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Title: Removal of benzoylecgonine from water matrices through UV254/H2O2 process: reaction kinetic modeling, ecotoxicity and genotoxicity assessment

Article Type: Research Paper

Keywords: benzoylecgonine, illicit drugs, AOP, kinetic modeling, ecotoxicological assessment

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Abstract: Benzoylecgonine (BE), the main cocaine metabolite, has been detected in numerous surface water and treatment plants effluents in Europe and there is urgent need for effective treatment methods. In this study, the removal of BE by the UV254/H2O2 process from different water matrices was investigated. By means of competition kinetics method, the kinetic constant of reaction between BE and the photogenerated hydroxyl radicals (*OH) was estimated resulting in kOH/BE = 5.13*109 M-1*s-1. By-products and water matrices scavengers effects were estimated by numerical modeling of the reaction kinetics for the UV254/H2O2 process and validated in an innovative microcapillary film (MCF) array photoreactor and in a conventional batch photoreactor. The ecotoxicity of the water before and after treatment was evaluated with four organisms Raphidocelis subcapitata, Daphnia magna, Caenorhabditis elegans, and Vicia faba. The results provided evidence that BE and its transformation by-products do not have significant adverse effects on R. subcapitata, while D. magna underwent an increase of lipid droplets. C. elegans was the most sensitive to BE and its by-products. Furthermore, a genotoxicity assay, using V. faba, showed cytogenic damages during the cell mitosis of primary roots.

DICATECh – Politecnico di Bari
Via Orabona, 4, 70125, Bari, Italy

Prof. Gerasimos Lyberatos

Editor of Journal of Hazardous Materials

Dear Prof. Lyberatos

please find enclosed a copy of the original manuscript:

"Removal of benzoylecgonine from water matrices through UV_{254}/H_2O_2 process: reaction kinetic modeling, ecotoxicity and genotoxicity assessment"

by Danilo Spasiano, Danilo Russo, Marianna Vaccaro, Antonietta Siciliano, Raffaele Marotta, Marco Guida, Nuno Reis, Gianluca Li Puma, and Roberto Andreozzi.

Number of words: 4998.

In this study, we present a kinetic model of the UV_{254}/H_2O_2 process for the removal of benzoylecgonine from different water matrices (milli-Q water, synthetic wastewater, real wastewater, and real surface water) by means of an innovative microcapillary film (MCF) array photoreactor, which allows very fast experimentation with minimal sample volumes. Benzoylecgonine is the major metabolite of cocaine and an emerging contaminant often detected in wastewater treatment plants, effluents and surface waters in higher concentrations than its parent compound. The MCF is an effective experimental tool to study oxidative removal of highly priced, uncommon, or regulated substances such as BE. Competition kinetics in the presence of benzoic acid was used to determine the second-order rate constant ($k_{OH/BE}$) for the reaction of BE with $^{\bullet}OH$. The effect of by-products and water matrices scavengers was estimated by numerical modeling of the reaction kinetics for the UV_{254}/H_2O_2 process in the MCF array photoreactor dissolving BE in milli-Q water, synthetic wastewater, real wastewater, and real surface water. Ecotoxicological bioassays with *R. subcapitata*, *D. magna* and *C. elegans* were further used to provide information

for environmental health and to investigate the effects of BE before and after the proposed treatment. These biomarkers were selected since have not been used in BE toxicity tests. Several endpoints were monitored on terrestrial and aquatic organisms to expand the range of effect expression due to differences in species sensitivity and exposure. Furthermore, the potential genotoxicity of BE and its by-products was investigated by means of the count of micronucleus observed in *V. faba* roots.

We believe that this topic is of high interest to the readership of Journal of Hazardous Materials, since it deals with the removal of a hazardous contaminant of emerging concern widely found in wastewater effluents and surface waters.

This is an original manuscript not submitted elsewhere. All the authors confirm that the present manuscript has been prepared in compliance with the Ethics in Publishing Policy as described in the Guide for Authors of Journal of Hazardous Materials.

We look forward to receiving your editorial response in due course.

Yours sincerely

Danilo Spasiano, PhD

Dear Prof. Gerasimos Lyberatos,

please find enclosed a copy of the paper "Removal of benzoylecgonine from water matrices through UV_{254}/H_2O_2 process: reaction kinetic modeling, ecotoxicity and genotoxicity assessment" by Danilo Spasiano, Danilo Russo, Marianna Vaccaro, Antonietta Siciliano, Raffaele Marotta, Marco Guida, Nuno M Reis, Gianluca Li Puma and Roberto Andreozzi (**Manuscript No. HAZMAT-D-16-02632**) which has been revised according to the Referees' minor revisions (all the corrections and new insertions have been printed in **red bold** typefaces).

We thank the Referees for their valuable suggestions that helped us to ameliorate the text and mostly you for the assistance and cooperation.

The Authors

Reviewer #1:

The study determines kinetics on removal/degradation of BE, a main cocaine metabolite, by UV/H_2O_2 process. The authors test the process on different water matrices such as wastewater and surface water, as well as evaluate the effect of by-products and scavenging process. At the end, the authors make a preliminary toxicity tests (before and after process) in a batch reactor because of work facilities.

If well the degradation of BE through UV/H_2O_2 process it has been studied, this manuscript has special emphasis on determine kinetics of the process and, specially, on kinetics by-products scavengers, which is an interesting contribution. Moreover, the use of an innovative photoreactor for this purposes makes a remarkable study.

I write below some suggestions for easy understand the whole manuscript, and some little mistakes I founded on it.

- Specific comments by sections:

ABSTRACT:

It is well write and easy resume of the manuscript.

HIGHLIGHTS:

The meanings of the highlights are un-precise; authors should consider improving the highlights with emphasis on results.

Reply: The highlights are completely rearranged in agreement with reviewer's suggestions.

INTRODUCTION:

· Second Paragraph: Authors explain impact of BE in different aquatic organisms. I think the effect on cat brains is out of the context in this study.

Reply: The paragraph and the relative reference are removed from the manuscript as suggested by the reviewer.

· Fourth paragraph: "Furthermore, the ecotoxicity and genotoxicity of the transformation by-products of the UV_{254}/H_2O_2 process remains unclear". Some reference?

Reply: The cited sentence is referred to the BE by-products. It has been rearranged with the aim of clarify its meaning. At the Authors knowledge, there are no references about the ecotoxicity and genotoxicity of BE by-products deriving from UV_{254}/H_2O_2 process. The text (page 3) was revised as follows:

Furthermore, the ecotoxicity and genotoxicity of the BE transformation by-products generated through the UV_{254}/H_2O_2 process have not been reported.

METHODS:

·Analytical Methods:

-"Algal densities were determined by an indirect procedure using a spectrophotometer…" At which absorbance? Did you relate them with concentration measure? I guess you obtained a calibration curve, so will be good if you state at least the correlation between absorbance and concentration measure.

Reply: The text on page 5 was revised as follows:

Algal densities were followed by optical density measurements at 670 nm (OD_{670nm}) using a spectrophotometer (Hach Lange DR5000) and a 5 cm cuvette ($OD_{670nm} = 1.69 \cdot 10^6$ algae·ml^{-1} + 105857; $R^2 = 0.99$).

- Maybe a little explain about how relate H2O2 actinometry with fluence/dose on UV reactor will be helpful.

Reply: The experimental procedure for the determination of photonic flux at 254 nm emitted by low-pressure mercury vapor lamp is well described in the references [41,42] of the manuscript. Furthermore, a complete description about the estimation of UV_{254} photon flux emitted by the lamp coupled with the MCF used in this experimental campaign was already reported in a previous paper we recently published and cited in this manuscript as the 21st reference (see sections 3.2 and 3.3). We consider further explanation in this paper as redundant.

- I think authors should write a little paragraph about the initial concentration of H_2O_2, how to determine it, and if they remove the residual peroxide for post-analysis in the way residual H_2O_2 can still be active. It is clear for V. faba seeds, but what about the rest?
I see on results that there is no degradation of it during the process, so H_2O_2 should be removed before post-treatments analysis.

Reply: We are grateful to the referee for these valuable suggestions. H_2O_2 concentrations were monitored by means of HPLC analysis (see section 2.2.). As reported in section 2.3.4. "Negative tests were carried out on aqueous solutions containing 6.3 mM of hydrogen peroxide and 80 $\mu L \cdot L^{-1}$ of catalase (used to destroy the residual hydrogen peroxide), to verify that the mixture did not exert negative effects on the target organisms." On the other hand, for the position of the sentence it seems that this blank tests were carried out only for the V. faba seed and not for all the tested living organisms. Moreover, we omitted to report in the paper that the same amount of catalase from Micrococcus lysodeikticuswas was also rapidly added to the fresh samples at the three different treatment times. In fact, our goal was to estimate the toxic effect of BE and its by-products only and not that of the mixture of these compounds with 6.3 mM of H_2O_2. For this purpose, the sentence we cited was moved from its initial position and modified in order to overcome all misunderstandings highlighted by the referee (second paragraph of section 2.3.4) now reads as follows:
80 $\mu L \cdot L^{-1}$ of catalase (used to destroy the residual hydrogen peroxide) were diluted into the fresh samples collected from the batch reactor at the three different reaction times with the aim of neglecting the toxic effect of H_2O_2 present into the solutions at the concentration of 6.3 mM. Negative tests were carried out on aqueous solutions containing 6.3 mM of hydrogen peroxide and 80 µL·L-1 of catalase (used to destroy the residual hydrogen peroxide), to verify that the mixture did not exert negative effects on the four target organisms.

RESULTS AND DISCUSSION

-Table 1 is a little bit confuse: Each column is a different run? Please, specify which each column means.

Reply: Thank you for the precious suggestion. The table has been modified in agreement with the referee' suggestion.

-Explanation of Eq. 18: "$k_{HO/BP}$ was thee only unknown parameter". "The" instead of "thee"
Reply: It has been corrected.

- "The UV_{254}/H_2O_2/BE reaction kinetics model was validated by predicting the results of four additional runs carried out at a different photon flux (Table 2, runs 1s-4s)". Table 2 is actually table 3.
Reply: It has been corrected.

- "The standard deviations calculated for both BE and H_2O_2 (Table 3) were found to be below 1.06%". It is below 1.35% according to values of table 3.
Reply: The sentence the referee highlighted was related only to the runs used in the simulation mode: that explains the reason why we reported the 1.06% as the maximum standard deviation value. On the other hand, in agreement with the reviewer suggestion, it is more correct to refer this sentence to all the experimental runs used for both the optimization and simulation mode. At this purpose, we substitutes the 1.06 % with 1.41% (standard deviation value on BE, run 6, table 3) as the maximum found standard deviation value.

- On figure 4 does not appear the predicted line for H_2O_2, please plot it.
Reply: Done.

- "…MCF has been observed [22] and Following the same optimization methodology reported earlier". "f" instead of "F" on Following.
Reply: Done.

CONCLUSIONS
Conclusions are according to the experimental results. The authors explain the necessity of further investigations on toxicity field, which is a realistic requirement.

REFERENCES

References are up to date. I suggest some fresh references (year 2016) about UV/H_2O_2 process application and optimization and BE genotoxicity:

-Parolini, M., Magni, S., Castiglioni, S., Binelli, A. (2016). Genotoxic effects induced by the exposure to an environmental mixture of illicit drugs to the zebra mussel Ecotoxicology and Environmental Safety, 132, pp. 26-30. http://doi.org/10.1016/j.ecoenv.2016.05.022

-Moreno-Andrés, J., Romero-Martínez, L., Acevedo-Merino, A., &Nebot, E. (2016). Determining disinfection efficiency on E. faecalis in saltwater by photolysis of H_2O_2: Implications for ballast water treatment. Chemical Engineering Journal, 283, 1339 - 1348. http://doi.org/10.1016/j.cej.2015.08.079

Reply: We appreciated the reviewer suggestion. These new papers have been cited into the manuscript as the 13th and 24th reference respectively. Moreover, considering the referee' comments, we also changed the 58th reference with the following updated review: J. Schmidhuber, Deep learning in neural networks: An overview, Neural Networks 61 (2015) 85-117.

Reviewer #2

The manuscript submitted by Spasiano et al. is a novel piece of work which includes unpublished data related to benzoylecgonine kinetics. This work shows a deep kinetic study with a thorough statistical analysis and a modelling approach. I recommend its publication in Journal of Hazardous Materials, provided some minor issues are addressed:

- The Introduction would be enriched if some background around the state of the art on BE ecotoxicity is included

Reply: As also suggested by Reviewer #1, we added an additional reference regarding the BE genotoxic effect on zebra mussel (Parolini et al., 2016), reference 13th.

