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

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Sorption

A B S T R A C T

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 $\mu\text{g L}^{-1}$ [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 $5 \mu\text{g L}^{-1}$ 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 ($220 \mu\text{g L}^{-1}$) 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 $1750 \text{ mg}_{\text{COD}} \text{ L}^{-1}$, $125 \text{ mg}_{\text{TN}} \text{ L}^{-1}$ and $25 \text{ mg}_{\text{TP}} \text{ L}^{-1}$ [7]. The raw urban wastewater ($\text{COD} = 760 \text{ mg L}^{-1}$, $\text{TN} = 65 \text{ mg L}^{-1}$, $\text{TP} = 10 \text{ mg L}^{-1}$) was collected after the sand trap of a WWTP (Ginestous, France, 800 000 person-equivalent) then screened at $200 \mu\text{m}$ and stored at 4°C . The synthetic solution was composed of $\text{C}_6\text{H}_{12}\text{O}_6$ ($22.6 \text{ g}_{\text{COD}} \text{ L}^{-1}$), NaCH_3COO ($22.6 \text{ g}_{\text{COD}} \text{ L}^{-1}$), NH_4Cl ($2.75 \text{ g}_{\text{N}} \text{ L}^{-1}$) and KH_2PO_4 ($0.6 \text{ g}_{\text{P}} \text{ L}^{-1}$).

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 $0.2 \mu\text{m}$ (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 $4.5 \text{ mg O}_2 \text{ L}^{-1}$. 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 $5 \mu\text{g L}^{-1}$.

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 $5000g$ for 20 min. Then, the supernatant was filtrated ($1.2 \mu\text{m}$) 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 $3 \text{ ng g}^{-1}_{\text{TSS}} \text{ ng g}^{-1}_{\text{TSS}}$ 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 $50 \mu\text{g L}^{-1}$ respectively. Flasks were shaken at 150 rpm at room temperature during 4 h in order to reach equilibrium. Then sludge were centrifuged to measure the quantity of CP sorbed on the solid phase of the sludge (in $\mu\text{g kg}^{-1}_{\text{TSS}}$) and the concentration of CP in the aqueous phase (in $\mu\text{g L}^{-1}$) 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 ($5 \mu\text{g L}^{-1}$) 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

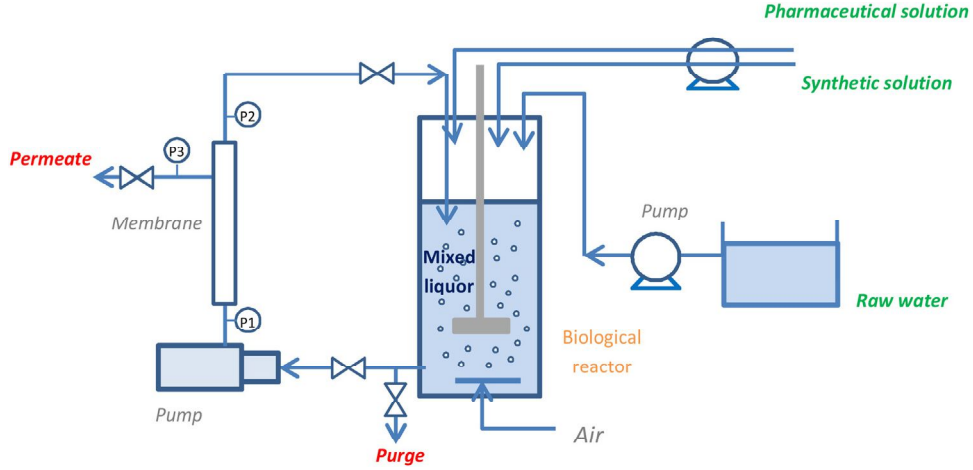


Fig. 1. Schematic diagram of the 20 L membrane bioreactor.

from the reactor, a spike of CP was made. Samples were collected from the permeate and inside the reactor after 2, 4, 6 and 10 h and analysed as described in Section 2.4.

