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Electron beam treatment for tackling the escalating problems of antibiotic resistance: Eliminating the antimicrobial activity of wastewater matrices originating from erythromycin

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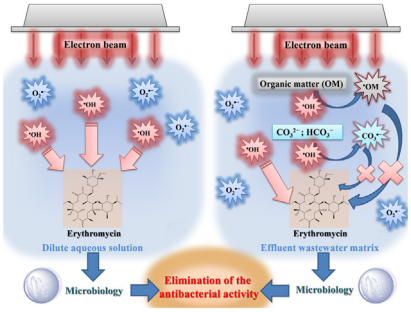
HIGHLIGHTS

- EB eliminates the antimicrobial activity of erythromycin in a wastewater matrix.
- Model effluent wastewater matrix is designed to mimic chemical kinetics.
- ·OH solely governs the inactivation process and not the secondary radicals.
- EB technology needs to be designed to ensure stoichiometric presence of ·OH.

GRAPHICAL ABSTRACT



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ABSTRACT

A synthetic effluent wastewater matrix was designed to be a kinetically appropriate reflection of a real sample while containing the antibiotic erythromycin at sufficiently high concentration for the microbiological assay. Following preliminary experiments on *S. aureus* isolates resistant and susceptible subtypes could be obtained against erythromycin. Agar diffusion and broth microdilution assays indicated antibacterial activity of the products forming in treated dilute aqueous solution of the antibiotic, however, also showed the applicability of EB treatment to eliminate their activity. In the complex wastewater matrix despite the high amount of secondary radicals forming from the matrix components (e.g. humic acid, HCO_3^-) the elimination of the antibacterial activity is suggested to follow the stoichiometry of ·OH in respect to the antibiotic in line with dilute aqueous solution. Although there will be cases when secondary radicals will help degrading a molecule, the technology needs to be designed to the stoichiometric presence of ·OH according to the worst-case scenario approach.

1. Introduction

Implementation of advanced technological processes that can help tackling the evolution and spread of antibiotic resistance in the aquatic environments is an issue of high scientific interest [1,2]. Such technology is worth the investment since bacterial

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resistance has plagued the global economy via extra healthcare expenditures and productivity losses (associated with human casualties) that have been estimated to amount to more than €1.5 billion/year in Europe and $55 billion/year in the US (health care and societal costs) [1]. The problem can only be urgently handled on an interdisciplinary way involving the tight collaboration of engineers and microbiologists.

Critical control points for antibiotic resistance management have been identified where the chain of events is expected to be efficiently intercepted [2]. These reservoirs, including animal/human microbiota, hospital settings, sewage treatment plants and natural waters, are considered to be hotspots (also referred to as "genetic reactors") for the widespread dissemination of antibiotic resistance in the biosphere [3]. The intimate connection of these ecological niches makes antibiotic resistance a direct threat to the humankind and the controllability highly depends on our ability to overcome the flow of antimicrobial agents, antibiotic resistant bacteria and resistance genes within these environments [3–5]. An easily accessible and practically reasonable intervention location within the "genetic reactor cascade" is represented by the effluent of an urban wastewater treatment plant, which is evidenced to be one of the fountains fostering the environmental resistome [6,7].

The holistic goal of our group is to demonstrate the applicability of electron beam (EB) technology for the treatment of effluents of wastewater treatment plants in order to reduce the factors that contribute to the evolution and spread of antibiotic resistance in receiving natural water environments. As far as we know, our research is the first that uses EB technique for this purpose. Advanced oxidation/reduction processes, including EB treatment, are based on free radical reactions. In EB technology high energy electrons – produced by an electron accelerator – are introduced into the medium initiating water radiolysis and thereby formation of oxidizing (·OH) and reducing (·H, e^-_{aq}) radical species according to Eq. (1) [8,9]. The yield, defined as the amount of species formed/decomposed following the absorption of 1 J energy (*G*-value, given in μmol J^{-1}), of each species is shown in brackets in Eq. (1). EB treatment has already been applied for removal of a wide range of water contaminants [10–23]. The advantage of EB treatment compared to other AOP techniques reside in the direct utilization of the invested energy to generate the reactive species without any additives. Therefore, the economic feasibility highly depends on the absorbed energy requirements that will also be the focus of our study. It should be noted that the EB facility can easily be implemented into a conventional treatment train and operated in continuous mode due to its simplicity [8].