- How can the toxicity tests under taken in this work be extrapolated to real situations, with lower pollutant concentrations? How do the Authors' findings signify? Can the evolution of toxicity during degradation be extrapolated to lower pollutant concentrations? Please discuss in the text

Reply: E.E. Kenaga [70] summarized 125 acute chronic ratio (ACR) values for 84 chemicals 11 species of aquatic organisms. This parameter, expressed as the ratio among the acute EC50 and the chronic toxicity (maximum acceptable toxicant concentration, MATC), was find to be in the range $1.0\text{-}1.8\cdot10^4$. Considering an ACR equal to 10^3 and the EC50 of BE on nematodes evaluated in this work (0.6 ppm), a chronic toxic effect could be observed on nematodes at the BE concentration equal to 0.6 ppb. The predicted MATC value is in agreement with the results previously reported [13-15] regarding the negative effect of BE contaminated water solutions (0.5 and 1.0 ppb) on *Dreissena polymorpha*. Moreover, the ecotoxicity and genotoxicity tests carried out with the sample withdrawn at tf suggest, considering the same ACR value, that it is possible that the by-products, deriving by the UV_{254}/H_2O_2, treatment of a solution containing BE at the concentration of 1 ppb, could exert a negative chronic effect on living organisms.

All these considerations could be obviously validated through dedicated chronic tests, but the results regarding the acute toxicity of BE and its by-products on *C. elegans* and *V. faba*, suggest that chronic negative effects could be observed on living organisms subjected to the BE concentrations founded in STP effluents and surface waters.

All these consideration were added at the end of the section 3.3, as follows:

In a previous paper [70], 125 acute chronic ratio (ACR) values for 84 chemicals and 11 species of aquatic organisms were summarized. The ACR, expressed by the ratio of the acute EC50 and the chronic toxicity (maximum acceptable toxicant concentration, MATC), was found to be in the range 1.0-1.8·104. Considering an ACR equal to 103 and the EC50 of BE on C. elegans evaluated in this work (0.6 ppm), a chronic toxic effect could be observed on nematodes at the BE concentration equal to 0.6 ppb. In particular, the predicted MATC value is in agreement with the results previously reported [13-15] regarding the negative effect of BE contaminated water solutions (0.5 and 1.0 ppb) on Dreissena polymorpha. Moreover, the ecotoxicity and genotoxicity tests carried out with the sample withdrawn at tf suggest, considering the same ACR value, that it is possible that the by-products, deriving by the UV254/H2O2, treatment of a solution containing BE at the concentration of 1 ppb, could exert a negative chronic effect on living organisms.

All these considerations could be certainly validated through dedicated chronic tests, but the results regarding the acute toxicity of BE and its by-products on C. elegans and V. faba, suggest that chronic negative effects could also be observed on living organisms subjected to the BE concentrations founded in STP effluents and surface waters.

- Please add the limit of detection of the HPLC method and retention times

Reply: We have added this information on Section 2.2 as follows:

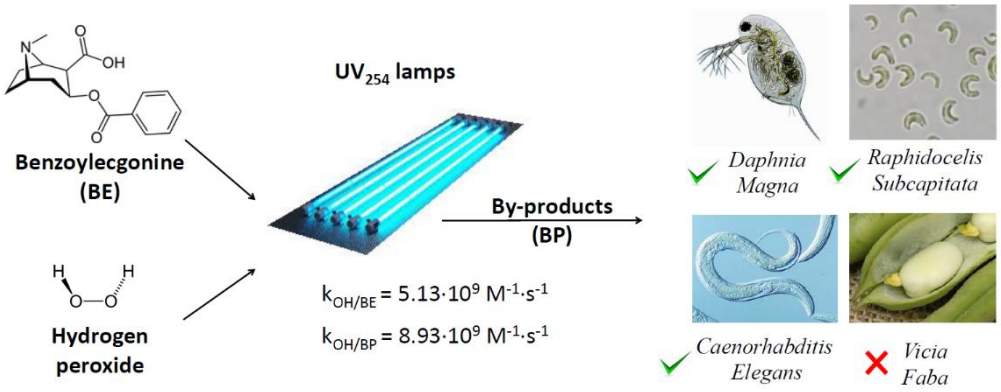
The retention times for H_2O_2, BE, and BA were 4.9, 13.8 and 17.1 min, respectively. Moreover, H_2O_2, BE, and BA concentrations down to 0.01 mM, $1.2 \cdot 10^{-4}$ mM and $2.08 \cdot 10^{-3}$ mM were successfully measured.

- What is the MCF reactor volume?

Reply: The MCF reactor (microcapillary film array photoreactor, see introduction) is a tubular continuous flow reactor so that its volume is strictly dependent on the length exposed to the UV radiation (only this can be properly defined as "reactor"). That is, for this experimental campaign, a volume ranging from 0 to 0.045 mL. If you are asking for the whole volume of the microcapillary film strip adopted, this is about 0.66 mL, for a length of about 2 m. The geometric properties among with the fluid dynamics of the reactor are reported in a previous paper we recently published and cited in this manuscript as the 21st reference. The information on the reactor volume was added in the manuscript as follows:

The MCF reactor volume exposed to UV irradiation varied according to the space time between 0 and 0.045 ml.

Graphical Abstract



Abstract

Benzoylecgonine (BE), the main cocaine metabolite, has been detected in numerous surface water and treatment plants effluents in Europe and there is urgent need for effective treatment methods. In this study, the removal of BE by the UV_{254}/H_2O_2 process from different water matrices was investigated. By means of competition kinetics method, the kinetic constant of reaction between BE and the photogenerated hydroxyl radicals ($^{\bullet}OH$) was estimated resulting in $k_{OH/BE} = 5.13 \cdot 10^9\ M^{-1} \cdot s^{-1}$. By-products and water matrices scavengers effects were estimated by numerical modeling of the reaction kinetics for the UV_{254}/H_2O_2 process and validated in an innovative microcapillary film (MCF) array photoreactor and in a conventional batch photoreactor. The ecotoxicity of the water before and after treatment was evaluated with four organisms *Raphidocelis subcapitata*, *Daphnia magna*, *Caenorhabditis elegans*, and *Vicia faba*. The results provided evidence that BE and its transformation by-products do not have significant adverse effects on *R. subcapitata*, while *D. magna* underwent an increase of lipid droplets. *C. elegans* was the most sensitive to BE and its by-products. Furthermore, a genotoxicity assay, using *V. faba*, showed cytogenic damages during the cell mitosis of primary roots.

Highlights

- **UV/H_2O_2 oxidation process is effective for BE removal from aqueous matrices**
- **MCF photoreactor technology is a valid and useful tool in AOPs studies**
- **The kinetic constants of HO radicals attack to BE and its by-products are estimated**
- **BE removal through UV/H_2O_2 process is modeled in different aqueous matrices**
- **Genotoxicity tests suggest the risk of long term effects of BE by-products**

Removal of benzoylecgonine from water matrices through UV_{254}/H_2O_2 process: reaction kinetic modeling, ecotoxicity and genotoxicity assessment

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Keywords: benzoylecgonine, illicit drugs, AOP, kinetic modeling, ecotoxicological assessment

Abstract

Benzoylecgonine (BE), the main cocaine metabolite, has been detected in numerous surface water and treatment plants effluents in Europe and there is urgent need for effective treatment methods. In this study, the removal of BE by the UV_{254}/H_2O_2 process from different water matrices was investigated. By means of competition kinetics method, the kinetic constant of reaction between BE and the photogenerated hydroxyl radicals ($^{\bullet}OH$) was estimated resulting in $k_{OH/BE} = 5.13 \cdot 10^9$ $M^{-1} \cdot s^{-1}$. By-products and water matrices scavengers effects were estimated by numerical modeling of the reaction kinetics for the UV_{254}/H_2O_2 process and validated in an innovative microcapillary film (MCF) array photoreactor and in a conventional batch photoreactor. The ecotoxicity of the water before and after treatment was evaluated with four organisms *Raphidocelis subcapitata*, *Daphnia*

magna, *Caenorhabditis elegans*, and *Vicia faba*. The results provided evidence that BE and its transformation by-products do not have significant adverse effects on *R. subcapitata*, while *D. magna* underwent an increase of lipid droplets. *C. elegans* was the most sensitive to BE and its by-products. Furthermore, a genotoxicity assay, using *V. faba*, showed cytogenic damages during the cell mitosis of primary roots.

1. Introduction

BE is a contaminant of emerging concern deriving from the consumption of cocaine, one of the most widely used illicit drugs [1]. The human metabolism of cocaine is dominated by hydrolytic ester cleavage resulting in urine metabolites consisting mostly of BE (45%), ecgonine methyl ester (40%), and a residue of unchanged cocaine (1-9%) [2]. Consequently, BE represents the primary cocaine metabolite and it is detected in almost all surface water (SurW) and sewage treatment plants (STP) effluents in Europe always at levels higher than cocaine [3,4]. Although the removal of BE in conventional STP is typically 80% [5,6], the effluent concentrations is in the range 0.1-3275 $ng \cdot L^{-1}$ [7,8]. BE in SurW ranges between 0.3-530 $ng \cdot L^{-1}$ [9-11] and in river sediments is reported as 1.0 $ng\ g^{-1}$ [12].

The exposure of freshwater mussel, *Dreissena polymorpha*, to BE contaminated water solutions (0.5 and 1.0 ppb), yielded a 3.5-fold increase in oxidative stress and increased or inhibited antioxidant and detoxifying enzymes activity depending on BE levels and exposure time [13-15]. Significant lipids peroxidation and protein carbonylation, DNA damage, and cellular apoptotic death were observed in experiments carried out up to 14 days [16]. Another study showed the negative effect of BE on fishes and plants [17].

The UV_{254}/H_2O_2 is an emerging STP tertiary treatment process, which is increasingly being used for water reuse in public works and in agriculture [18-20]. In previous studies [21,22] we have investigated the BE removal with both UV_{254} and UV_{254}/H_2O_2 using an innovative microcapillary film (MCF) array photoreactor, which allowed extremely rapid experimentation with minimal

sample volumes. The feasibility of BE removal by direct photolysis (UV_{254}) in STP during a conventional water disinfection treatment is questionable due to the low molar adsorption coefficient and quantum yield [21]. However, BE removal in STP is a promising proposition using the UV_{254}/H_2O_2 process, as we determined much faster BE oxidation in different water matrices: milliQ water, synthetic wastewater (SWW), real wastewaters (RWW), and SurW [22]. Other studies have demonstrated that Fenton, Fenton-like, and potassium ferrate treatment are also effective methods for BE removal in STP effluents [23] although their practical implementation can be problematic.

In this study, we therefore focused on the UV_{254}/H_2O_2 process as a potential tertiary treatment of STP effluents [24-31] since it appears of more feasible implementation.

In the UV_{254}/H_2O_2 process the homolytic cleavage of H_2O_2 leads to the formation of $^{\bullet}OH$ radicals (1) that unselectively attack dissolved organic substances such as BE (2) [32]:

$$H_2O_2 \xrightarrow{hv} 2\ {}^{\bullet}OH \qquad (1)$$

$$^{\bullet}OH + BE \xrightarrow{k_{OH/S}} By - products \qquad (2)$$

However, the second-order rate constant ($k_{OH/BE}$) for the reaction of BE with $^{\bullet}OH$ is unknown. **Furthermore, the ecotoxicity and genotoxicity of the BE transformation by-products generated through the UV_{254}/H_2O_2 process have not been reported.**

In this study, competition kinetics in the presence of benzoic acid (BA) [33,34], was used to determine the second-order rate constant ($k_{OH/BE}$) for the reaction of BE with $^{\bullet}OH$, which was evaluated in the MCF array photoreactor and validated in a batch photoreactor. The effect of by-products and water matrices scavengers was estimated by numerical modeling of the reaction kinetics for the UV_{254}/H_2O_2 process in the MCF array photoreactor dissolving BE in milli-Q water, SWW, RWW, and SurW. Ecotoxicological bioassays with *R. subcapitata*, *D. magna* and *C. elegans* were further used to provide information for environmental health and to investigate the effects of BE before and after the proposed treatment. Several endpoints were monitored on terrestrial and

aquatic organisms to expand the range of effect expression due to differences in species sensitivity and exposure. Furthermore, the potential genotoxicity of BE and its by-products was investigated by means of the count of micronuclei observed in *V. faba* roots.

This study was based on the "effect-driven approach" [35] in which a parent compound is undergoing degradation and is analyzed with eco-bioassays to follow the toxicity evolution during a transformation process.

