Journal of Hazardous Materials xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

On the capacity of ozonation to remove antimicrobial compounds, resistant bacteria and toxicity from urban wastewater effluents

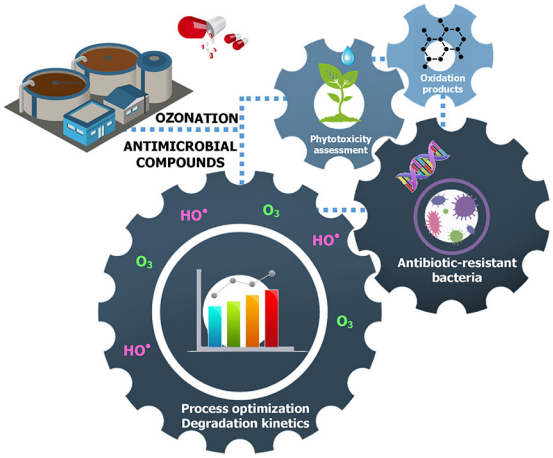
I. Michael-Kordatou[a], R. Andreou[a], M. Iacovou[a], Z. Frontistis[a,b], E. Hapeshi[a], C. Michael[a], D. Fatta-Kassinos[a,*]

[a] Department of Civil and Environmental Engineering and Nireas-International Water Research Centre, School of Engineering, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus
[b] Department of Chemical Engineering, University of Patras, Caratheodory 1, University Campus, GR-26504 Patras, Greece

HIGHLIGHTS

- Erythromycin and ethylparaben were rapidly eliminated within 2 min at low O_3 doses.
- 5 Transformation products (TPs) were identified retaining the ERY lactone ring.
- EtP structural alterations took place at the ethyl ester chain yielding 15 TPs.
- Phytotoxicity can be attributed to the organic matter and its oxidation products.
- ERY- and EtP-resistant *E. coli* were eliminated after 15 min of ozonation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 6 February 2016
Accepted 9 February 2016
Available online xxx

Keywords:
Antimicrobials
Bacterial resistance
Ozonation
Phytotoxicity
Transformation products

ABSTRACT

The degradation of erythromycin (ERY) and ethylparaben (EtP) in urban wastewater effluents at low concentration level during ozonation was investigated under different experimental conditions. Both substrates were rapidly eliminated within 2 min at low ozone dose of 0.3 mg L^{-1} and the experimental data were well fitted in the pseudo-first-order kinetic model. The ratio of HO•- and O_3-exposure (R_{ct}) at the inherent pH was found to be 1.9×10^{-8}. The degradation of ERY and EtP was pronounced at pH 8 compared to acidic pH conditions, while the degradation rate of both substrates was found to be matrix-depended. It was also shown that both O_3- and HO•-mediated pathways are involved in the degradation of EtP, whereas the saturated-rich structure of ERY renders it O_3-recalcitrant. Under the optimum O_3 dose, the BrO_3^- concentration was found to be lower than 10 μg L^{-1}. Five and fifteen transformation products were elucidated during ERY and EtP oxidation, respectively. The root and shoot inhibition can be attributed to the oxidation products formed upon dissolved effluent organic matter transformation. *Escherichia coli* harbouring resistance to ERY survived ozonation better than EtP-resistant *E. coli*. However, neither ERY- nor EtP-resistant *E. coli* were detected after 15 min of ozonation.

* Corresponding author.
E-mail address: dfatta@ucy.ac.cy (D. Fatta-Kassinos).

http://dx.doi.org/10.1016/j.jhazmat.2016.02.023
0304-3894/

1. Introduction

Whilst wastewater reuse is nowadays considered as an indispensable practice to cope with water scarcity, a number of wastewater "quality" challenges are associated with this practice [1]. The ubiquitous occurrence of antimicrobials in conventionally treated wastewater effluents, e.g. antibiotics [2] and preservatives used in various formulations of personal care products such as parabens [3,4], elevates the environmental and human health significance of this problem considering their potential biological effects.

The selective pressure exerted by antibiotics' improper use has been the major driving force behind the emergence and spread of antibiotic-resistant bacteria and resistance genes (ARB&ARG) in both medical settings and environmental compartments [5]. Even though bacteria are continuously exposed to sub-inhibitory concentration levels of antibiotics in wastewater, recent evidence suggests that these levels can indeed impose selective pressure on ARB [6]. However, the potential of sub-inhibitory levels of antibiotics to select ARB can be limited given the complexity of the wastewater environment [7]. In addition, although the knowledge on the contribution of biocides (e.g. parabens) in antibiotic resistance is scanty, the Scientific Committee on Emerging and Newly Identified Health Risks of the EU [8] pointed out that prolonged exposure of bacteria to these compounds can also select for biocide-resistant strains. To date, only one study has reported the presence of paraben-resistant bacteria in urban wastewater effluents [9]. Since conventional activated sludge (CAS) treatment can promote the horizontal gene transfer of antibiotic resistance among bacteria, it can be postulated that wastewater treatment facilities could create a potential risk of development of cross-resistance (i.e. the presence of one resistance mechanism that counteracts two or more antibacterial agents) between antibiotics and biocides.

Three macrolide antibiotics (i.e. erythromycin, clarithromycin and azithromycin), have been recently selected for inclusion in the EU *Watch List* of substances known to potentially pose environmental implications to the aquatic environment [10]. Among these, erythromycin (ERY) has been shown to significantly inhibit the photosynthesis-related processes of aquatic organisms even at $\mu g\,L^{-1}$ concentrations [11], while acute and chronic toxic effects of this antibiotic towards various species have been reported [12,13].

