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Research article

Bacterial degradation of moxifloxacin in the presence of acetate as a bulk substrate

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ABSTRACT

Fluoroquinolones constitute a group of emerging pollutants and their occurrence in different environmental compartments is becoming object of increasing public concern due to their ecotoxicological effects and the potential to develop resistant bacteria. This study aimed to investigate the biodegradation of moxifloxacin (MOX), for which studies in the literature are very scarce. An activated sludge (AS) consortium and three bacterial strains able to degrade fluoroaromatic compounds – strains F11, FP1 and S2 – were tested. Biodegradation studies were conducted using acetate as a bulk carbon source. Strain F11 showed the highest biodegradation capacity, being able to completely consume and dehalogenate 7.5 μM of the target antibiotic when daily co-supplemented with acetate present as a readily degradable organic substrate in wastewaters. MOX could be used by strain F11 as a sole nitrogen source but the presence of an external nitrogen source in the culture medium was essential for complete biodegradation. Strain F11 was capable of completely consuming MOX in a range between 2 and 11 μM, although stoichiometric fluoride release was not obtained for the highest tested concentration. The antibacterial activity of residual MOX and of the metabolic products potentially resultant from the biodegradation process was investigated by agar diffusion tests, demonstrating that MOX biodegradation is associated with the elimination of the antibacterial properties of the target antibiotic and of the produced metabolites, which is an important result, as the activity of antibiotics and/or their metabolites in the environment, even at low levels, may lead to the development of resistant bacterial strains. Overall, the results obtained in this study suggest that strain F11 is a promising microorganism for the treatment of waters contaminated with MOX, where it could be used for bioaugmentation/bioremediation purposes. To the best of our knowledge, this is the first study reporting complete removal and dehalogenation of MOX by a single microorganism.

1. Introduction

Pharmaceutical compounds are an important class of complex organic molecules that, due to their extensive use in a variety of human and animal applications, are becoming emerging environmental contaminants. These compounds have been detected at very low concentrations (ranging from the ng/L up to the μg/L level) in several environmental compartments including influents and effluents of wastewater treatment plants (WWTP), surface waters, groundwater and soils (Ashton et al., 2004; Fent et al., 2006; Heberer, 2002; Kummerer, 2009). Conventional WWTP are not designed to deal with the presence of these often recalcitrant micropollutants, resulting in their incomplete or even no removal and in their subsequent release to the environment (Gracia-Lor et al., 2012). The awareness of the potential negative effects caused by pharmaceutical contaminants has prompted a deeper research on the environmental presence, effect and removal of these compounds and calls to the necessity of finding effective

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remediation technologies for their removal (Fatta-Kassinos et al., 2011; Kummerer, 2009). The occurrence of antibiotics in the environment pose public health risks associated with the development of resistant bacterial strains (Le-Minh et al., 2010).

Fluoroorganic compounds constitute a relevant group of chemicals that are increasingly used in several applications, thriving in virtually all areas of society (Key et al., 1997). The unique properties of the fluorine atom, such as high electronegativity and the capacity to create extremely strong and highly polarised C–F bonds, confer to organic molecules particular characteristics that make them commercially attractive but also turn them into highly recalcitrant compounds, with low susceptibility to microbial degradation (Key et al., 1997; O'Hagan, 2008). Due to characteristics such as improved pharmacokinetics, higher metabolic stability and lower toxicity, many fluorinated pharmaceuticals have become available in the healthcare market (Ismail, 2002). Fluoroquinolones (FQs) constitute the fourth largest class of antibiotics used in human and veterinary medicine for the treatment of serious bacterial infections (Khetan and Collins, 2007). This family of compounds has been reported to occur worldwide in WWTPs, soils and rivers (Golet et al., 2002; Pico and Andreu, 2007; Zhang and Li, 2011).

