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Removal of the anti-cancer drug methotrexate from water by advanced oxidation processes: Aerobic biodegradation and toxicity studies after treatment

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HIGHLIGHTS

- AOP's were compared with respect to degree and kinetics of elimination and mineralization of MTX.
- The parent compound was easily eliminated in all the treatments.
- None of the treatments fully mineralized MTX and formation of stable intermediates was observed.
- MTX and its photolytic mixture were not biodegradable.
- Photolytic mixture was less toxic than MTX itself.

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ABSTRACT

Anti-cancer drugs are discussed as high risk substances in regard to human health and considered as problematic for the environment. They are of potential environmental relevance due to their poor biodegradability and toxicological properties. Methotrexate (MTX) is an antimetabolite that was introduced in the pharmaceutical market in the 40's and still today is one of the most consumed cytotoxic compounds around the world. In the present study MTX was only partially biodegraded in the closed bottle test (CBT). Therefore, it was submitted to three different advanced oxidation processes (AOPs): UV/H_2O_2, UV/Fe^{2+}/H_2O_2 and UV/TiO_2. The irradiation was carried out with a Hg medium-pressure lamp during 256 min whereas the analytical monitoring was done through LC-UV–MS/MS and DOC analysis. MTX was easily removed in all the irradiation experiments, while the highest mineralization values and rates were achieved by the UV/Fe^{2+}/H_2O_2 treatment. The lowest resulted from the UV/H_2O_2 reactions. The UV/H_2O_2 treatment resulted in little biodegradable transformation products (TPs). However, the same treatment resulted in a reduction of the toxicity of MTX by forming less toxic TPs. Analysis by LC-UV–MS/MS revealed the existence of nine TPs formed during the photo-catalytic treatments. The pH of the solutions decreased from 6.4 (*t* 0 min) to 5.15 in the UV/H_2O_2 and from 6.4 (*t* 0 min) to 5.9 in the UV/TiO_2 at the end of the experiments. The initial pH of the UV/Fe^{2+}/H_2O_2 experiments was adjusted to 5 and after the addition of H_2O_2 the pH decreased to around 3 and remained in this range until the end of the treatments.

1. Introduction

Pharmaceuticals are ubiquitous substances and several studies have detected their presence in wastewaters (Kümmerer, 2001; Escher et al., 2011), surface waters (Buerge et al., 2006; Fatta-Kassinos et al., 2011), drinking waters (Heberer, 2002; Mompelat et al., 2009) and ground waters (Heberer, 2002). Among the various classes of pharmaceuticals, the anti-cancer drugs require a special attention because of an increasing demand for the chemotherapy treatment (Hoppe-Tichy, 2010). Anti-cancer drugs are discussed

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as high risk substances in regard of human health and considered as problematic for the environment because of their special properties in combination with poor biodegradability (Toolaram et al., 2014). Methotrexate (MTX) is a mutagen and a teratogen anticancer drug which belongs to the subgroup of the antimetabolites and acts by blocking enzyme activity and disrupting the DNA synthesis.

It is widely used at high dose for the chemotherapy of various forms of cancer (bronchial, breast and ovarian cancer, lymphomas, leukemia) and has been sold since the 1940s (Rubino, 2001). MTX is a highly polar compound with negligible bioaccumulation and sorption to organic matter (Table S1). Up to 90% of the unchanged drug can be excreted by urine and feces. Therefore, it can be expected in the water cycle. Some studies reveal the presence of MTX in hospital and sewage treatment plant (STP) effluents, and even in surface waters at a concentration range of ng/L (Halling-Sørensen et al., 1998; Castiglioni et al., 2005; Yin et al., 2010; Besse et al., 2012).

These findings demonstrate that the traditional biological, physical and chemical water and wastewater treatments are ineffective in removing or mineralizing MTX. In those cases, advanced oxidation processes (AOP's) may be suitable methods, which may yield good elimination. Besides, the AOP's represent an interesting alternative, since they can be employed in association with biological treatments for wastewater remediation, as a pre-treatment, increasing the biodegradability by a partial oxidation, or as a post-treatment for the degradation of persistent compounds (De la Cruz et al., 2012).

Over the last years many studies have reported the application of UV/H_2O_2, $UV/Fe^{2+}/H_2O_2$ and UV/TiO_2 systems to degrade pharmaceutical compounds (Arslan-Alaton and Dogruel, 2004; Elmolla and Chaudhuri, 2010; De la Cruz et al., 2012; Sleman et al., 2012). However, often incomplete mineralization can result in the formation of unwanted reaction products (so called transformation products, TPs) of unknown properties.