2.7. Performance parameters and mass balances

2.7.1. Apparent removal efficiency

Apparent removal efficiency (RE) referred to the elimination of COD, TN or CP by the membrane bioreactor. It was calculated by comparing the quantity remaining at the outlet (permeate) to the inlet quantity:

$$RE = \frac{Q_{in} \cdot S_{in} - Q_{out} \cdot S_{out}}{Q_{in} \cdot S_{in}} \times 100 \quad (1)$$

where S_{in} and S_{out} are the concentration in the influent and permeate respectively (in $\mu\text{g L}^{-1}$). Q_{in} and Q_{out} are the flow rates of the influent and permeate (in L d^{-1}).

2.7.2. Solid-liquid partition coefficient

The solid-liquid partition coefficient (K_d , $\text{L kg}^{-1} \text{TSS}$) of the micropollutant was calculated as follows:

$$K_d = \frac{S_{CP,sor}}{S_{CP,aq}} \quad (2)$$

where $S_{CP,sor}$ is the concentration of CP sorbed on the solid phase of the sludge (in $\mu\text{g kg}^{-1} \text{TSS}$) and $S_{CP,aq}$ is the concentration of CP in the aqueous phase of the sludge (in $\mu\text{g L}^{-1}$) at equilibrium conditions.

2.7.3. Mass balance

By supposing that the biological reactor was completely mixed, the mass balance for CP could be expressed by Eqs. (3) and (4):

$$Q_{in} \cdot S_{CP,in} - Q_{out} \cdot S_{CP,out} - Q_p \cdot S_{CP} = V \cdot \frac{dS_{CP}}{dt} + V \cdot r_{bio} + V \cdot r_{abiot} \quad (3)$$

$$S_{CP} = S_{CP,aq} + S_{CP,sor} \cdot \frac{X_{TSS}}{1000} \quad (4)$$

where S_{CP} , $S_{CP,in}$ and $S_{CP,out}$ are respectively the concentrations (in $\mu\text{g L}^{-1}$) of CP in the mixed liquor, the influent and the permeate. X_{TSS} is the concentration of total suspended solids (in g L^{-1}). Q_p is the purge flow rate (L d^{-1}). V is the bioreactor volume. r_{bio} ($\mu\text{g}_{CP} \text{L}^{-1} \text{d}^{-1}$) is the biodegradation rate. r_{abiot} ($\mu\text{g}_{CP} \text{L}^{-1} \text{d}^{-1}$) is the degradation rate due to abiotic reactions. r_{abiot} was previously experimentally estimated for CP and was equal to 7% of the inlet quantity of CP. Thus, r_{abiot} was calculated as follow:

$$r_{abiot} = \alpha \cdot \frac{S_{CP,in}}{HRT}, \text{ with } \alpha = 0.07 \quad (5)$$

By combining Eqs. (3)–(5), the quantity of micropollutant entering the reactor during the interval of time dt is equal to:

$$\begin{aligned} \bar{Q}_{in} \cdot \bar{S}_{CP,in} \cdot dt = & \bar{Q}_{out} \cdot \bar{S}_{CP,out}(t) \cdot dt + \bar{Q}_p \cdot (\bar{S}_{CP,aq} + \bar{S}_{CP,sor} \cdot \frac{X_{TSS}}{1000}) \cdot dt + \alpha \cdot \bar{Q}_{in} \cdot \bar{S}_{CP,in} \cdot dt \\ & + V \cdot dS_{CP} + V \cdot r_{bio} \cdot dt \end{aligned} \quad (6)$$

