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Elimination of an anticancer drug (cyclophosphamide) by a membrane bioreactor: Comprehensive study of mechanisms

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ABSTRACT

The mechanisms of elimination of an anticancer drug (cyclophosphamide) by a membrane bioreactor were investigated. The membrane bioreactor was run for 153 days with a sludge retention time (SRT) of 20 days. A removal efficiency of 60% was observed despite some variations in the influent. This removal was higher than reported in most of the studies in the literature. Biodegradation was the predominant removal mechanism and sorption onto sludge could be neglected.

1. Introduction

The occurrence and fate of human pharmaceuticals (HP) and their residues in treated wastewater and in aquatic environments have attracted increasing interest in the last two decades. Several studies have shown the presence of different classes of drugs at the outlet of wastewater treatment plants (WWTP), in surface water and sometimes in drinking water, with concentrations ranging from ng L^{-1} to µg L^{-1} [1–5]. The main reason is that these pharmaceutical compounds have complex structures and most of them are recalcitrant to biodegradation in conventional wastewater treatment (i.e. activated sludge). However, little attention has been paid to chemotherapy drugs, especially cytostatics (or antineoplastics) [6,7] even though they are potentially highly dangerous to human health and the environment because of their cytotoxicity, genotoxicity, mutagenicity and teratogenicity [6,8,9]. Anticancer drugs have frequently been detected in hospital effluent, WWTP influent and effluent, and sometimes in surface water, indicating a very low removal rate by conventional activated sludge systems [6,10]. The cytostatics most commonly reported in WWTP effluent are two alkylating agents: cyclophosphamide (CP) and ifosfamide (IF), and one hormone: tamoxifen (TAM) [6]. The efficiency with which these cytostatics are removed is largely dependent on their physicochemical properties and on WWTP operating parameters. However, the reasons for their relatively low removal are still unclear. Membrane bioreactors (MBRs) which generally operate with higher sludge retention time (SRT) have been reported to enhance biodegradation of some micropollutants [11–13]. A long SRT can favour the proliferation of slowing growing bacteria (such as nitrifying bacteria), thus improving the microbial diversity and achieving better biodegradation [11,13,14]. However, recent reviews [6,7] reported only 3 studies dealing with the elimination of cytostatics by MBR. Two of them [15,16] used hospital wastewater as a matrix and showed large variations in CP removal from 12% [16] to less than 20% [15], while the third one [17] reported a CP removal efficiency of 75% from a semi-synthetic wastewater. Differences in the composition of the effluent or in the operating conditions that influence biotic treatment, such as hydraulic retention time (HRT), sludge retention time (SRT) or temperature, could explain the variations observed in these experiments. However, the influence of operating parameters is still not clearly understood and any attempt has been made

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to identify removal mechanisms of cytostatics. Some studies have investigated the biodegradation and sorption of pharmaceuticals in MBR [12,18] but cytostatics were not considered. Removal can be affected by three main mechanisms: volatilization, sorption and biodegradation. Because of the low values of the Henry constant and vapour pressure of most cytostatics, the fraction removed by volatilization can be neglected [10]. Thus there is a need to understand the contribution of sorption on sludge and biodegradation in cytostatic drugs removal by MBR. This is essential for improving process performance and characterizing the impact of these micropollutants on the environment.

Therefore, the aim of this work was to confirm MBR efficiency for the removal of a cytostatic drug (cyclophosphamide) and to characterize the mechanisms (sorption and/or biodegradation). For that purpose, a laboratory scale (20 L) membrane bioreactor was run for 153 days to eliminate cyclophosphamide (CP) with inlet concentration of 5 $\mu g\,L^{-1}$ which was in the range of CP concentration in wastewaters [6]. Measurements of CP concentration in the inlet, the permeate and both the aqueous and solid phases of the MBR sludge allowed sorption coefficient and biodegradation rate to be estimated throughout the process run.

2. Material and methods

2.1. Micropollutants

CP was purchased from Sigma Aldrich (France). A stock solution of 1 $g\,L^{-1}$ in methanol was prepared every 4 months and stored at −20 °C. A solution of CP in milliQ water (220 $\mu g\,L^{-1}$) was prepared every 3 or 4 days from stock solutions and kept in the dark at 4 °C.

2.2. Wastewater

Semi-synthetic wastewater was used as a model for hospital effluent of average pollutant strength [7]. It was composed of a raw urban wastewater supplemented with a synthetic solution (2.3% v/v) in order to increase the chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) contents up to 1750 $mg_{COD}\,L^{-1}$, 125 $mg_{TN}\,L^{-1}$ and 25 $mg_{TP}\,L^{-1}$ [7]. The raw urban wastewater (COD = 760 $mg\,L^{-1}$, TN = 65 $mg\,L^{-1}$, TP = 10 $mg\,L^{-1}$) was collected after the sand trap of a WWTP (Ginestous, France, 800 000 person-equivalent) then screened at 200 μm and stored at 4 °C. The synthetic solution was composed of $C_6H_{12}O_6$ (22.6 $g_{COD}\,L^{-1}$), $NaCH_3COO$ (22.6 $g_{COD}\,L^{-1}$), NH_4Cl (2.75 $g_N\,L^{-1}$) and KH_2PO_4 (0.6 $g_P\,L^{-1}$).

