeping a constant flow of water and ozonating gas and changing the concentration of ozone. The amount of ozone per litre of water introduced varied with the purpose of determining the efficiency of ozone usage from 4.5 mg L^{-1} (0.44 g O_3 g TOC^{-1}), a low concentration at which ozone acts as a limiting reagent, to 93 mg L^{-1} (9.0 g O_3 g TOC^{-1}). Fig. 1a represents the evolution of TOC and consumed ozone as a function of the amount of ozone supplied. Up to 46 mg L^{-1}, TOC declined with ozone dosage up to a value for which it remained essentially constant. This initial zone (zone 1 in Fig. 1a) corresponded with the reaction of the more readily oxidizable acids. In it, ozone was the limiting reagent and the reaction was mass-transfer controlled as revealed by the fact that no dissolved ozone (<0.01 mg L^{-1}) was detected in solution (see Fig. S1, supplementary data). In zone 2, ozone consumption slightly increased up to a value of 71 mg L^{-1}. In this intermediate zone, TOC depletion stabilized and the increased consumption of ozone indicated the presence of organic matter oxidized but not mineralized. This zone corresponded to chemical control and, accordingly, an increase in the concentration of dissolved ozone concentration was detected. At higher ozone dosages, above 71 mg L^{-1} (zone 3 Fig. 1a), ozone consumption was almost constant and in parallel the concentration of ozone at the reactor outlet increased (Fig. S1, supplementary data). This value was considered the upper operational limit. The maximum TOC depletion with non-catalytic ozonation was low, at about 35%, a figure that corresponds with the well-known behaviour of direct ozonation processes [2].

Fig. 1b represents the evolution of the concentration of individual carboxylic acids with ozone dosage. A good agreement was observed between the experimental TOC and the theoretical TOC calculated from the concentration of the acids detected with ion chromatography (>90%). Other organic reaction by-products were not detected. Maleic and formic acids were completely removed, acetic acid concentration was slightly reduced and the amount of oxalic acid increased during treatment, the latter being the main component of the final mixture (around 60% TOC). Glyoxalic acid, an acid not present in the initial mixture, was detected as a reaction by-product. The glyoxalic acid concentration was detected for low ozone dosages to further reach a plateau and decrease thereafter with increased ozone input.

Maleic acid was the most reactive component and it was the only one oxidized for the lowest ozone dosages with the simultaneous evolution of glyoxalic acid. Under these conditions, maleic acid depletion (30.2 μmol L^{-1}) generated 30.3 μmol L^{-1} of glyoxalic acid, displaying an almost stoichiometric conversion. This oxidation from maleic to glyoxalic acid has been previously reported together with the formation of formic acid [10,30,31]. In this study, a TOC reduction of 69.2 μmol L^{-1} (almost two-fold maleic depletion) was observed. These facts suggest that roughly half of the maleic acid was converted to glyoxalic acid, with the rest being mineralized to CO_2. A tentative reaction pathway is presented in supplementary data (Scheme S1).

At a higher ozone dosage, other reactions took place, such as the depletion of formic acid. Maleic and formic acids totally disappeared after dosing 46 mg L^{-1} of ozone, after which no further TOC depletion took place (Fig. 1a). Final TOC removal, 3.41 mg L^{-1}, was in good agreement with the total mineralization of formic acid, 6.57 mg L^{-1} (1.75 mg TOC L^{-1}), and the above-explained elimination of two CO_2 moles per mol of maleic acid depleted (1.67 mg TOC L^{-1}), suggesting that both acids essentially account