Indeed, it was chosen an initial concentration of BE higher than usually found in effluents or SurW [7-12] to better assess ecotoxicological effects of by-products solutions, as reported in previous studies [36-38]. Furthermore, such high concentrations are not of limited relevance because they are suitable to determine the median effective concentration (EC50), an indicator of by-products toxicity and the time onset of the effects.

The investigation on the relative toxicities of BE and its by-products is relevant to future discussions regarding the treatment, control and fate of BE and BE-derivatives in the environment.

2. Materials and methods

2.1. Materials

Hydrogen peroxide (30% v/v), benzoylecgonine (≥99% w/w), acetonitrile (≥99.9% v/v), formic acid (≥95% v/v), benzoic acid (≥99.5% w/w), catalase from Micrococcus lysodeikticus were purchased from Sigma-Aldrich. Milli-Q water (18 MΩ resistivity) was prepared with a Millipore Elix water purification system.

In agreement with the OECD guidelines [36], SWW was prepared with peptone (32 ppm), meat extract (22 ppm), urea (6 ppm), K_2HPO_4 (28 ppm), $CaCl_2{\cdot}H_2O$ (4 ppm), NaCl (7 ppm) and Mg_2SO_4 (0.6 ppm) in milli-Q water. These substances were from Sigma-Aldrich and were used as received. RWW was sampled from the Severn Trent Sewage Water Treatment Plant of Festival Drive, Loughborough, Leicestershire (UK). SurW was collected from the Grand Union Canal in the same

area. RWW and SurW samples were filtered through Whatman nylon filters (0.45 µm) to avoid clogging of the MCF and of the high performance liquid chromatography (HPLC).

For toxicity assessment, reference toxicants (potassium dichromate, cupric chloride, maleic hydrazide) and salts for the preparation of artificial freshwater ($CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7\ H_2O$, KCl, $NaHCO_3$, $NaNO_3$, NH_4Cl, $MgCl_2 \cdot 6(H_2O)$, K_2HPO_4, KH_2PO_4, $FeCl_3 \cdot 6(H_2O)$, $Na_2EDTA \cdot 2(H_2O)$ H_3BO_3, $MnCl_2 \cdot 4(H_2O)$ $ZnCl_2$, $CoCl_2 \cdot 6(H_2O)$, $Na_2MoO_4 \cdot 2(H_2O)$, $CuCl_2 \cdot 2(H_2O)$, NaCl) were used. All chemicals were analytical grade supplied by Sigma Aldrich. Double distilled water (Microtech) was used to prepare dilution water and treatments.

2.2. Analytical methods

Hydrogen peroxide, BE, and benzoic acid were assayed by HPLC (1100 Agilent) equipped with a Gemini C18 (Phenomenex) reverse phase column and a diode array detector (λ = 232 nm). The mobile phase was a mixture of formic acid aqueous solution (25 mM) (A) and acetonitrile (B) flowing at 0.6 mL min^{-1} with a gradient 7% B to 28% B in 9 min, then 50% B in 5 min, constant for 2 min, and then to 7% B in 3 min. **The retention times for H_2O_2, BE, and BA were 4.9, 13.8 and 17.1 min, respectively. Moreover, H_2O_2, BE, and BA concentrations down to 0.01 mM, $1.2 \cdot 10^{-4}$ mM and $2.08 \cdot 10^{-3}$ mM were successfully measured.** The pH of the reacting solutions and the total organic carbon (TOC) of water samples were measured with an Accumet Basic AB-10 pH-meter and a TOC-5000A TOC analyzer (Shimadzu), respectively.

Crustaceans and nematodes viability were observed with a stereomicroscope (LEICA EZ4-HD) and visualized on a computer. Nuclei and micronuclei were observed through 40x/0.50 objective lenses in a microscope (Nikon, Eclipse E1000) equipped with a digital camera (Nikon, DXM 1200 F) and acquisition software (Nikon ACT1). **Algal densities were followed by optical density measurements at 670 nm (OD_{670nm}) using a spectrophotometer (Hach Lange DR5000) and a 5 cm cuvette ($OD_{670nm} = 1.69 \cdot 10^6$ algae$\cdot ml^{-1} + 105857$; $R^2 = 0.99$).**

2.3. Experimental apparatus and procedures

2.3.1. MCF photoreactor

The water matrices (milli-Q, SWW, RWW, and SurW) were spiked with BE and H_2O_2. The concentrations of BE (0.6 – 18.5 ppm) were higher than those found in RWW and SurW [3,4,7,8] to allow a more precise evaluation of the kinetic parameters. BE and H_2O_2 under dark conditions did not react. BA (BA:BE molar ratio = 2.5) was added in the runs performed to determine $k_{OH/BE}$ by competition kinetics.

The UV_{254}/H_2O_2 BE oxidation kinetics were determined in a MCF array photoreactor (Figure 1a) containing 10 microcapillaries with a mean hydraulic diameter (D) of 194 μm. The description and validation of the MCF array photoreactor has been reported elsewhere [21,40]. The microcapillaries were coiled around a UV monochromatic lamp (Germicidal G8T5, 8W) emitting at 253.7 nm. Segments not coiled were kept in the dark with aluminum foil. Experiments were performed in continuous flow through the reactor at different space times (i.e., the reaction time) with capillaries of different length exposed to the UV_{254} lamp. The fluid velocity in the capillaries was $5.56 \cdot 10^{-2}$ $m \cdot s^{-1}$ and the MCF flow rate was $1.0 \cdot 10^{-3}$ $l \cdot min^{-1}$. **The MCF reactor volume exposed to UV irradiation varied according to the space time between 0 and 0.045 ml.** Samples were collected from the feed and at the MCF outlet, after reaching the steady-state condition (i.e., five times the reactor volume processed) and analyzed. Experiments were carried out isothermally at room temperature (~ 25 °C) and the fluid temperature flowing through the reactor remained unchanged. The pH of the feed solution during the each experimental run was constant. In addition, BE removal was insensitive to pH in the range 4.0-8.0 [22].

The lamp irradiance and thus the incident flux in the capillaries was varied by changing the electrical power supplied to the lamp from 4.5 W to 8.0 W using a variable power supply unit. The photon fluxes per unit volume emitted by the UV lamp ($I_{0/MCF}/V_{MCF}$) for each power setting were estimated with an indirect approach by H_2O_2 actinometry [41,42] and the calculated values were

$1.26 \cdot 10^{-2}$ ein$\cdot s^{-1} \cdot L^{-1}$ and $1.92 \cdot 10^{-2}$ ein$\cdot s^{-1} \cdot L^{-1}$, respectively. The MCF average optical path length (l_{MCF}) was 152 μm (Figure 1a).

2.3.2. Cylindrical batch photoreactor

In the cylindrical batch photoreactor (V_{batch} = 0.480 l) the BE/H_2O_2 aqueous solution was irradiated with a low-pressure mercury monochromatic lamp (Helios Italquartz, HGL10T5L, 17W nominal power emitting at 253.7 nm) immersed in the center axis. The photon path length (l_{batch}) was 2.20 cm (Figure 1b). The reactor was thermostated at 25 °C and wrapped with aluminum foil. At the top, the reactor had two inlets for feeding reactants and for collecting samples. The photon flux emitted by the UV lamp at 253.7 nm by H_2O_2 actinometry was $I_{0/batch} = 2.86 \cdot 10^{-6}$ ein$\cdot s^{-1}$. The primary use of the cylindrical batch photoreactor was to provide sufficient liquid volumes to carry out the toxicity tests on samples collected at different reaction times.

2.3.3. Optimization and validation procedures

The unknown kinetic parameters were estimated from the results obtained from the MCF photoreactor. $k_{OH/BE}^{\cdot}$ was evaluated trough the competition kinetics method in the presence of benzoic acid [33,34]. Subsequently, the reaction rate constants for the radical scavengers (k'_{sca}) and by-products ($k_{OH/BE}$) were estimated by numerical modelling.

After the determination of the reaction rate constants of the elementary reactions in the proposed reaction mechanism, additional experimental runs were executed, in both MCF and cylindrical batch photoreactors, to validate the proposed kinetic model, without further adjustment of the model parameters. Figure 2 summarizes graphically the optimization and validation procedures used.

2.3.4. Ecotoxicity assessment apparatus and procedures

Ecotoxicity assessments of untreated and treated water samples were carried out with four different organisms including two primary producers, the freshwater alga *R. subcapitata* and the plant *V.*

faba and two primary consumers, the cladoceran *D. magna* and the nematode *C. elegans*. These essential components of the food chain are highly sensitive analytical tools to screen the toxicity of environmental chemicals [43-48].

80 µL·L^{-1} of catalase (used to destroy the residual hydrogen peroxide) were diluted into the fresh samples collected from the batch reactor at the three different reaction times with the aim of neglecting the toxic effect of H_2O_2 present into the solutions at the concentration of 6.3 mM. Negative tests were carried out on aqueous solutions containing 6.3 mM of hydrogen peroxide and 80 µL·L-1 of catalase (used to destroy the residual hydrogen peroxide), to verify that the mixture did not exert negative effects on the four target organisms.

An ISO protocol [49] was used to measure the algal growth inhibition with *R. subcapitata*. For each sample, six replicates were inoculated with 10^7 algal cells·L^{-1} in well plates and incubated for 72 h test at 23 ± 2 °C under continuous illumination (in the irradiance range of 120-60 µein· m^{-2} s^{-1}).

The specific growth rate (µ) of *R. subcapitata* in each replicate was calculated from the logarithmic increase in cell density in the interval 0 to 72 h as follows:

$$\mu = \frac{\ln N_i - \ln N_0}{t_i - t_0} \qquad (3)$$

where N_i represents the cell concentration at time t_i. Results were expressed as the mean (± standard deviation) of the percentage inhibition of cell growth *vs.* negative controls.

The toxicity of the samples on *Daphnia magna* was assessed by viability, morphological alterations, heartbeat frequency, and immobilization endpoints. ISO 6341 protocol [50] was followed to evaluate the acute bioassay at 24 and 48 hours. Test daphnids were generated from a laboratory breeding facility operating according to OECD 202 [51]. For each sample, four replicates with 20 daphnids (<24 h old) in each well were tested in static tests. The immobilization frequencies were recorded at 24 and 48 hours. The tests were considered valid if the immobilization in the control did not exceed 10%. The daphnids heart rate was counted with the stereomicroscope after an

acclimation period (120 s). The heart rate readings were taken per daphnids, at regular intervals (30 s) for total time of 60 min.

Toxicity tests in *C. elegans*, wild-type strain N2 variant Bristol, were performed using an age-synchronous L4-larval nematodes method [52]. Ten worms at a time were placed into 24-well tissue culture plates containing 0.5 mL of water sample and 0.5 mL of K-medium as negative control. All treatments were done in triplicate and without feeding the worms. The worms were exposed for 24 hr at 20°C. The number of dead worms was determined by absence of movement monitored by the stereomicroscope.

The samples genotoxicity potential was performed on *Vicia faba* root tips [53]. Bioassays used 90 mm Petri dishes with one Whatman No. 1 filter paper as support. After adding 5 seeds and 6 mL of the sample (previously equilibrated for 24 h in the dark), the Petri dishes were incubated in the dark at 22 °C for 96 h. After exposure, the *V. faba* root tips were immersed for 24 h in 1:3 acetic acid:ethanol solutions, then were cut, stained in Schiff's Reagent using Feulgens method [54] and squashed on microscope slides. The micronucleus frequency (MN) was evaluated in $1 \cdot 10^3$ cells of *V. faba* seeds. Positive tests on *V. faba* ecotoxicity were perfomed with an aqueous solution of maleic hydrazide (5 ppm). Whenever possible, ecotoxicity data were expressed as the EC50 values and its 95% confidence intervals calculated by non-linear regression.

The significance of the differences between the mean values of different tests and controls was assessed by Student's test and analysis of variance (ANOVA) with a 0.05 significance level. Moreover, *post-hoc* analysis were carried on with Dunnett's method and Tukey's test [55-56].

3. Results and discussions

3.1. Competition kinetic method for $k_{HO/BE}$ value evaluation

Competition kinetics was used to evaluate the second-order rate constant $k_{OH/BE}$ between $^{\bullet}OH$ radicals and BE which compares the substrate concentration decay to that of the reference compound, benzoic acid (BA) [33,34]. With this procedure, the estimation of $k_{OH/BE}$ was not

influenced by the reactor geometry and by the presence of scavengers such as water impurities since both BE and BA were exposed to the same $^{\bullet}OH$ radical concentration. The BE and BA mass balances in the MCF are:

$$\frac{d[BE]}{d\tau} = -k_{OH/BE} \cdot [BE] \cdot [^{\bullet}OH] \quad (4)$$

$$\frac{d[BA]}{d\tau} = -k_{OH/BA} \cdot [BA] \cdot [^{\bullet}OH] \quad (5)$$

where τ is the space-time and $k_{OH/BA}$ = $5.9 \cdot 10^9$ $M^{-1} \cdot s^{-1}$ the kinetic rate constant of the reaction between the $^{\bullet}OH$ radicals and BA [33,34].