\bar{Q} and \bar{S} indicate the mean value of Q and S respectively during the interval of time dt . The term $V \cdot dS_{CP}$ represents the accumulation of CP in the reactor. At steady state, this term of accumulation was equal to zero and the fate of CP entering the reactor could be calculated as follow:

$$\begin{aligned} \bar{Q}_{in} \cdot \bar{S}_{CP,in} \cdot dt = & \bar{Q}_{out} \cdot \bar{S}_{CP,out}(t) \cdot dt + \bar{Q}_p \cdot \bar{S}_{CP,aq} \cdot dt \\ \text{(entering the reactor)} & \quad \text{(recovered in the permeate)} \quad \text{(recovered in the purge sorbed or sludge)} \\ & + \bar{S}_{CP,sor} \cdot \frac{X_{TSS}}{1000} \cdot dt + \alpha \cdot \bar{Q}_{in} \cdot \bar{S}_{CP,in} \cdot dt + \frac{V \cdot r_{bio} \cdot dt}{\quad} \\ \text{(recovered in the purge in aqueous phase)} & \quad \text{(eliminated by abiotic reactions)} \quad \text{eliminated by biodegradation} \end{aligned} \quad (7)$$

2.7.4. Biodegradation rate

Biodegradation rate (r_{bio} , in $\mu\text{g}_{CP} \text{L}^{-1} \text{d}^{-1}$) was estimated from the mass balance (Eq. (7)) of micropollutant in the reactor during the contaminated phase and the specific biodegradation rate $r_{S,bio}$ ($\mu\text{g}_{CP} \text{g}^{-1} \text{VSS} \text{d}^{-1}$) could be calculated as:

$$r_{S,bio} = \frac{r_{bio}}{X_{VSS}} \quad (8)$$

3. Results and discussion

3.1. Biomass growth

Prior to the addition of anticancer drug (day 76), the MBR was acclimated under stable operating conditions (Table 1). During the first 10 days, a phase of adaptation to environmental conditions (shear stress due to the pump) resulted in a decrease of TSS due to biomass death (Fig. 2). Then, biomass grew to $6 \text{ g}_{TSS} \text{L}^{-1}$ and the process stabilized after 31 days, which corresponded to 1.5 times of the SRT.

During this stationary phase (phase I.b) between day 31 and day 76, the sludge production rate was $0.11 \text{ g}_{TSS} \text{L}^{-1} \text{d}^{-1}$ ($0.09 \text{ g}_{VSS} \text{L}^{-1} \text{d}^{-1}$), the specific growth rate (μ) was 0.018 d^{-1} (Fig. 2). The observed sludge yield was low ($0.08 \text{ g}_{VSS} \text{g}^{-1} \text{COD}$) but within the range of values reported in the MBR literature for similar

Table 1
MBR operating conditions.

Micropollutant	Phase I		Phase II		
	I.a no pharmaceutical	I.b	II.a pharmaceutical	II.b	II.c
Days	0–31	31–76	76–106	106–140	140–153
Operating conditions	Stable	Stable	Stable	Variable	Variable
HRT (d)	1.5 ± 0.1		1.5 ± 0.1	2.2 ± 0.5	1.9 ± 0.2
SRT (d)	19.9 ± 0.2		20.0 ± 0.5	18.1 ± 3.9	11.4 ± 1.6
COD loading (g d ⁻¹)	23.8 ± 2.8		23.4 ± 0.9 ^a	15.7 ± 3.9	21.0 ± 2.7
TN loading (g d ⁻¹)	1.7 ± 0.2		1.46 ± 0.2	1.13 ± 0.2	1.3 ± 0.1
TSS (g L ⁻¹)	increasing	constant	constant	decreasing	decreasing

^aWithout the value at 93d (with this value COD = 26.4 ± 6.6 g L⁻¹).

