Catalytic ozonation by metal ions for municipal wastewater disinfection and simulataneous micropollutants removal

Jacqueline A. Malvestiti[(a)], Alberto Cruz-Alcalde[(b)], Núria López-Vinent[(b)], Renato F. Dantas*[(a)], Carme Sans[(b)]

[(a)] School of Technology, University of Campinas – UNICAMP, Paschoal Marmo 1888, 13484332, Limeira-SP, Brazil.
[(b)] Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, C/Martí i Franqués 1, 08028 Barcelona, Spain.

*Corresponding author. E-mail address: renatofalcaod@ft.unicamp.br, phone: +55 19 21133365, fax: +55 19 21133305.

Abstract

This work evaluated the effects of Fe^{2+}, Co^{2+} and Al^{3+} in municipal wastewater disinfection through *E.coli* and *Pseudomonas spp* inactivation along with cellular adenosine triphosphate (ATP) depletion. Simultaneously, the effect of catalytic ozonation of secondary effluent on the removal of selected micropollutants with different ozone kinetics (acetamiprid, dichlorvos and atrazine) was evaluated. *E.coli* and *Pseudomonas spp* inactivation increased almost 20% with 1 mgL^{-1} Fe^{2+}, Co^{2+}, Al^{3+} and 40% with 10 mgL^{-1} Fe^{2+} compared with single ozonation. The bacteria reactivation after the treatments showed that Fe^{2+} was the most effective metal ion on inhibiting regrowth. The cellular ATP followed the same trend as the indicators microorganisms inactivation, with significant reduction of ATP over the treatment compared to single ozonation. Finally, the $HO^{\bullet}$ exposure per consumed ozone ratio was applied for single ozonation, Fe^{2+}/O_3, Co^{2+}/O_3 and Al^{3+}/O_3 processes to evaluate and compare the contribution of radical pathway on micropollutants abatement.

Keywords: Metals ions; *E. coli*; *Pseudomonas spp*; R_{OHO3}; O_3-resistant micropollutants.

1. Introduction

Due to the excessive population growth and rapid urbanization, especially in developing countries, there is an accelerated wastewater generation and the lack of convenient wastewater treatment plants (WWTP) and drainage facilities. This context leads to the pollution of the water resources, which makes their recycling and self-regulating capability very limited [1]. Effluents coming from inefficient WWTP may cause serious problems to human health and the environment since it may contain undesirable chemical constituents and pathogens [2]. Therefore, wastewater disinfection is the most important step to protect ecosystem safety and human health when reuse is the final goal [3,4].

Besides to disinfection, attention should also be given to chemical pollutants which have great diversity and high degree of ecosystem toxicity [5,6]. Many of these compounds are mostly present in very small concentrations ranging from μg/L to below ng/L [7,8]. The incapability of conventional wastewater treatments to effectively remove many pollutants such as nonbiodegradable and toxic pollutants makes imperative that innovative methods for wastewater treatment must continuously being explored, especially when the aim is the wastewater recycle and reuse.

Advanced oxidation processes such as ozonation have been widely used for microorganism's inactivation [9,10] demonstrating to be an efficient technique for urban effluents disinfection, even in the inactivation of more resistant organisms than *Escherichia coli* [11,12]. Besides it appears as one of the most promising treatments for removal of organic contaminants even in low concentrations [7]. However, its reactivity toward organic pollutants is selective. Ozone reacts mainly with compounds by specific reaction pathways such as electrophilic, nucleophilic or dipolar addition reactions (direct reaction with O_3) [13]. In contrast, the hydroxyl radical ($HO^\bullet$) formed by O_3 decomposition is a nonselective oxidant and highly reactive with organic pollutants [13, 14], which promotes the complete degradation of organic pollutants, even reaching the mineralization of many micropollutants [15]. Therefore, ozone-resistant micropollutants are abated almost exclusively by $HO^\bullet$ oxidation during ozonation [16].

One of the strategies to promote greater generation of $HO^\bullet$ is the catalytic ozonation, through the insertion of transition metals in solution (homogeneous catalytic ozonation) or through the addition of dispersed solid metal oxides (heterogeneous catalytic ozonation). The mechanism of homogeneous catalytic ozonation follows two main pathways. The first one is based on the acceleration of ozone decomposition by the generation of the $^\bullet O_2^-$ and $^\bullet O_3^-$ radicals and subsequently $HO^\bullet$ formation [11,17]. The other one is based on formation of complexes between the catalyst and the organic compound, followed by a final oxidation reaction [18]. Therefore, metal ions are able to enhance the efficiency of single ozonation for the removal of different organic compounds in aqueous solution, particularly those recalcitrant to direct ozone oxidation [19].

Several works on literature demonstrated that metal-catalyzed homogeneous ozonation is efficient in treating wastewater, especially regarding the oxidation of micropollutants [20-22] while others are devoted to microorganisms inactivation [23,24]. However, there are few studies describing the microbial inactivation and micropollutants removal simultaneously in WWTP effluent samples [23,25].

Pesticides acetamiprid (ACMP), dichlorvos (DDVP) and atrazine (ATZ), considered as priority substances, have an uncontrolled use in several countries. These compounds call attention to the potential environment damages because these substances are toxic, persistent and able to bioaccumulate [26]. Due to the widespread employment of these pesticides, almost any water and wastewater stream contains such compounds [6, 27], therefore, the removal of this type of contaminant must be effective.

ACMP and ATZ are resistant to direct oxidation by ozone [28,29], while DDVP has a medium resistance [30]. Due to variation of micropollutants reactivity with ozone, the use of kinetic models to predict micropollutants removal such as the dynamic second order kinetic-based method that includes $HO^{\bullet}$ exposure ($\int [HO\ \bullet]_{dt}$) and total O_3 exposure ($\int [O_3]_{\boldsymbol{dt}}$) could be used (Equation 1), however the radicals availability remains still undetermined.

$$-\ln([MP]/[MP]_0) = k_{MP,O_3} \int [O_3]\, dt + k_{MP,OH\cdot} \int [OH\ \cdot]\, dt \quad (1)$$

The kinetic modeling of removal of pesticides in the secondary effluent is also very important as it provides details of how they behave when they undergo treatment such as ozonation. Recently, Kwon et al. [31] developed a new kinetic parameter defined as the $HO^{\bullet}$ exposure per consumed O_3 (Transferred Ozone Dose) (Equations 2 and 3).

$$\mathrm{R_{OHO_3}} = \frac{\int [\mathrm{HO}\cdot]\mathrm{dt}}{\mathrm{TOD_t}} \qquad (2)$$

$$TOD = \int_0^t \frac{F_g}{V_{liq}} \cdot ([O_3]_{in} - [O_3]_{out}) \cdot \mathrm{d}t \quad (3)$$

where F_g, V_{liq} represent the gas flow and the volume of the liquid phase respectively; t is the contact time; and $[O_3]_{in}$ and $[O_3]_{out}$ correspond to the inlet and outlet ozone concentrations in the gas phase, respectively.

A higher $R_{OH,O3}$ value indicates a higher $HO^{\bullet}$ formation, so the $HO^{\bullet}$ yield from O_3 decomposition for different operation conditions or different wastewaters can be easily compared using the $R_{OH,O3}$ value. It can be easily determined through the monitoring of a $HO^{\bullet}$ probe compound during ozonation [32], based on its very slow direct reaction with ozone and fast reaction with $OH^{\cdot}$ radicals [33], and is particularly interesting to be applied in the abatement performance prediction of ozone recalcitrant micropollutants, like the ones included in this study, through Equation 4 [34].

$$-\ln\left(\frac{[MP]}{[MP_0]}\right) = k_{MP,OH^{\cdot}} * R_{OH,O_3} * TOD \quad (4)$$

The main objective of this work was to evaluate the catalytic effect of Fe^{2+}, Co^{2+} and Al^{3+} on ozone disinfection (through *E.coli* and *Pseudomonas spp* inactivation and cellular ATP depletion) and micropollutants removal (ACMP, DDVP and ATZ) in municipal wastewater samples. The catalytic ozonation effect on the inhibition of bacterial regrowth after the treatments was also studied. Finally, the influence of Fe^{2+}, Co^{2+} and Al^{3+} through the $R_{OH,O3}$ parameter was verified along with the utility of the $R_{OH,O3}$ concept in the modelling of the catalytic ozonation. As an OH radical probe compound was used the ACMP, which has $k_{O3:}$ 0.25 $M^{-1}s^{-1}$ and $k_{HO\bullet:}$ 2.1 x 10^9 $M^{-1}s^{-1}$ Cruz-Alcalde et al. [29].

2. Materials and methods

2.1. Chemicals and reagents

Acetamiprid, atrazine and 121 dichlorvos analytical standards used as target compounds, iron(II) sulphate, cobalt(II) nitrate hexahydrate and aluminium sulphate hydrate, employed as a catalysts, were acquired from Sigma-Aldrich (Germany). Ultrapure water was produced by a filtration system (Millipore, USA). Pure oxygen 123 (≥ 99.999%) was supplied by Abelló Linde (Spain). Acetonitrile and orthophosphoric acid were used as mobile phase for HPLC. GranuCult™ and Chromocult® agar were acquired from Merck (Spain) and BacTiter-Glo™ Microbial Cell Viability Assay from Promega (Spain).

2.2 Wastewater effluent

The secondary wastewater effluent was collected from a wastewater treatment plant (WWTP) in the province of Barcelona (Spain), after an Integrated Fixed-Film Activated Sludge (IFAS) treatment. The main quality parameters are listed in Table 1. (TOC: total organic carbon; COD: chemical oxygen demand; BOD: Biochemical oxygen demand; UV_{254}: specific absorbance at 254 nm; TSS: total suspended solids). All the effluent samples were stored at 4 ºC prior to be used in the experiments.

Table 1. Characterization of secondary effluent samples

Parameters	Units	Values	Parameters	Units	Values
E.coli	CFU (Log_{10})	4.34 ± 0.46	K^+	mg L^{-1}	42.11 ± 1.36
Pseudomonas spp	CFU (Log_{10})	3.46 ± 0.21	Na^+	mg L^{-1}	270.28 ± 18.2
pH	-	7.20 ± 0.33	S^{2-}	mg L^{-1}	61.65 ± 5.03
Turbidity	NTU	10.4 ± 1.06	Fe^{2+}	mg L^{-1}	0.18 ± 0.03
UV_{254}	Abs	0.40 ± 0.05	Al^{3+}	mg L^{-1}	-
COD	mgO_2 L^{-1}	60.75 ± 1.02	Co^{2+}	mg L^{-1}	-
BOD	mgO_2 L^{-1}	27.62 ±1.77	Cu^{2+}	mg L^{-1}	0.09 ± 0.03
TOC	mg L^{-1}	16.35 ±10.2	SO_4^-	mg S L^{-1}	160.88 ± 20.17
TSS	mg L^{-1}	68.0 ± 0.21	NO_2^-	mg N L^{-1}	0.1 ± 0.03
Alkalinity	$CaCO_3$ L^{-1}	405.54 ± 49.33	NO_3^-	mg N L^{-1}	0.55 ± 0.21
Mg^{2+}	mg L^{-1}	40.82 ± 5.02	Ca^{2+}	mg L^{-1}	118.12 ± 7.98

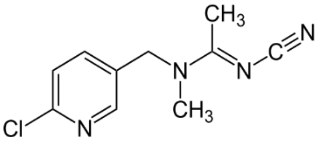
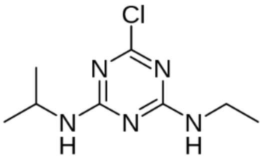
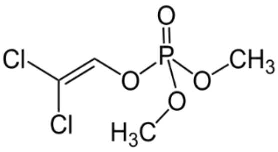
2.3 Ozonation of wastewater effluents

Ozonation experiments were performed in a 1.5 L jacketed reactor, operated in semi-continuous mode, without pH adjustment and unfiltered wastewater. The experiments were carried out at the natural pH of the wastewater and was followed during the reaction (Supplementary information Table S-1). Disinfection analysis were carried out with the indicator microorganisms naturally present in the effluent. The pesticides ATZ, DDVP and ACMP (Table 2) were spiked simultaneously to the sample and the same method was used for their analysis. For the micropollutants quantification, the linearity between the concentration of each pesticide and the area peaks were calculated and different concentrations in order to produced peaks of similar area, being 50, 200 and 100 μgL^{-1} of ATZ, DDVP and ACMP, respectively. Blank tests were performed to assess if the target micropollutants affected the bacterial inactivation and quantification. For this, triplicate tests were performed with the effluent without any type of treatment and then, the quantification of the indicator microorganisms were compared in the presence and absence of the contaminants.