Integrating Eqs. (4) and (5) yields the linear relation between the logarithms of the normalized BE and BA concentrations:

$$\ln\left(\frac{[BE]}{[BE]_0}\right) = \frac{k_{OH/BE}}{k_{OH/BA}} \cdot \ln\left(\frac{[BA]}{[BA]_0}\right) \quad (6)$$

Three experimental runs varying the H_2O_2 concentration were performed to evaluate $k_{OH/BE}$ (Table 1). On the basis of the results reported in Table 1, an average value of $k_{OH/BE}$ = $5.13 \cdot 10^9 \pm 2.6 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ was calculated.

3.2. Evaluation of by-products and scavenging effect

3.2.1 Reaction kinetics model development and validation in the MCF photoreactor and evaluation of the reaction by-products effect

A simplified reaction kinetics mechanism was developed to describe the removal of BE by UV_{254}/H_2O_2 process in water in the absence of scavengers (milli-Q water) (Table 2). It takes into account both, direct BE photolysis (r_1) with the consequent production of by-products (BPs) and hydrogen peroxide photolysis (r_2) with the generation of $^{\bullet}OH$ radicals, which further attack H_2O_2 (r_3), BE (r_4), and all the generated by-products, considered as a pseudo-component (BPs) (r_5). Finally, the peroxyl radicals ($HO_2^{\bullet}$) produced through the reaction r_4 undergo radical termination reaction to generate hydrogen peroxide (r_6).

The mole balances on hydroxyl and peroxyl radicals in the MCF are:

$$\frac{d[^{\bullet}OH]}{d\tau} = 2F_{H_2O_2} - [^{\bullet}OH] \cdot \left(k_h \cdot [H_2O_2] - k_{OH/BE} \cdot [BE] - k_{OH/BP} \cdot [BPs]\right) \quad (7)$$

$$\frac{d[HO_2^{\bullet}]}{d\tau} = k_h \cdot [^{\bullet}OH] \cdot [H_2O_2] - 2k_t \cdot [HO_2^{\bullet}]^2 \quad (8)$$

where $F_{H_2O_2}$ is the rate of photolysis of H_2O_2 expressed as:

$$F_{H_2O_2} = \frac{\Phi_{254}^{H_2O_2} \cdot I_{0/MCF}}{V_{MCF}} \cdot \left(1 - \exp\left(-2.3 \cdot l_{MCF} \cdot \left(\varepsilon_{254}^{BE} \cdot [BE] + \varepsilon_{254}^{H_2O_2} \cdot [H_2O_2]\right)\right)\right) \cdot f_{H_2O_2} \quad (9)$$

where $f_{H_2O_2}$ is the UV_{254} radiation fraction absorbed by H_2O_2.

Assuming steady-state of all radical species [58], the concentrations of $HO^{\bullet}$ and $HO_2^{\bullet}$ are:

$$[^{\bullet}OH]_{ss} = \frac{2 \cdot F_{H_2O_2}}{k_h \cdot [H_2O_2] + k_{OH/BE} \cdot [BE] + k_{OH/BP} \cdot [BPs]} \quad (10)$$

$$[HO_2^{\bullet}]_{ss}^2 = \frac{k_h}{k_t} \cdot \frac{2 \cdot F_{H_2O_2}}{k_h \cdot [H_2O_2] + k_{OH/BE} \cdot [BE] + k_{OH/BP} \cdot [BPs]} \quad (11)$$

On the other hand, the mole balances on BE and H_2O_2 are:

$$\frac{d[BE]}{d\tau} = -F_{BE} - k_{OH/BE} \cdot [BE] \cdot [^{\bullet}OH] \quad (12)$$

$$\frac{d[H_2O_2]}{d\tau} = -F_{H_2O_2} - k_h \cdot [^{\bullet}OH] \cdot [H_2O_2] + k_t \cdot [HO_2^{\bullet}]^2 \quad (13)$$

The term F_{BE} in Eq. (12), is the rate of BE direct photolysis expressed as:

$$F_{BE} = \frac{\Phi_{254}^{BE} \cdot I_{0/MCF}}{V_{MCF}} \cdot \left(1 - \exp\left(-2.3 \cdot l_{MCF} \cdot \left(\varepsilon_{254}^{BE} \cdot [BE] + \varepsilon_{254}^{H_2O_2} \cdot [H_2O_2]\right)\right)\right) \cdot f_{BE} \quad (14)$$

where f_{BE} is the fraction of UV_{254} radiation absorbed by BE.

Replacing Eqs. (10-11) into (12-13) yields:

$$\frac{d[BE]}{d\tau} = -F_{BE} - \frac{2 \cdot k_{OH/BE} \cdot F_{H_2O_2} \cdot [BE]}{k_h \cdot [H_2O_2] + k_{OH/BE} \cdot [BE] + k_{OH/BP} \cdot ([BE]_0 - [BE])} \quad (15)$$

$$\frac{d[H_2O_2]}{d\tau} = -F_{H_2O_2} - \frac{k_h \cdot F_{H_2O_2} \cdot [H_2O_2]}{k_h \cdot [H_2O_2] + k_{OH/BE} \cdot [BE] + k_{OH/BP} \cdot ([BE]_0 - [BE])} \quad (16)$$

where the by-product concentration equals the amount of BE reacted.

The integration of two ODEs (Eqs. 15-16) results in the BE and H_2O_2 concentration profiles vs space time in the MCF. The unknown rate constant $k_{HO/BP}$ was estimated by an iterative optimization procedure that minimized the square of the differences between the calculated (y) and experimental (c) concentrations of each species (optimization mode) [59]. Specifically, the objective function (Φ) was expressed as:

$$\Phi = \sum_{g=1}^{h}\sum_{i=1}^{l}\sum_{m=1}^{n}\left(y_{g,i,m} - c_{g,i,m}\right)^2 \quad (17)$$

where *h*, *l* and *n* respectively represent the number of experimental data recorded in each experiment, the number of the reacting species, and the number of experiment used in the optimization procedure. The percentage standard deviation on the i^{th} species was calculated as follows:

$$\sigma_i(\%) = 100 \cdot \frac{1}{\bar{c}_i}\sqrt{\sum_{g=1}^{h}\frac{\left(y_{i,g}-c_{i,g}\right)^2}{h-p}} \quad (18)$$

where $\bar{c}_i$ represents the average measured concentration of the i^{th} species and *p* the number of the unknown parameters to be estimated. In this case, *p* = 1, since $k_{HO/BP}$ was **the** only unknown parameter.

Table 3 (runs 1-6) show the experimental conditions of the runs used in the optimization procedure and the corresponding standard deviation (σ_i) on BE and H_2O_2 from the model. The unknown rate constant was therefore estimated as $k_{OH/BP} = 8.93 \cdot 10^9 \pm 1.0 \cdot 10^9\ M^{-1}s^{-1}$ (95% confidence interval).

The UV_{254}/H_2O_2/BE reaction kinetics model was validated by predicting the results of four additional runs carried out at a different photon flux (Table **3**, runs 1s-4s) without further adjustment of the reaction rate constant estimated (simulation mode). Figure 3 show excellent prediction of the concentrations of BE and H_2O_2 at different space time. The standard deviations calculated for both BE and H_2O_2 (Table 3) were found to be below **1.41**% which demonstrated a very close agreement between the kinetic model and the experimental results.

3.2.1.1 Model validation on a batch photoreactor

The cylindrical batch reactor, reported in Figure 1b, is characterized by a drastically different geometry compared to the MCF, but Eqs. (15-16) of the reaction kinetics model are still valid once the parameters related to the reactor geometry F_{BE} and F_{H2O2} were changed (Eqs. 19-20) and space-time τ replaced by reaction time t:

$$F_{BE} = \frac{\Phi_{254}^{BE} \cdot I_{0/batch}}{V_{batch}} \cdot \left(1 - \exp\left(2.3 \cdot l_{batch} \cdot \left(\varepsilon_{254}^{BE} \cdot [BE] + \varepsilon_{254}^{H_2O_2} \cdot [H_2O_2]\right)\right)\right) \cdot f_{BE} \quad (19)$$

$$F_{H_2O_2} = \frac{\Phi_{254}^{H_2O_2} \cdot I_{0/batch}}{V_{batch}} \cdot \left(1 - \exp\left(2.3 \cdot l_{batch} \cdot \left(\varepsilon_{254}^{BE} \cdot [BE] + \varepsilon_{254}^{H_2O_2} \cdot [H_2O_2]\right)\right)\right) \cdot f_{H_2O_2} \quad (20)$$

An experimental UV_{254}/H_2O_2/BE run was carried out in the cylindrical batch photoreactor to further validate the reaction kinetics model of BE oxidation by the UV_{254}/H_2O_2 process. The profiles of BE and H_2O_2 concentrations against reaction time were compared with those obtained by the proposed reaction kinetic model, without further adjustment of the previously estimated parameters (validation mode). The results in Figure 4 show a robust prediction capability by the model of the experimental results even though the reactor geometry radically changed in comparison to the MCF. σ_i(%) of BE and H_2O_2 were 0.56% and 0.28%, respectively.

3.2.2. Evaluation of scavenging effect

The role of radical scavenging species [60] was evaluated in three further water matrices including synthetic (SWW) and real (RWW) wastewater, and surface water (SurW) to provide a realistic analysis of the effectiveness of the UV_{254}/H_2O_2 process on the removal of BE. For this purpose the reaction kinetics model of BE oxidation was modified (Eqs. 21-22) to include the effect of scavengers:

$$\frac{d[BE]}{d\tau} = -F_{BE} - \frac{2 \cdot k_{HO/BE} \cdot F_{H_2O_2} \cdot [BE]}{k_h \cdot [H_2O_2] + k_{OH/BE} \cdot [BE] + k_{OH/BP} \cdot ([BE]_0 - [BE]) + k'_{sca}} \quad (21)$$

$$\frac{d[H_2O_2]}{d\tau} = -F_{H_2O_2} - \frac{k_h \cdot F_{H_2O_2} \cdot [H_2O_2]}{k_h \cdot [H_2O_2] + k_{OH/BE} \cdot [BE] + k_{OH/BP} \cdot ([BE]_0 - [BE]) + k'_{sca}} \quad (22)$$

where $k'_{sca} = k_{sca} \cdot [SCA]$ is the *pseudo*-first order rate constant of the reaction between the $^{\bullet}OH$ radicals and the scavenger species. Since similar removal rates of BE in SWW and RWW in the MCF has been observed [22] and **following** the same optimization methodology reported earlier, $k'_{sca/SWW} = k'_{sca/RWW} = 4.01 \cdot 10^5 \pm 1.8 \cdot 10^4\ s^{-1}$ was estimated from three experimental runs in the MCF (run 1-3, Table 4) with a 97.5% confidence interval.

The model was validated by predicting the results of four additional runs carried out with both SWW and RWW (Table 4, runs 1s-4s) without further adjustment of the reaction rate constant estimated (validation mode). Although a small systematic error appeared on BE trends of run 2s and run 3s, the comparison between experimental and theoretical results (Figure 5) and the percentage standard deviations on the measured species (Table 4) highlights a good capability of the model of predicting the BE and H_2O_2 consumption in both SWW and RWW.

The procedure was repeated for two further runs executed with SurW in the MCF (Table 4, runs 4-5, optimization mode) yielding of $k'_{sca/SurW} = 6.82 \cdot 10^5 \pm 3.8 \cdot 10^4\ s^{-1}$, which were validated with two further experiments (Table 4, runs 4s-6s, and Figure 6, validation mode) showing a good model prediction capability of the experimental results.

3.3. Ecotoxicity and genotoxicity of treated water

Ecotoxicological tests were performed on samples collected from UV_{254}/H_2O_2/BE experiments performed in the cylindrical batch reactor with an aqueous solution containing BE and H_2O_2 at initial concentrations of $3.5 \cdot 10^{-3}$ M (10 ppm) and $6.3 \cdot 10^{-3}$ M (214 ppm) respectively. In addition to the sample (t_0) collected at time zero, two further samples were collected at reaction time of 1.5 min (t_f) corresponding to almost complete BE conversion (Figure 4) and at 3.0 min ($2t_f$) reasonably representative of a solution containing secondary oxidation by-products. Within the reaction range chosen (0 - 3.0 min), TOC analysis did not evidenced any noticeable mineralization phenomena.

