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Heterogeneous photocatalysis of moxifloxacin in water: Chemical transformation and ecotoxicity

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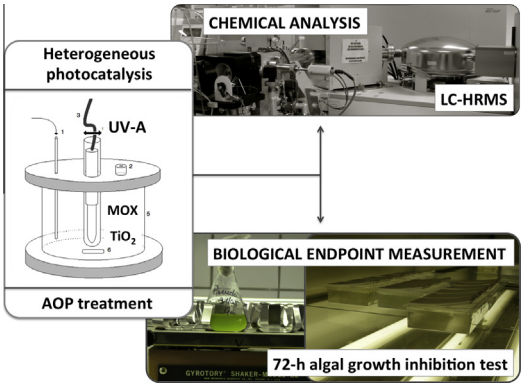
HIGHLIGHTS

- Moxifloxacin's EC-50 for *P. subcapitata* is 7 times lower than that of ciprofloxacin.
- Photocatalytic treatment of aqueous moxifloxacin decreases algal growth inhibition.
- Moxifloxacin is converted into degradation products without carbon mineralization.
- Moxifloxacin contributes more to growth inhibition than its degradation products.
- Possible explanations are decreased cell permeation and lower biological activity.

GRAPHICAL ABSTRACT



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ABSTRACT

This work provides new insights on the impact of TiO_2/UV catalyzed chemical transformation of moxifloxacin on ecotoxicity effects towards the green alga *Pseudokirchneriella subcapitata*. The moxifloxacin median effect concentration (EC-50 = 0.78 [0.56, 1.09] mg L^{-1}), determined in accordance to the OECD 72-h growth inhibition test guideline, was 7 times lower than that of the older and widely used fluoroquinolone ciprofloxacin (EC-50 = 5.57 [4.86, 6.38] mg L^{-1}).

Applying heterogeneous photocatalysis as an advanced oxidation technique to degrade moxifloxacin in aqueous solution decreased the average growth inhibition from 72% to 14% after 150 min of treatment. No significant carbon mineralization was observed and liquid chromatography mass spectrometry analysis revealed the formation of 13 degradation products for which a chemical structure could be proposed based on accurate mass determination. Combined chemical and ecotoxicological analysis showed that as long as moxifloxacin is present in the reaction solution, it is the main compound affecting algal growth inhibition. However, also the contribution of the degradation products to the observed ecotoxicity cannot be neglected.

Photocatalytically induced modifications of moxifloxacin mainly occur at the diazobicyclo-substituent as ring opening, oxidation into carbonyl groups, and hydroxylation. This results into the formation of

Abbreviations: AOP, advanced oxidation processes; CI, confidence interval; CIP, ciprofloxacin; DP, degradation product; EC, effect concentration; ESI, electrospray ionization; FQ, fluoroquinolone; HRMS, high resolution mass spectrometry; LOD, limit of detection; MOX, moxifloxacin; PDA, photodiode array; TOC, total organic carbon; WWTP, wastewater treatment plant.

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more hydrophilic compounds with a decreased biological activity compared with moxifloxacin. The change in lipophilicity, and possibly a modified acid–base speciation, most probably also affect the cell membrane permeation of the degradation products, which might be another factor explaining the observed lower residual ecotoxicity of the photocatalytically treated reaction solutions.

1. Introduction

Antibiotics are detected in American, Asian, African and European surface waters as a result of their extensive use and their inefficient removal in conventional wastewater treatment plants (WWTPs), resulting into a continuous discharge into the natural environment (Kolpin et al., 2002; Santos et al., 2010; K'Oreje et al., 2012). One group of antibiotics which is frequently detected in surface waters are the fluoroquinolones (FQ) (Speltini et al., 2010). Despite their low environmental concentrations (ng to $\mu g\ L^{-1}$), these biologically active molecules can affect aquatic organisms, especially through long-term exposure (Robinson et al., 2005; Ebert et al., 2011).

The limitations of conventional WWTPs in removing these biorecalcitrant molecules indicate the urgent need for improved water treatment techniques such as advanced oxidation processes (AOPs). Among the available AOPs, heterogeneous photocatalysis has proven its potential in degrading FQs from aqueous matrices (Paul et al., 2010; Sturini et al., 2012; Van Doorslaer et al., 2011, 2012). Since it cannot be excluded that photocatalytic degradation products (DPs) can still exert adverse biological activity, the elimination of the mother compound does not necessarily result in toxicity removal. It is therefore important to evaluate the ecotoxicity of a reaction solution after an AOP treatment, and not solely determine process efficiency on a compound removal basis (Li et al., 2008). Ecotoxicity test procedures are available for many aquatic organisms. However, since micro-algae are the basis of the food web in aquatic ecosystems, they are often used as first screen for possible adverse effects of xenobiotic pollution (Martins et al., 2012; Gonzalez-Pleiter et al., 2013).