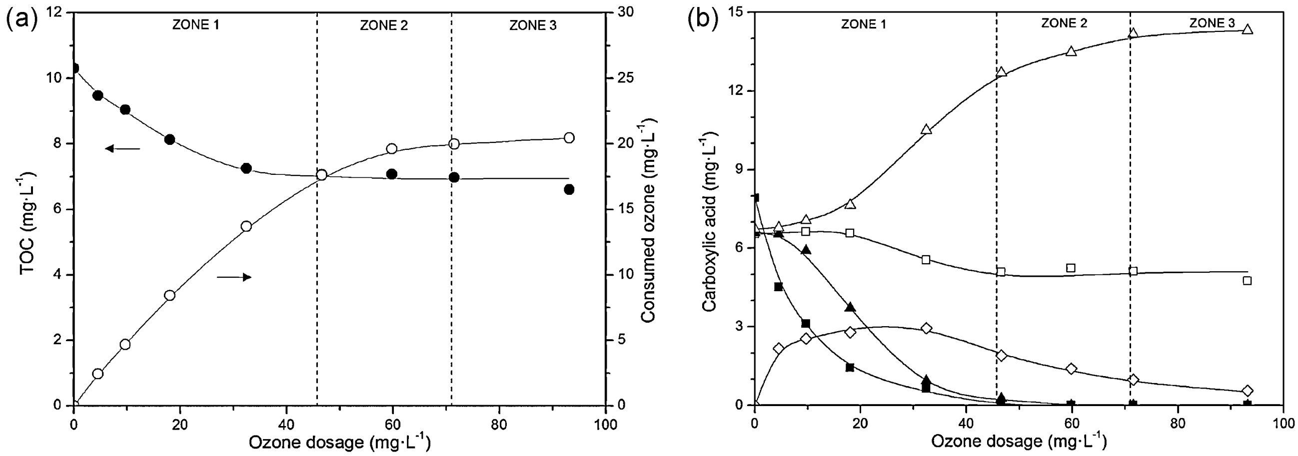


Fig. 1. Evolution of (a) TOC (●), consumed ozone (○) and (b) the concentration of acetic (□), glyoxalic (◇), formic (▲) maleic (■) and oxalic (△) acid with ozone dosage in the synthetic water matrix.

for all TOC reduction. In spite of the reaction of glyoxalic acid, the concentration of which was reduced, it was not mineralized but rather oxidized to oxalic acid. This fact is well-documented and explains the fate of both acids [10,32,33]. Initially, glyoxalic concentration increased due to maleic acid oxidation, reducing at higher ozone dosages due to its oxidation to oxalic acid (Scheme S1, supplementary data). Acetic, glyoxalic and, particularly, oxalic acid were the main contribution to final TOC in treated water (>90%), which is compatible with their well-known refractory character [22,34,35].

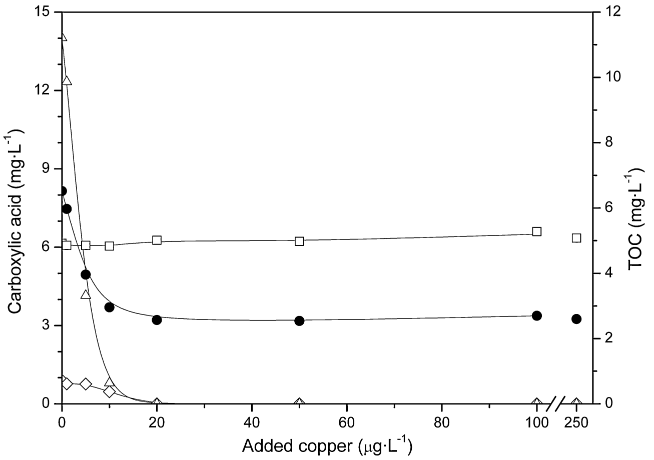


Fig. 2. Evolution of TOC (●) and the concentration of acetic (□), glyoxalic (◇) and oxalic (△) acid with added copper in the synthetic water matrix. Ozone dosage 71 mg L^{-1}.

3.1.2. Catalytic ozonation

Copper-catalysed continuous ozonation was carried out with increasing amounts of copper (from 1 to 250 µg L^{-1}) for a fixed ozone dosage of 71 mg L^{-1}, which represented the maximum conversion obtained with non-catalytic ozonation. Fig. 2 displays TOC reduction as copper concentration increased. A remarkable TOC depletion was observed even with the lowest concentration (1 µg L^{-1}), which increased with increasing copper concentration up to 20 µg L^{-1}, for which TOC removal reached around 75% (two-fold higher than that observed in non-catalysed reaction). Fig. 2 also represents the concentration of individual carboxylic acids. The strong influence of copper is apparent over oxalic and glyoxalic acids, the concentration of which decreased with the amount of added copper and became completely removed for 20 µg L^{-1} of the catalyst. The depletion of both acids fitted well with the observed TOC reduction indicating the mineralization of both acids. After the removal of these acids, no more TOC depletion took place and the only acid detected was acetic acid, whose contribution to final TOC