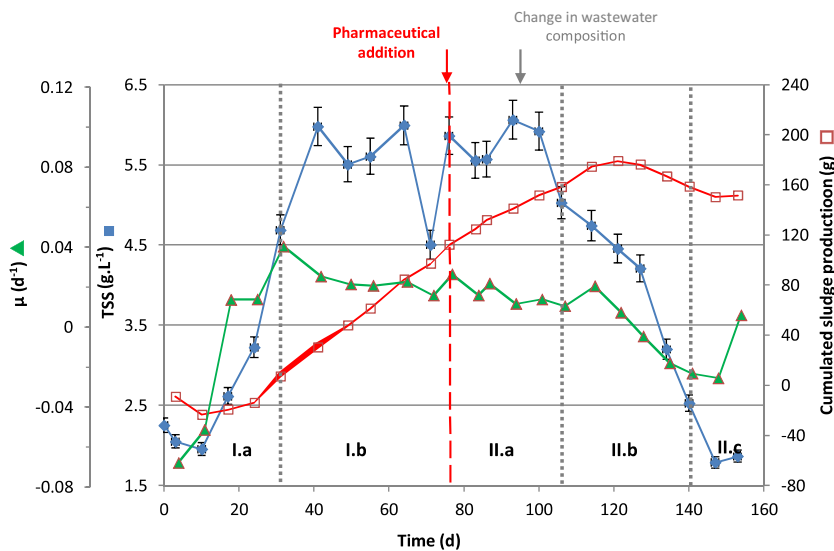


Fig. 2. Evolution of TSS (■), total sludge production (□) and specific growth rate μ (▲) in MBR in continuous mode (TSH = 36 h and SRT = 20 d).

SRT [21–24]. The organic loading rate under stable operating conditions (phase I.b) was 0.25 g_{COD} g⁻¹_{VSS} d⁻¹ (volumetric loading rate 1.25 g_{COD} L⁻¹ d⁻¹). This rate was in the range of those reported in the literature, between 0.1 and 0.3 g_{COD} g⁻¹_{VSS} d⁻¹ [25–28].

After 76 days, pharmaceutical was introduced into the MBR with the same stable running conditions as before until day 106 (phase II.a, Table 1). During this phase, biomass growth did not seem to be disturbed by the addition of the drug (Fig. 2) and sludge production occurred at the same rate of 2 g_{TSS} d⁻¹, even though a modification in raw wastewater composition should be noted between days 93 and 97 (COD = 1700 mg L⁻¹ instead of 800 mg L⁻¹ and TSS = 1000 mg L⁻¹ instead of 400 mg L⁻¹). Delgado et al. [17] reported that addition of CP and some of its metabolites did not affect sludge production or observed yield when the circulation rate was similar (4 m s⁻¹), whereas a slight increase was observed for higher circulation rate (5 m s⁻¹).

After 106 days (phase II.b, Table 1), the running of the MBR was perturbed. At this time, a sudden increase to 4 bar was observed in the transmembrane pressure (TMP), indicating clogging of the membrane, maybe due to an increase in exopolysaccharide (EPS) concentration [17]. The reason may have been the introduction of modified raw wastewater that brought more EPS and/or a secretion of more EPS by microorganisms in response to stress due to introduction of pharmaceuticals [17]. The consequence was a decrease in the feed flow rate of the MBR, and so a decrease in COD volumetric loading rate from 1.2 g L⁻¹ d⁻¹ at day 106–0.6 g L⁻¹ d⁻¹ at day 140. During the same period, the sludge concentration decreased from 5.5 g_{TSS} L⁻¹ to 2.5 g_{TSS} L⁻¹ (Fig. 2).

Finally, from day 140 to day 153 (Phase II.c), TSS concentration stabilized at 1.9 g L⁻¹ while the specific growth rate increased (Fig. 2), suggesting more favourable conditions for microorganism growth. During these last two phases (II.b and II.c), the MBR was kept under supervision to investigate the elimination of drugs under disturbed operating conditions since, in real treatment plants, concentrations of pharmaceuticals and/or carbon and nitrogen sources often vary over time. Little information is available in the literature on process responses to these variations.