For comparison purposes single ozonation and catalytic ozonation (Fe^{2+}/O_3, Co^{2+}/O_3 and Al^{3+}/O_3) with 1 mgL^{-1} of Fe^2, Co^{2+} and $Al^{3+\ +}$ and 10 mgL^{-1} of Fe^{2+}were carried out.

Table 2. Pesticides characteristics

Pesticide	Group	Chemical formula	Structure
Acetamiprid		$C_{10}H_{11}ClN_4$	

	Neonicotinoid		
Atrazine	Triazines	$C_8H_{14}ClN_5$	
Dichlorvos	Chlorinated organophosphorus	$C_4H_7Cl_2O_4P$	

Ozone was produced by a lab ozonator (Sander, Germany) and injected at the bottom of the reactor by means of a porous diffuser. A mechanical mixing system ensured the good contact between liquid and gas phases. The gas flow rate and the inlet ozone concentration were maintained at 0.2 $Lmin^{-1}$ and 10 mgL^{-1} respectively. Inlet and outlet gas-phase ozone concentrations were continuously monitored by two BMT 964 ozone analyzers (BMT Messtechnik, Germany) placed up and downstream of the reactor, respectively. The ozone concentration in the aqueous phase was measured by means of a Q45H/64 dissolved O_3 probe (Analytical Technology, USA) which was connected to a liquid recirculation stream. A complete description of the ozonation system can be found elsewhere [33]. The reaction medium was maintained at a temperature of 20 ± 2 °C and under stirring conditions. Samples were withdrawn at 0, 3, 5, 10, 20, 30, 40, 50 and 60 min and the microbiological parameters were immediately analyzed; the remaining samples were stored at 4 ºC.

The dead volume of the reactor was corrected by means of TOD calculations [33]. To ensure reproducibility the runs were repeated three times and the results were presented as average with standard deviation (SD). The Student’s t-test was used to compare the experimental data and the significance was accepted when the null hypothesis (p) was less than 0.05.

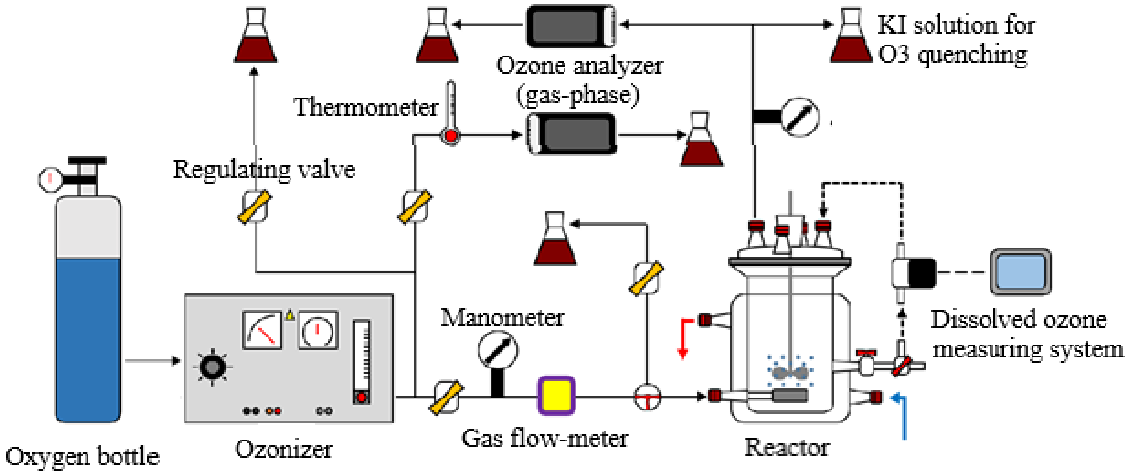


Figure 1. Ozonation setup schematic.

2.4 Analytical methods

After collection in bottles of 25 L, the samples were transported to the laboratory and stored at 4 °C. The characterization was performed as soon as samples arrived at the laboratory and experiments were performed with less than 1 hour interval between the triplicates.

Bacterial inactivation measurements were carried out with Chromocult® TBX agar for the *Escherichia coli* bacteria count and GranuCult™ CFC agar for *Pseudomonas spp* bacteria count. The respective agars were prepared in plates according to indications of the manufacturer and subsequently incubated for 24 hours at 40 °C.

Samples for regrowth experiments were taken from the middle to the end (25, 30 and 36 mgL^{-1} of TOD) of disinfection experiments and were incubated at 22 ±2 ºC for 24, 48 and 72 h and plated on Chromocult® TBX agar and GranuCult™ CFC agar, for *E.coli* and *Pseudomonas spp* bacteria count after regrowth, respectively.

To the cellular ATP analysis, a measuring kit BacTiter-Glo™ Microbial Cell Viability Assay (Promega, Barcelona, Spain) was used. The BacTiter-GloTM reagent containing the ATP releasing agents and the luciferase enzymes was prepared according to the manufacturer’s guidelines. The data were collected as relative light units (RLU) and converted to ATP (M) by means of a calibration curve employing a series of rATP standards (Promega Corp.) ranging from 10^{-7} µM to 1 µM. Extracellular ATP was quantified by measuring ATP after filtering each sample through a 0.20 µm sterile syringe filter and then, cellular ATP was calculated by subtracting extracellular ATP from total ATP. All experiments were carried out in triplicates and average values and standard deviation were plotted as colony forming units (CFU) per mL for bacteria and as C/C_0 for ATP depletion.

The concentrations of ACMP, DDVP and ATZ were quantified by means of a high-performance liquid chromatograph (HPLC) equipped with a diode array detector (DAD), all supplied by Agilent (1260 Infinity) and Teknokroma Mediterranea Sea18 (250 mm × 4.6 mm and 5 µm size packing) column. The mobile phase consisted on a 35:65 volumetric mixture of acetonitrile and Milli-Q water acidified at pH 3 by the addition of H_3PO_4. The flow rate was maintained at 0.7 mL min^{-1}, and the detection wavelength was set to 205, 220 and 250 nm for DDVP, ATZ and ACMP, respectively. Finally, UV absorbance data was determined with a DR6000 UV VIS spectrophotometer (Hach, USA).

During the experiments, the pH was monitored being that it remained 7.2±1.3. The concentration of 10 mgL^{-1} Fe^{2+} was also analysed with the purpose of verifying if there was no precipitation of the same, which would entail in the decrease of the efficiency of the treatment. To quantify soluble iron, analyses of Fe^{2+} and total Fe were performed during the experiments. The iron content was determined according to the 1,10-phenantroline standardized procedure (ISO 6332) by spectrophotometer Hach Lange DR 3900 at 510nm.

3. Results and discussions

3.1 Wastewater disinfection

The kinetic study of inactivation of microorganisms by ozone in wastewater disinfection was carried out by estimating the inactivation through established mathematical model of pseudo-first order.

$$\ln\left(\frac{[N]}{[N_0]}\right) = -kt \quad (4)$$

where N_0 is the number of microorganisms at time t=0; N the number of vital microorganisms at time t; k the rate constant for the inactivation; t: the contact time.

The blank tests performed in presence and absence of the pesticides did not present significant statistical differences (see Supplementary information F S-2).

Figure 2 shows the inactivation of *E. coli and Pseudomonas spp* after ozonation of secondary effluent samples spiked with 50, 200 and 100 μgL^{-1} of ATZ, DDVP and ACMP, respectively in absence and in presence of 1 and 10 mgL^{-1} of Fe^{2+}, 1 mgL^{-1} of Co^{2+} and 1 mgL^{-1} of Al^{3+}. The tests performed with 10 mgL^{-1} of Co and Al are not presented, since the performance improvement compared with single ozonation was not significant for this concentration of ions (see Supplementary information F S-3).

According to Student's t-test, the results indicated that the inactivation of *E. coli* and *Pseudomonas spp* could be enhanced by the presence of Fe^{2+}, Co^{2+} and Al^{3+} and consequently there is a synergism in the combined use of these metal ions and O_3 in disinfection of real wastewaters. Inactivation of *E. coli* and *Pseudomonas spp* in the presence of metals was significantly different from single ozonation ($p < 0.05$) and 10 mgL^{-1} of Fe was highly significant ($p < 0.01$), as can be seen in figure 2 A and B.

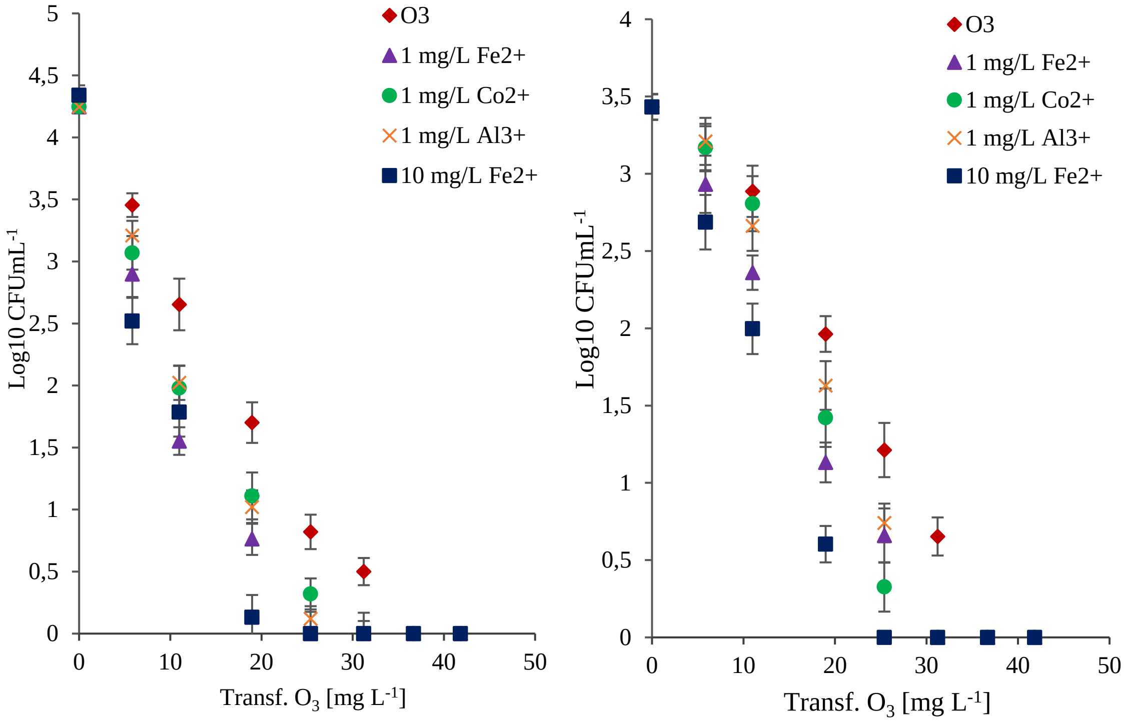


Figure 2. Disinfection of wastewater by ozonation, 1 $mgL^{-1}Fe^{2+}/O_3$, 1 $mgL^{-1}Co^{2+}/O_3$, 1 mgL^{-1} Al^{3+}/O_3 and 10 $mgL^{-1}Fe^{2+}/O_3$. Inactivation of *E. coli* (A) and *Pseudomonas spp* (B).