$$H_2O \rightsquigarrow [0.28]{\cdot}OH + [0.28]e^-_{aq} + [0.062]H^{\cdot} + [0.073]H_2O_2 + [0.28]H_3O^+ + [0.047]H_2 \quad (1)$$

As a first stage of our research we focus on the elimination of the antibacterial activity in wastewater effluent matrices (being one of the aforementioned factors). While there are several advanced oxidation techniques applied for the removal of a specific antimicrobial agent [7], it is clear that oxidation of the parent compound does not necessarily imply the elimination of biological activity. The number of studies focusing on this issue is growing [24–37], however, limited information has been brought to light in respect to the role of the complex wastewater matrix [38]. Nevertheless, spiking real effluent samples with antibiotics at concentrations of appropriate potency for the microbial test leads to erroneous results since the course of reactions differs from reality (ng L^{-1} or μg L^{-1} antibiotic concentrations) due to chemical kinetics reasons.

Therefore, we have placed special emphasis on the construction of a matrix that is anticipated to be a kinetically relevant reflection of a real wastewater effluent sample while containing the antibiotic at sufficiently high concentration for the microbiological assay. We have also performed experiments to demonstrate the contribution of secondary radical species forming from the wastewater matrix to the elimination of the antibacterial activity. To the best of our knowledge, this study is the first addressing these issues and using a matrix designed according to the reaction kinetics of the ongoing processes.

2. Materials and methods

2.1. Materials

Erythromycin (CAS 114-07-8) and humic acid (HA, CAS 1415-93-6) were purchased from Sigma-Aldrich and used as received without purification. Sodium bicarbonate ($NaHCO_3$, CAS 144-55-8), dipotassium hydrogen phosphate (K_2HPO_4, CAS 7758-11-4), magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$, CAS 10034-99-8) and ammonium sulfate ($(NH_4)_2SO_4$, CAS 7783-20-2) were obtained from Reanal.

The materials used in microbiological work such as sodium chloride (NaCl, Cat. No. 1.06404.1000), glucose (Cat. No. 1.08346.9029), peptone (Cat. No. 1.11931.1000), bacteriological agar (Cat. No. 1.01615.1000), yeast extract (Cat. No. 1.11926.1000) and Mueller-Hinton agar (MH agar, Cat. No. 1.05437.0500) were from Merck. Trypto-casein soy broth (CASO, Product Code BK048HA) was obtained from Biokar Diagnostics. Tryptone-glucose-yeast extract agar (TGE) contained 1 g L^{-1} glucose, 5 g L^{-1} peptone, 2.5 g L^{-1} yeast extract and 7.5 g L^{-1} bacteriological agar.

2.2. EB irradiation

Irradiation was performed using a vertically mounted Tesla Linac LPR-4 type linear electron accelerator (Centre for Energy Research, Budapest, Hungary), which delivers short pulses of electrons with 4 MeV energy, 800 ns duration and 50 Hz repetition frequency. The average pulse current was 10 μA in our experiments. The penetration depth of 4 MeV electrons in pure water is somewhat more than 20 mm [39].

Samples were irradiated in a 150 mL glass beaker having a diameter of 57 mm. The sample volume was 50 mL resulting in a liquid phase with 25 mm thickness. The solution was continuously stirred and bubbled with air during the treatment to avoid diminution (dissolved oxygen) of the sample, which is expected not to be the case when wastewater is treated under continuous flow. The glass beaker was placed beneath the exit window of the electron gun 16 cm away from that at the focus of the electron beam.

Absorbed dose was determined using 0.25 M L-alanine solution, which was irradiated under exactly the same conditions as the samples of interest. The dosimeter was evaluated according to the oscillometric method elaborated by Kovács et al. [40].

2.3. Sample preparation

Stock solutions were prepared in purified water (conductivity of 0.055 μS cm^{-1} and a total organic carbon content <2 ppb) produced by an Adrona B30 system. 0.5 mM erythromycin stock solution has a pH of ~9.

When creating the synthetic effluent wastewater matrix, the work of Keen et al. [41] has been taken into account. They report on a reaction rate constant of $k = 2.5 \times 10^8$ M_C^{-1} s^{-1} (M_C = moles of carbon × L^{-1}) for the effluent organic matter + ·OH reaction. It has been shown that there are some limitations in applying commercial humic substances instead of the natural isolates [42].