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Electron beam treatment for eliminating the antimicrobial activity of piperacillin in wastewater matrix

László Szabó[a,b,*], Orsolya Gyenes[c], Júlia Szabó[c], Krisztina Kovács[a], András Kovács[a], Gabriella Kiskó[c], Ágnes Belák[c], Csilla Mohácsi-Farkas[c], Erzsébet Takács[a,d], László Wojnárovits[a]

[a] Institute for Energy Security and Environmental Safety, Centre for Energy Research, Hungarian Academy of Sciences, Konkoly-Thege Miklós út 29-33, H-1121 Budapest, Hungary
[b] Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Szent Gellért tér 4, H-1111 Budapest, Hungary
[c] Department of Microbiology and Biotechnology, Szent István University, Somlói út 14-16, H-1118 Budapest, Hungary
[d] Faculty of Light Industry and Environmental Engineering, Óbuda University, Doberdó utca 6, H-1034 Budapest, Hungary

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ABSTRACT

Effluents of wastewater treatment plants represent critical control points for antibiotic resistance management. To meet strict regulations coming into effect in the future advanced technologies need to be implemented that can remove the factors contributing to the development of resistance in receiving natural environments. By performing microbiological assays we show that electron beam treatment of a complex synthetic effluent wastewater matrix is able to eliminate one of these factors, the antimicrobial activity of the β-lactam antibiotic piperacillin present at environmentally relevant concentration. Since •OH governs the antibacterial inactivation the technology needs to be designed to the stoichiometric presence of •OH.

Introduction

Antibiotic resistance certainly poses a threat to the global healthcare that has been established in the past decades. The emergence of resistant bacterial species escalated to such an extent that negative impact on human health and the environment can be measured in tens of dollar billions per year [1]. The origins of the problem are not only confined to hospital settings but also involve the environment, e.g. different water bodies [2,3]. The issue calls for the introduction of new regulations and therefore, for new technologies that can meet these requirements necessitating an interdisciplinary action of engineers and microbiologists.

The water cycle enables the movement of water and sometimes its inhabitants to different environments that has a profound impact on the omnipresence of resistant bacteria in the biosphere. Four sites have been identified in water environments including animal/human microbiota, hospitals/farms, wastewater treatment plants and natural waters, in which ideal conditions are given for the genetic evolution of resistance [4]. From our point of view these “genetic reactors” (a term introduced by Baquero et al. [4]) represent critical control points for antibiotic resistance management [5,6]. A vital issue is to prevent the overflow of several factors to the downstream environments that facilitates the emergence of resistance. The factors of concern in water bodies are antibiotic agents, resistant bacteria and resistance determinants (genetic information) [4–8]. Since harvesting natural waters for drinking water production represents one of the main paths for the transmission of antibiotic resistance to the upstream human systems, the purification of the effluent of wastewater treatment plants is a key issue in order to protect the receiving environment [9]. It is a major concern that several antibiotics are able to pass through the biological process in wastewater treatment plants (without change in their antibacterial activity) and persist in the aquatic environment [10]. Implementation of electron beam treatment appears to be a promising measure since the feasibility of the technique for the degradation of several water pollutants has already been confirmed [11–19]. The applicability of the technique for wastewater treatment purposes is testified by several

* Corresponding author at: Institute for Energy Security and Environmental Safety, Centre for Energy Research, Hungarian Academy of Sciences, Konkoly-Thege Miklós út 29-33, H-1121 Budapest, Hungary. Fax: +36 1 392 2548.
E-mail address: szabo.laszlo@energia.mta.hu (L. Szabó).

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industrial-scale realizations of the electron beam treatment [20]. Our main purpose is to probe the applicability of EB technique to efficiently remove the antimicrobial activity of a complex wastewater effluent matrix thereby eliminating one of the factors that facilitates the development of antibiotic resistance in receiving water environments.

EB treatment is grouped together with advanced oxidation/reduction techniques that are based on free radical reactions initiated by oxidizing ($^{\bullet}OH$) and sometimes along with reducing ($^{\bullet}H$, e_{aq}^-) reactive species e.g. photocatalytic treatment [21–23]. The pitfalls of using aggressive oxidizing/reducing free radicals reside in their reactivity towards several constituents of the wastewater effluent matrix. Since the residual antibiotic concentration of the effluent of a wastewater treatment plant falls in the ng L^{-1} range [24] and other constituents that are also reactive towards free radicals (effluent organic matter, natural alkalinity) have a concentration on the mg L^{-1} level [25], only a small fraction of free radicals is available for antimicrobial inactivation purposes. Furthermore, the reactivity profile of secondary radicals forming from the matrix components remains mostly unclear even though they might help in degrading the molecule of interest. There is a lack of knowledge on this field as most of the studies address antibiotic solutions prepared in purified water [26–31] and only some of them apply wastewater matrix spiked with antibiotics, however, at concentrations irrelevant to a real wastewater effluent [32,33]. In these studies the antimicrobial inactivation was slightly compromised by degradation products still retaining some biological activity when $^{\bullet}OH$ was scavenged only by the antibiotic.