As shown in Figure 2A, a faster decrease in *E. coli* (CFU log10) concentration occurs after the addition of metals ions. Due to the characteristics of this effluent, the instantaneous ozone dose (IOD) is high (31 mgL^{-1} TOD). Therefore, at the beginning of the treatment, the catalytic effect of the metals is not perceptible (see Supplementary information F S-4).

After 20 mgL^{-1} of TOD, a reduction of 3.3 and 4.2 logs of *E. coli* was observed with 1 and 10 mgL^{-1} Fe^{2+} respectively, 3.2 logs with 1 mgL^{-1} Co^{2+} and 2.8 logs with 1 mgL^{-1} Al^{3+}, while only 2.5 logs reduction was measured for single ozonation. For *Pseudomonas spp* (Figure 2B) for the same TOD a reduction about 2.3 and 3.0 logs with 1 and 10 mgL^{-1} Fe^{2+} respectively, 2.4 logs with 1 mgL^{-1} Co^{2+} and 2.2 logs with 1 mgL^{-1} Al^{3+} were observed, while only 1.7 logs reduction was measured for single ozonation. Considering complete inactivation of *E.coli and*

Pseudomonas spp, while single ozonation required 36 mgL^{-1}of TOD, in presence of 1 mgL^{-1} Fe^{2+}, Co^{2+} and Al^{3+} required 30 mgL^{-1} and with 10 mgL^{-1} Fe just 22 mgL^{-1}. Positively, the presence of studied metals ions increased ozone disinfection capability, having almost doubled in the presence of Fe^{2+}.

The main consequence of this catalytic effect is the saving in energy requirements for disinfection of wastewaters. The energy costs were estimated considering operational costs related to industrial ozone production, which according to Katsoyiannis et al. has an average value of 15 kWh [34], the energy consumption is reduced from 0.54 kWh/m^3 for single ozonation, to 0.45 kWh/m^3 for 1 mgL^{-1} Fe^{2+}, Co^{2+} and +Al^{3+} and just to 0.33 kWh/m^3 for 10 mgL^{-1} Fe^{2+} representing an energy savings of almost 40% in the particular case of 10 mgL^{-1} Fe^{2+}. Based on the work of Nielsen et al. [35] which estimates 0.0027 €/m^3 for 1mgL^{-1} TOD it can be estimate the savings in euros/m^3 (Table 3).

Table 3. Estimated treatment costs per cubic meter in Euro and energy requirements in kWh for the treatments tested.

Treatment	TOD [mgL^{-1}]	Operational costs [€/m^3]	Energy requirements [kWh/m^3]	Savings %
Single ozonation	36	0.097	0.54	-
1mgL^{-1}metals ions/O_3	30	0.081	0.45	17
10 mgL^{-1}Fe^{2+}/O_3	22	0.060	0.33	39

From these results it is clear that the effect of Fe^{2+} addition on the ozone oxidation efficiency becomes more significant. The results of Fe^{2+} in solution fit well with those of disinfection (Figure 3). With initial addition of 1 mgL^{-1}, Fe^{2+} disappeared from solution at 20 mgL^{-1} TOD, whereas with initial addition of 10 mgL^{-1} Fe^{2+} it was still present until 36 mgL^{-1} TOD, maintaining its catalytic improvement along the treatment. At the same time, total iron content decreased because, as Fe^{3+} was generated, it precipitated and/or coordinated with organic compounds present in the water matrix.

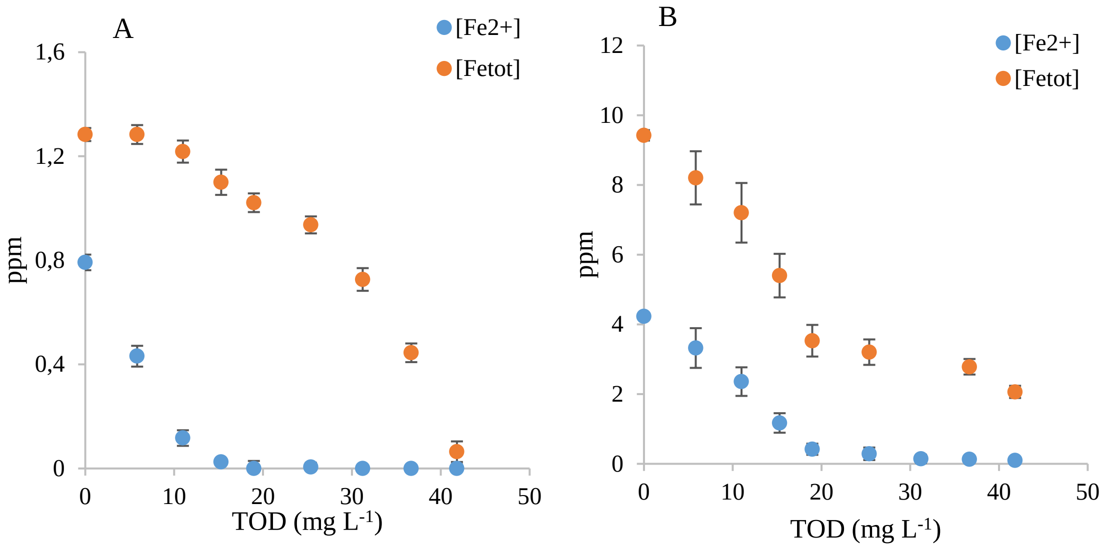


Figure 3. Fe^{2+} and total Fe quantification during the experiments in function of TOD. (A): 1 mgL^{-1} Fe^{2+}, (B): 10 mgL^{-1} Fe^{2+}

What must also be observed in Figure 2 is the higher resistance of *Pseudomonas spp* to the treatment by ozone, since it have lower inactivation rate than the *E. coli* (1 log less). These results agree with bibliography [38-40], where it was also demonstrated that *E. coli* was more susceptible to the ozone disinfection compared to *Pseudomonas spp*.

All these observations are clearly reflected when compared the pseudo-first-order reaction rate constants k (min^{-1}), as shown in Figure 4. It seems that ozonation in presence of 1 mgL^{-1} of Fe^{2+}, Co^{2+} and Al^{3+} ions as well as 10 mgL^{-1} of Fe^{2+}, kinetics of disinfection for both *E. coli* and *Pseudomonas spp* have increased significantly ($p<0.05$), between 1.2 and 3.2 times for *E. coli* and 1.15 and 2.8 times for *Pseudomonas spp*. The lower disinfection kinetics of *Pseudomonas spp* presented in this study confirm their higher resistance to disinfection compared with *E. coli*, as already discussed above.

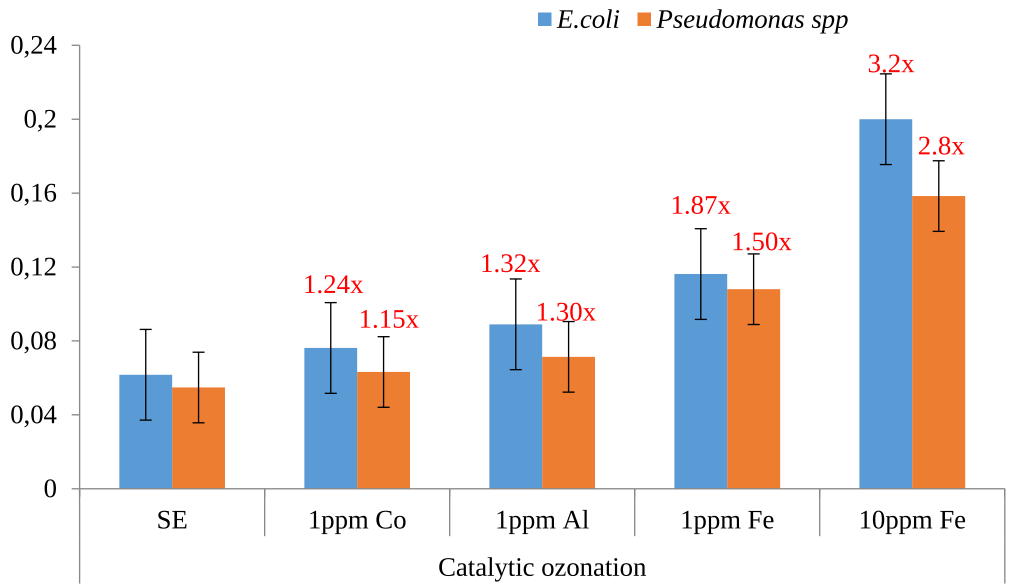


Figure 4. Pseudo first order kinetic constants calculation for the ozonation wastewater disinfection in the presence and absence of catalysts. (SE: secondary effluent).

The analysis of cellular ATP is used for the indirect evaluation of disinfection. It consists in the measurement of ATP, a molecule used in all cells as a carrier of free energy and phosphate groups to drive many chemical reactions. It intervenes in all the energy transactions that take place in cells and can therefore be used as an indicator for microbial activity [41].

The decline in microbial activity (as measured though cellular ATP determination) also followed the same trend as the inactivation of *E. coli and Pseudomonas spp*. (Figure 5). It is noted that the addition of Fe^{2+}, Co^{2+} and Al^{3+} ions to the reaction media provided a significant reduction ($p<0.05$) of ATP over the treatment compared with single ozonation.

The total elimination of cellular ATP occurred again at 36 mgL^{-1} of TOD for single ozone, while in the presence of 1 mgL^{-1} of metals and 10 mgL^{-1} of Fe^{2+} the ozone dose needed decreased to 30 and 20 mgL^{-1}, respectively. In both methods, the $CFUmL^{-1}$ (log 10) and the microbial ATP cells concentration decreased with the increased of ozone dosage. Rauch et al. [42] found that the response of the biomass recovery-ATP method indicated a significant direct correlation to the microbial population reduction observed in heterotrophic plate count (HPC) and Colilert® methods using both pure *E. coli* culture and secondary municipal wastewater effluent. Shawn et al. [43] demonstrated the correlation between ATP bioluminescence measurements and quantitative microbiology.

