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Occurrence of cyclophosphamide and ifosfamide in aqueous environment and their removal by biological and abiotic wastewater treatment processes

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HIGHLIGHTS

- Cyclophosphamide (CP) and ifosfamide (IF) were detected in Slovene wastewaters.
- Hydrodynamic cavitation did not result in any significant CP nor IF removal.
- Biological treatment removed 59% and 35% of CP and IF, respectively.
- A combination of selected AOP ($UV/O_3/H_2O_2$ at 5 g L^{-1}) removed 99% (CP) and 94% (IF).
- Coupling biological treatment to a selected AOP removed >99% of CP and IF.

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ABSTRACT

Cytostatic drug residues in the aqueous environment are of concern due to their possible adverse effects on non-target organisms. Here we report the occurrence and removal efficiency of cyclophosphamide (CP) and ifosfamide (IF) by biological and abiotic treatments including advanced oxidation processes (AOPs). Cyclophosphamide was detected in hospital wastewaters (14–22,000 ng L^{-1}), wastewater treatment plant influents (19–27 ng L^{-1}) and effluent (17 ng L^{-1}), whereas IF was detected only in hospital wastewaters (48–6800 ng L^{-1}). The highest removal efficiency during biological treatment (attached growth biomass in a flow through bioreactor) was 59 ± 15% and 35 ± 9.3% for CP and IF, respectively. Also reported are the removal efficiencies of both compounds from wastewater using hydrodynamic cavitation (HC), ozonation (O_3) and/or UV, either individually or in combination with hydrogen peroxide (H_2O_2). Hydrodynamic cavitation did not remove CP and IF to any significant degree. The highest removal efficiencies: 99 ± 0.71% for CP and 94 ± 2.4% for IF, were achieved using $UV/O_3/H_2O_2$ at 5 g L^{-1} for 120 min. When combined with biological treatment, removal efficiencies were >99% for both compounds. This is the first report of combined biological and AOP treatment of CP and IF from wastewater with a removal efficiency >99%.

1. Introduction

According to the International Agency for Research on Cancer (IARC), there are an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths per year worldwide, which makes cancer the second leading cause of death (IARC, 2013). Amongst the many pharmaceuticals used to treat cancer, cyclophosphamide (CP) and ifosfamide (IF) are two of the oldest and widely prescribed alkylating cytostatic medicines. The current trend in chemotherapy is towards the non-hospitalization of patients i.e., only receiving the chemotherapy

Abbreviations: AOP, advanced oxidation process; COD, chemical oxygen demand; CP, cyclophosphamide; DO, dissolved oxygen; DOC, dissolved organic carbon; GC–MS, gas chromatography coupled to mass spectrometry; HC, hydrodynamic cavitation; HRT, hydraulic retention time; H_2O_2, hydrogen peroxide; IARC, International Agency for Research on Cancer; IF, ifosfamide; NH_4–N, ammonia ion; NO_3–N, nitrate ion; O_3, ozone; OECD, Organisation for Economic Co-operation and Development; SPE, solid-phase extraction; TFAA, trifluoroacetic acid; UV, ultraviolet (irradiation); WHO, World Health Organisation; WWTP, wastewater treatment plant; ε, molar extinction coefficient.

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at the hospital, although hospitals, where chemotherapies are administered daily, remain a significant source of anticancer drug residues. The levels of CP and IF have been reported in hospital wastewaters from the current limits of detection up to 15 μg L^{-1} and 11 μg L^{-1} for CP and IF, respectively (Ferrando-Climent et al., 2013; Gómez-Canela et al., 2012; Kim et al., 2009a; Kümmerer et al., 1997; Negreira et al., 2013, 2014a, 2014b; Steger-Hartmann et al., 1996, 1997; Yin et al., 2010). Hospital wastewaters are usually untreated and discharged directly into the sewerage system, where they eventually arrive at a wastewater treatment facility (Zhang et al., 2013). The concern is that if not completely removed, residues of CP and IF, which are known to have cytotoxic, mutagenic, teratogenic and genotoxic properties, could have adverse effects on aquatic organisms.

Various biological treatment technologies have been applied to reduce the presence of CP and IF in wastewater. Literature review reveals inconsistent removal efficiencies for CP and IF (0–72%) by biological treatment (Buerge et al., 2006; Delgado et al., 2011; Kiffmeyer et al., 1998; Kovalova et al., 2012; Köhler et al., 2012; Kümmerer et al., 1997, 2000; Steger-Hartmann et al., 1997). Amongst them only Kovalova et al. (2012), Köhler et al. (2012) and Delgado et al. (2011) used a non-conventional biological treatment by applying membrane bioreactors. Kovalova et al. (2012) and Köhler et al. (2012), who used hospital wastewater as a matrix, were able to remove 20% and 12% of CP, respectively, while Delgado et al. (2011) removed 80% of CP from an artificial wastewater. To our knowledge there have been no published studies of a non-conventional biological treatment to remove IF. A removal efficiency of 80% for CP using membrane bioreactors suggests that attached growth biomass could be a promising treatment technology for the removal of CP and IF.