Throughout the process running time, the ratio VSS/TSS was almost constant (83 ± 3%). This value was in the usual range for MBR [28] and indicated no accumulation of inorganic matter in the bioreactor.

3.2. Organic carbon and nitrogen removal

As soon as the experiment started, the membrane bioreactor showed high COD removal efficiency around 98 ± 1% (Fig. 3) and efficient nitrogen elimination of 94 ± 5% (Fig. 4). Mean permeate concentration was about 38 ± 12 mg L⁻¹ for COD and 8 ± 6 mg L⁻¹ for TN. Both of these values were below those set by the European Standards (91/271/CEE) for effluent discharge. These high COD and nitrogen removal efficiencies were maintained even when pharmaceutical was added at day 76, as reported for other MBR treating wastewater containing pharmaceuticals [17,23]. High removal efficiencies were also maintained whatever the variations in the inlet water composition (at day 93) or loading rate (during phases II.b and II.c), confirming the robustness of a membrane bioreactor

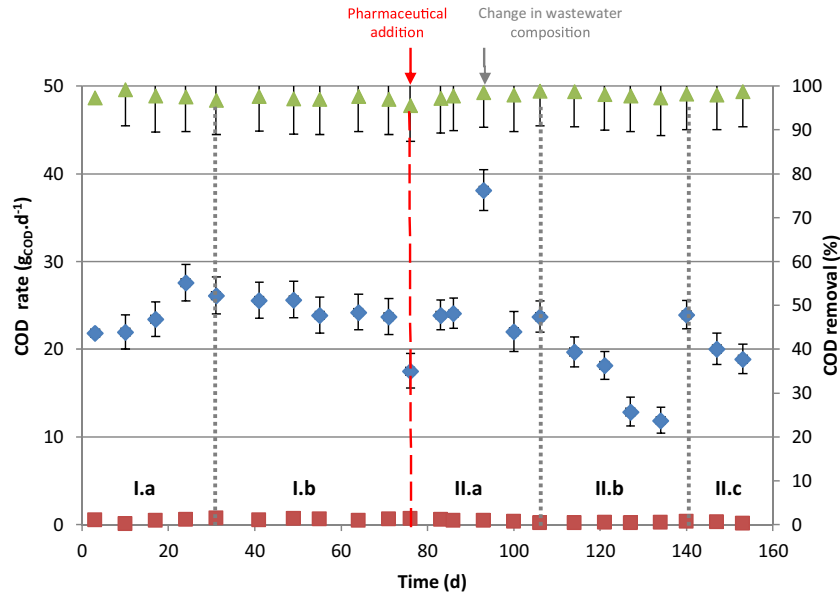


Fig. 3. Evolution of COD rate in the inlet (◆), in the permeate (■) and removal efficiency (▲) in MBR in continuous mode (TSH = 36 h and SRT = 20 d).