Our aim is to demonstrate that EB treatment of a complex wastewater matrix polluted with a β-lactam antibiotic is efficient in eliminating the antimicrobial activity and can be recommended for antibiotic resistance management purposes in wastewater treatment plants. Piperacillin is one of the most important β-lactam antibiotics used here as a model compound. By comparing the pure aqueous system with the synthetic matrix both spiked with piperacillin, we are also about to reveal the impacts of secondary radicals on the process efficiency.

Experimental

Materials

Piperacillin sodium salt (CAS 59703-84-3) and humic acid (HA, CAS 1415-93-6) were obtained from Sigma Aldrich. The inorganic salts for the synthetic wastewater matrix ($NaHCO_3$; K_2HPO_4; $MgSO_4$; $(NH_4)_2SO_4$) were purchased from Reanal.

Glucose (Cat. No. 1.08346.9029), yeast extract (Cat. No. 1.11926.1000), bacteriological agar (Cat. No. 1.01615.1000), peptone (Cat. No. 1.11931.1000) and sodium chloride (NaCl, Cat. No. 1.06404.1000) for microbiological practice were provided by Merck. Trypto-casein soy broth (CASO, European Pharmacopoeia/USP/NF EN ISO 9308-1:2000-09, Product Code BK048HA) were from Biokar Diagnostics. Tryptone-glucose-yeast extract agar (TGY) was obtained by mixing 5 g peptone, 2.5 g yeast extract, 1 g glucose and 7.5 g bacteriological agar in 1 L distilled water. All the microbiological work was carried out under sterile conditions.

Sample preparation

Stock solutions for irradiation were prepared in distilled water provided by an Adrona B30 system ($\kappa = 0.055\ \mu S\ cm^{-1}$; total organic carbon content <2 ppb). The synthetic effluent wastewater matrix was engineered using Sigma–Aldrich humic acid (HA) as a model for dissolved organic matter. The composition of the matrix is shown in Table 1, for further explanation the authors refer to their previous study [34].

Table 1
Composition of the synthetic effluent wastewater matrix.

Component	Concentration (mg L^{-1})
Piperacillin	0.002
Humic acid	7
$NaHCO_3$	81.5
K_2HPO_4	7
$(NH_4)_2SO_4$	7.1
$MgSO_4.7H_2O$	0.71

EB treatment

EB treatment was conducted using a Tesla Linac LPR-4 type linear electron accelerator operated by the Radiation Chemistry Laboratory, Centre for Energy Research (Budapest, Hungary). The details of experimental setup and dosimetry are given in our previous study [34].

The absorption of the energy delivered by the electron pulse occurs according to the relative masses of the constituents of the medium. It follows that in dilute aqueous solutions water absorbs most of the energy initiating water splitting (water radiolysis) and thereby formation of reactive radical and molecular species according to Eq. (1):

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

In brackets the radiation chemical yields (G-values) of each species are given in μmol J^{-1} units. The amount of species [X] generated within a certain treatment time (t) can be calculated according to Eq. (2):

$$[X]\ (\mu M) = \dot{D}\ (1\ Gy\ min^{-1} = 1\ J\ kg^{-1}\ min^{-1}) \times t\ (min) \times G\ (\mu mol\ J^{-1}) \times \rho\ (kg\ dm^{-3}) \quad (2)$$

where $\dot{D}$ represents the absorbed dose rate, G is the radiation chemical yield and ρ is the density of the solution.

Sample analysis

The concentration of piperacillin in treated samples was determined by HPLC–MS analysis. Chromatographic separation was done using an Agilent 1200 liquid chromatograph (LC) with a Phenomenex Kinetex XB-C18 capillary column (2.1 mm × 100 mm, 2.6 μm particle size). Gradient elution was applied using water with 0.1% formic acid (A) and acetonitrile (B) as mobile phase according to the following settings: 5% B at the beginning, B was increased to 20% in 5 min and maintained for 23 min at this proportion, then increased to 90% in 2 min and maintained for 4 min under this condition, thereafter it was decreased to 5% in 1 min and kept under this condition for another 5 min. The elution rate was 0.25 mL min^{-1} and 10 μL sample was injected into the column that was kept at 25 °C. The LC system was coupled to an Agilent 6410 triple quadrupole MS/MS using an electrospray ionization (ESI) interface. The mass spectrometer was operated according to the following settings: fragmentor voltage, 130 V; gas temperature, 300 °C; gas flow, 12 L min^{-1}; nebulizer, 30 psi; capillary voltage, +3000 V; chamber current, 0.13 μA. Mass spectral analysis was performed in single ion monitoring mode

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