The objectives of the present work were: (i) to evaluate the biodegradability of MTX, (ii) to compare the efficiency of the elimination of MTX as well as its degree of mineralization by UV/H_2O_2, $UV/Fe^{2+}/H_2O_2$ and UV/TiO_2 (iii) to evaluate the biodegradability of its photo-TPs, and (iv) to assess the toxicity of MTX and its photo TPs.

2. Materials and methods

As methotrexate exhibits mutagenic and teratogenic properties, the work with this compound requires strict safety precautions (Allwood et al., 2002).

2.1. Chemicals

All solvents used in our studies were of HPLC grade and all chemicals were of analytical reagent grade or higher (Text S1).

2.2. Advanced photo treatments

The assays were performed in an 800 mL batch photo-reactor containing 600 mL of MTX with an initial concentration of 20 mg/L. The high concentration was used in order to allow for performing subsequent biodegradation testing. A medium-pressure mercury lamp (TQ150, UV Consulting Peschl, Mainz) with an ilmasil quartz immersion tube was used to irradiate the samples. A measured lamp output of 2057 mJ/cm^2 was obtained using an UVpad Spectral Radiometer (Opsytec Dr. Gröbel GmbH, Ettlingen, Germany). The emission of the used UV lamp was measured with UV-pad Spectral Radiometer (Opsytec Dr. Gröbel GmbH, Ettlingen, Germany) at a distance of 4 cm from the emission source in an aluminum box in the range of 200–440 nm (Fig. S1).

The solutions were constantly stirred in order to ensure homogeneity and a circulating cooling system (WKL230, LAUDA, Berlin) was used to keep the temperature between 20 ± 2 °C. The pH values were also monitored during all the treatments. The experiments were performed during 256 min with samplings at regular time points in a geometrical row (2, 4, 8, 16, 32, 64, 128 and 256 min) to check the degradation and mineralization rates of the compounds.

Nonlinear regression analyses were performed using an exponential decay model with the statistical software Prism 5 (Graphpad Inc., CA, USA). The functions "one phase decay" and "two phase decay" were used to calculate the rate constants and the half-lifes of the three processes. A more detailed description of data analysis is available in the Supplementary Material (Text S2).

2.2.1. UV/H_2O_2

Three different concentrations of hydrogen peroxide (9.8 mM, 14.7 mM and 19.6 mM) were tested in order to determine the optimum concentration range in our experiments. The mineralization degrees were used to evaluate the performance of the processes. Aiming to avoid any direct oxidation of MTX by hydrogen peroxide, the lamp was firstly turned on and afterwards the hydrogen peroxide was added. The pH of the samples collected during the assays was adjusted to 7 (±1) with NaOH, whereas the residual, unreacted H_2O_2 was destroyed by the addition of catalase made from bovine liver (1 unit will decompose 1.0 μmole of H_2O_2 per min at pH 7.0 at 25 °C).

2.2.2. $UV/Fe^{2+}/H_2O_2$

The experiments were performed based on the mineralization results of the UV/H_2O_2 experiments. The initial concentrations of H_2O_2 and $FeSO_4{\cdot}7H_2O$ were 9.8 mM and 1.56 mM respectively. The pH was adjusted to 5 with H_2SO_4 (2 M, 98%) (although the literature defines the optimal pH for the Fenton reactions at around 3, preliminary experiments showed no better results than the ones performed in pH 5). Furthermore two additional experiments varying the concentrations of hydrogen peroxide (15.5 mM and 19.6 mM) and of the catalyst (2.14 mM) were also performed. In order to precipitate the ferric ions after the irradiation was finished the pH of the aliquots was adjusted to 7 ± 1 with NaOH. Afterwards catalase was used to destroy the residual, unreacted H_2O_2 and the samples were then centrifuged at 4000 rpm for 5 min and subsequently filtered through 0.2-μm filter membranes (CHROMAFIL® Xtra.Typ: PES 20/25, Macherey–Nagel, Germany).

A simple and practical spectrophotometric method based on the reaction of H_2O_2 with ammonium metavanadate in acidic medium was used for monitoring the H_2O_2 consumption during the tests (Nogueira et al., 2005). The concentrations of ferric and ferrous ions were determined using a colorimetric method with 1–10 phenantroline (APHA/AWWA/WPCF, 2012).

2.2.3. UV/TiO_2

Preliminary experiments with different concentrations (100, 500 and 1000 mg/L) were performed in order set the best titanium dioxide concentration. Before starting the photo-catalytic assays, solutions containing MTX and TiO_2 were constantly stirred for 30 min in the dark to reach adsorption equilibrium on the TiO_2 surface. The samples collected during the treatments were centrifuged at 4000 rpm for 5 min and then filtered through 0.2-μm membrane filters (CHROMAFIL® Xtra.Typ: PES 20/25, Macherey–Nagel, Germany).