PHOTOCATALYTIC DEGRADATION OF THE ANTIBIOTIC MOXIFLOXACIN:
A DEEPER LOOK AT THE DEGRADATION PRODUCTS AND RESIDUAL
ANTIBACTERIAL ACTIVITY

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EXTENDED ABSTRACT
Fluoroquinolones (FQ) are a family of synthetic, broad-spectrum antibacterial compounds, used in a variety of human and veterinary applications. Due to their increasing consumption more of the compounds are detected in effluent waters of wastewater treatment plants. Since activated sludge and adsorption using activated carbon come short in the efficient removal of these rather persistent molecules, other techniques like advanced oxidation processes (AOPs) are needed. In house experiments with AOPs like ozonation and sonolysis show that these physical-chemical techniques are promising for the treatment of effluent waters loaded with biorecalcitrant antibiotics (Dewitte et al., 2008; De Bel et al., 2009).
In this study, heterogeneous photocatalysis is used for the degradation of the third generation fluoroquinolone moxifloxacin (MOX) in a lab-scale batch reactor. In a first part, the objective is to bring new insights in the photocatalytic degradation pathway of this antibiotic. During photocatalytic treatment, different degradation products are detected by liquid chromatography coupled to low resolution mass spectrometry. Identification of the detected products is done using high resolution magnetic sector mass spectrometry, and chemical structures are elucidated using accurate mass, double bond equivalent and the molecular composition of the mother compound.
In a second part, the residual antibacterial activity is investigated by means of agar diffusion tests for four bacterial species selected from the antibacterial spectrum of MOX. From a dosis-respons test using a range of MOX concentrations and the agar diffusion tests with the photocatalytic samples, it can be concluded that the residual concentration of mother compound in solution correlates well with the residual antibacterial activity. Only for E. coli at lower liquid concentrations a higher inhibition is noticed. This could be the result of the formed degradation products which still exert some antibacterial activity toward E. coli. After 12 minutes of photocatalytic degradation using UVA irradiation, no residual inhibition could be observed for the selected bacterial species.

Keywords: Heterogeneous photocatalysis, fluoroquinolone, degradation products, antibacterial activity

1. INTRODUCTION

Fluoroquinolones (FQs) are a class of synthetic broad-spectrum antibacterial agents. They have gained substantial popularity since their introduction in the 1980s. Consumed antibiotics are not fully metabolized in the body and are partially (> 50%) excreted in the
pharmaceutically active form (Stass, 1999). Due to the limited biodegradability and the widespread use of these antibiotics, an increasing amount of FQ antibiotic residues is detected in effluent waters of common wastewater treatment plants (WWTPs) (Kummerer, 2009). Low concentrations in WWTPs provide suitable conditions for the selective pressure towards resistant organisms (Jury et al., 2011). Next to resistance formation, the possible toxic effects provoked on ecosystems is of increased concern (Ebert et al., 2011).

Therefore, physical-chemical removal technologies are needed such as advanced oxidation processes (AOP) like heterogeneous photocatalysis, ozonation and sonification (De Bel et al., 2009; De Witte et al., 2010; Van Doorslaer et al., 2011). AOPs oxidize the compounds through active species like hydroxyl radicals generated at ambient conditions. The photocatalytic degradation of FQs is not widely studied, especially for the more recently introduced FQs like moxifloxacin (MOX), which accounted for 81.3 % of the European third generation quinolone outpatient consumption in 2009 (Adriaenssens et al., 2011). To evaluate the efficiency of an AOP towards antibiotic removal, not only kinetics and process parameters are of interest, it is equally important to study the formed degradation products and residual antibacterial activity after an AOP treatment (De Witte et al., 2011).

2. MATERIALS AND METHODS

Photocatalytic degradation experiments are performed in a lab scale batch slurry reactor, see Figure 1. A UV-A pen ray is used as the light source during photocatalytic degradation experiments and is positioned axially in the reactor.

![Figure 1. Experimental set-up with oxygen input (1), sampling port (2), UV Pen Ray lamp (3), quartz tube (4), 300 mL reaction vessel (5) and stir bar (6).](image)

Operational variables applied during the photocatalytic degradation experiments are mentioned in Table 1.

The solution is continuously stirred and sparged with air. Adsorption/desorption equilibrium is attained within 30 minutes in the dark before switching on the lamp to start the degradation reaction. A sample of the solution is taken at 0 minutes reaction time, representing the solution before catalyst addition, and 0’ minutes reaction time, representing the starting point for the reaction after adsorption/desorption equilibrium. During degradation, samples are taken at regular time intervals, filtered and analyzed by HPLC–PDA/MS.
Table 1. Experimental conditions applied during heterogeneous photocatalytic degradation of MOX.

