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Degradation of 5-FU by means of advanced (photo)oxidation processes: UV/H_2O_2, $UV/Fe^{2+}/H_2O_2$ and UV/TiO_2 — Comparison of transformation products, ready biodegradability and toxicity

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HIGHLIGHTS

- Full primary elimination of 5-FU was achieved in all the treatments.
- None of the processes were able to fully mineralize 5-FU.
- Six transformation products (TPs) were identified during the treatments.
- Photolytic mixture was more biodegradable and non-toxic against *V. fisheri*.
- Several of the formed TPs showed less mutagenic and genotoxic activities.

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ABSTRACT

The present study investigates the degradation of the antimetabolite 5-fluorouracil (5-FU) by three different advanced photo oxidation processes: UV/H_2O_2, $UV/Fe^{2+}/H_2O_2$ and UV/TiO_2. Prescreening experiments varying the H_2O_2 and TiO_2 concentrations were performed in order to set the best catalyst concentrations in the UV/H_2O_2 and UV/TiO_2 experiments, whereas the $UV/Fe^{2+}/H_2O_2$ process was optimized varying the pH, Fe^{2+} and H_2O_2 concentrations by means of the Box–Behnken design (BBD). 5-FU was quickly removed in all the irradiation experiments. The $UV/Fe^{2+}/H_2O_2$ and UV/TiO_2 processes achieved the highest degree of mineralization, whereas the lowest one resulted from the UV/H_2O_2 treatment. Six transformation products were formed during the advanced (photo)oxidation processes and identified using low and high resolution mass spectrometry. Most of them were formed and further eliminated during the reactions. The parent compound of 5-FU was not biodegraded, whereas the photolytic mixture formed in the UV/H_2O_2 treatment after 256 min showed a noticeable improvement of the biodegradability in the closed bottle test (CBT) and was nontoxic towards *Vibrio fischeri*. In silico predictions showed positive alerts for mutagenic and genotoxic effects of 5-FU. In contrast, several of the transformation products (TPs) generated along the processes did not provide indications for mutagenic or genotoxic activity. One exception was TP with m/z 146 with positive alerts in several models of bacterial mutagenicity which could demand further experimental testing. Results demonstrate that advanced treatment can eliminate parent compounds and its toxicity. However, transformation products formed can still be toxic. Therefore toxicity screening after advanced treatment is recommendable.

Abbreviations: 5-FU, 5-fluorouracil; AI, acute inhibition; ANOVA, analysis of variance; AOP, advanced oxidation process; BBD, Box Behnken design; BOD, biochemical oxygen demand; CAP, capecitabine; CBT, closed bottle test; CI, chronic inhibition; COD, chemical oxygen demand; DOC, dissolved organic carbon; DOM, dissolved organic matter; ESI, electrospray ionization; GI, growth inhibition; H_2O_2, hydrogen peroxide; HPLC, high performance liquid chromatography; LC–IT-MS/MS, liquid chromatography tandem mass spectrometry; LBT, luminescent bacteria test; LC, liquid chromatography; mM, milli-molar; MS, mass spectrometry; nm, nanometers; OECD, organization for economic co-operation and development; $HO^{\bullet}$, hydroxyl radicals; QSAR, quantitative structure activity relationship; ThOD, theoretical oxygen demand; TOC, total organic carbon; UV, ultraviolet; IT, ion trap; HRMS, high resolution mass spectrometry; TiO_2, titanium dioxide; TOC, total organic carbon; TPs, transformation products; UHPLC, ultra high performance liquid chromatography.

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1. Introduction

Anticancer drugs are substances specially designed to act by inhibiting cell growth or directly killing cells (Besse et al., 2012; Mahnik et al., 2004; Kümmerer et al., 2014). Because of the non-selective mode of action, affecting both cancerous and healthy cells, anticancer drugs are reported as potentially carcinogenic, genotoxic, mutagenic and teratogenic compounds (Allwood et al., 2002; Kümmerer et al., 2014). Although, in comparison to other groups of pharmaceuticals, the relatively low consumption rates and thus, relatively low environmental concentrations, it is not possible to establish threshold values for lowest effective concentrations for some of these compounds (Kosjek and Heath, 2011; Kümmerer et al., 2014). Due to their toxicological properties, some authors believe that anticancer drugs might inflict adverse effects on any growing eukaryotic organism, even at very low concentrations (Allwood et al., 2002; Besse et al., 2012; Johnson et al., 2008).

The antimetabolite 5-fluorouracil (5-FU) was introduced into the pharmaceutical market in the late 1950s and, globally, is the most commonly consumed anticancer drug (Kosjek and Heath, 2011; Straub, 2010). 5-FU is also the active substance in the prodrug capecitabine (CAP), which is rapidly metabolized and converted to the active compound (5-FU) in the human body (Deboever et al., 2013). It acts by blocking enzyme activity and disrupting DNA synthesis, and has been widely used in the treatment of solid tumors, such as colorectal and breast cancers. Approximately 15% of the parent compound of 5-FU is excreted unchanged (Zhang et al., 2013). 5-FU may therefore reach different water compartments. Furthermore, it is a very polar compound, which is unlikely to be eliminated by sorption into sewage sludge and is not expected to bioaccumulate in aquatic organisms (Table S1) (Wang and Lin, 2014; Zhang et al., 2013). Contradictory results have been published regarding the biodegradation of 5-FU (Kümmerer and Al-Ahmad, 1997; Straub, 2010). Thus, a general conclusion about its biodegradability cannot be made.