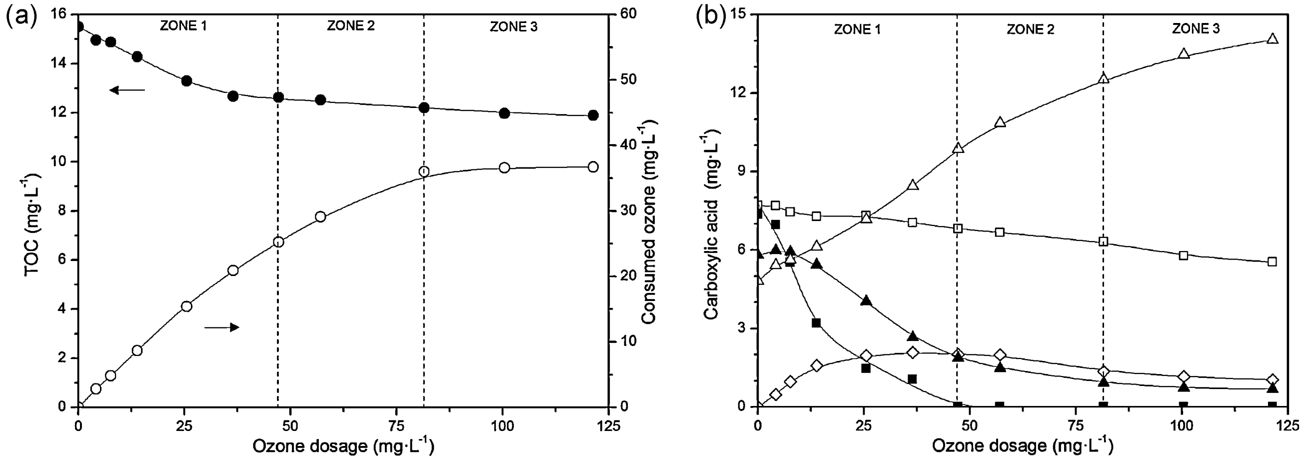


Fig. 3. Evolution of (a) TOC (●), consumed ozone (○) and (b) the concentration of acetic (□), glyoxalic (◇), formic (▲) maleic (■) and oxalic (△) acid with ozone dosage in STP effluent.

was essentially 100%. Oxalic acid accumulated in direct ozonation and got depleted in catalytic ozonation due the ability of copper in catalyzing its decomposition.

The two major mechanisms proposed in the literature for the homogeneous catalytic ozonation are the decomposition of ozone by metal ions leading to the generation of radicals and the formation of complexes between catalysts and the organic molecule followed by the oxidation of the former [7]. In order to elucidate the reaction pathway of copper oxalate, catalytic ozonation runs were carried out using *t*-butanol (30 mM) as a radical scavenger. The presence of *t*-butanol did not inhibit oxalate depletion, confirming that the catalysed reaction does not proceed by radical pathway but via complex formation [7]. Some authors claim that oxalic acid reacts relatively slow with hydroxyl radicals [8,12]. Other works suggest that the catalytic ozonation of oxalate occurs via complex formation, which is in good agreement with our findings [8,11]. MINTEQ chemical equilibrium model was used to calculate the chemical speciation of copper [36]. The modelling results of the synthetic water matrix showed that most copper concentration was present as oxalate complexes in the initial mixture (13 out of 20 μg L^{-1}, see Table S2, supplementary data), which displaced bicarbonate, the predominant complexing anion in the absence of oxalate. This fact is interesting because of the ubiquitous presence of radical scavengers in natural water and wastewater (mainly carbonates and bicarbonates), which could hamper the oxidation through hydroxyl radicals [5,12]. Copper catalyst is highly active for the depletion of oxalic acid and, contrary to other transition metals, it is active at the natural pH of most surface waters and wastewaters [7,11]. It is also interesting to point out that the low concentration of homogeneous copper necessary for oxalic depletion should be taken into account while testing heterogeneous copper catalysts because a small leaching of the active could represent an important contribution.