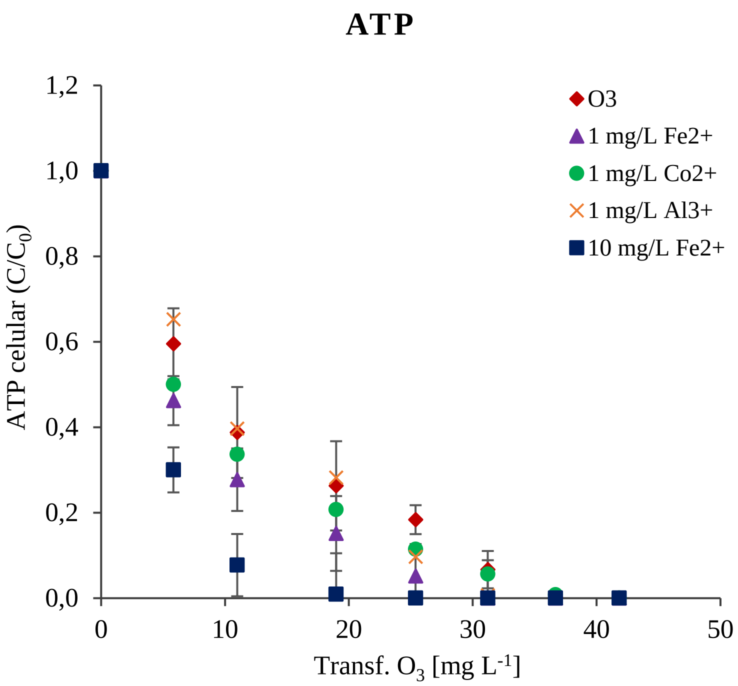


Figure 5. Quantification of ATP cellular in function of TOD.

Depending on the effluent quality, the ozone dose needed to achieve complete inactivation of indicator organisms varies extensively [44]. Complex water matrices with high loads of pathogens and chemical pollutants negatively affect the efficiency of disinfection and pollutant removal [45]. This indicates how important it is to save ozone in the disinfection of wastewater, unlike what happens in drinking water. Reported ozone needs in literature for secondary effluents with high TSS and COD, to meet the totally indicators inactivation, such as the effluent under study, were consistently very high, from 15 to 40 mg L^{-1} [46-49]. That represents energy requirements between 225 to 600 kWh/m^3. However, in this study, the addition of 1 mgL^{-1} of metals resulted in an economy of 17% (540 to 450 kWh/m^3) and with 10 mgL^{-1} of Fe of almost 40% (540 to 330 kWh/m^3) in the ozone energy requirement (kWh/m^3).

As demonstrated by Arslan et al. [36] and Sauleda and Brillas [37], Fe^{2+} catalyzes the decomposition of O_3 to generate hydroxyl radicals following a distinct mechanism. The direct reaction of Fe^{2+} with ozone in the Fe^{2+}/O_3 system resulting in the production of $HO^{\bullet}$ (Eqs 5 – 12):

$$Fe^{2+} + O_3 \rightarrow FeO^{2+} + O_2 \quad (5)$$

$$FeO^{2+} + H_2O \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \quad (6)$$

FeO^{2+} is also able to oxidize Fe^{2+} to Fe^{3+}, at a slower rate, with the termination of the chain reaction:

$$FeO^{2+} + Fe^{2+} + 2H^{+} \rightarrow 2Fe^{3+} + H_2O \quad (7)$$

Ozone is an excellent disinfectant and is able to inactivate even more resistant pathogenic microorganisms such as protozoa where conventional disinfectants fail [13]. Among the mechanisms of microorganisms inactivation, it can be cited the oxidation of the cell wall, reactions with $HO^{\bullet}$ by-products, damage of cell components: enzymes, proteins, DNA, RNA and breakage of C-N bonds [50-52]. There are also many mechanisms of microorganisms inactivation by ions metals, which includes: deterioration in membrane cell structure or oxidation of membrane lipids, interference with cellular vital functions by the affinity for ionic metals form with sulfo hydroxyl group inside the cell, damage or destruction to several intracellular components, i.e.DNA and protein damage by reactive oxygen species formed by reduced metal cations [53,54]. However, in this study, the concentrations of metal ions used were very low, being far from those able to cause inhibition in the bacteria (MIC), as it can be observed in Morrison et al. [55] and Schoonen et al. [56] works.

Wu et al. [57] and Gracia et al. [58] reported that catalytic ozonation using metal ion increases the $HO^{\bullet}$ generation from ozone in aqueous solution and the oxidation of the compound occurs both directly and indirectly. Khuntia et al. [19] determined the enhancement of $HO^{\bullet}$ radical generation from ozone for the catalytic ozonation using Fe(III), Fe(II), Mn(II) and Cu(II) and the results showed that catalytic ozonation was more efficient than that the single ozonation.

The iron-catalysed ozonation, in addition to the equations 6-8, also may share reactions with the classical Fenton homogeneous process. The interaction of ozone and water is known to produce hydrogen peroxide, which may produce hydroxyl radicals [59]:

$$O_3 + H_2O \rightarrow H_2O_2 + O_2 \quad (8)$$

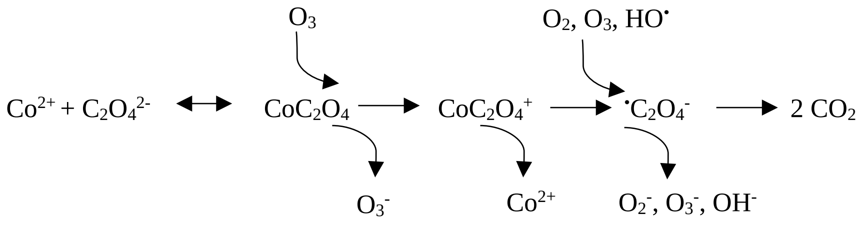
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-} \quad (9)$$

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^{-} \quad (10)$$

$$RH + HO^{\bullet} \rightarrow H_2O + R^{\bullet} \quad (11)$$

$$R^{\bullet} + Fe^{3+} \rightarrow R^{+} + Fe^{2+} \quad (12)$$

Besides to reacting directly with ozone there is evidence that metals can form complexes with compounds and generate radicals that catalyze ozonation, as can the example of Pines and Reckhow [18] for cobalt(II)oxalate/ozone (Scheme 1).



Schem 1: Oxalic acid catalytic ozonation mechanism by means of the Co(II)/O_3 system.

Besides complex formation, cobalt can interact directly with ozone (Eq. 13-15).

$$Co^{2+} + O_3 + H_2O \rightarrow Co(OH)^{2+} + O_2 + HO^{\bullet} \quad (13)$$

$$HO + O_3 \rightarrow HO^{\bullet}_2 + O_2 \quad (14)$$

$$HO^{\bullet}_2 + Co(OH)^{2+} \rightarrow Co^{2+} + H_2O + O_2 \quad (15)$$

There were very few studies involving Al^{3+} as a catalyst for ozonation and therefore its mechanism of action was not described. von Sonntag and von Gunten [16] report that for metals ions with both valence 2 and 3, the reactions are governed by O-transfer reactions and ozone adducts are likely intermediates.

Okawa et al. [60] found in their work that the degradation rate in addition of Al^{3+} to the ozonation system was almost the same as that with no metal ion. Al_Defiery and Gopal [61] reported that with 10 mgL^{-1} of Al^{3+} no catalytic effects were found, but when adding 100 mgL^{-1} this metal exerted catalytic effect. The works cited differ from the results found in this study, since with the addition of 1 mgL^{-1} there was an increase in the production of hydroxyl radicals (R_{OH}) and with that, an improvement in the rate of removal of pesticides.

3.1.2 Bacterial Regrowth

Ozone doses should eliminate and/or reduce the concentration of pathogenic microorganisms to values that exclude any risk to human health. There are pathogenic bacteria such as *E. coli* that have a "dormancy" mechanism during wastewater treatments [62] and may then become reactive under certain conditions. In this context, in order to reuse the treated effluent, it is necessary to include a more detailed evaluation of the role of treatment. If the treatment is not efficient, the reuse can be an agent of propagation and dissemination of pathogens. Therefore, it is very important to evaluate the regrowth capacity of the disinfection indicator microorganisms.

E.coli and *Pseudomonas spp*. regrowth tests were carried out at effluents treated with TODs of 25, 30 and 36 mgL^{-1} and after 24, 48 and 72 hours from the completion of treatment. In Figure 6 only 25 mgL^{-1} (A and B) and 30 mgL^{-1} (A’ and B’) of TODs are presented for both indicator microorganisms, because at the dosage of 36 mgL^{-1} TODs there was no regrowth in any sample for both indicator microorganisms. In the presence of both concentrations of Fe^{2+}, for the both indicator microorganisms tested, the criteria required for non-potable purposes, such as agriculture, landscape, public parks, and golf course irrigation according to EPA was reached [63].

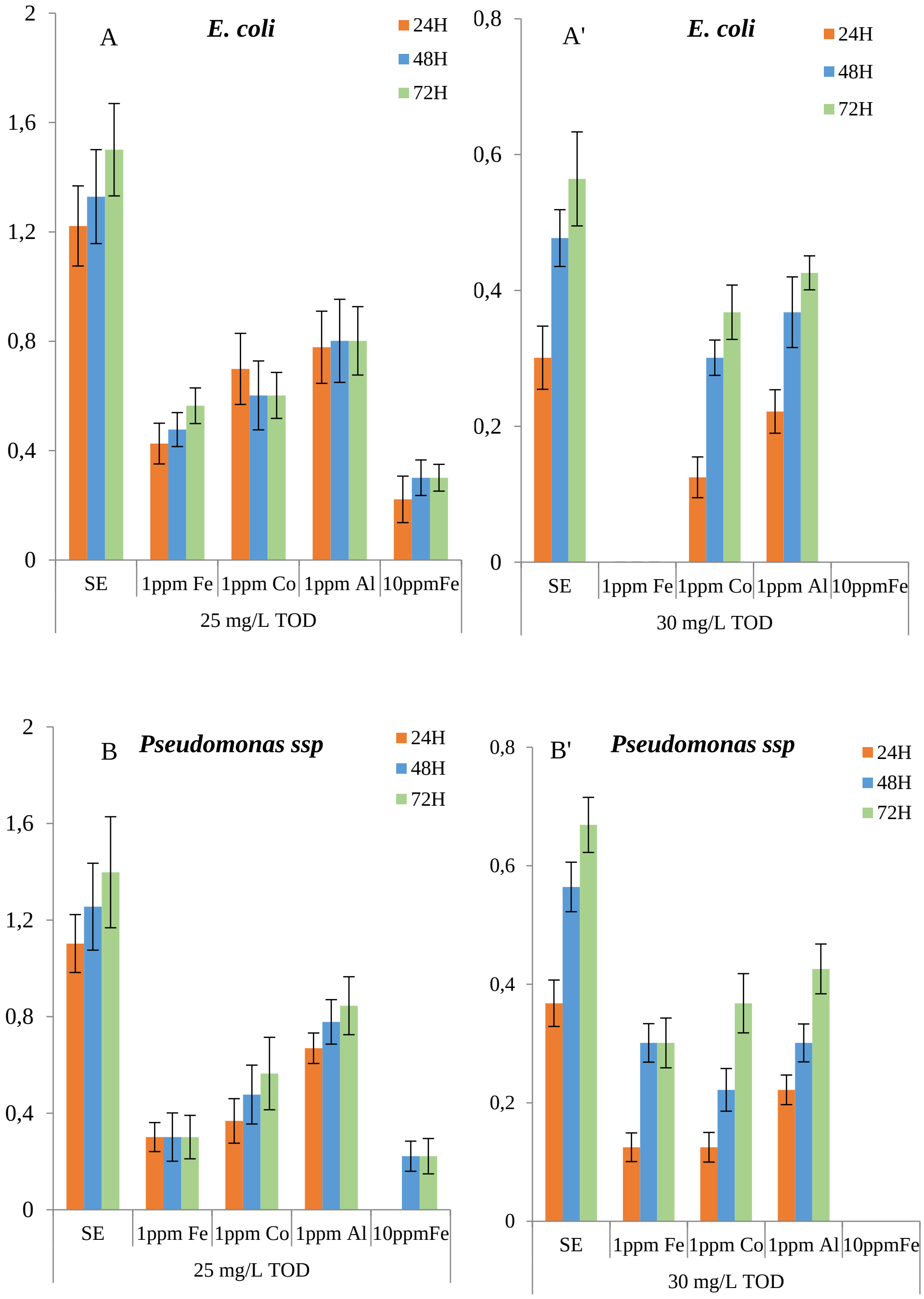


Figure 6. Bacterial regrowth (A) *E. coli* and (B) *Pseudomonas spp* at 25 mgL^{-1} and 30 mgL^{-1} TODs.