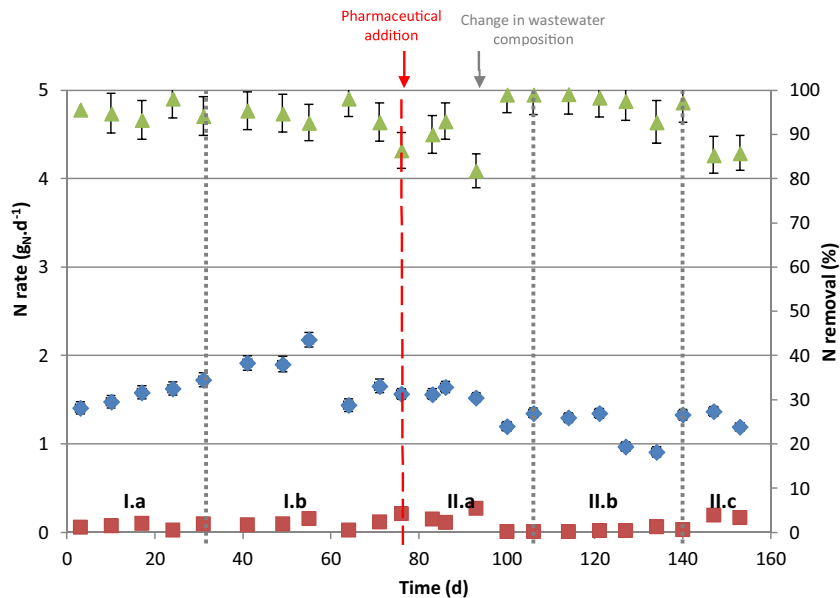


Fig. 4. Evolution of TN rate in the inlet (◆), in the permeate (■) and removal efficiency (▲) in MBR in continuous mode (TSH = 36 h and SRT = 20 d).

regardless of the variations in running conditions. The robustness of the MBR process was mainly due to its high sludge concentration and the complete retention of sludge by the membrane. Moreover, high SRT (20 d) was favourable to the development and persistence of slow-growing bacteria (such as nitrifying bacteria) and allowed the microbial community to be more diversified [13]. It was noted that variations in removal efficiency were higher for nitrogen than for COD. Nitrogen was mostly removed through biological reaction by slow-growing bacteria [22], so a decrease in SRT could lead to their leaching, which could explain the observed changes.

3.3. Cyclophosphamide removal mechanisms

3.3.1. Global removal of CP

As soon as anticancer drug was introduced into the reactor, elimination from the aqueous phase was observed (Fig. 5). The removal efficiency stabilized at day 100, 24 days after the addition of the

pharmaceutical, which corresponded to about one sludge retention time. After this time, the mean concentration in the permeate was $2.8 \pm 0.6 \mu\text{g L}^{-1}$, corresponding to a mean apparent removal efficiency $RE = 60\%$. This efficiency was not impacted by the modification of raw wastewater composition at day 93 nor by the variation in COD loading after 106 days. In the literature, only three studies have dealt with removal of CP from wastewater effluents by MBR with different SRT. The first one concerned a lab-scale pilot similar to ours, feeding with supplemented domestic wastewater spiked with CP ($5 \mu\text{g L}^{-1}$) [17]. The removal efficiency was 75% for $SRT = 70$ d. The two others studies were performed in pilot-scale MBR with hospital wastewater ($CP = 0.161 \mu\text{g L}^{-1}$ [15]) and SRT varying from 30 to 50 d [15,16]. In these studies, apparent removal efficiency varied from 12 to 25%. The highest removal efficiency (25%) was obtained with the higher SRT of 50 d. Comparison between lab-scale and pilot-scale studies was difficult because of differences in the running conditions and in the influent composi-