3.2. Wastewater matrix

3.2.1. Non-catalytic ozonation

In this study we used real biologically treated wastewater as an alternative matrix for the carboxylic acids ozonation. The organic compounds present in the matrix before adding the organic acids were essentially refractory to ozonation under the working condition used in this study, achieving a mineralization value lower than 5% (Fig. S2, supplementary data). However, ozone was consumed up to 15 mg L^{-1} as a result of partial oxidation reactions, which can be traced by the reduction (65%) of the specific ultraviolet absorption at 254 nm ($SUVA_{254}$); the parameter that provided an indirect measure of the aromaticity of the dissolved organic matter. The refractory character of wastewater TOC was previously reported [22]. The evolution of TOC and the ozone consumption during ozonation in the wastewater matrix spiked with organic acids are represented in Fig. 3a. A similar behaviour to the synthetic matrix (Fig. 1a) was observed. For lower ozone dosages (zone 1) TOC decreased with increasing ozone up to a value of 47 mg L^{-1} for the latter and remained constant afterwards. After the ozone dosage of 81 mg L^{-1} mineralization did not further proceed. This value was taken as a reference for the treatments in the wastewater matrix described below. The maximum TOC removal was 22%, considerably lower than that observed in the synthetic matrix. Taking into account the refractory character of organic natural matter present in wastewater, it can be argued that TOC removal corresponded essentially to the depletion of the acids added to the matrix. Maximum ozone consumption was 37 mg L^{-1}, which was higher than the value obtained in the synthetic matrix and close to the sum of consumed ozone by the matrix, 15 mg L^{-1} (Fig. S2, supplementary data), and by the depletion of carboxylic acids, 20 mg L^{-1} (Fig. 1a). Fig. 3b represents the evolution of individual acids with increasing ozone dosage. The pattern was similar to that found in the synthetic water matrix with maleic acid being readily eliminated. Glyoxalic acid also appeared as an oxidation by-product and was further oxidized to oxalic acid, which

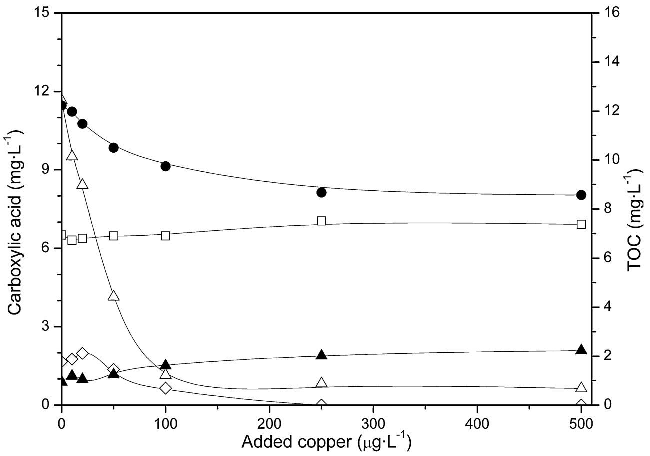


Fig. 4. Evolution of TOC (●) and the concentration of acetic (□), glyoxalic (◇), formic (▲) and oxalic (△) acid with added copper in STP effluent. Ozone dosage 81 mg L^{-1}.

Table 1
Effects of acid mixture addition and copper EC_{50} in three water matrices on *V. fischeri*, *P. putida*, *P. subcapitata*, *T. thermophila* and *D. magna*. (Mean ± 95% confidence interval).

Inhibition/immobilization (%)	*V. fischeri*	*P. putida*	*P. subcapitata*	*T. thermophila*	*D. magna*
Synthetic matrix[a]	−2 ± 1	−10 ± 3	−10 ± 1	8 ± 1	6 ± 2
STP effluent	13 ± 4	−21 ± 1	−40 ± 5	15 ± 3	3 ± 1
Acid mixture in MQ water[b]	4 ± 1	−5 ± 1	5 ± 2	5 ± 3	12 ± 3
Acid mixture in synthetic matrix	−8 ± 1	−13 ± 4	−12 ± 2	10 ± 1	5 ± 1
Acid mixture in STP effluent	5 ± 2	−17 ± 3	−45 ± 4	9 ± 3	0 ± 1
Ozonated acid mixture in synthetic matrix[c]	−14 ± 5	9 ± 6	−15 ± 7	8 ± 3	10 ± 3
Ozonated acid mixture in STP effluent[d]	5 ± 3	−16 ± 4	−42 ± 6	6 ± 2	10 ± 2
Copper EC_{50} (μg L^{-1})					
In MQ water	820 ± 90	29.5 ± 3.5	20.6 ± 2.6	400 ± 38	20.5 ± 3.9
In synthetic matrix	1750 ± 180	21.9 ± 2.6	29.8 ± 3.5	306 ± 41	51.1 ± 8.8
In STP effluent	1730 ± 210	19.1 ± 3.6	53.9 ± 9.5	284 ± 50	293 ± 33