So far, ecotoxicity data for the most recent generation fluoroquinolone antibiotics such as moxifloxacin (MOX) are scarce or missing, making their environmental risk assessment difficult. It is also unclear to what extent their photocatalytic DPs contribute to the reaction solution's toxicity. In the present paper, algal growth inhibition experiments, using the fresh water alga *Pseudokirchneriella subcapitata* as a non-target model organism, are performed to: (i) determine the 72-h algal growth inhibition of MOX compared with that of the older FQ ciprofloxacin (CIP), and (ii) evaluate the effect of a heterogeneous photocatalytic treatment on the toxicity of a MOX solution. The contribution of both MOX and its photocatalytic DPs to the overall reaction solution's toxicity is assessed, and the effect of chemical transformation on cell permeation and biological activity is discussed in the context of the observed residual ecotoxicity.

2. Materials and methods

2.1. Photocatalytic degradation of MOX: experimental set-up

Photocatalytic degradation of a 50 $mg\ L^{-1}$ MOX (Moxifloxacin. HCl, BAY12-80369, Bayer, Berlin, CAS: 0151096-09-2) solution is performed in a batch reactor of 300 mL (Fig. S-1). This rather high concentration is chosen to enable the detection of both the mother compound and its degradation products with the applied analytical instruments without any (selective) preconcentration.

The reactor is kept at a constant temperature of 298 ± 1 K by a thermostated water bath. A UV-A (4 $mW\ cm^{-2}$ at 0.5 cm, 300–440 nm with main peak at 365 nm) pen ray is used as a light source during photocatalytic degradation and positioned axially in the reactor (UVP, United Kingdom). A phosphate buffer of 10 mM, prepared with KH_2PO_4 (Sigma Aldrich, 99%) and K_2HPO_4 (Acros, $\geqslant$98%), and NaOH (Acros) are applied for pH control during degradation. All stock and buffer solutions are prepared with demineralized water, and all reagents are used as received without any purification. As a photocatalyst, commercial Degussa P25 TiO_2 is used with a BET specific surface area of 48.3 ± 0.7 $m^2\ g^{-1}$ (TRISTAR Micromeretics), 86.7 ± 0.6% of anatase, and primary anatase and rutile particle sizes of 18.7 ± 0.1 nm and 23.3 ± 1.2 nm, respectively (Siemens D5000 scintillation counter, $\theta = 0.02°$) (Spurr and Myers, 1957).

After catalyst addition (1.0 $g\ L^{-1}$), the solution is stirred (13.2 rps) under darkness and sparged with dry air at a flow of 60 $mL\ min^{-1}$ (20 ± 1% O_2, Air Liquide, Belgium) to attain adsorption/desorption equilibrium prior to degradation. Also during irradiation, the solution is continuously stirred and sparged. For chemical analysis, aliquots of 2 mL are collected with a spinal needle syringe and filtered over a Whatman 0.2 μm Spartan mini disk filter to remove the residual TiO_2.

More information on the photocatalytic set-up and conditions can be found in recent work (Van Doorslaer et al., 2011, 2012, 2013) focusing on MOX adsorption and photocatalytic degradation mechanisms, effects of operational variables, and antibacterial activity.

2.2. Chemical analysis

MOX is measured by liquid chromatography (HPLC) coupled to a photodiode array detector (PDA, Surveyor, Thermo Scientific, USA). A Luna C18(2) column (150 mm × 3.0 mm, 3 μm, Phenomenex, USA) with a mobile phase containing water (0.1% formic acid added to improve peak shape, Tang et al. (2012)) and acetonitrile is used for the chromatographic analysis of MOX. Quantification of MOX is performed at 296.0 ± 4.5 nm, with a LOD and LOQ of 0.3 and 1 $mg\ L^{-1}$, respectively, based on a signal to noise ratio of 3 and 10.

The total organic carbon content (TOC) of the photocatalytically treated samples is analyzed using a Shimadzu TOC analyzer equipped with a non-dispersive infrared detector. TOC values of demineralized water with and without catalyst are taken into account as background TOC values.

DPs are measured using liquid chromatography coupled to a double focusing magnetic sector MAT95XP-TRAP mass spectrometer (Thermo Finnigan, Bremen, Germany) equipped with an electrospray ionization (ESI) source operating in positive-ion mode. Low resolution magnetic scan (150–450 Da) is used to screen for DPs while their elemental composition is determined at high resolution (R = 10000, 10% valley) mass separation. Given the molecular formula of the protonated MOX ($C_{21}H_{25}O_4N_3F$), chemical formulae containing 0–21 carbon atoms, 0–30 hydrogen atoms, 0–10 oxygen atoms, 0–3 nitrogen atoms, and 0–1 fluoro atoms are taken into account for DP identification. More details on the