The exposure to *D. magna* for 24 h and 48 h of contact time to untreated and treated solutions were not showed since no significant immobilization was induced compared to negative controls. A detailed inspection of daphnids at 48 h under static conditions confirmed the absence of clear morphological changes, as well as, a comparable growth in all groups of exposure (t-test). Microscopically observations showed an increase, compared to the control groups, in the size of lipid droplets in juveniles of *D. magna* after the exposure to t_0 and t_f samples (Figure 7). These droplets were already observed when daphnids were exposed to various toxic substances such as insecticide or nanoparticles [61-63]. Generally, the accumulated lipid droplets indicate abnormal synthesis or metabolism of lipids and it is plausible that samples corresponding to t_0 and t_f reaction times could alter lipid homeostasis in *D. magna*. When exposed to samples treated for $2t_f$, daphnids showed a yellow pigmentation of the body (Figure 7) which indicates BE toxicity [64]. The heartbeat rate of daphnids exposed to samples collected at different treatment times was similar to that observed for the control group (t-test). A previous study, carried out on the exposure of zebrafish (*Danio rerio*) embryo to BE contaminated solutions, confirms this findings since any appreciable change of the cardiac rate of the tested specie was observed [17].

Growth inhibition data for algae *R. subcapitata* exposed to BE contaminated solution at different reaction times are shown in Figure 8. The maximum effect (12.01%) was observed when algal suspensions were exposed to the sample collected at t_f, but even lower inhibitory effects were detected in t_0 and $2t_f$ (10.9 and 8.3 % of inhibition respectively), with significant differences of the treated samples to the control. A similar effect was reported on green algae exposure to **anatoxin** [65], a neurotoxin structurally similar to benzoylecgonine [66].

When *C. elegans* was exposed for 24 h at 20 °C to undiluted samples, the archived percentage mortality of worms ranged from 90 and 100% for all tested samples (Figure 9). The viability of *C. elegans* increased after exposure of $2t_f$ undiluted solution, however this effect was not statistically different than t_0 and t_f samples. In contrast, the exposure of *C. elegans* to diluted samples showed that 50% survival of nematodes occurred at 6%, 8% and 22% dilution for the t_0, t_f and $2t_f$ samples

respectively. On the basis of these results the EC_{50} was estimated as 0.6 ± 0.1 ppm. The nematodes offered higher sensitivity to BE and its reaction by-products, recognizing the role of *C. elegans* for drug discovery and neurobehavioral toxicity [67-69].

V. faba was exposed to the t_0, t_f and $2t_f$ samples though liquid phase exposure routes. All samples showed similar responses, but significantly different in relation to the negative control. A remarkable increase of MN was found for exposures to t_f sample that was comparable to MN in the positive control (Ctr+) (Figure 10) suggesting that the first generation of BE by-products induce a higher MN generation into *V. faba* root tips. Additional tests with diluted samples at a ratio of 1:2 showed a MN frequency not significantly different compared to the negative control.

In a previous paper [70], 125 acute chronic ratio (ACR) values for 84 chemicals and 11 species of aquatic organisms were summarized. The ACR, expressed by the ratio of the acute EC50 and the chronic toxicity (maximum acceptable toxicant concentration, MATC), was found to be in the range 1.0-1.8·10^4. Considering an ACR equal to 10^3 and the EC50 of BE on *C. elegans* evaluated in this work (0.6 ppm), a chronic toxic effect could be observed on nematodes at the BE concentration equal to 0.6 ppb. In particular, the predicted MATC value is in agreement with the results previously reported [13-15] regarding the negative effect of BE contaminated water solutions (0.5 and 1.0 ppb) on *Dreissena polymorpha*. Moreover, the ecotoxicity and genotoxicity tests carried out with the sample withdrawn at t_f suggest, considering the same ACR value, that it is possible that the by-products, deriving by the UV_{254}/H_2O_2, treatment of a solution containing BE at the concentration of 1 ppb, could exert a negative chronic effect on living organisms.

All these considerations could be certainly validated through dedicated chronic tests, but the results regarding the acute toxicity of BE and its by-products on *C. elegans* and *V. faba*, suggest that chronic negative effects could also be observed on living organisms subjected to the BE concentrations founded in STP effluents and surface waters.

Conclusions

Benzoylecgonine removal by the UV_{254}/H_2O_2 treatment was modeled using the results collected in ultrarapid experiments performed in the MCF reactor and using very small water volumes. The reaction kinetics model was successfully validated on an experimental run carried out on a radically different and much bigger cylindrical batch reactor, thus demonstrating the possibility of using a MCF reactor for ultrarapid photochemistry investigations. By competition kinetics the rate constant of the reaction between BE and the photogenerated HO radicals in milli-Q water was estimated as $k_{OH/BE} = 5.13 \cdot 10^9\ M^{-1}\ s^{-1}$. The reaction kinetic constant of the reaction between the HO radicals and BE transformation by-products resulted be equal to $8.93 \cdot 10^9\ M^{-1}\ s^{-1}$ while the ones between the HO radicals and the scavengers present into RWW and SurW were estimated as $4.01 \cdot 10^5\ s^{-1}$, and $6.82 \cdot 10^5\ s^{-1}$ respectively.

The growth of *R. subcapitata* and the immobility of *D. magna* were not significantly influenced during the exposure to all the samples. However, an increase in lipid droplets was observed in *D. magna,* probably related to the presence of BE and its photodegradation by-products, but the significance of the response needs further investigations.

The survival of *C. elegans* dramatically decreased in presence of the untreated and treated BE solutions. Marked DNA damages on *V. faba* were found in samples collected at the reaction time corresponding to the complete conversion of BE, thus indicating a toxic effect ascribed to the presence of primary by-products. Our results herein demonstrate that this photocatalytic process is very effective, with the prospect development of technologies for BE detoxification by second generation of BE by-products that reduce the acute eco-toxicity of treated solutions.

Even though the concentrations of BE were higher than those found into the environment, the results on acute toxicity suggest further investigations on the long-term effects of BE and its UV_{254}/H_2O_2 transformation by-products on environmental health.

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References

[1] World Drug Report, United Nations Office on Drugs and Crime, Vienna 1-112, 2012.

[2] S. Castiglioni, E. Zuccato, C. Chiarabrando, R. Fanelli, R. Bagnati, Mass spectrometric analysis of illicit drugs in wastewater and surface water, Mass Spectrom. Rev. 27 (2008) 378-394.

[3] R.J. Irvine, C. Kostakis, P.D. Felgate, E.J. Jaehne, C. Chen, J.M. White, Population drug use in Australia: a wastewater analysis, Forensic Sci. Int. 210 (2011) 69-73.

[4] F. Yin-Lai, R. Bruno, H.W. Leung, P.K. Thai, C. Ort, S. Carter, K. Thompson, P.K.S. Lam, J.F. Mueller, Estimating daily and diurnal variations of illicit drug use in Hong Kong: a pilot study of using wastewater analysis in an Asian metropolitan city, Forensic Sci. Int. 233 (2013) 126-132.

[5] B. Du, A.E. Price, W.C. Scott, L.A. Kristofco, A.J. Ramirez, C.K. Chambliss, J.C. Yelderman, B.W. Brooks, Comparison of contaminants of emerging concern removal, discharge, and water quality hazards among centralized and on-site wastewater treatment system effluents receiving common wastewater influent, Sci. Total Environ. 466-467 (2014) 976-984.

[6] C. Repice, M. Dal Grande, R. Maggi, R. Pedrazzani, Licit and illicit drugs in a wastewater treatment plant in Verona, Italy, Sci. Total Environ. 463−464 (2013) 27-34.

[7] M. Huerta-Fontela, M.T. Galceran, J. Martin, F. Ventura, Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain, Sci. Total Environ. 397 (2008) 31-40.

[8] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters, Water Res. 43 (2009) 363-380.

[9] J.D. Berset, R. Brenneisen, C. Mathieu, Analysis of llicit and illicit drugs in waste, surface and lake water samples using large volume direct injection high performance liquid chromatography – electrospray tandem mass spectrometry (HPLC–MS/MS), Chemosphere 81 (2010) 859-866.

[10] A.L.N. Van Nuijs, B. Pecceu, L. Theunis, N. Dubois, C. Charlier, P.G. Jorens, L. Bervoets, R. Blust, H. Neels, A. Covaci, Cocaine and metabolites in waste and surface water across Belgium, Environ. Pollut. 157 (2009) 123-129.

[11] E. Zuccato, S. Castiglioni, R. Bagnati, C. Chiabrando, P. Grassi, R. Fanelli, Illicit drugs, a novel group of environmental contaminants, Water Res. 42 (2008) 961-968.

[12] R. Álvarez-Ruiz, M.J. Andrés-Costa, V. Andreu, Y. Picó, Simultaneous determination of traditional and emerging illicit drugsin sediments, sludges and particulate matter, J. Chromatogr. A 1405 (2015) 103-115.

[13] **M. Parolini, S. Magni, S. Castiglioni, A. Binelli, Genotoxic effects induced by the exposure to an environmental mixture of illicit drugs to the zebra mussel, Ecotoxicology and Environmental Safety 132 (2016) 26-30.**

[14] M. Parolini, A. Pedriali, C. Riva, A. Binelli, Sub-lethal effects caused by the cocaine metabolite benzoylecgonine to the freshwater mussel Dreissena polymorpha, Sci. Total Environ. 444 (2013) 43-50.

[15] A. Pedriali, C. Riva, M. Parolini, S. Cristoni, D. Sheehan, A. Binelli, A redox proteomic investigation of oxidative stress caused by benzoylecgonine in the freshwater bivalve Dreissena polymorpha, Drug Test. Anal. 5 (2013) 646-656.

[16] A. Binelli, I. Marisa, M. Fedorova, R. Hoffmann, C. Riva, First evidence of protein profile alteration due to main cocaine metabolite (benzoylecgonine) in a freshwater biological model, Aquat. Toxicol. 140-141 (2013) 268-278.

[17] J.P. García-Cambero, H. García-Cortés, Y. Valcárcel, M. Catalá, Environmental concentrations of the cocaine metabolite benzoylecgonine induced sublethal toxicity in the development of plants but not in a zebrafish embryo–larval model, J. Hazard. Mater. 300 (2015) 866-872.

[18] G. Orona, C. Campos, L. Gillerman, M. Salgot, Wastewater treatment, renovation and reuse for agricultural irrigation in small communities, Agric. Water Manage. 38 (3) (1999) 223–234.

[19] D. Christova-Boal, R.E. Eden, S. McFarlane, An investigation into greywater reuse for urban residential properties, Desalination 106(1–3) (1996) 391-397.

[20] G.Grissop, Orange county water district groundwater replenishment system, Journal of the New England Water Works Association 123(3) (2009) 230-237.

[21] D. Russo, D. Spasiano, M. Vaccaro, R. Andreozzi, G. Li Puma, N.M. Reis, R. Marotta, Direct photolysis of benzoylecgonine under UV irradiation at 254 nm in a continuous flow microcapillary film (MCF) array photoreactor, Chem. Eng. J. 283 (2016) 243-250.

[22] D. Russo, D. Spasiano, V. Vaccaro, K.H. Cochran, S.D. Richardson, R. Andreozzi, G. Li Puma, N.M. Reis, R. Marotta, Investigation on the removal of the major cocaine metabolite (benzoylecgonine) in water by UV_{254}/H_2O_2, process by using a flow microcapillary film array photoreactor as an efficient experimental tool, Water Res. 89 (2016) 375-383.

[23] T. Mackulak, L. Birošová, I. Bodík, R. Grabic, A. Takáčová, M. Smolinská, A. Hanusová, J. Híveš, M. Gál, Zerovalent iron and iron(VI): Effective means for the removal of psychoactive pharmaceuticals and illicit drugs from wastewaters, Sci. Total Environ. 539 (2016) 420-426.

[24] **J. Moreno-Andrés, L. Romero-Martínez, A. Acevedo-Merino, E. Nebot, Determining disinfection efficiency on E. faecalis in saltwater by photolysis of H_2O_2: Implications for ballast water treatment, Chem. Eng. J. 283 (2016) 1339-1348.**

[25] B.S. Souza, R.F. Dantas, M. Agullo-Barcelò, F. Lucena, C. Sans, S. Esplugasa, M. Dezotti, Evaluation of UV/H_2O_2 for the disinfection and treatment of municipal secondary effluents for water reuse, J. Chem. Technol. Biotechnol. 88 (2013) 1697-1706.