<table>
<thead>
<tr>
<th>Process variable</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial MOX concentration</td>
<td>µM</td>
<td>37.4a and 124.6b</td>
</tr>
<tr>
<td>Temperature</td>
<td>K</td>
<td>298</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>3.0, 7.0 and 10.0</td>
</tr>
<tr>
<td>Stirring speed</td>
<td>rps</td>
<td>13.2</td>
</tr>
<tr>
<td>Reactor volume</td>
<td>mL</td>
<td>200</td>
</tr>
<tr>
<td>Catalyst loading</td>
<td>g L⁻¹</td>
<td>1.0</td>
</tr>
<tr>
<td>Air flow</td>
<td>mL min⁻¹</td>
<td>60</td>
</tr>
<tr>
<td>Phosphate buffer concentration</td>
<td>mM</td>
<td>10</td>
</tr>
<tr>
<td>Average light intensity UV-A</td>
<td>mW/cm²</td>
<td>5.15</td>
</tr>
</tbody>
</table>

aconcentration applied during photocatalytic degradation for the antibacterial diffusion tests
bconcentration applied during photocatalytic degradation for the determination of degradation products
creserved at 0.3 cm distance from the light source

3. RESULTS AND DISCUSSION

3.1. IDENTIFICATION OF PHOTOCATALYTIC DEGRADATION PRODUCTS

At the conditions mentioned in Table 1, more than 90% of the MOX is degraded within 60 minutes of photocatalytic degradation time. During photocatalytic treatment, different degradation products of MOX are detected by liquid chromatography coupled to low resolution mass spectrometry with masses ranging from 429 to 292 Da (Figure 2). The detected degradation products show a maximum peak area during photocatalytic degradation with a decrease after longer reaction times. These maxima differ from product to product.

Figure 2. Peak areas of the major products in the liquid phase during the photocatalytic degradation of MOX. Nominal masses (Da) are (+) 292; (○): 306; (■): 306; (×): 320; (●): 417; (□): 429 and (★): 429 (left y-axis), with (●): the liquid concentration of MOX (C_{MOX,liq,mg L⁻¹}) during photocatalytic degradation.
Identification of the detected products is done using high resolution magnetic sector mass spectrometry, and chemical structures are elucidated using accurate mass, double bond equivalent and the molecular composition of the mother compound. Similar major photoproducts are identified for all investigated pH levels and the most prominent degradation products are depicted in Figure 3.

**Figure 3.** Identified degradation products in the liquid phase during photocatalytic degradation of MOX (n_{rep} = 1).

All identified compounds show a limited change in the UV-spectrum compared to MOX, which indicates that the quinolone core is unaffected and, by consequence, degradation occurs at the R_7 substituent. Newly identified major photocatalytic degradation products of MOX are 1, 2, 3, 4, 5, 6, 7, 10 and 11. Based on their maximum appearance during photocatalytic degradation, an initial photocatalytic degradation pathway is proposed.

### 3.2. RESIDUAL ANTIBACTERIAL ACTIVITY

Since no full mineralization is obtained during photocatalytic degradation of MOX and degradation products are detected which still contain the active quinolone core, the reaction solution can still exert some antibacterial activity. This quinolone core contains a carboxyl and a keto group which are considered to be essential for the antibacterial activity since they are necessary for the FQ-DNA gyrase binding. To evaluate this residual antibacterial activity, agar diffusion tests are performed towards *E.coli* (Van Doorslaer et al. 2013, in press). Comparing the inhibition zone diameter for *E. coli* of a MOX concentration range with the inhibition zone diameter from the photocatalytic degradation samples, it can be concluded that, initially, the residual concentration of the mother compound in solution correlates well with the residual antibacterial activity.

At lower MOX solution concentrations a higher inhibition zone diameter is observed for the photocatalytic samples. This indicates that the formed degradation products, containing the quinolone moiety as mentioned in Section 3.1, still exert some antibacterial activity towards *E.coli*.

During the presentation, also the evaluation of the residual antibacterial activity towards three other bacterial species will be discussed.
The residual percentage of inhibition, based on the measured inhibition zone diameters, for the selected bacteria during photocatalytic degradation of MOX at pH 3.0, 7.0 and 10.0 are also compared. After 12 minutes of photocatalytic treatment of MOX at pH 7.0 no residual antibacterial activity is noticed for all investigated bacteria. Residual activity is still observed after 14 minutes of reaction under acidic and alkaline conditions, probably due to the slower photocatalytic degradation rate of MOX at these pH levels (Van Doorslaer et al. 2011).

4. CONCLUSIONS
In a first part, the detection and identification of degradation products was performed at pH 3.0, 7.0 and 10.0. All the detected degradation products in this study retained the base quinolone moiety and no pH dependent degradation product formation is noticed. During degradation the residual antibacterial activity of the reaction solution is determined by linking inhibition zone diameters resulting from a MOX concentration range with the inhibition zone diameters of photocatalytic samples, it is clear that the residual concentration of mother compound in solution correlates well with the residual antibacterial activity at higher liquid concentrations. The formed degradation products still exert some antibacterial activity towards E.coli. After 12 minutes of degradation time at neutral reaction conditions no residual antibacterial activity could be observed. In acidic and alkaline conditions residual antibacterial activity is noticed due to a slower degradation of the mother compound. Photocatalysis is therefore a promising technique to degrade the antibiotic MOX and to reduce its antibacterial activity in wastewater effluents, decreasing the resistance formation potential.

REFERENCES