Parabens have been shown to possess endocrine disrupting activity [14] exhibiting both estrogenic- [15] and antiandrogenic-like properties [16,17]. Moreover, a potential relationship between breast cancer and prolonged skin exposure to paraben-containing products was speculated, since *in vitro* studies provided evidence of intact parabens being present in human breast cancer tissue (20.6 ± 4.2 ng g_{tissue}^{-1}) [18]. Even though this hypothesis has not been verified and additional studies are needed to confirm their potential carcinogenicity, a new generation of paraben-free cosmetic products has emerged in the market recently to appease public concern. Despite the numerous reports on their estrogenic activity, few studies were conducted to assess the toxic effects exerted by parabens towards various aquatic organisms with the lowest-observed-effect concentration (LOEC) values being in the range of $\mu g\,L^{-1}$ to $mg\,L^{-1}$ depending on the type of organism and exposure conditions [3,19,20].

Over the last decades, a great deal of interest has been focused on the application of ozone-based systems from laboratory to pilot- and later on to full-scale studies, which have been demonstrated to be effective in achieving significant abatement (> 90%) of various microcontaminants, while also providing sufficient disinfection of wastewater at reasonable specific ozone doses (e.g. $g_{O_3}/g_{DOC} = 0.5–1$) and short contact times [21–24]. Ozone can react directly or via a hydroxyl radical-mediated mechanism [25] with certain microcontaminants prevailing in the dissolved effluent organic matter (dE_fOM), resulting in the generation of new organic entities, which in some cases can be more biologically active than the compounds originally present in dE_fOM [26]. According to the authors' knowledge, a limited number of studies is available in the literature, dealing with the degradation of ERY [27,28] and ethylparaben (EtP) [29] by ozonation. Ternes et al. [27] reported that 92% degradation of ERY ($0.62\ \mu g\,L^{-1}$) was achieved at O_3 doses ranging between 5 and 15 $mg\,L^{-1}$, while the removal of ERY ($40\ mg\,L^{-1}$) exceeded 99% within 45 min at O_3 rate of 0.17 $g_{O_3}\,min^{-1}$. Tay et al. [29] determined the second-order rate constants during the degradation of parabens (including EtP) by ozonation. Nevertheless, ecotoxicological evaluation of the treated flow and assessment of the process in inactivating bacteria harbouring resistance to ERY and EtP have not been addressed in these studies.

For this purpose, a systematic study on the evaluation of the performance of ozonation in removing ERY and EtP present in secondary wastewater effluents at low concentration level ($100\ \mu g\,L^{-1}$) was carried out. An experimental methodology was put into place to assess the effect of various operating parameters on the removal and degradation rate of the substrates. Under the optimum experimental conditions, the contribution of both O_3 and $HO^{\bullet}$ in ERY and EtP degradation was determined. Also, bromate (BrO_3^-) formation was determined under various O_3 doses and pH values. The main transformation products (TPs) derived from the oxidation of each substrate during ozonation were tentatively elucidated. A phytotoxic evaluation of the treated samples was carried out given that wastewater was taken from a treatment plant whose treated wastewater is reused in agricultural irrigation. Finally, the feasibility of the ozonation process in eliminating *Escherichia coli* that are resistant to ERY and EtP was evaluated. This is an assessment performed for the first time according to the authors' knowledge. Given the increasing interest in reusing treated wastewater and the fact that ozonation finds an increasing application acceptance during the last years, this paper constitutes the first integrated approach regarding the evaluation of the efficiency of this treatment technology to remove selected antimicrobials, dE_fOM, phytotoxicity and resistant bacteria.

2. Materials and methods

2.1. Chemicals

Reference standards for the selected compounds (ERY [CAS number: 114-07-8] and EtP [CAS number: 120-47-8]) were purchased from Sigma Aldrich. Anhydrous sodium sulfite (Sigma Aldrich) was used to quench the reactions in each sample for the chromatographic analysis and for the determination of the dissolved organic carbon (DOC). O_3 trap relying on the reaction of O_3 with potassium iodide (KI, Fluka) was used for the decomposition of gas O_3. Potassium indigo trisulfonate (Sigma Aldrich), sodium dihydrogen phosphate (Fluka) and phosphoric acid (85%, Fluka) were used for the preparation of the indigo solution [30]. Humic acid (HA) was obtained from Sigma Aldrich. *tert*-butyl alcohol (*t*-BuOH) was supplied by Sigma Aldrich and used to suppress $HO^{\bullet}$ reactions allowing thus only reactions with O_3 to take place. For the chromatographic analysis, LC/MS-grade MeOH (Sigma Aldrich) and formic acid (98%, Fluka) were used. *p*-chlorobenzoic acid (*p*CBA, Sigma Aldrich) was used as a probe compound for the determination of $HO^{\bullet}$ in the treated samples. The pH of the treated solutions intended for phytotoxicity and microbiological analyses was adjusted to 7 ± 0.5 using 2N NaOH solution (Merck). Tryptone Bile X-Glucuronide (TBX) medium agar (Sigma Aldrich) was used for the enumeration of *E. coli*.

Wastewater samples were collected downstream of CAS treatment (i.e. secondary clarifier) with their main qualitative

Please cite this article in press as: I. Michael-Kordatou, et al., On the capacity of ozonation to remove antimicrobial compounds, resistant bacteria and toxicity from urban wastewater effluents, J. Hazard. Mater. (2016), http://dx.doi.org/10.1016/j.jhazmat.2016.02.023