[26] D. Rubio, E. Nebot, J.F. Casanueva, C. Pulgarin, Comparative effect of simulated solar light, UV, UV/H_2O_2 and photo-Fenton treatment (UV-Vis/H_2O_2/$Fe^{2+,3+}$) in the Escherichia coli inactivation in artificial seawater, Water Res. 47 (2013) 6367-6379.

[27] J.C. Kruithof, P.C. Kamp, B.J. Martijn, UV/H_2O_2 Treatment: A Practical Solution for Organic Contaminant Control and Primary Disinfection, Ozone Sci. Eng. 29 (2007) 273-280.

[28] U. Alkan, A. Teksoy, A. Atesli, H.S. Baskaya, Efficiency of the UV/H_2O_2 process for the disinfection of humic surface waters, J. Environ. Sci. Health., Part A 42 (2007) 497-506.

[29] A. Lopez, A. Bozzi, G. Mascolo, J. Kiwi, Kinetic investigation on UV and UV/H_2O_2 degradations of pharmaceutical intermediates in aqueous solution, J. Photochem. Photobiol., A 156(1-3) (2003) 121-126.

[30] D. Vonga, R. Marotta, A. Napolitano, R. Andreozzi, M. d'lschia, Advanced oxidation of the pharmaceutical drug diclofenac with UV/H_2O_2 and ozone, Water Res. 38(2) (2004) 414-422.

[31] V.J. Pereira, K.G. Linden, H.S. Weinberg, Evaluation of UV irradiation for photolytic and oxidative degradation of pharmaceutical compounds in water. Water Res., 41(19) (2007) 4413-4423.

[32] R. Andreozzi, V. Caprio, A. Insola, R. Marotta, Advanced oxidation processes (AOP) for water purification and recovery, Catal. Today 53(1) (1999) 51-59.

[33] G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (OH/O) in aqueous solution, J. Phys. Chem. Ref. Data, 17 (1988) 513-886.

[34] P. Onstein, M.I Stefan, J.R. Bolton, Competition kinetics method for the determination of rate constants for the reaction of hydroxyl radicals with organic pollutants using the UV/H_2O_2 advanced oxidation technology: the rate constants for the tert-buthyl formate ester and 2,4-dinitrophenol, J. Adv. Oxid. Technol. 4(2) (1999) 231-236.

[35] B.I Escher, K. Fenner, Recent advances in environmental risk assessment of transformation products, Environ. Sci. Technol. 45 (2011) 3835 – 3847.

[36] C. Postigo, C. Sirtori, I. Ollerb, S. Malatob, M. I. Maldonado, M. L. de Aldaa, D. Barceló, Solar transformation and photocatalytic treatment of cocaine in water: Kinetics, characterization of major intermediate products and toxicity evaluation, Applied Catalysis B: Environmental 104 (2011) 37–48.

[37] T. An, J. An, Y. Gao, G. Li, H. Fanga, W. Song, Photocatalytic degradation and mineralization mechanism and toxicity assessment of antivirus drug acyclovir: Experimental and theoretical studies, Applied Catalysis B: Environmental 164 (2015) 279–287

[38] J. Xu, Z. Hao, C. Guo, Y. Zhang, Y. He, W. Meng, Photodegradation of sulfapyridine under simulated sunlight irradiation: Kinetics, mechanism and toxicity evolvement, Chemosphere 99 (2014) 186–191

[39] Organisation for Economic Cooperation and development (OECD), Guidelines for testing of Chemicals, Simulation Test-Aerobic Sewage Treatment, 1999, 303A.

[40] N.M. Reis, G. Li Puma, A novel microfluidic approach for extremely fast and efficient photochemical transformations in fluoropolymer microcapillary films, Chem. Commun. 51 (2015) 8414-8417.

[41] I. Nicole, J. De Laat, M. Doré, J.P. Duguet, C. Bonnel, Use of UV radiation in water treatment: measurement of photonic flux by hydrogen peroxide actinometry, Water Res. 24 (1990) 157-168.

[42] S. Goldstein, D. Aschengrau, Y. Diamant, J. Rabani, Photolysis of aqueous H_2O_2: quantum yield and applications for polychromatic UV actinometry in photoreactors, Environ. Sci. Technol. 41 (2007) 7486-7490.

[43] K. Ura, T. Kai, S. Sakata, T. Iguchi, K. Arizono, Aquatic acute toxicity testing using the nematode Caenorhabditis elagans, J. Health Sci. 48 (2002) 583-586.

[44] P.L. Williams, D.B. Dusenbery, A promising indicator of neurobehavioral toxicity using the nematode Caenorhabditis elegans and computer tracking, Toxicol. Ind. Health 6 (1990) 425-440.

[45] G. Persoone, R. Baudo, M. Cotman, C. Blaise, K.C. Thompson, M. Moreira-Santos, B. Vollat, A. Törökne, T. Han, Review on the acute Daphnia magna toxicity test - Evaluation of the

sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs, Knowl. Managt. Aquatic Ecosyst. 393 (2009) 01.

[46] A. Siciliano, R. Gesuele, G. Pagano, M. Guida, How Daphnia (Cladocera) Assays may be used as Bioindicators of Health Effects? J. Biodivers. Endanger Species S1 (2015) 005.

[47] I. Blinova, Use of freshwater algae and duckweeds for phytotoxicity testing, Environ. Toxicol. 19 (2004) 425-428.

[48] M. Iqbal, Vicia faba bioassay for environmental toxicity monitoring: A review. Chemosphere 144 (2016) 785-802.

[49] International Organization for Standardization (ISO) 8692, Water quality - Freshwater algal growth inhibition test with unicellular green algae (2012).

[50] International Organization for Standardization (ISO) 6341, Water quality - Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) - Acute toxicity test (2012).

[51] Organisation for Economic Cooperation and development (OECD), Guidelines for the Testing of Chemicals, Section 2, 2008, 202.

[52] S.G. Donkin, P.L. Williams, Influence of developmental stage, salts and food presence on various end points using Caenorhabditis elegans for aquatic toxicity testing, Environ. Toxicol. Chem. 14 (1995) 2139-2147.

[53] T.H. Ma, Vicia faba cytogenetic test for environmental mutagen. A report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutation Research 99 (1982) 257-271.

[54] J. Alexander, K.S. Mccarty, E. Alexanderjackson, Rapid method of preparing Schiff reagent for the Feulgen test, Science 111 (1950) 13-13.

[55] C.W. Dunnett, A multiple comparison procedure for comparing several treatments with a control, J. Amer. Statist. Assoc. 50 (1955) 1096-1121.

[56] J. W. Tukey, Comparing individual means in the analysis of variance, Biometrics 5 (1949) 99–114.

[57] B.H. Bielski, D.E. Cabelli, R.L. Aruda, A.B. Ross, Reactivity of HO_2/O_2 radicals in aqueous solution, J. Phys. Chem. Ref. Data 14 (1985) 1041-1077.

[58] J. De Laat, P. Berger, T. Poinot, N.K. Vel Leitner, M. Dorè, Proceedings of the 12th Ozone World Congress of the International Ozone Association, Lille, May 1995, 373.

[59] **J. Schmidhuber, Deep learning in neural networks: An overview, Neural Networks 61 (2015) 85-117.**

[60] B.A. Wols, C.H.M. Hofman-Caris, Review of photochemical reaction constants of organic micropollutants required for UV advanced oxidation processes in water, Water Res. 46 (2012) 2815-2827.

[61] B. Sosak-Swiderska, D. Tyrawska, D. Dzido, Daphnia magna ecotoxicity test with parathion, Chemosphere 37(14) (1998) 2989-3000.

[62] M.C. Artal, R.D. Holtz, F. Kummrow, O.L. Alves, G. de A. Umbuzeiro, The role of silver and vanadium release in the toxicity of silver vanadate nanowires toward Daphnia similis, Environ. Toxicol. Chem. 32 (2013) 908-912.

[63] S. Asghari, S.A. Johari, J.H. Lee, Y.S. Kim, Y.B. Jeon, H.J. Choi, M.C. Moon, I.J. Yu, Toxicity of various silver nanoparticles compared to silver ions in Daphnia magna, J. Nanobiotechnology 10 (2012) 14.

[64] V.A. Lakhnova, Influence of aniline on Daphnia magna Straus, Freshwater Biological Association, Windermere, UK, 1982.

[65] K.D. Kearns, M.D. Hunter, Green algal extracellular products regulate antialgal toxin production in a cyanobacterium, Environ. Microbiol. 2 (2000) 291-297.

[66] J.S. Metcalf, G.A. Codd, Cyanobacteria, neurotoxins and water resources: Are there implications for human neurodegenerative disease? Amyotroph. Lateral Scler. 10(s2) (2009) 74-78.

[67] M. Artal-Sanz, L. de Jong, N. Tavernarakis, C. elegans: A versatile platform for drug discovery, Biotechnol. J. 1 (2006) 1405-1418.

[68] L. Ségalat, Invertebrate animal models of diseases as screening tools in drug discovery, ACS Chem. Bio. 2 (2007) 231-236.

[69] P.L. Williams, D.B. Dusenbery, Aquatic toxicity testing using the nematode, Caenorhabditis elegans, Environ. Toxicol. Chem. 9 (1990) 1285-1290.

[70] E.E. Kenaga, Predictability of chronic toxicity from acute toxicity of chemicals in fish and aquatic invertebrates, Environ. Toxicol. Chem. 1 (1982) 347-358.

Table

	Run 1	Run 2	Run 3
$[H_2O_2]_0$ (10^{-3} M)	17.9	6.4	2.3
$k_{OH/BE}$ (10^{-9} $M^{-1}\cdot s^{-1}$)	5.37	5.17	4.85
R^2	0.9998	0.9975	0.9971

Table 1

r_1)	$BE \xrightarrow{h\nu} BPs$	$\Phi_{254}^{BE} = 6.22 \cdot 10^{-3}\ mol \cdot ein^{-1}$ $\varepsilon_{254}^{BE} = 1684\ M^{-1} \cdot cm^{-1}$	[21]
r_2)	$H_2O_2 \xrightarrow{h\nu} 2HO^{\bullet}$	$\Phi_{254}^{H_2O_2} = 0.55\ mol \cdot ein^{-1}$ $\varepsilon_{254}^{H_2O_2} = 18.6\ M^{-1} \cdot cm^{-1}$	[42]
r_3)	$HO^{\bullet} + H_2O_2 \xrightarrow{k_h} H_2O + HO_2^{\bullet}$	$k_h = 2.7 \cdot 10^7\ M^{-1} \cdot s^{-1}$	[33]
r_4)	$BE + HO^{\bullet} \xrightarrow{k_{OH/BE}} BPs$	$k_{OH/BE} = 5.13 \cdot 10^9\ M^{-1} \cdot s^{-1}$	(estimated in this study)
r_5)	$BPs + HO^{\bullet} \xrightarrow{k_{OH/BP}} BP$	$k_{OH/BP}$	(estimated in this study)
r_6)	$2HO_2^{\bullet} \xrightarrow{k_t} H_2O_2 + O_2$	$k_t = 8.3 \cdot 10^5 M^{-1} \cdot s^{-1}$	[57]

Table 2

Run	$[BE]_0$ (M)	$[H_2O_2]_0$ (M)	$I_{0/MCF}/V_{MCF}$ (ein·s^{-1}·L^{-1})	σ_{BE} (%)	$\sigma_{H_2O_2}$ (%)
1	$6.39\cdot10^{-5}$	$5.03\cdot10^{-3}$	$1.92\cdot10^{-2}$	1.35	0.08
2	$2.52\cdot10^{-5}$	$1.04\cdot10^{-3}$		0.92	0.69
3	$2.87\cdot10^{-5}$	$0.42\cdot10^{-3}$		1.01	0.61
4	$2.62\cdot10^{-5}$	$5.00\cdot10^{-3}$		0.39	0.31
5	$3.35\cdot10^{-5}$	$10.4\cdot10^{-3}$		0.16	0.13
6	$2.98\cdot10^{-5}$	$20.4\cdot10^{-3}$		1.41	0.18
1s	$2.82\cdot10^{-5}$	$0.42\cdot10^{-3}$	$1.26\cdot10^{-2}$	0.56	0.52
2s	$2.82\cdot10^{-5}$	$1.10\cdot10^{-3}$		0.45	0.21
3s	$2.84\cdot10^{-5}$	$2.31\cdot10^{-3}$		0.77	0.19
4s	$2.35\cdot10^{-5}$	$5.05\cdot10^{-3}$		1.06	0.98