The metals addition had a clear inhibitory effect on the reactivation of *E. coli and Pseudomonas spp* when compared with single ozonation. According to the Student's t test for *E. coli*, treatments with the presence of metals differed significantly ($p < 0.05$) from the single O_3 tests for both 25 and 30 mgL^{-1} of TOD. However, for *Pseudomonas spp* only treatments with 25 mgL^{-1} of TOD differed significantly ($p < 0.05$) from single O_3 tests.

With 25 mgL^{-1} of TOD the *E.coli* (Figure 6A) regrowth rate decreased 4.6 times with 1 mgL^{-1} of Fe^{2+}, 2.5 times with 1 mgL^{-1} of Co^{2+} and 1.7 times with 1 mgL^{-1} of Al^{3+}, compared with single ozonation and 2.4 times with 1 mgL^{-1} of Fe^{2+} and Co^{2+} and 1.4 times with 1 mgL^{-1} of Al^{3+} for 30 mgL^{-1} of TOD (Figure 6A'). Meanwhile, for *Pseudomonas spp*. the decreased was 2.7, 2.5 and 1.9 times for 25 mgL^{-1} of TOD (Figure 6B) and 2.2, 1.8 and 1.6 for 30 mgL^{-1} of TOD (Figure 6B') for Fe^{2+}, Co^{2+} and Al^{3+}, respectively.

Iron was the metal that had the greatest influence on the inhibition of both indicator microorganisms' reactivation. It is observed for the treatments with Fe^{2+} in the Figure 6A' that 30 mgL^{-1} TOD there was no regrowth of *E. coli* and in the Figure 6B' the regrowth of *Pseudomonas spp* did not increase from 48 to 72 hours, while for the other metals there is a minimal increase with the each analyzed time.

Kim et al. [64] and Rodrigues et al. [59] also related that Fe^{2+} can generate oxidants such as $HO^{\bullet}$, Fe(IV), O_2^{-}, and H_2O_2 if oxygen is present, and these reactive oxidants generated are responsible for microorganisms' inactivation. It is also reported that these oxidants cause serious damage to cell membrane integrity and respiratory activity.

According to the purpose of reuse, it is necessary to meet the standards required in the legislation. The low regrowth rates presented, after the addition of metals, particularly Fe, would allow the treated effluent reuse in different activities, according to EPA [63], Spanish Royal Decree [65] and Brazilian legislation [66] since the regrowth rate did not reach 1 log10 for any of the treatments. This means that with only 25 mgL^{-1} of TOD (375 kWh/m^3) it is possible to reuse this effluent on the cited areas, representing an energy saving of almost 30% of the overall energy requirement.

3.2 Pesticides degradation

Another concern in recycled effluent is the removal of micropollutants. Figure 7 shows the degradation of ACMP, DDVP and ATZ with single ozonation, O_3/1mgL^{-1} Fe^{2+}, O_3/1mgL^{-1} Co^{2+}, O_3/1mgL^{-1} Al^{3+} and O_3/10mgL^{-1} Fe^{2+}, as a function of TOD.

It can be observed in figure 7B that DDVP was degraded much more easily by single ozone than ACMP and ATZ (Figure 7A and 7C), even though it is present in a concentration 2 times higher than ACMP and 4 times higher than ATZ. Comparing the pesticides kinetic constants with molecular ozone, which are listed in Table 4, it is observed that the DDVP shows moderate reactivity with molecular ozone while ACMP and ATZ are ozone-resistance compounds. On the other hand, all compounds have high reactivity with the $HO^{\bullet}$, with a magnitude order of 10^9 $M^{-1}s^{-1}$.

It is also perceived that the metals have a catalytic effect in the three pesticides removal. Thus, for each pesticide, according to the Student's t test, when the means of the different treatments (absence and presence of 1 and 10 mgL^{-1} Fe^{2+}, 1 mgL^{-1} Co^{2+} and Al^{3+}) were compared, the metals ions presence showed statistically significant differences ($p<0.05$) and this significance increased with 10 mgL^{-1} Fe ($p<0.01$). The ACMP degradation increased between 1.45 to 1.66 times, DDVP increased between 2.70 to 5.0 times and ATZ between 1.5 to 2.1 times with the addition of 1 mgL^{-1} Fe^{2+}, Al^{3+}, Co^{2+} and 10 mgL^{-1} Fe^{2+}.

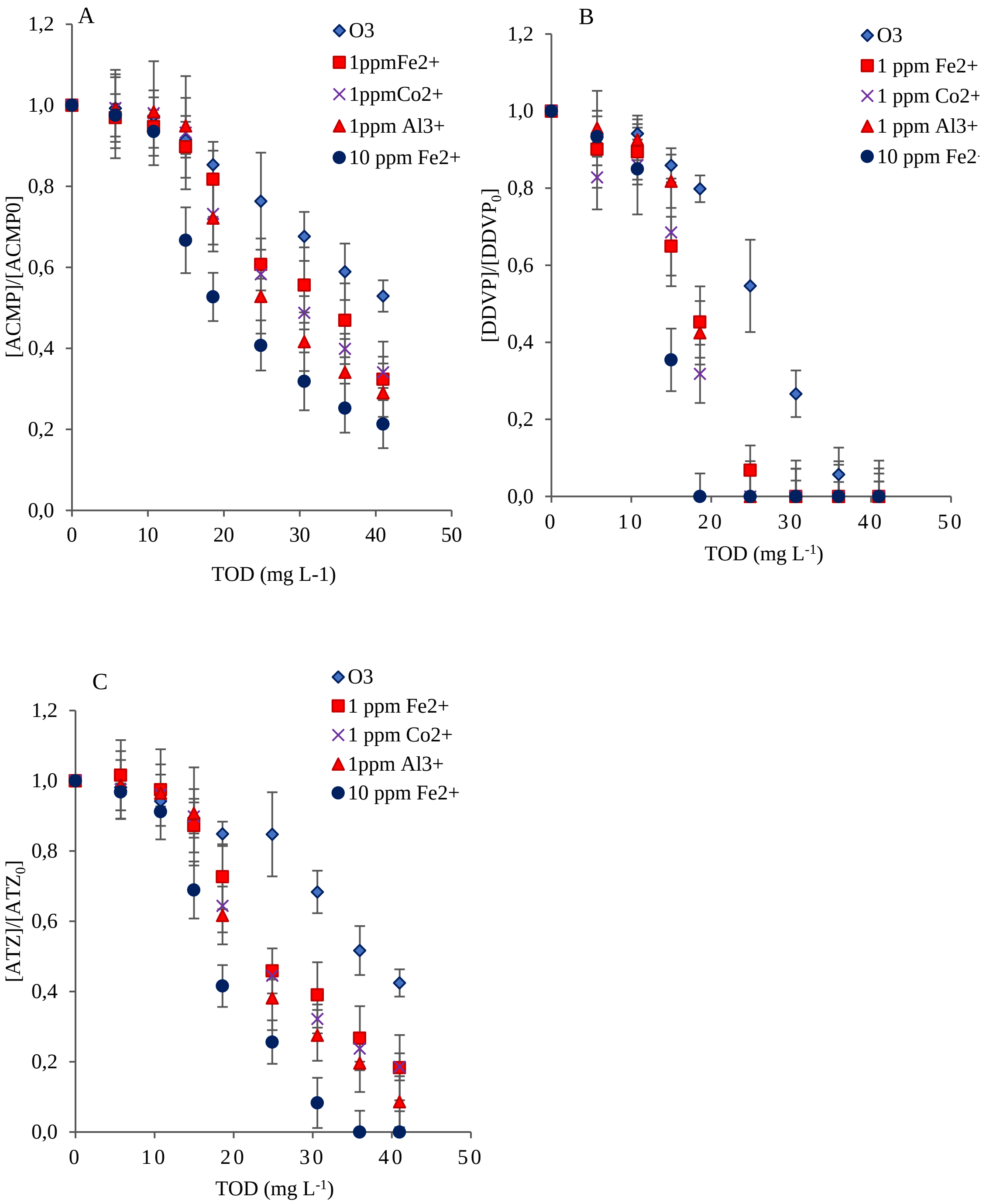


Figure 7. Degradation of (A) ACMP, (B) DDVP and (C) ATZ. ◊ O$_3$; □ O$_3$/1mgL^{-1} Fe^{2+}; Δ O$_3$/1mgL^{-1} Al^{3+}; × O$_3$/1mgL^{-1} Co^{2+}; ○ O$_3$/10 mgL^{-1}Fe^{2+}.

Table 4. Kinetic constant of ACMP, ATZ, DDVP with HO $^•$ and O_3

	k_{O3}	$k_{HO•}$	References
ACMP	0.25 $M^{-1}s^{-1}$	2.1 x 10^9 $M^{-1}s^{-1}$	Cruz-Alcalde et al. [29]
ATZ	6 $M^{-1}s^{-1}$	3.0 x10^9 $M^{-1}s^{-1}$	Acero et al. [28]
DDVP	590 $M^{-1}s^{-1}$	2.2 x 10^9 $M^{-1}s^{-1}$	Cruz-Alcalde et al. [30]

In general, when ozone is dissolved, it is consumed in two steps: first the rapid ozone consumption step which can be represented by the parameter IOD (instantaneous ozone demand) and the rather slower decay step or second stage. In the first stage the direct reaction to ozone predominates and in the second stage (after IOD) the radical pathway, mainly hydroxyl radical predominates [32].

At the beginning of ozonation, the degradation of all pesticides was slow, since wastewater had more reacting compounds with molecular ozone, minimizing ozone decomposition into HO$^•$. However, for TOD higher than 30 mgL^{-1} and particularly in the presence of metals ions, pesticides depletion rates had a significant improvement ($p < 0.05$). This behavior also applies to DDVP, which despite having a higher kinetic constant with molecular ozone, had its degradation potentiated by metals as well. The micropollutants degradation enhancement was significantly increase in the presence of Fe^{2+} ions. Again, metals ions catalyze the decomposition of molecular O_3 intoHO $^•$, particularly after IOD completion, causing a much faster reaction of the pesticides with HO$^•$ and consequently an overall faster degradation.

In the work of Cruz-Alcalde et al. [29] the removal of ACMP by the both possible transformation routes was analyzed, that is, direct by molecular ozone and indirect by the HO$^•$. It was found that the degradation by means of direct reaction barely occurred, therefore the ozone decomposition into HO$^•$ is the key of ACMP removal, while another work [30] reported that both ozone and HO$^•$ can play an important role in DDVP abatement. Zhu et al.[22] compared the effect of single, homogeneous and heterogeneous catalytic ozonation on ATZ degradation and its results showed that homogeneous catalytic oxidation by leached Fe ions increased the atrazine degradation when compared with single ozonation.