Due to its low absorption at wavelengths above 290 nm, 5-FU (maximum of absorption is 265 nm in ultrapure water) is unlikely to undergo direct solar photolysis. Therefore, it is likely that 5-FU will be found in the water cycle. Accordingly, 5-FU has been detected in the aquatic environment at concentrations ranging from ng/L up to μg/L (Kovalova et al., 2009; Mahnik et al., 2007; Mahnik et al., 2004; Mullot et al., 2009; Weissbrodt et al., 2009).

Since the removal of 5-FU and other pharmaceuticals by conventional wastewater treatment is often incomplete and inefficient (Wang and Lin, 2014; Zhang et al., 2013), other alternatives need to be investigated. The so-called advanced oxidation processes (AOPs) may be suitable methods, which may result in satisfactory elimination. Moreover, they represent an interesting alternative, since they can be employed in association with biological treatments for wastewater remediation, as a pre-treatment, increasing the biodegradability through partial oxidation, or as a post-treatment for the degradation of persistent compounds (De la Cruz et al., 2012). However, a possible incomplete mineralization can generate unwanted, and often unknown, transformation products (TPs) of unknown properties which can be recalcitrant and even more toxic than the parent compound itself (Lutterbeck et al., 2015; Mahmoud et al., 2013; Rastogi et al., 2014). Therefore, the identification of the main TPs generated in the processes is an important issue. Furthermore, the degree of mineralization, the kinetics of formation of TPs and their chemical structure may depend on the type of treatment.

In this way, the present study aims to (i) compare the efficiency of the elimination of 5-FU, as well as its degree of mineralization by means of three different AOPs (UV/H_2O_2, $UV/Fe^{2+}/H_2O_2$ and UV/TiO_2); (ii) identify and elucidate the structure of TPs formed during degradation processes; (iii) evaluate the biodegradability of 5-FU and the mixture of its post-process TPs by standard OECD tests; (iv) assess the bacterial toxicity of 5-FU and its photo-TPs and (v) assess the mutagenic and genotoxic activities of 5-FU and the TPs formed in the advanced (photo)oxidation processes.

2. Materials and methods

Based on its mode of action 5-FU is a potentially mutagenic and teratogenic agent and thus, should be handled with care by taking the appropriate safety measures (Allwood et al., 2002; Eitel et al., 1999). All the waste generated during the experiments was treated and then disposed as hazardous waste, and the instruments and glassware used were carefully cleaned before and after usage.

2.1. Chemicals

Stock solutions of 5-FU (5-fluoro-1H, 3H-pyrimidine-2,4-dione, CAS Nr: 51-21-8) were kindly provided and handled by the pharmacy of the Hospital Lüneburg, Germany (therapeutic infusions bags were prepared on demand). In order to avoid any scavenging effect of other chemical species, and also to evaluate only the photo degradation of 5-FU, all the solutions were prepared using ultrapure water (Q_1:16.6 MΩ and Q_2:18.2 MΩ Ultra Clear Water, Evoqua Water Technologies GmbH, Barsbüttel, Germany).

All solvents used were of HPLC grade, and all chemicals were of analytical reagent grade or higher. Acetonitrile (HiPerSolv CHROMANORM, LC–MS grade, BDH Prolabo) and formic acid (analytical grade) were acquired from VWR International GmbH (Darmstadt, Germany). Sodium hydroxide (NaOH 1 mol/L), sulfuric acid (H_2SO_4 2 mol/L, 98%) was purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Titanium dioxide (TiO_2 P25) was purchased from Evonik Degussa GmbH (Frankfurt, Germany). Hydrogen peroxide (H_2O_2 30% w/w) was obtained from Merck-Group (Darmstadt, Germany). Iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), catalase from bovine liver (2000–5000 units/mg protein), ammonium metavanadate (NH_4VO_3), and 1,10-phenantroline ($C_{12}H_8N_2$) were all acquired from Sigma-Aldrich Biochemie GmbH (Hamburg, Germany).

2.2. Setup of the advanced photo degradation experiments

The advanced (photo)oxidation experiments were carried out in an 800 mL reactor, containing 600 mL of a 5-FU solution with an initial concentration of 20 mg/L. The higher initial concentrations were used to allow subsequent biodegradation experiments and enable the identification of transformation products (TPs) that could be formed during the photo treatment, and finally to evaluate the possible effects on *Vibrio fischeri*.

The irradiation source used was a mercury medium pressure lamp (TQ150, UV Consulting Peschl, Mainz) with an ilmasil quartz immersion tube (Fig. S1). An UV-pad Spectral Radiometer (Opsytec Dr. Gröbel GmbH, Ettlingen, Germany) was used to estimate the total photon flow rate of the polychromatic lamp (200–440 nm). The radiometer was positioned at a distance of 4 cm from the emission source in an aluminum box and the integration of the total photon flow rate of the lamp for each wavelength (200–440 nm) was estimated to be 5.71×10^6 mol·photons/$cm^2 \cdot s^1$ (Fig. S2).

The reactor was placed on a magnetic stirrer to maintain the homogeneity of the solution, and the temperature was kept constant at 20 ± 1 °C by using a cooling system (WKL230, LAUDA, Berlin). The experiments were carried out for 256 min and samples (approx. 20 mL) were withdrawn according to a determinate time schedule (2, 4, 8, 16, 32, 64, 128 and 256 min) for further analysis.

The kinetic rates of the three advanced photo treatments were assessed through nonlinear regression models using the software Prism 5 (Graphpad Inc., CA, USA). Exponential decay models with the functions "one phase decay" and "two phase decay" were used to calculate the rate constants and half-lives of the reactions, and to take into account possible first order or second order kinetics. A more detailed