Table 3

Run	Matrix	$[BE]_0$ (M)	$[H_2O_2]_0$ (M)	σ_{BE} (%)	$\sigma_{H_2O_2}$ (%)
1	SWW	$2.1 \cdot 10^{-6}$	$5.0 \cdot 10^{-4}$	0.37	0.37
2	SWW	$3.26 \cdot 10^{-5}$	$7.4 \cdot 10^{-3}$	0.48	0.17
3	SWW	$3.08 \cdot 10^{-5}$	$5.0 \cdot 10^{-3}$	0.71	0.40
4	SurW	$2.80 \cdot 10^{-5}$	$1.4 \cdot 10^{-3}$	0.49	0.52
5	SurW	$2.88 \cdot 10^{-5}$	$6.7 \cdot 10^{-3}$	0.15	0.43
1s	SWW	$3.08 \cdot 10^{-5}$	$1.5 \cdot 10^{-3}$	0.59	0.98
2s	RWW	$3.34 \cdot 10^{-5}$	$7.6 \cdot 10^{-3}$	1.53	0.15
3s	RWW	$3.36 \cdot 10^{-5}$	$14.9 \cdot 10^{-3}$	1.82	0.22
4s	RWW	$3.37 \cdot 10^{-5}$	$21.9 \cdot 10^{-3}$	0.55	0.24
5s	SurW	$3.21 \cdot 10^{-5}$	$13.1 \cdot 10^{-3}$	0.56	0.16
6s	SurW	$3.05 \cdot 10^{-5}$	$19.2 \cdot 10^{-3}$	0.65	0.15

Table 4

Figure

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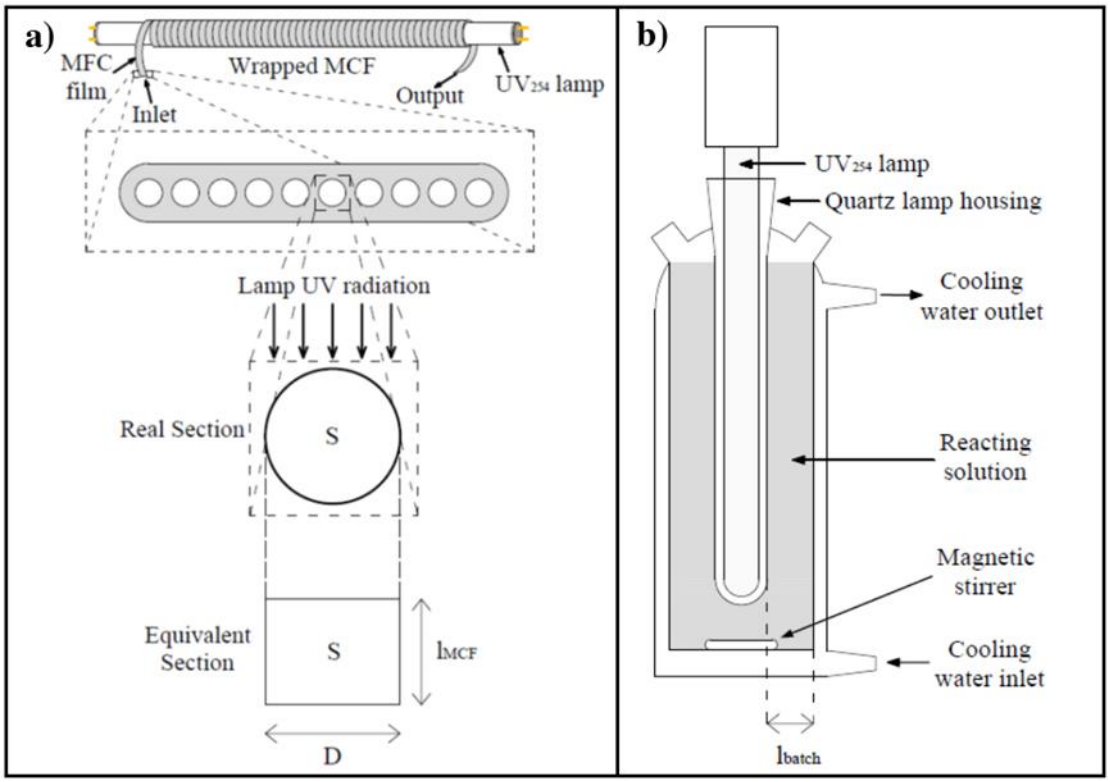

Figure 1

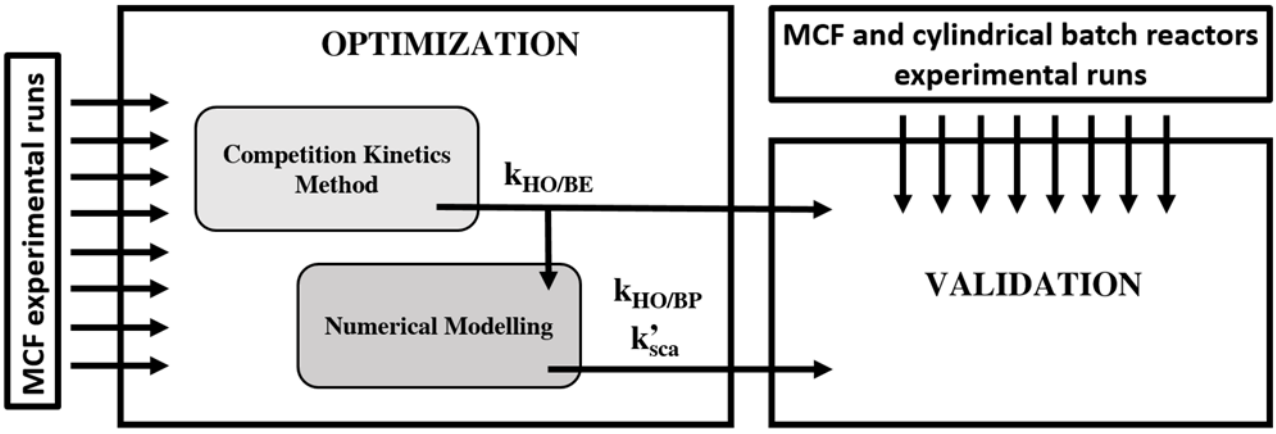
MCF experimental runs
OPTIMIZATION
Competition Kinetics Method
$k_{HO/BE}$
Numerical Modelling
$k_{HO/BP}$
k'_{sca}
MCF and cylindrical batch reactors experimental runs
VALIDATION

Figure 2

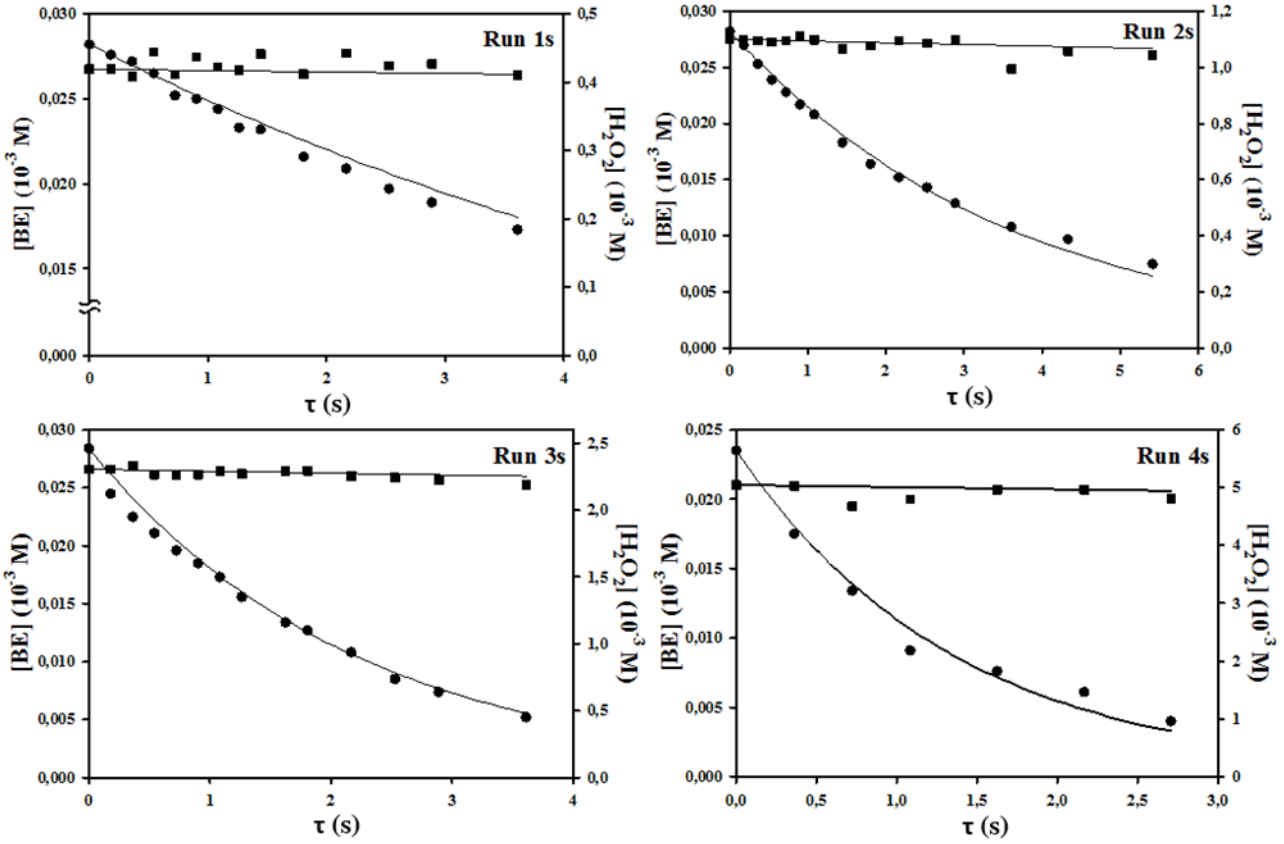

Figure 3

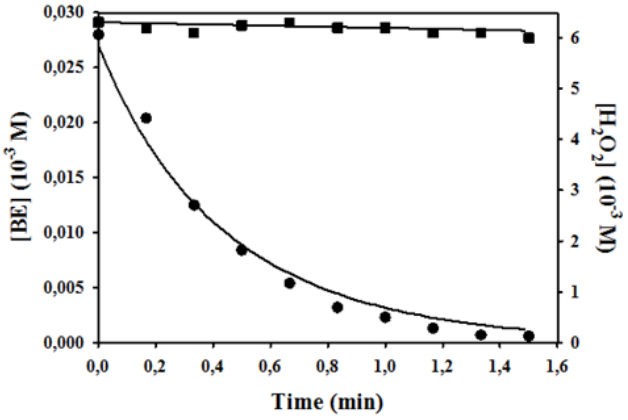
0,030
0,025
0,020
0,015
0,010
0,005
0,000
[BE] (10-3 M)
6
5
4
3
2
1
0
[H2O2] (10-3 M)
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
1,6
Time (min)