[a] Milli-Q water buffered with 276 mg L^{-1} of $NaHCO_3$.
[b] Mixture of formic, acetic, oxalic and maleic acid with a concentration of 7 mg L^{-1} each.
[c] Ozone dosage in non-catalytic process 71 mg L^{-1}.
[d] Ozone dosage in non-catalytic process 81 mg L^{-1}.

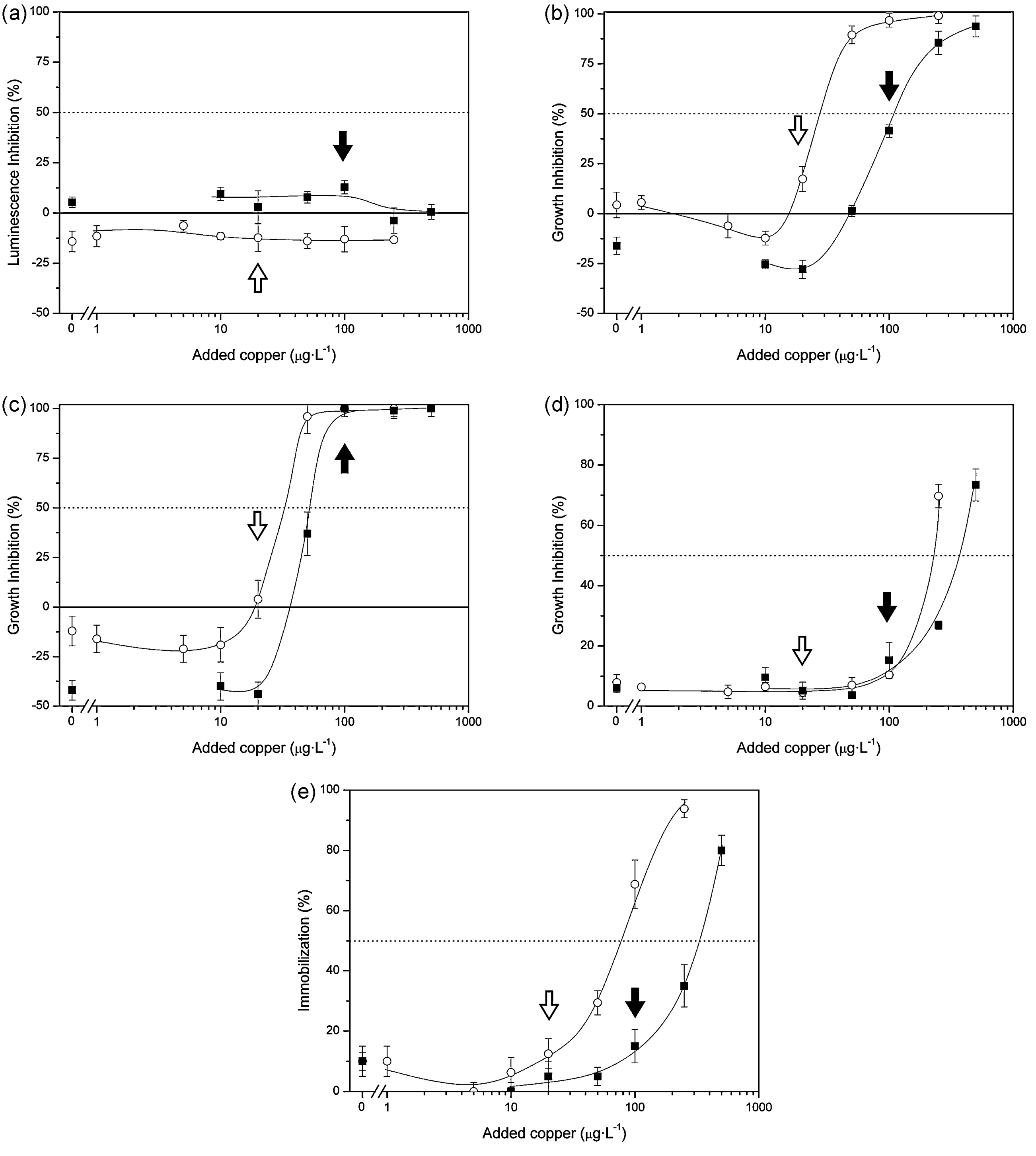


Fig. 5. Evolution of the effects of catalytic ozonated samples for different amount of added copper in the synthetic water matrix and STP effluent on (a) *V. fischeri*, (b) *P. putida*, (c) *P. subcapitata*, (d) *T. thermophila* and (e) *D. magna*, (mean ± 95% confidence interval). White and black arrows represent the operational concentration of added copper for synthetic and STP effluent.

accumulated steadily in treated wastewater. The final concentration of glyoxalic acid was noticeably higher in STP effluent and, contrary to the synthetic matrix, formic acid was only partially removed.

3.2.2. Catalytic ozonation in wastewater

The catalytic ozonation in wastewater was carried out using copper concentrations ranging from 10 to 500 μg L^{-1} and a fixed ozone dosage of 81 mg L^{-1}. Fig. 4 displays the evolution of TOC with the increasing copper concentration. Similarly to the synthetic matrix, a strong improvement of TOC depletion (around 45%) was achieved with the increasing catalyst concentration. The evolution of TOC can be explained as following that of individual acids also shown in Fig. 4. The concentration of glyoxalic acid increased initially to decrease when a higher amount of copper was added. The concentration of formic acid slightly increased with the copper concentration probably indicating that formic acid is a by-product of the oxidation of organic matter present in wastewater. Nevertheless, the main contribution to TOC depletion was due to the removal of oxalic acid. It is noteworthy that total oxalic acid depletion was not achieved even at the highest copper concentration (500 $\mu g\,L^{-1}$). No improvement was found in oxalic acid depletion for copper concentration above 100 $\mu g\,L^{-1}$, which is five-fold the concentration required in the synthetic