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Photocatalytic degradation of the antibiotic chloramphenicol and effluent toxicity effects

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ABSTRACT

Chloramphenicol sodium succinate (CAP, $C_{15}H_{15}Cl_2N_2\ Na_2O_8$) is a broad-spectrum antibiotic exhibiting activity against both Gram-positive and Gram-negative bacteria as well as other groups of microorganisms only partially removed by conventional activated sludge wastewater treatment plants. Thus, CAP and its metabolites can be found in effluents. The present work deals with the photocatalytic degradation of CAP using TiO_2 as photocatalyst. We investigated the optimization of reaction contact time and concentration of TiO_2 considering CAP and its by-products removal as well as effluent ecotoxicity elimination. Considering a CAP real concentration of 25 mg L^{-1}, kinetic degradation curves were determined at 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 g L^{-1} TiO_2 after 5, 10, 30, 60 and 120 min reaction time. Treated samples were checked for the presence of by-products and residual toxicity (*V. fischeri*, *P. subcapitata*, *L. sativum* and *D. magna*). Results evidenced that the best combination for CAP and its by-products removal could be set at 1.6 g L^{-1} of TiO_2 for 120 min with an average residual toxicity of approximately 10%, that is the threshold set for negative controls in most toxicity tests for blank and general toxicity test acceptability.

1. Introduction

Emerging contaminants are continually discharged into the aquatic environment without any restriction posing potential risks for public health and the environment. Antibiotics have been increasingly detected in sewage water, natural water, surface water and groundwater (Chatzitakis et al., 2008; Fatta-Kassinos et al., 2011, Van Doorslaer et al., 2015). Antibiotics are readily available on the market being used to treat diseases in humans and in animals, promote animal growth and improve nutritional efficiency of feed (Sarmah et al., 2006). Despite their low environmental concentrations (from ng L^{-1} to μg L^{-1}), the continuous input and persistence into the aquatic ecosystem make antibiotics one of the most urgent environmental issue, primarily due to the potential for the development of antimicrobial resistance (Dunlop et al., 2015).

The limitations of conventional wastewater treatment plants (WWTPs) in removing these bio-recalcitrant molecules point toward the urgent need for improved wastewater treatments such as Advanced Oxidation Processes (AOPs), a special class of oxidation techniques characterized by production of •OH radicals. Amongst several AOPs, heterogeneous photocatalysis has proven its potential in degrading antibiotics from aqueous matrices (Zhang et al., 2010; Lofrano et al., 2014, Van Doorslaer et al., 2015). The elimination of mother compounds does not necessarily result in toxicity removal, since the photocatalytic degradation can produce intermediate by-products, which can still exert adverse biological effects. Therefore to evaluate the overall behavior and efficiency of the process, it is worth to assess not only the removal of a specific compound, but also of the whole ecotoxicity potential (Rizzo et al., 2009; Libralato et al., 2010a, 2016; Carotenuto et al., 2014; Lofrano et al., 2014). So far, ecotoxicity data for AOPs treated solutions of antibiotics are scarce or missing, making their environmental risk assessment difficult.

In the present study, the photocatalytic degradation of chloramphenicol sodium succinate (CAP, $C_{15}H_{15}Cl_2N_2\ Na_2O_8$), which is a

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representative antibiotic applied to inhibit Gram-positive and Gram-negative bacteria, was investigated at various aqueous suspensions of TiO_2. Its photo-degradation by-products as well as the toxicity of the final treated effluent were assessed as well. CAP has been widely used due to its low cost and high efficiency in the treatment of various infectious diseases. Due to its carcinogenic effects and other serious adverse reactions, such as bone marrow depression, aplastic anemia and severe blood disorders, CAP has been banned from China, Japan, Canada, United States, Australia and European Union in animals used for human consumption, even if it is still legally used in Brazil and other countries, or illegally, in livestock, due to the easy access, low price and steady antibacterial effectiveness (Andrade et al., 2006). As consequence CAP has been found in concentrations between 0.001 and 0.031 $\mu g\,L^{-1}$ in surface waters in Singapore and Korea, respectively. Average CAP concentrations between 2.08 and 26.6 $\mu g\,L^{-1}$ were found in effluents of sewage treatment plants in China (Choi et al., 2008; Liu et al., 2009; Peng et al., 2006; Xu et al., 2011). The degradation of CAP has been evaluated by several AOPs such as UV/H_2O_2 (Baeza et al., 2007), photo-Fenton (Trovó et al., 2013), photoelectron Fenton (Garcia-Segura et al., 2014), photocatalysis (Chatzitakis et al., 2008; Zhang et al., 2010), electrochemical degradation (Rezende et al., 2010). None of these carried out ecotoxicity tests on the TiO_2 photo-catalytically treated effluent. Data on CAP ecotoxicological effects are available only for single species like for *Vibrio fischeri* ($EC50 = 20.68\ mg\,L^{-1}$) (Choi et al., 2008) and *Daphnia magna* ($EC50 = 1086\ mg\,L^{-1}$ (Calleja et al., 1994); $EC50 = 227–600\ mg\,L^{-1}$ (Müller, 1982); $EC50 = 542.86\ mg\,L^{-1}$ (Lilius et al., 1994)). Currently, no toxicity data are available for widely used photosynthetic biological models like the microalga *Pseudokirchneriella subcapitata* and dicotyledonous macrophyte *Lepidium sativum*.