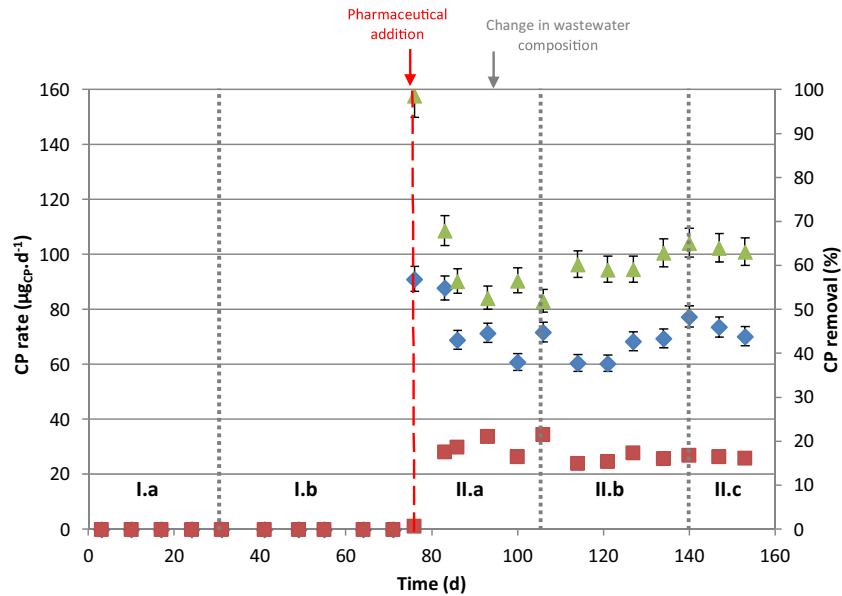


Fig. 5. Evolution of CP rate in the inlet (◆), in the permeate (■) and removal efficiency (▲) in MBR in continuous mode (TSH = 36 h and SRT = 20 d).

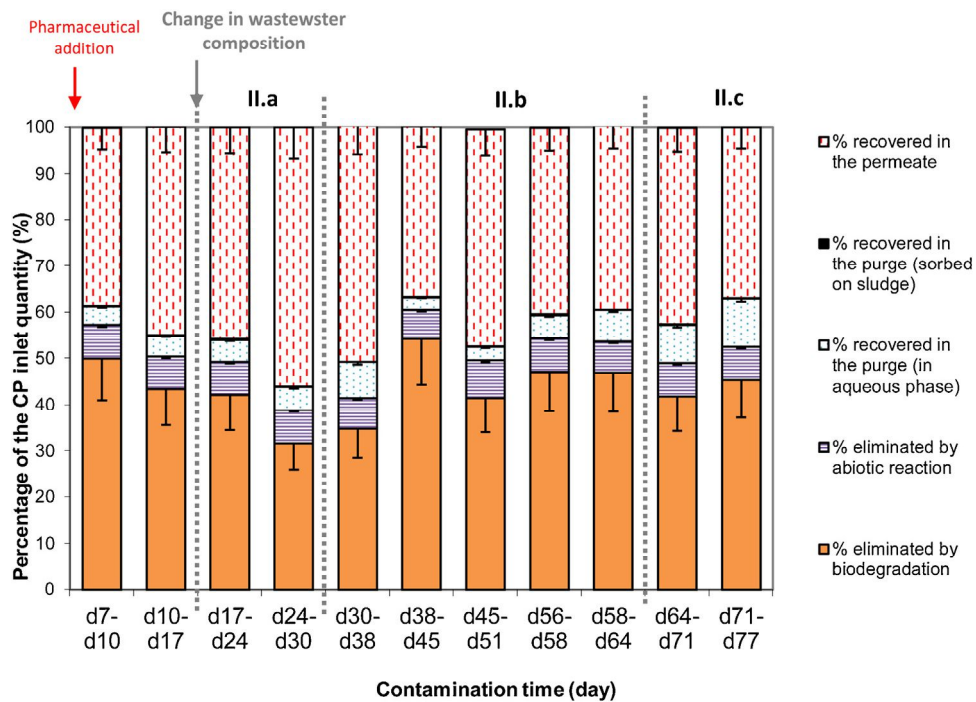


Fig. 6. Fate and distribution of CP entering the MBR in continuous mode (TSH = 36 h and SRT = 20 d) calculated from Eq. (7).

tion. For instance, the biodegradation of a specific molecule could be less efficient when it is in mixture with other cytostatic compounds [29]. However, it seemed that higher SRT was favourable for CP elimination as it is reported for some other micropollutants [14,28]. High SRT allows for the enrichment of slow-growing bacteria and also the establishment of a more diversified population able to degrade some specific pollutants [13,30]. It also increases the contact time between microorganisms and micropollutants.

3.3.2. Distribution of CP

Removal mechanisms of CP have never been investigated in the literature. So the distribution of inlet CP was calculated from

Eq. (7) and presented in Fig. 6. After stabilization, 40% of the inlet CP was recovered in the permeate, 6% was recovered in the purge, 7% was eliminated by abiotic reactions and 47% was biodegraded. So biodegradation was the main mechanism of elimination.