water matrix. The chemical copper speciation (MINTEQ model) in STP effluent using the available data (Table S1, supplementary data) and common assumptions on the nature of organic matter in wastewater effluents [37], leads to a concentration of copper-oxalate complexes of 12 $\mu g\,L^{-1}$ at operational copper concentration of 100 $\mu g\,L^{-1}$ (see Table S3, supplementary data). This value was near to the amount of copper-oxalate complexes (13 $\mu g\,L^{-1}$) obtained for the synthetic matrix adding 20 $\mu g\,L^{-1}$of copper.

3.3. *Aquatic toxicity assessment*

The minimum amount of copper used in this work which achieved the highest TOC depletion for synthetic matrix and STP effluent, 20 and 100 $\mu g\,L^{-1}$, were well below the standard water quality regulated or recommended for different uses of reclaimed water. US EPA recommends a maximum of 200 (long-term) or 5000 $\mu g\,L^{-1}$ (short-term) of copper in water reused for irrigation [38]. Nonetheless, in spite of the good activity of copper catalysts in ozonation processes, concern about toxicity of treated water must be addressed in order to ensure the absence of negative impacts on receiving water bodies.

Aquatic toxicity data show that both water matrices, the mixture of organic acids and non-catalytically ozonated water did not present noticeable toxic effects on single species tests (Table 1). On the contrary, the studied organisms were sensitive to copper presence as demonstrated by the low EC_{50} values in the three water matrices: Milli-Q water, synthetic matrix and STP effluent. For ultrapure water, the reported aquatic toxicity values are in agreement with the data in the literature for *V. fischeri* ($EC_{50} = 640\ \mu g\,L^{-1}$ in Heinlaan et al. [39] and 740 $\mu g\,L^{-1}$ in Lappalainen et al. [40]), *P. subcapitata* ($EC_{50} = 16.5\ \mu g\,L^{-1}$ in Heijerick et al. [41] and 20 $\mu g\,L^{-1}$ in Aruoja et al. [42]), *T. thermophila* ($EC_{50} = 470\ \mu g\,L^{-1}$ in Gallego et al. [19]) and *D. magna* ($EC_{50} = 18\ \mu g\,L^{-1}$ in Kim et al. [43] and 24 $\mu g\,L^{-1}$ in Postma et al. [44]).