The aim of this study was to elucidate the photocatalytic degradation kinetics of CAP (increasing contact times and photocatalytic agent concentrations, *i.e.* TiO_2) and to assess the efficiency of degradation processes through the removal of ecotoxicological effects related to the potential by-product residues applying the principles of the whole effluent assessment (WEA) (OSPAR Commission, 2007; Libralato et al., 2010b) also in order to meet the goal of the best available technology (BAT). A battery of acute (A) and chronic (C) toxicity tests was used including biological models belonging to various trophic levels like *V. fischeri* (A), *P. subcapitata* (C), *L. sativum* (A) and *D. magna* (A). The toxicity of CAP as a pure substance was investigated on *P. subcapitata* and *L. sativum* due to missing data.

2. Materials and methods

2.1. Reagents and analytical procedures

All reagents were of analytical grade. Photocatalytic degradation experiments were carried out using gravimetrically measured aliquots of TiO_2 Degussa P25. The decay of CAP dispersed in ultrapure water was followed by HPLC-UV (Finnigan Surveyer) equipped with a reversed phase C18 analytical column (Vydac, 5 μm, 150 mm × 3.0 mm). The injection volume was 10 μL and the wavelength set for the quantification was 275 nm according to the maximum light absorption of CAP. The limit of quantification (LOQ) was 0.5 $\mu g\,mL^{-1}$.

HPLC grade water and methanol were supplied from Sigma Aldrich. The compounds were separated using as mobile phase a mixture of methanol/ultrapure water (30%/70%) at flow rate of 1 $mL\,min^{-1}$. The UV–Vis spectra were recorded using a spectrophotometer (Varian Cary 50). Chlorides and nitrates were determined by ion chromatography (Dionex 2000).

Electrospray ionization mass spectrometry (ESI-MS, Micromass Quattro *micro*™) was used to detect CAP by-products. Samples were introduced into the electrospray (ESI) source by continuous flow injection. The following conditions were found to provide the optimum signal: ion source temperature 100 °C, desolvation temperature 250 °C, desolvation gas 500 $L\,h^{-1}$, cone voltage 30 V, and capillary voltage 3.5 KV. The instrument was run in the negative ion mode.

2.2. Experimental design

Preliminary investigations took into consideration the effects of TiO_2 in dark conditions to set the background level of CAP removal and potential adsorption. Photolysis experiments were carried out at 20 ± 2 °C in a 250 mL magnetic stirred cylindrical Pyrex vessel filled with 200 mL ultra-pure aqueous solution (25 $mg\,L^{-1}$ of CAP). In photocatalysis experiments various TiO_2 concentrations (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 $g\,L^{-1}$) were added to the solution at natural pH of 5.5.

The CAP concentration was selected after an initial screening assessment not reported here and allowed to clearly follow CAP degradation kinetics. The reaction vessel was placed in a chamber and illuminated for 5, 10, 30, 60, 120 min with a xenon arc lamp (450 W, LotOriel Group, Italy) equipped with special glass filters restricting the transmission of wavelengths below 300 nm. The light intensity determined by the potassium ferrioxalate actinometry (Hatchard and Parker, 1956) was 4.5×10^{-7} Einstein s^{-1}. After the photocatalysis process, samples were slowly filtered through 0.45 μm polymer membrane filters (Whatman) to remove the catalyst.

2.3. Ecotoxicity

Toxicity tests were carried out on untreated 25 $mg\,L^{-1}$ CAP solution (pure substance, only for *P. subcapitata* and *L. sativum*) and after the photocatalytic treatment with various TiO_2 concentrations (0.1, 0.2, 0.4 and 0.6 $mg\,L^{-1}$) according to various photo-oxidation times (5, 10, 30, 60 and 120 min). For *V. fischeri*, the toxicity of treated CAP solutions was investigated only after 120 min. Treated CAP solutions were assessed for ecotoxicity collecting samples' aliquots after each treatment interval. All toxicity tests included the assessment of negative and positive controls in accordance with the specific reference method.

The acute bioluminescence inhibition assay was carried out using *V. fischeri* (NRRL-B-11177) according to ISO (2007). The luminescence was measured with a Microtox® analyzer (Model 500, AZUR Environmental) after 5 and 15 min at 15 °C. Tests were carried out in duplicate. Data were analyzed with Microtox Omni software and the result expressed as percentage of bioluminescence inhibition (%).

The chronic growth inhibition test with *P. subcapitata* was carried out according to ISO (2012). Cultures were kept in Erlenmeyer flasks. The initial inoculum contained 10^4 cells mL^{-1}. The specific growth inhibition rate was calculated considering 6 replicates exposed at 20 ± 1 °C for 72 h under continuous illumination (6000 lx). Effect data were expressed as percentage of growth inhibition.

The acute bioassay with *L. sativum* evaluated the potential toxicity considering the root elongation according to OECD (2006). Experiments were conducted in triplicate at 25 ± 1 °C for 72 h in aqueous solutions. The root elongation inhibition normalized on negative control data were expressed as percentage of effect.

Acute toxicity tests with *D. magna* were carried out according to ISO (2013). Newborn daphnids (< 24 h old) were exposed in four replicates for 24 and 48 h at 20 ± 1 °C under continuous illumination (1000 lx). Before starting the test they were fed with *P.*