From the works found in the literature dealing with catalytic ozonation and disinfection, most are involved with heterogeneous catalysis, thus this work demonstrated the importance of the catalytic effect of metals in simultaneous disinfection and elimination of O_3 recalcitrant micropollutants.

3.3 Comparison of oxidation efficiency: Determination of $R_{OH,O3}$

The methodology described by Cruz-Alcalde et al. [33] was applied to calculate the $R_{OH,O3}$ parameter for each experimental conditions assayed. ACMP is the most ozone recalcitrant compound among the three tested pesticides (see Table 4), thus the wastewater effluent was spiked with 100 µgL^{-1} of ACMP as HO$^\bullet$ probe compound, and then ozonized for 60 min under the different studied operational conditions (in presence and absence of 1 and 10 mgL^{-1} Fe^{2+}, 1 mgL^{-1} Co^{2+} and 1 mgL^{-1}Al^{3+}). Table 4 data was used to deduct the accumulated hydroxyl radical exposure according to equation 4. Experimental plots of $\int$[HO$^\bullet$]dt versus TOD (consumed ozone, according to the employed experimental methodology) were performed to obtain the R_{OHO3} values for each experimental conditions (see Supplementary information F S-5). Table 5 shows the R_{OHO3} values resulted, which were different before (Stage 1) and after (Stage 2) IOD (31 mgL^{-1}).

Table 5. R_{OHO3} plot obtained during ozonation of wastewater effluent samples and TOD to 50% of ACMP degradation. [ACMP]$_0$ = 100 µg L^{-1}, as flow rate: 0.2 L min^{-1}; Inlet (gas) ozone concentration: 10 mg O$_3$ L^{-1} at STP conditions. Fist stage: before IOD (31 mgL^{-1}); second stage: after IOD.

Process	R_{OHO3}	
	Stage 1	Stage 2
Single ozonation	1.51 x10^{-7}	4.82x10^{-7}
1 mgL^{-1} Co^{2+}/O$_3$	2.23x10^{-7}	9.43x10^{-7}
1 mgL^{-1} Al^{3+}/O$_3$	1.05 x10^{-7}	9.60 x10^{-7}
1 mgL^{-1} Fe^{2+}/O$_3$	2.64 x10^{-7}	9.10 x10^{-7}
10 mgL^{-1} Fe^{2+}/O$_3$	1.25 x10^{-6}	8.20 x10^{-7}

R_{OHO3} values increased in the second stage for conditions tested as expected, which means that more hydroxyl radicals were available for oxidation. During early stage of ozone-based processes, there is an almost instantaneous ozone consumption exerted by O$_3$ direct reacting matter, both organic and inorganic, present in the highly polluted secondary wastewater effluent. Once this matter is partially oxidized, this consumption decreased, increasing the ozone decomposition into hydroxyl radicals. Moreover, metals ions addition, excepting Al^{3+} (Table 5), caused a significantly increased of R_{OHO3} values in the first stage of ozonation ($p<0.05$), particularly the presence of 10 mgL^{-1} Fe^{2+} ($p<0.01$), although it was in the second stage where all metals ions presence remarkably increased of R_{OHO3} values. Metals ions accelerated the decomposition of molecular ozone into hydroxyl radicals, resulting in more radical formation per transferred ozone. This increase was almost 1.8 and 8.2 times higher than the single ozonation for

1 and 10 mgL^{-1} Fe and 1.5 times more for 1 mgL^{-1} Co (first stage) and almost 2.0 times for all metals ions addition (second stage) (see Table 4).

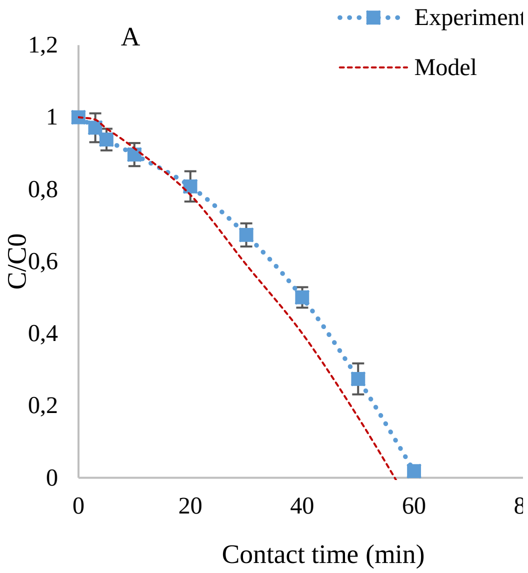
It should be noted that in the second stage of ozonation 10 mgL^{-1} of Fe has less effect on R_{OH} values than 1 mgL^{-1}. This may be related to the fact that the oxidation of Fe^{2+} into Fe^{3+} generated precipitates that decrease the catalytic effect of this metal (see Figure 3). This result agrees with Kishimoto and Ueno [67] study, which Fe^{2+} appears to be associated with no significant effect in the second stage of ozonation due to the formation of precipitates throughout the treatment.

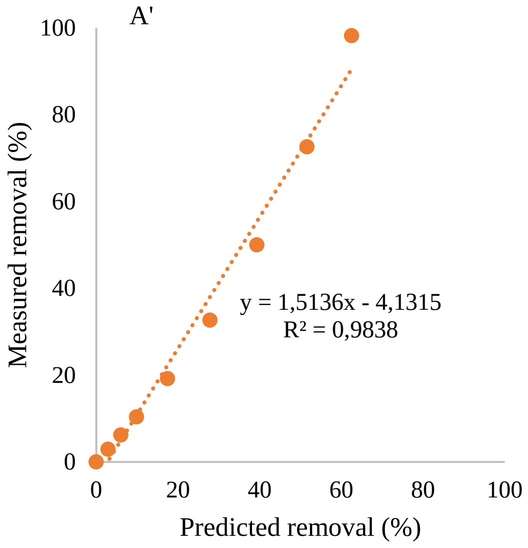
3.4 Prediction of micropollutants removal by homogeneous catalytic ozonation based on R_{OHO3} concept

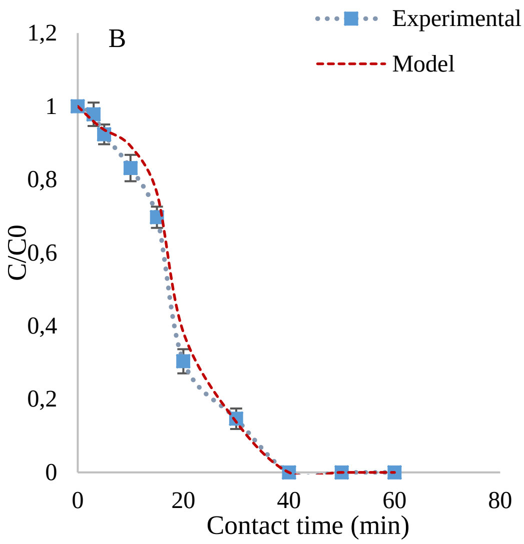
According to the R_{OHO3} definitions (Eq 1 and 2), the removal of the ATZ O_3-resistant micropollutant can be predicted by Equation 4 [33]. The prediction of DDV was also performed, however it has a moderate reaction with molecular ozone, so the model underestimating the degradation of itself (Supplementary F S-6).

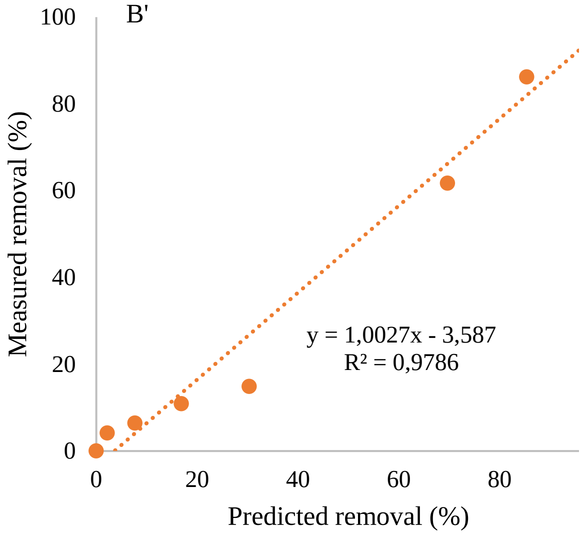
A comparison was made between the experimental results and the model predictions for the ATZ removal and its correlation in the two stages for all metals tested (Figure 8). All the experimental results presented an excellent correlation with the values of the model ($R^2>0.98$).

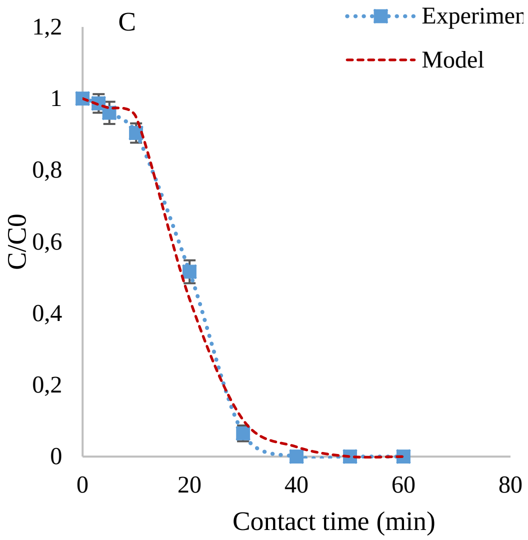
It is noted that for both single ozonation and metals ions/O_3, the model can be used satisfactorily to predict the abatement efficiency of ozone-recalcitrant micropollutants. It can be seen in Figures 8A, (single ozonation), that the model overestimated and, in the Figure 8E (10mgL-1Fe^{2+}), underestimated ATZ degradation, however these estimation deviations were not significant ($p> 0.05$).