2.3. Experimental set-up

The laboratory-scale MBR had a working volume of 20 L (Fig. 1) and was equipped with a Rushton turbine (200 rpm). The membrane module consisted of a ceramic tubular Membralox® (MF) membrane with surface area of 0.0055 m^2 and pore size of 0.2 μm (Pall Exekia, France) located in an external loop. The tangential velocity in the membrane was maintained at 4 $m\,s^{-1}$.

The wastewater and permeate flow were set to 13.3 $L\,d^{-1}$. The average values of hydraulic retention time (HRT) and sludge retention time (SRT) were respectively 36 h and 20 d.

Aerobic/anoxic conditions were maintained to allow nitrification and denitrification of the influent. Dissolved oxygen levels were kept between 0 and 4.5 mg $O_2\,L^{-1}$. The aeration cycle was 3 min aeration/30 min without aeration which corresponded to 10 $h\,d^{-1}$ of aerobic and 14 $h\,d^{-1}$ of anoxic periods.

Temperature, dissolved oxygen and pH in the bioreactor were monitored. For all experiments, the temperature varied from 25 to 32 °C and pH varied between 7 and 8.

The bioreactor was seeded with an activated sludge from a real WWTP (Ginestous, France) with a suspended solids concentration (X_{TSS}) of 2 $g\,L^{-1}$.

The experiment was first carried out without addition of pharmaceutical in order to reach stationary performance of the bioreactor (Phase I: from day 0 to day 76). After 76 days of operation, the solution of CP was supplied (Phase II: from day 76 to day 153) at 0.3 $L\,d^{-1}$ to obtain concentration in wastewater of 5 $\mu g\,L^{-1}$.

2.4. Sampling and analytical methods

Samples of the feeding solution, membrane permeate, purge and mixed liquor were taken once a week at the end of the anoxic phase. Concentrations of COD, TN, total suspended solids (TSS), volatile suspended solids (VSS) and CP concentrations were determined.

COD and TN concentrations were measured by spectrophotometric methods with reagent kits (Method HACH 8000 and HACH 395N). The concentrations of total suspended solids (X_{TSS}) and volatile suspended solids (X_{VSS}) were measured according to standard methods 2540D and 2540E [19].

For CP analysis, aqueous and solid phases of the mixed liquor (200 mL) were separated by centrifugation at 5000*g* for 20 min. Then, the supernatant was filtrated (1.2 μm) and the solid phase was frozen at −20 °C, lyophilized and ground. Both aqueous and solid phases were spiked with a deuterated compound (CP-d_4). The aqueous phase was extracted, concentrated by solid phase extraction (SPE) and quantified by Ultra Performance Liquid Chromatography coupled with tandem Mass Spectrometry (UPLC–MS/MS). The solid phase was extracted with a Pressurized Liquid Extraction (PLE) system. Extracts were purified with SPE and quantified in the same way as for the liquid phase [20]. The limits of quantification (LOQ) were 80 $ng\,L^{-1}$ in the aqueous phase and 3 $ng\,g^{-1}{}_{TSS}$ $ng\,g^{-1}{}_{TSS}$ in the solid phase.

2.5. Sorption isotherms

Batch experiments were carried out in flasks containing 100 mL of sludge. Sludge was subjected to bubbling with O_2 flow for 15 min, followed by an anoxic step of 30 min and then bubbling with N_2 flow to exhaust carbon and nitrogen sources and O_2 traces. This procedure limited the biodegradation activity during the test without the use of a chemical inhibitor, which could have modified the sludge structure. Then 7 flasks were spike with CP solution in order to obtain concentration of 0 (blank), 1.25, 2.5, 5, 10, 25 and 50 $\mu g\,L^{-1}$ respectively. Flasks were shaken at 150 rpm at room temperature during 4 h in order to reach equilibrium. Then sludge were centrifugated to measure the quantity of CP sorbed on the solid phase of the sludge (in $\mu g\,kg^{-1}{}_{TSS}$) and the concentration of CP in the aqueous phase (in $\mu g\,L^{-1}$) at equilibrium.

2.6. Biodegradation tests

2.6.1. Without carbon and nitrogen supplement

A 1500 mL sample of the mixed liquor was collected from the membrane bioreactor during the acclimated period (phase II) and put into a 2 L-fermentor for two hours with successive aerobic and anoxic phases in order to exhaust any residual substrate. Then samples were spiked with CP (5 $\mu g\,L^{-1}$) without nitrogen or carbon addition in aerobic conditions for 10 h. Samples were taken after 2, 4, 6 and 10 h and analysed as described in Section 2.4.

2.6.2. With carbon and nitrogen supplement

This experiment was carried out in the MBR after the addition of CP had been stopped (at day 153) meanwhile the reactor was still fed with raw water and synthetic solution. After a period of 3 days during which traces of the anticancer drug were eliminated