Figure 4

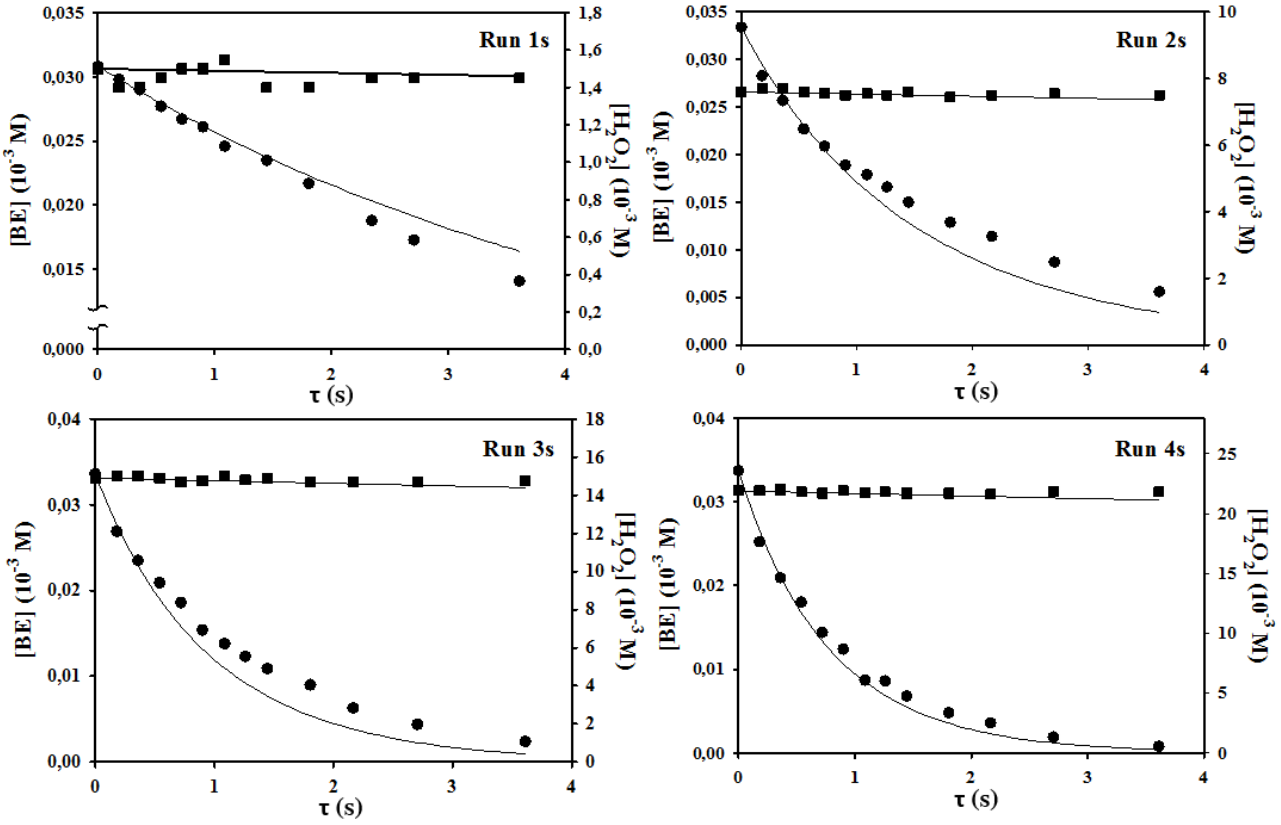

Figure 5

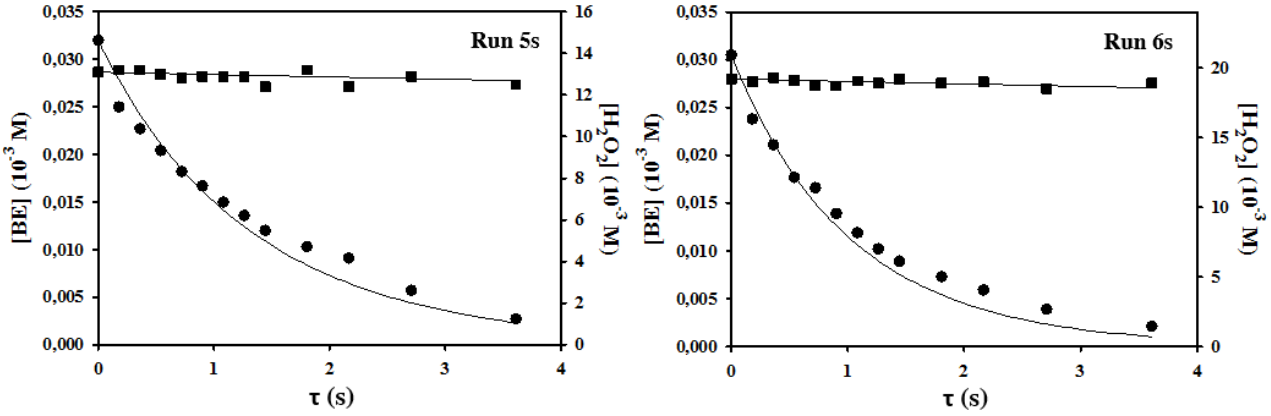

Figure 6

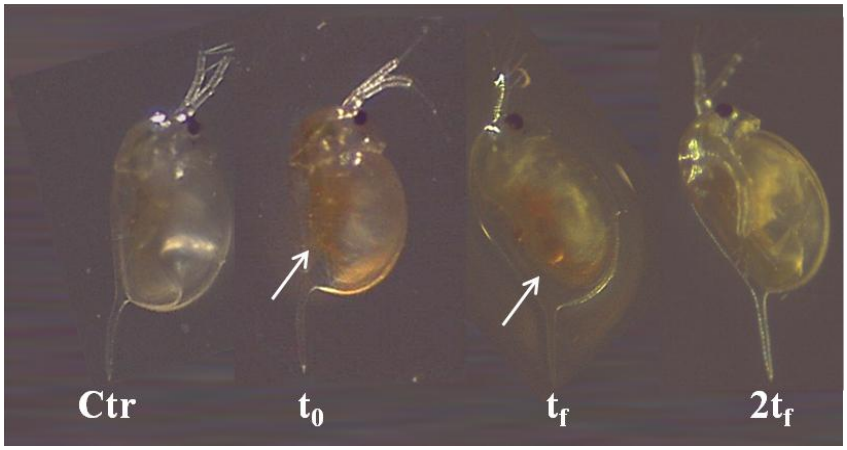

Figure 7

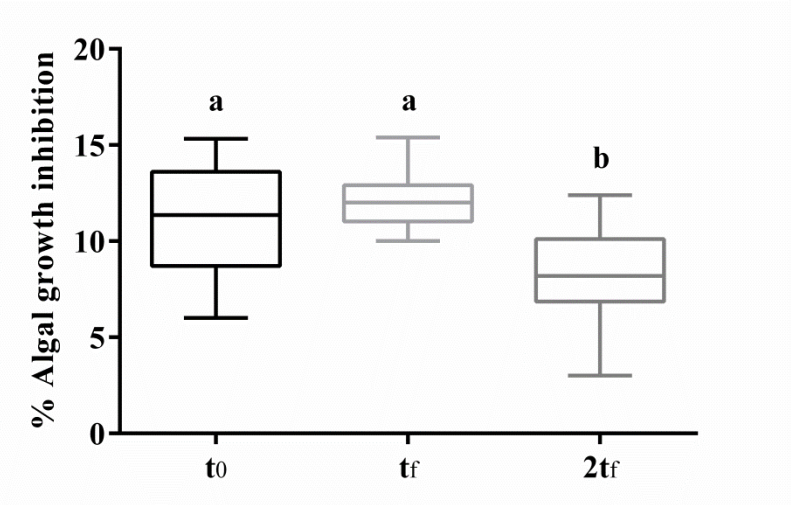

Figure 8

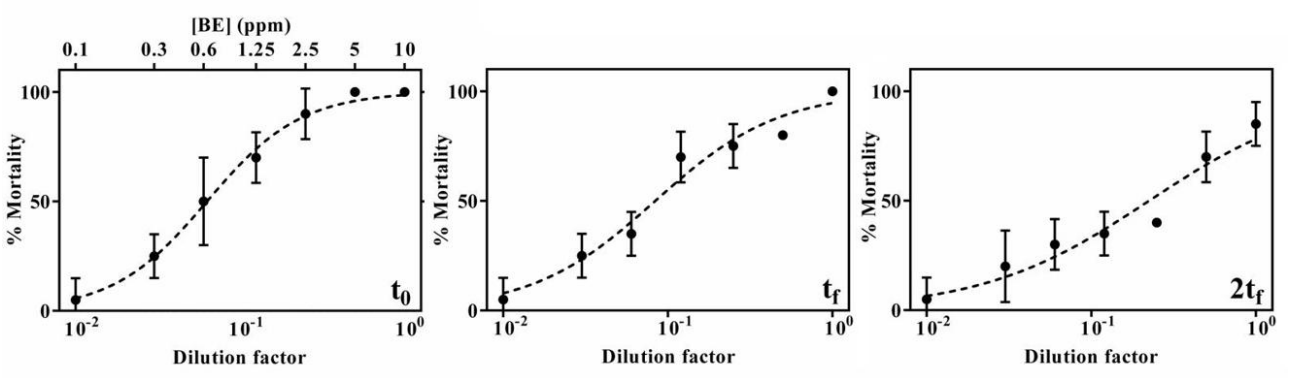
[BE] (ppm)
0.1
0.3
0.6
1.25
2.5
5
10
% Mortality
100
50
0
10^{-2}
10^{-1}
10^{0}
Dilution factor
t_0
t_f
$2t_f$

Figure 9

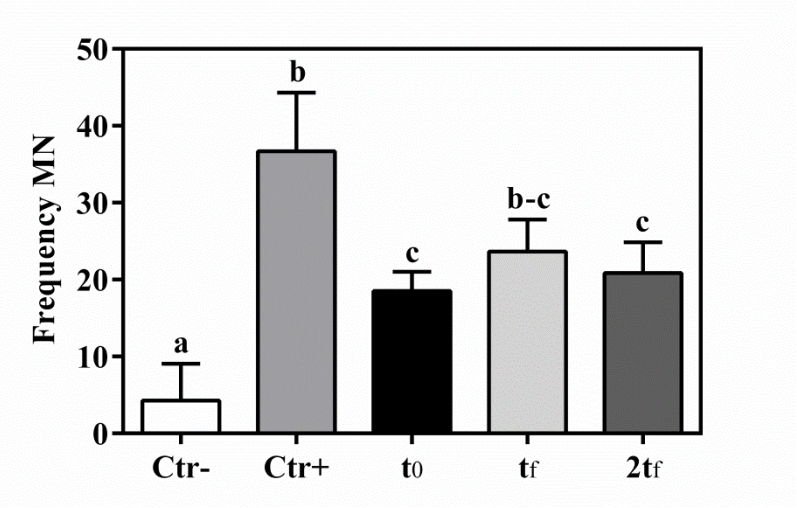

Figure 10

Figure
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Fig. 1. MCF photoreactor (a) and cylindrical batch photoreactor (b).

Fig. 2. Optimization and validation procedures scheme.

Fig. 3. UV_{254}/H_2O_2 removal of BE from milli-Q water in the MCF at different space times. Predicted (continuous lines) and experimental (symbols). (●) [BE], (■) [H_2O_2]. pH=6.0. T=25 °C.

Fig. 4. UV_{254}/H_2O_2 removal BE from milli-Q water in the cylindrical batch photoreactor. Predicted (continuous lines) and experimental (symbols). (●) [BE], (■) [H_2O_2]. $[BE]_0=3.5\cdot10^{-5}$ M, $[H_2O_2]_0=6.3\cdot10^{-3}$ M. pH=6.0. T=25 °C.

Fig. 5. UV_{254}/H_2O_2 removal of BE from SWW (run 1s) and RWW (run 2s-4s) in the MCF at different space times. Predicted (continuous lines) and experimental (symbols). (●) [BE], (■) [H_2O_2]. $I_{0/MCF}/V_{MCF}=1.92\cdot10^{-2}$ ein$\cdot s^{-1}\cdot L^{-1}$. pH_{SWW}= 6.0. pH_{RWW}=7.6. T = 25 °C.

Fig. 6. UV_{254}/H_2O_2 removal of BE from SurW in the MCF at different space times. Predicted (continuous lines) and experimental (symbols). (●) [BE], (■) [H_2O_2]. $I_{0/MCF}/V_{MCF}=1.92\cdot10^{-2}$ ein$\cdot s^{-1}\cdot L^{-1}$. pH=7.8. T=25 °C.

Fig. 7. *D.magna* exposed to BE samples treated at different reaction times. The arrows indicate the presence of lipid droplets in t_0 and t_f samples.

Fig. 8. Growth inhibition of algae *R. subcapitata* exposed to BE samples treated at different reaction times. Data with different letters (a and b) are significantly different ($p<0.05$).

Fig. 9. Dose-response curve describing the inhibitory effects of BE and its UV_{254}/H_2O_2 by-products on survival of *C. elegans*.

Fig. 10. Mean micronucleus (MN) frequencies (expressed per 1000 cells) detected in *V. faba* root tip cells after exposure to BE samples treated at different reaction times. Data with different letters (a-c) are significantly different ($p<0.05$).

Table 1 Competition kinetics results. $[BE]_0=4\cdot10^{-5}$ M. $[BA]_0=1.0\cdot10^{-4}$ M. pH=6.0. T=25 °C.

Table 2 Simplified reaction kinetics mechanism of BE oxidation by UV_{254}/H_2O_2 process.

Table 3 Experimental conditions of the runs used for the optimization (runs 1-6) and validation (runs 1s-4s) procedures and the percentage standard deviations.

Table 4 Experimental conditions of the runs used for the optimization (runs 1-5) and validation (runs 1s-6s) modes and percentage standard deviations.

Statement of novelty

At the best of our knowledge, this is the first investigation about the evaluation of the kinetic constants of the reactions between hydroxyl radical, generated by the UV_{254}/H_2O_2 advanced oxidation process, and benzoyecgonine and its by-products deriving from this process. Furthermore, this investigation was carried out by using an innovative microcapillary film array photoreactor as an effective experimental tool to study oxidative removal of highly priced, uncommon, or regulated substances. The submitted manuscript is also enriched by an ecotoxicity and a genotoxicity assessment of the benzoylecgonine contaminated water solutions before and, for the first time, after the proposed treatment.