Studies on FQs degradation mainly focus on physical–chemical processes (Golet et al., 2003; Jia et al., 2012; Lindberg et al., 2006), while biodegradation of these compounds is scarcely reported and targets essentially second generation FQs, like ciprofloxacin (CPF), ofloxacin (OFL) and norfloxacin (NOR) (Adjei et al., 2006; Adriaenssens et al., 2011; Amorim et al., 2014b; Kim et al., 2011; Wetzstein et al., 1999). The present work has as objective the study of the biodegradation of a fourth generation FQ – moxifloxacin (MOX). This antibiotic belongs to the so-called group of respiratory quinones which, together with levofloxacin, accounts for more than 10% of the total FQs used (Pico and Andreu, 2007). The use of MOX in Europe in 2009 was superior to 80% (Adriaenssens et al., 2011). MOX has been found to occur in the influents and effluents of WWTP and in surface waters at a ng/L level, but concentrations up to several μg/L have already been detected in the effluents of hospital wastewaters (Beier et al., 2010; Jia et al., 2012; Xiao et al., 2008; Zhang and Li, 2011). Biodegradation studies of this compound are extremely rare and complete removal of MOX has not yet been reported (Dorival-Garcia et al., 2013; Jia et al., 2012). Biodegradation of MOX by a mixture of three bacterial strains capable of degrading fluoroaromatic compounds has been already subject of study in our laboratory, but mineralization was not achieved, with 80.5% of the compound being degraded (Maia et al., 2014). A deeper investigation on the biodegradation of MOX is now presented in this study, using the previously tested bacterial strains (Maia et al., 2014), both as a mixture or individually, and an activated sludge consortium (AS). The influence of parameters such as acetate supplementation regime, absence/presence of a nitrogen source and of different MOX concentrations was evaluated. In addition, the antibacterial activity of residual MOX and of its biodegradation products was investigated.

2. Materials and methods

2.1. Reagents

All chemicals used were of the highest purity grade available (Sigma–Aldrich Chemie, Steinheim, Germany; Merck, Darmstadt, Germany). MOX (>99% in purity) was a gift of Bayer HealthCare AG (Leverkusen, Germany). A concentrated stock solution 10 000 mg L^{-1} in water was prepared for this compound. HPLC grade ethanol and triethylamine (≥99% purity) were purchased from Merck and Sigma Aldrich, respectively. Acetic and trifluoroacetic acids obtained from Panreac and Acros Organics. The ultrapure water was supplied by a Milli-Q water system.

2.2. Microorganisms and culture conditions

The biodegradation of MOX was tested using AS, collected from the aerated tanks of a municipal WWTP (Parada, Maia, Portugal), and three previously isolated bacterial strains able to degrade fluoroaromatic compounds – strains F11, FP1 and S2. Strain F11 is a fluorobenzene degrading bacterium, identified as *Labrys portucalensis*, with a high versatility to biodegrade fluoroaromatic compounds (Carvalho et al., 2008, 2005). *Rhodococcus* sp. strains FP1 and S2 have the capacity to degrade 2-fluorophenol and 4-fluorocinnamic acid, respectively, as sole carbon source (Amorim et al., 2014a; Duque et al., 2012). The strains were periodically streaked on nutrient agar (NA) plates and incubated for 2 days at 25 °C before serving as inoculum. The purity of the tested microbial cultures was tested at the end of each biodegradation assay by plating serial dilutions in NA plates.

Prior to the experiments, the AS inoculum was centrifuged (5000 rpm for 10 min at 4 °C), washed twice with a 0.85% saline solution and resuspended in the same solution in one tenth of the original culture volume.

2.3. Biodegradation assays

MOX biodegradation was investigated, in batch mode, in sealed flasks, filled to one fifth of their volume, containing a sterile mineral salts medium (MM) (Caldeira et al., 1999) supplemented with 7.5 μM of MOX (unless otherwise indicated) and 6 mM of acetate as an additional carbon source. The flasks were inoculated with the respective culture to give an optical density (OD) at 600 nm in the range of 0.1–0.2 and were incubated in a rotary shaker (140 rpm) at 30 °C. Whenever necessary, the cultures were transferred to new sterilised flasks in order to secure enough oxygen for aerobic degradation. Samples were taken at regular intervals to analyse bacterial growth and MOX biodegradation. Depletion of the target compound and fluoride ion release were used as indicators of substrate utilization. The experiments were conducted under sterile conditions and protected from light in order to prevent photodegradation of MOX. Abiotic degradation assays were performed under the same conditions used in the microbial experiments, supplementing MM with 7.5 μM of MOX and 6 mM of acetate without bacterial inoculation. Photolytic degradation of MOX was evaluated by incubating MM supplemented with 7.5 μM of MOX and 6 mM of acetate under artificial light (2000 lux). MOX degradation rate constants (k, day^{-1}) were estimated by fitting the model presented in equation (1) to experimental data through non-linear regression analysis applying least square estimation, using the program IBM SPSS statistics (version 2.0). Confidence intervals at 95% were used to evaluate the precision of the estimated parameters and the quality of the regressions was ascertained by analysis of the coefficient of determination (R^2) and by visual inspection of the randomness of the residuals.

$$C = C_0 e^{-kt} \quad (1)$$

In equation (1) C_0 represents the concentration at the beginning of the experiment and C the concentration at time t (day).

2.4. Analytical methods

2.4.1. Optical density measurement

Growth of the cultures was measured in a spectrophotometer (Helios Gamma, Unicam Instruments, UK) by reading the absorbance of culture samples at 600 nm.