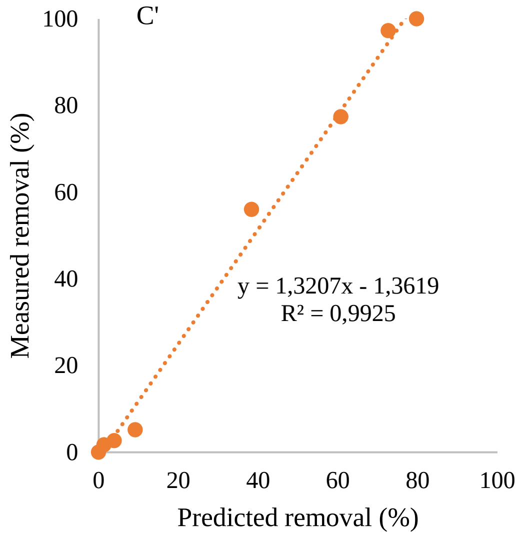

A
Experiment
Model
1,2
1
0,8
0,6
0,4
0,2
0
C/C0
0
20
40
60
8
Contact time (min)


A'
100
80
60
40
20
0
Measured removal (%)
y = 1,5136x - 4,1315
R² = 0,9838
0
20
40
60
80
100
Predicted removal (%)


B
Experimental
Model
1,2
1
0,8
0,6
0,4
0,2
0
C/C0
0
20
40
60
80
Contact time (min)


B'
100
80
60
40
20
0
Measured removal (%)
y = 1,0027x - 3,587
R² = 0,9786
0
20
40
60
80
Predicted removal (%)


C
Experimer
Model
1,2
1
0,8
0,6
0,4
0,2
0
C/C0
0
20
40
60
80
Contact time (min)


C'
100
80
60
40
20
0
Measured removal (%)
y = 1,3207x - 1,3619
R² = 0,9925
0
20
40
60
80
100
Predicted removal (%)

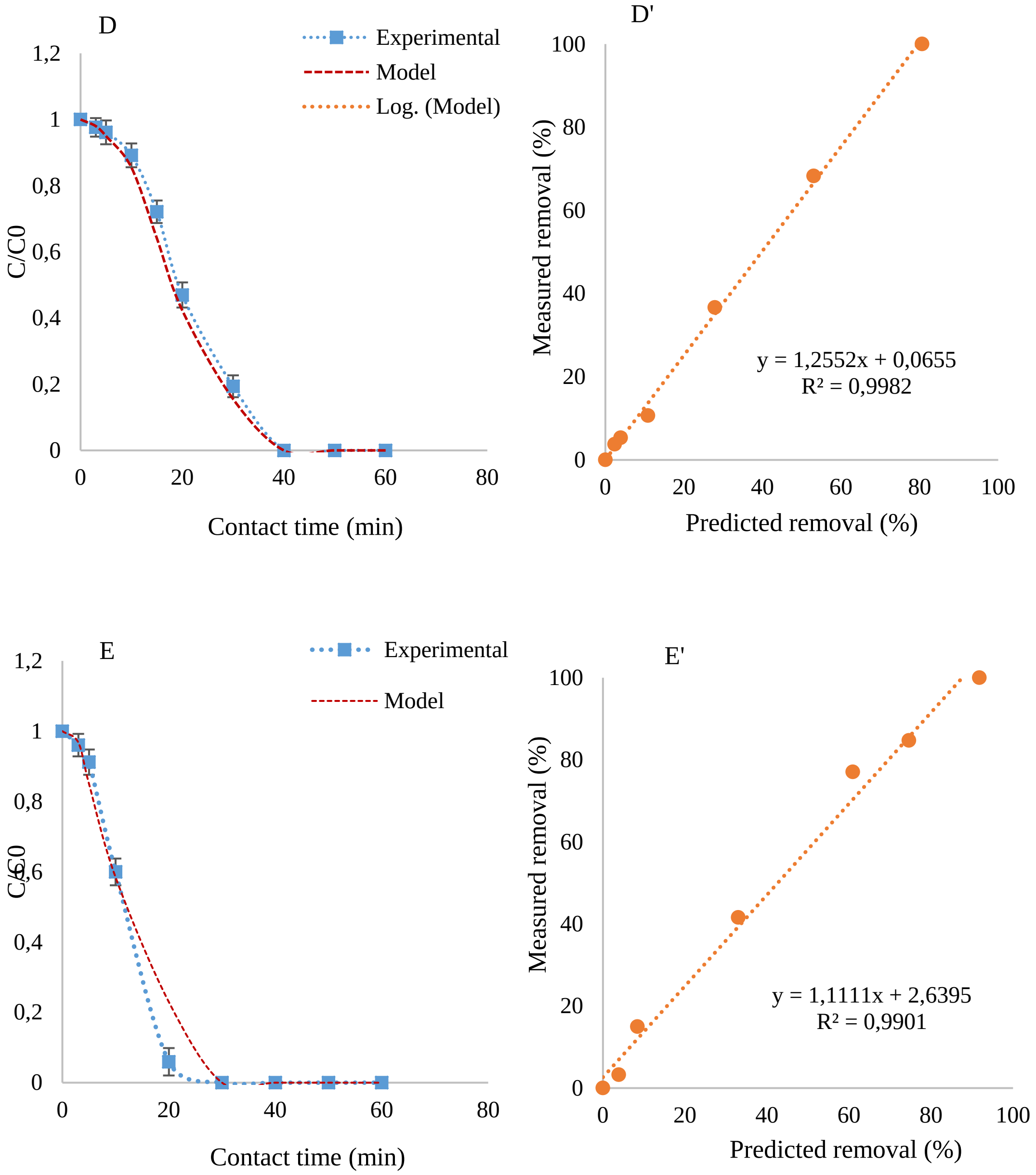


Figure 8. Comparison between the experimental results and the model predictions for the ATZ removal and your correlation. (A and A': Single ozone), (B and B': O_3 + 1 mgL^{-1} Fe^{2+}), (C and C': O_3 + 1 mgL^{-1} Al^{3+}), (D and D': O_3 + 1 mgL^{-1} Co^{2+}), (E and E': O_3 + 10 mgL^{-1} Fe^{2+}).

4. Conclusions

According to the results some conclusions are drawn as following:

(1) The disinfection performance was improved by metals ions/O_3, as can be observed through *E. coli* and *Pseudomonas spp* inactivation and ATP cellular depletion. It was also clear that the addition of the ions to ozonation acts as a regrowth inhibitor of both indicator microorganisms.

(2) The improvement of metal ions on R_{OHO3} values occurred significantly for both stages of ozonation (before and after initial ozone demand), for all tested metals.

(3) The modelling results based on R_{OHO3} values demonstrate that, for both single ozonation and metals ion/O_3, the atrazine depletion can be predicted satisfactory (R^2> 0.97), being a useful tool for the generalized prediction of ozone resistant micropollutants abatment.

(4) Of the metal ions tested, Fe^{2+} was the one that exerted the most significant effect in both in disinfection and micropollutants removal.

(5) The metals ions addition to the ozonation system provided saving in the ozone energy requirement (kWh/m^3) for disinfection and pesticides abatement. To achieve 50% degradation of ACMP (most O_3-resistant compound) using 10 mgL^{-1} Fe, only 22 mgL^{-1} of TOD were needed, almost 50% less than that for single O_3. For the wastewater disinfection to reuse, it was necessary 25 mgL^{-1} of TOD, almost 30% less than the single O_3 to respect the limits of Brazilian, Spanish and American legislations. This means savings between 30-50% of total ozone requirements for the micropollutants depletion and disinfection.

Funding: This work was supported by the São Paulo Research Foundation (FAPESP) [2014/17774-1], [2016/50460-6], [2017/23642-9] and Spanish Ministry of Science, Innovation and Universities [project CTQ2017-86466-R].

5. References

[1] J. Blasco, A. DelValls. Impact of Emergent Contaminants in the Environment: Environmental Risk Assessment. In: Barceló D., Petrovic M. (eds), Emerging Contaminants from Industrial and Municipal Waste. The Handbook of Environmental Chemistry, Springer, Berlin, Heidelberg, 2008, vol 5/5S/5S/1, pp169-188.

[2] I. Papadopoulos. Wastewater Management for Agriculture Protection in the Near East Region, Technical Bulletin, FAO, Regional Office for the Near East, Cairo, Egypt, 1995.

[3] A.N. Angelakis, S.A. Snyder. Wastewater Treatment and Reuse: Past, Present, and Future. Water. 7 (2015) 4887-4895.

[4] S. Naidoo, A.O. Olaniran. Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources. Int. J. Environ. Res. Public Health. 11(1) (2014) 249-270.

[5] J. Fenik, M. Tankiewicz, M. Biziuk. Properties and determination of pesticides in fruits and vegetables. Trends in Analytical Chemistry. 30(6) (2011) 814-826.

[6] M. Köck-Schulmeyer, M. Villagrasa, M. L. de Alda, R. Céspedes-Sánchez, F. Ventura, D. Barceló. Occurrence and behavior of pesticides in wastewater treatment plants and their environmental impact. Science of the Total Environment. 458-460 (2013) 466-476.

[7] R. Zhao, Y. Deng. Advanced Oxidation Processes (AOPs) in Wastewater Treatment, Water Pollution. 1(2015)167–176.

[8] C. Ameta. Advanced Oxidation Processes for Waste Water Treatment. Emerging Green Chemical Technology. Chapter 1 – Introduction. Elsevier. 2018, pp1-12.

[9] B. Kasprzyk-Hordern, M. Ziółek, J. Nawrocki. Catalytic ozonation and methods of enhancing molecular ozone reactions in water treatment. Appl. Catal. B: Environ. 46 (4) (2003) 639–669.

[10] K. Ikehata, Y. Li. Emerging Green Chemical Technology. Chapter 5 - Ozone-Based Processes. In: Advanced Oxidation Processes for Waste Water Treatment. 2018, pp115-134.

[11] M. Agulló-Barceló, M.I. Polo-López, F. Lucena, J. Jofre, P. Fernández-Ibáñez. Solar Advanced Oxidation Processes as disinfection tertiary treatments for real wastewater: Implications for water reclamation. Applied Catalysis B: Environmental. 136-137 (2013)341-350.

[12] R. Etchepare, R. Zaneti, A. Azevedo, J. Rubio. Application of flocculation-flotation followed by ozonation in vehicle wash wastewater treatment/disinfection and water reclamation. Desalination and Water Treatment, 56 (7) (2015) 1728-1736.

[13] U. von Gunten. Ozonation of drinkingwater: Part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. Water Research 37 (2003) 1469–1487.

[14] Y. Lee, U. von Gunten. Oxidative transformation of micropollutants during municipal wastewater treatment: comparison of kinetic aspects of selective (chlorine, chlorine dioxide, ferrate VI, and ozone) and non-selective oxidants (hydroxyl radical). Water Research 44(2) (2009) 555-66.

[15] 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 Environment Research 81(2) (2009) 201-205.

[16] C. von Sonntag, U.von Gunten. Chemistry of ozone in water and wastewater treatment: From basic principles to applications. London, UK: IWA Publishing. 2012, 312p.

[17] R. Gracia, J.L. Aragües, S. Cortés, J.L. Ovelleiro. in: Proceedings of the 12th World Congress of the International Ozone Association, Lille, France, 15–18 May 1995, p. 75.

[18] D.S. Pines, D.A. Reckhow. Effect of Dissolved Cobalt(II) on the Ozonation of Oxalic Acid. Environ. Sci. Technol. 36 (2002) 4046- 4051.

[19] S. Khuntia, S.K. Majudar, P. Ghosh. Catalytic ozonation of dye in a microbubble system: hydroxyl radical contribution and effect of salt. J. Environ. Chem. Eng. 4 (2016) 2250-2258.

[20] X. Li, W. Chen, L. Ma, H. Wang, J.Fan. Industrial wastewater advanced treatment via catalytic ozonation with an Fe-based catalyst. Chemosphere. 195 (2018) 336-343.

[21] C.Y. Wu, Y.X. Zhou, Y. Wang, M.K. Guo. Innovative combination of Fe^{2+}-BAF and ozonation for enhancing phosphorus and organic micropollutants removal treating petrochemical secondary effluent, J. Hazard. Mater. 323 (2017) 654–662.

[22] H. Zhu, W. Ma, H. Han, Y. Han, W. Ma. Catalytic ozonation of quinoline using nano-MgO: Efficacy, pathways, mechanisms and its application to real biologically pretreated coal gasification wastewater. Chemical Engineering Journal, 327 (2017) 91-99.

[23] N. Khadgi, A.R. Upreti, Y. Li. Simultaneous bacterial inactivation and degradation of an emerging pollutant under visible light by ZnFe2O4 co-modified with Ag and rGO† RSC Adv., 7 (2017) 27007–27016.