The results of several abiotic treatment studies using UV, UV and hydrogen peroxide (UV/H_2O_2), ozone (O_3) and O_3/H_2O_2 to remove CP and studies using UV, UV/H_2O_2 and O_3 to remove IF have been published (Chen et al., 2008; Fernández et al., 2010; Garcia-Ac et al., 2010; Kim and Tanaka, 2009; Kim et al., 2009a, 2009b; Köhler et al., 2012; Lester et al., 2011; Lin et al., 2014, 2015; Lutterbeck et al., 2015; Wols et al., 2013, 2015). The removal of CP and IF by either UV or O_3 alone was disappointing, but by adding H_2O_2 removal efficiency was substantially improved (up to 90%). With the exception of Kim et al. (2009a), Köhler et al. (2012) and Lin et al. (2015) who used UV, UV/H_2O_2 or O_3 to remove CP and/or IF from a wastewater, others used more simple aqueous matrices like deionised and drinking water. In addition, because CP and IF are only partially removed by existing abiotic wastewater treatment technologies, alternative technologies must be investigated. An example is hydrodynamic cavitation (HC), for which promising results for removing pharmaceutical residues from a wastewater have been reported (Zupanc et al., 2014).

The aims of this study were to evaluate for the first time the occurrence of CP and IF in Slovene wastewaters and to investigate their removal from wastewater using biological treatment based on attached-growth biomass, abiotic treatments (HC, UV and/or O_3 with varying initial concentrations of H_2O_2) and combined biological and selected abiotic treatment.

2. Experimental

2.1. Standards, reagents and chemicals

Cyclophosphamide (99%, CAS 50-18-0) and IF (99%, CAS 3778-73-2) were purchased from Sigma Aldrich (Hong Kong, China). The surrogate standard deuterated cyclophosphamide (CP-d_6) was obtained from Niomech — IIT GmbH (Bielefeld, Germany), the derivatizing agent trifluoroacetic anhydride (TFAA, 99%, CAS 407-25-0) was purchased from Fluka (Buchs, Switzerland) and the solvents acetonitrile, ethyl acetate and methanol were purchased from J. T. Baker (Deventer, Netherlands), which were all of analytical grade purity. Hydrogen peroxide (30%; CAS 7722-84-1) was purchased from AppliChem (Darmstadt, Germany). The composition of the artificial wastewater influent used for biological and abiotic treatments is provided in SM-I and described in detail by Kosjek et al. (2007).

2.2. Sample preparation, chemical analysis and method validation

Sample preparation included solid-phase extraction (SPE) and derivatization of previously published methods (Kosjek and Heath, 2011). Briefly, 100 mL of hospital wastewater sample and samples from laboratory experiments and 200 mL of wastewater treatment plant (WWTP) influent and effluent sample were filtered through glass microfiber filters (minimal pore size 0.5 μm, Machery Nagel, Dueren, Germany) to remove solids and through cellulose-nitrate filters (0.45 μm; Sartorius Stedim Biotech, Göttingen, Germany) to remove bacteria. The samples were then pre-concentrated using Oasis HLB (60 mg, 3 cm^3) cartridges (Waters, Massachusetts, USA). Each cartridge was conditioned with 3 mL of ethyl acetate, 3 mL of methanol and equilibrated with 3 mL of water. The samples were then extracted at a flow rate of 3 mL min^{-1}. The sorbents were dried under vacuum before being eluted with 3 mL of ethyl acetate (3 × 1 mL). The remaining organic solvent was removed under a gentle stream of N_2. Finally, 100 μL of TFAA and 100 μL of ethyl acetate were added to the dried extract and left to derivatize for 1 h at 60 °C. The samples were then dried (with N_2) to remove any trifluoroacetic acid formed during derivatization and re-dissolved in 250 μL of ethyl acetate prior to analysis by GC–MS.

The samples were analysed using a HP 6890 series (Hewlett-Packard, Waldbron, Germany) gas chromatograph with a single quadrupole mass selective detector. The capillary column was a DB-5 MS 30 m × 0.25 mm × 0.25 μm (Agilent J&W, CA, US). The carrier gas was He set at a flow rate of 1 mL min^{-1}. One microlitre of sample extract was injected in splitless mode with the injector temperature set to 270 °C. The oven temperature programme was as follows: an initial temperature of 65 °C was ramped at 30 °C min^{-1} to 300 °C, where it was held for 2 min. Total runtime was 9.83 min. The MS was operated in electron impact (EI) mode with an ionization voltage of 70 eV. Selective ion monitoring (SIM) was used for qualitative and quantitative determination by monitoring the following ions: m/z 309, 307 and 150 for CP, m/z 307, 181 and 150 for IF and m/z 313 and 315 for CP-d_6. Instrumental control and data processing were performed using ChemStation software.

The analytical method was validated by determining the following parameters: SPE efficiency, linearity, instrumental and method repeatability, accuracy and sensitivity (LOD and LOQ). Full details are given in SM-II.

2.3. Collection of hospital wastewater and wastewater from WWTPs

Wastewater samples were collected from five Slovene hospitals, labelled as A, B, C, D and E in Table 1, where CP and/or IF are regularly administered to cancer patients. In addition, where possible corresponding WWTP influents and effluents were sampled (hospitals A, B, C and D). Samples were collected by grab and/or 24-h time proportional sampling (Table 1). Hospitals A and B are connected to the same WWTP, while hospital C is connected directly to a WWTP, hence, the hospital C sample corresponds to the WWTP influent. For hospital E, where the wastewater is untreated, only hospital wastewater was collected. Water treatment technologies included mechanical and conventional biological treatment with suspended biomass in case of the WWTP receiving wastewater from hospitals A and B (360,000 PE) and the WWTP receiving wastewater from hospital C (800 PE). The WWTP, which received wastewater from hospital D used mechanical and biological treatment with membrane bioreactors (attached growth biomass on filters; 55,000 PE). Further information about each hospital and WWTP is given in SM-I. All samples were immediately transferred on ice to the laboratory, filtered and stored at −20 °C prior to analysis.