The evolution of the effects of catalytically ozonated samples for different amounts of copper in synthetic water and STP effluent on the battery of biotests are presented in Fig. 5. Increased concentration of copper caused an increase in the toxic effects of studied organisms except for *V. fischeri*. These data suggest that, regardless of the possible combined effect of other compounds present or formed during ozonation, copper appeared to be the main source of toxicity. It is also interesting to note that very low amounts of copper led to a hormetic effect on both matrices with remarkable stimulation on *P. putida* and *P. subcapitata* growth, most probably due to the assimilable organic matter [45], bicarbonate [46] and/or extra amounts of nitrate and phosphate [47].

As can also be seen in Fig. 5, copper-catalysed samples caused a considerably lower toxicity in STP effluent than in the synthetic matrix, with the water matrix effect ratio (the ratio in terms of added copper in STP effluent and synthetic matrix in order to obtain a 50% of inhibition/immobilization) in the interval 1.6–4.4 except for *V. fischeri*. It is important to stress that the impact of copper on aquatic organisms does not only depend on its nominal concentration, but also on its bioavailability, which is influenced by water quality parameters such as pH, hardness, alkalinity and dissolved organic matter. Copper has been described as presenting high complexation capacity with both inorganic [48] and organic ligands [49,50], which influences its effect on biological organisms. It has also been noted that the presence of natural organic matter considerably reduces copper toxicity to *V. fischeri* [51,52]. Heijerick et al. revealed that copper toxicity in natural waters to *P. subcapitata* (32–245 $\mu g\,L^{-1}$) is mainly determined by the concentration of dissolved organic carbon [41]. Naddy et al. and De Schamphelaere and Jansen showed that copper EC_{50} values for *D. magna* in artificial media without organic matter vary between 4 and 57 $\mu g\,L^{-1}$ [53,54]. For natural water and wastewater, the presence of dissolved organic matter drastically decreases copper toxicity (34–1086 $\mu g\,L^{-1}$) as a consequence of copper-complexation [37,55]. Moreover, as the water matrix changes during the ozone treatment, the copper speciation also changes and consequently, so does the water toxicity. Thus, in the synthetic matrix, the sharp increase in the response curves (Fig. 5) started at a copper concentration of about 20 $\mu g\,L^{-1}$. For lower concentrations, oxalic and glyoxalic acid, whose copper complexation capacity is high [12], were present in the mixture and probably contributed to a reduced copper bioavailability. For increased amounts of copper the main organic acid was acetic, whose complexation capacity is low [56], and a sharp toxicity increase was obtained accordingly. In STP effluent, the steep toxicity increase takes place at doses above 100 $\mu g\,L^{-1}$ except for *P. putida* and *P. subcapitata*, the organisms with higher sensitivity for copper in this water matrix.

Focus on the effects of the minimum amount of copper used to achieve the highest TOC depletion on the single species tests (see arrows in Fig. 5); catalytic ozonation in the synthetic matrix adding 20 $\mu g\,L^{-1}$ generated treated water with no significantly different inhibition/immobilization with respect to samples obtained from non-catalytic ozonation, causing an inhibition/immobilization below 15% for all single species tests. Otherwise, catalytically ozonated water from STP effluent using 100 $\mu g\,L^{-1}$ was notably toxic to *P. putida* (42% growth inhibition) and, particularly, to *P. subcapitata* (100% growth inhibition). For the rest of the species, the effect was below 15%. Despite adding an amount of catalyst five-fold higher in STP effluent than in synthetic water, the toxicity was not affected in the same proportion as a consequence of the above-explained matrix effects.

4. Conclusions

Copper-catalysed continuous ozonation significantly improves organic acid mineralization, mainly due to its high performance in oxalic acid depletion at near neutral pH, with short reaction time and in water matrices with high scavenging rate.

The same copper concentration is less toxic in STP effluent than in the synthetic water matrix, an effect attributed to copper complexation with organic and inorganic compounds present in the wastewater that reduce its bioavailability.

Catalytic ozonation is also strongly influenced by the water matrix. The copper catalytic reaction proceeds through a selective complex reaction pathway so that complexation with STP effluent organic matter reduces the availability of metal for catalysis. Thus, in wastewater, a five-fold copper concentration is necessary to achieve similar oxalic depletion to that obtained in the synthetic water matrix.

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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.apcatb.2014.08.007.

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