[24] P. Kolosov, M-L Peyot, V. Yargeau. Novel materials for catalytic ozonation of wastewater for disinfection and removal of micropollutants. Science of the Total Environment 644 (2018) 1207–1218.

[25] N. Garkusheva, G. Matafonova, I. Tsenter, S. Beck, V. Batoev, K. Linden. Simultaneous atrazine degradation and E. coli inactivation by simulated solar photo-Fenton-like process using persulfate, Journal of Environmental Science and Health, Part A. 52:9 (2017) 849-855.

[26] The European Commission. Priority substances under the Water Framework Directive. 2016. http://ec.europa.eu/environment/water/water-dangersub/pri_substances.htm

[27] M.I. Badawy, M.Y. Ghaly, T.A. Gad-Allah. Advanced oxidation processes for the removal of organophosphorus pesticides from wastewater. Desalination. 194 (1–3) (2006) 166-175.

[28] J.L. Acero, K. Stemmler, U. von Gunten. Degradation kinetics of atrazine and its degradation products with ozone and OH radicals: a predictive tool for drinking water treatment. Environ Sci Technol. 34 (2000) 591–597.

[29] A. Cruz-Alcalde, C. Sans, S. Esplugas. Priority pesticides abatement by advanced water technologies: The case of acetamiprid removal by ozonation. Science of the Total Environment. 599–600 (2017) 1454–1461.

[30] A. Cruz-Alcalde, C. Sans, S. Esplugas. Priority pesticide dichlorvos removal from water by ozonation process: Reactivity, transformation products and associated toxicity. Separation and Purification Technology. 192 (2018a) 123–129.

[31] M. Kwon, H.K.Y. Jung, Y. Yoon, J.-W. Kang. Performance characterization and kinetic modeling of ozonation using a new method: $R_{OH,O3}$ concept. Water Research. 122 (2017) 172-182.

[32] M.S. Elovitz, U. von Gunten. Hydroxyl Radical/Ozone Ratios During Ozonation Processes. I. The Rct Concept. Ozone: Science & Engineering 21 (3) (1999) 239-260.

[33] Y. Pi, J. Schumacher, M. Jekel. The Use of para-Chlorobenzoic Acid (pCBA) as an Ozone/Hydroxyl Radical Probe Compound. Ozone: Science & Engineering, 27:6 (2005) 431-436.

[33] A. Cruz-Alcalde, S. Esplugas, C. Sans. Abatement of ozone-recalcitrant micropollutants during municipal wastewater ozonation: kinetic modelling and surrogate-based control strategies. Chemical Engineering Journal 360 (2019), 1092-1100.

[34] I.A. Katsoyiannis, S. Canonica, U. von Gunten. Efficiency and energy requirements for the transformation of organic micropollutants by ozone, O_3/H_2O_2 and UV/H_2O_2. Water Research, 45 (2011) 3811-3822.

[35] U. Nielsen, C. Hastrup, M.M. Klausen, B.M. Pedersen, G.H. Kristensen, J.L. Jansen, S.N. Bak, J. Tuerk. Removal of APIs and bacteria from hospital wastewater by MBR plus O_3, O_3+ H_2O_2, PAC or ClO_2. Water Sci Technol. 67(4) (2013) 854-62.

[36] I. Arslan, I.A. Balcioglu, T. Tuhkanen. Advanced treatment of dyehouse effluents by Fe(II) and Mn(II)-catalyzed ozonation and the H_2O_2/O_3 process. Water Sci Technol. 42(1-2) (2000) 13-18.

[37] R. Sauleda, E. Brillas. Mineralization of aniline and 4-chlorophenol in acidic solution by ozonation catalyzed with Fe^{2+} and UVA light. Appl. Catal., B. 29 (2001) 135-145.

[38] I. Lezcano, R. Pérez, C. Baluja, E. Sánchez. Ozone Inactivation of Pseudomonas aeruginosa, Escherichia coli, Shigella sonnei and Salmonella typhimurium in Water. Ozone: Science & Engineering. The Journal of the International Ozone Association. 21(3) (1999) 293-300.

[39] M. Prabakaran, S. Tamil Selvi, S. Merinal, A. Panneerselvam. Effect of ozonation on pathogenic bacteria.Advances in Applied Science Research. 3 (1) (2012) 299-302.

[40] R. Saha, N. Saha, R.S. Donofrio, L.L. Bestervelt. Microbial siderophores: a mini review : Microbial Siderophores. Ann Microbiol 64 (2014) 1607–1613.

[41] G. Liu, F.Q. Ling, V.D. Mark, X.D. Zhang, A. Knezev, J.Q.J.C Verberk, W.G.J. Van Der Meer, G.J. Medema, W.T. Liu, J.C. Van Dijk. Comparison of particle-associated bacteria from a drinking water treatment plant and distribution reservoirs with different water sources. Sci. Rep. 6 (2016) 20367.

[42] K.D. Rauch, A. L. Mackie, B. Middleton, X. Xie, A. Graham Gagnon. Biomass Recovery Method for Adenosine Triphosphate (ATP) Quantification Following UV Disinfection. Ozone: Science & Engineering. In Press, 2018.

[43] G.G. Shawn, H. Sayles, O. Chaika, A. Hewlett, E.M. Colbert, P.W. Smith. Evaluation of the relationship between ATP bioluminescence assay and the presence of organisms associated with healthcare-associated infections. Infection, disease & Health. 19(3) (2014) 101–107.

[44] J.A Malvestiti, R.F. Dantas. Disinfection of secondary effluents by O3, O3/H2O2 and UV/H2O2: Influence of carbonate, nitrate, industrial contaminants and regrowth. Journal of Environmental Chemical Engineering. 6(1) (2018) 560-567.

[45] O. Tsydenova, V. Batoev, A. Batoev. Solar Enhanced Advanced Oxidation Processes for Water Treatment: Simultaneous Removal of Pathogens and Chemical Pollutants. Scholz M. ed. International Journal of Environmental Research and Public Health.12 (8) (2015) 9542-9561.

[46] R. Gehr, M. Wagner, P. Veerasubramanian, P. Payment. Disinfection efficiency of peracetic acid, UV and ozone after enhanced primary treatment of municipal wastewater. Water Research 37(19) (2003) 4573-4586.

[47] L. Liberti, M. Notarnicola, A. Lopez. Advanced Treatment for Municipal Wastewater Reuse In Agriculture. III - Ozone Disinfection. Ozone Science and Engineering. 22 (2000) 151-166.

[48] P. Xu, M-L Janex, P. Savoye, A. Cockx, V. Lazarova. Wastewater disinfection by ozone: main parameters for process design. Water Research 36 (2002) 1043–1055.

[49] W. Yao, S. Wagi, Ur. Rehman, H. Wang, H. Yang, G. Yu, Y. Wang. Pilot-scale evaluation of micropollutant abatements by conventional ozonation, UV/O_3, and an electro-peroxone process. Water Research. 138 (2018) 106-117.

[50] S.C. Cho, B.N. Kim, Y.C. Hong, M.S.Shin, H.J. Yoo, J.W. Kim, S.Y. Bhang, I.H. Cho, H.W. Kim. Effect of environmental exposure to lead and tobacco smoke on inattentive and hyperactive symptoms and neurocognitive performance in children. J Child Psychol Psychiatry, 51 (2010) 1050-1057.

[51] G. Zeng, V. Morgenstern, C. Braesicke, J. A. Pyle. Impact of stratospheric ozone recovery on tropospheric ozone and its Budget. Geophysical Research Letters. 37(9) (2010) 1-5.

[52] J. Zhang, M.C. Duke, K. Northcott, M. Packer, M. Allinson, G. Allinson, K. Kadokami, J . Tan, S. Allard, J-P Croué, A. Knight, P. J. Scales, S R. Gray. Small Scale Direct Potable Reuse (DPR) Project for a Remote Area. Water. 9- 94 (2017) 1-25.

[53] D.H. Nies. “Microbial Heavy-Metal Resistance,” Applied Microbiology and Biotechnology. 51(6) (1999) 730-750.

[54] C. Cervantes, J. Campos-Garcia, S. Devars, F. Gutierrez-Corona, H. Loza-Tavera, J.C. Torres-Guzman; R. Moreno-Sanchez. “Interactions of Chromium with Microorganisms and Plants,” FEMS Microbiology Reviews. 25(3) (2001) 335-347.

[55] K.D. Morrison; R. Misra; L.B. Williams. Unearthing the antibacterial mechanism of medicinal clay: A geochemical approach to combating antibiotic resistance. Sci. Rep. 6 (2016) 19043.

[56] M.A. Schoonen, C.A. Cohn, E. Roeme, R. Laffer, S. R. Simon, T. O’Riordan. Mineral-induced formation of reactive oxygen species. Rev. Mineral. Geochem. 64 (1) (2006) 179−221.

[57] S.L.Wu, L.J. Mickley, D.J. Jacob, D. Rind, D.G. Streets. Effects of 2000-2050 changes in climate and emissions on global tropospheric ozone and the policy-relevant background surface ozone in the United States. J. Geophys. Res., 108(D18312) (2008) 1-12.

[58] R. Gracia, J.L. Aragües, J.L. Ovelleiro. Study of the Catalytic Ozonation of Humic Substances in Water and Their Ozonation Byproducts. Ozone: Science & Engineering.18(3) (1996) 195-208.

[59] A. Rodríguez, R. Rosal, J.A. Perdigón-Melón, M. Mezcua, A. Agüera, M.D. Hernando, P. Letón, A. R. Fernández-Alba, E. García-Calvo. Ozone-Based Technologies in Water and Wastewater Treatment. Hdb Env Chem. Springer-Verlag Berlin Heidelberg. 5 (2008) 1-49.

[60] K. Okawa, T-Y Tsai, Y. Nakano, W. Nishijima, M. Okada. Effect of metal ions on decomposition of chlorinated organic substances by ozonation in acetic acid. Chemosphere. 58 (2005) 523–527.

[61] M.E.J. Al_Defiery, G. Reddy. Influence of metal ions concentration on phenol degradation by Rhodococcus pyridinivorans GM3, Mesopotamia Environment Journal. 1(1) (2014) 30-38.

[62] A.R. Gupte, C.L.E. de Rezende, S.W. Joseph. Induction and resuscitation of viable but nonculturable Salmonella enterica serovar typhimurium DT104. Applied and Environmental Microbiology. 69 (11) (2003) 6669-6675.

[63] EPA. U.S. Environmental Protection Agency. Guidelines for Water Reuse. EPA/600/R-12/618. 2012. pp 643.

[64] J.Y. Kim, H-J Park, C. Lee, K.L. Nelson, D.L. Sedlak, J Yoon. Inactivation of Escherichia coli by Nanoparticulate Zerovalent Iron and Ferrous Ion. Appl Environ Microbiol. 76 (22) (2010) 7668–7670.

[65] BOE 2007. Royal Decree 1620/2007, 294, 50639-50661. http://www.boe.es/boe/dias/2007/12/08/pdfs/A50639-50661.pdf. Accessed 10 jan 2019.

[66] ABNT - NBR 13969/1997. Septic tanks - Units of complementary treatment and disposal end of the liquid effluents - Project, construction and operation. Brazilian Association of

Technical Standards. 60p. http://acquasana.com.br/legislacao/nbr_13969.pdf. Acessed 10 jan 2019.

[67] N. Kishimoto, S. Ueno. Catalytic Effect of Several Iron Species on Ozonation. Journal of Water and Environment Technology, 10 (2) (2012) 205-2015.