Concerning the purge, the quantity of CP sorbed on the solid phase of sludge was inferior to 3% of the total quantity in the mixed liquor. The maximal value of CP sorbed on the solid phase was $34 \mu\text{g}_{\text{CP}} \text{kg}^{-1}_{\text{TSS}}$. Thus the environmental impact of CP recovered in purge would be very limited if the aqueous phase of the purge (containing more than 97% of the total CP) is sent back into the MBR.

3.4. Sorption

The partition coefficient (K_d) for CP was calculated from Eq. (2) during the contaminated phase (phase II). K_d values were quite constant throughout the experiment, at about $5 \pm 2 \text{ L kg}^{-1} \text{ TSS}$. This was in accordance with the fact that sorption on sludge was low. Ternes et al. [31] indicated that for K_d values lower than 100 L kg^{-1} , sorption could be neglected. This K_d value obtained in MBR was compared to values obtained in batch assays from sludge collected from the MBR during the uncontaminated (phase I) and contaminated (phase II) periods. Sorption isotherms are presented in Fig. 7. It can be seen that sludge had similar sorption capacities during both uncontaminated and contaminated phases, probably because adsorption was low, so sorption sites were still available for other micropollutants. Moreover, K_d values were higher in batch tests (about $60 \text{ L kg}^{-1} \text{ TSS}$) than in the MBR but remained very low ($<100 \text{ L kg}^{-1}$). Even if precautions were taken to avoid modification of sludge properties, batch tests led to new environmental conditions for sludge that could little modify their structure or properties, and so did not allow to obtain proper values of K_d . In our case, the value was overestimated. This is an important point for the evaluation of micropollutant partition in bioreactors as some studies estimate sorbed quantities of micropollutant from batch tests or from the literature.

3.5. Biodegradation

3.5.1. Biodegradation rate

Biodegradation occurred as soon as drug introduction. So microorganisms with potential biodegradation of CP should already be present in the first part of the experiment (phase I). This could be due to the high diversity of microorganisms developed in membrane bioreactors. In particular, recent works have suggested that slow-growing bacteria like autotrophic ammonia oxidizers can be responsible for the degradation of a large variety of micropollutants via non-specific enzymes, such as AMO (ammonia monooxygenase) [11,30,33]. This could explain the higher performance of MBR compared to activated sludge in the elimination of micropollutants [6,7,11,14].

From Eqs. (7) and (8), the volumetric biodegradation rate (r_{bio}) and specific biodegradation rate ($r_{\text{S,bio}}$) were calculated. After 100 h (24 days after CP addition), r_{bio} was equal to $1.51 \pm 0.26 \mu\text{g}_{\text{CP}} \text{ L}^{-1} \text{ d}^{-1}$. Whatever the inlet running conditions of the MBR, microorganisms were able to maintain stable biodegradation of cyclophosphamide. During the same period, the specific biodegradation rate increased from $0.3 \mu\text{g}_{\text{CP}} \text{ g}_{\text{VSS}}^{-1} \text{ d}^{-1}$ to $1 \mu\text{g}_{\text{CP}} \text{ g}_{\text{VSS}}^{-1} \text{ d}^{-1}$. Microorganisms have increased their metabolic activity (due to acclimatation) or a higher fraction of microorganisms was able to degrade CP (growth of a specific biomass). Three days after pharmaceutical addition had been stopped; a new spike of CP was introduced. As can be seen on Fig. 8a, CP was again biodegraded and the specific biodegradation rate varied from 0.34 to $1.15 \mu\text{g}_{\text{CP}} \text{ g}_{\text{VSS}}^{-1} \text{ d}^{-1}$. These values were similar to those found in the MBR during phase II so the biomass did not lose its ability to biodegrade the micropollutant during this short period. This is an important point for the treatment of real effluent, in which the concentration of CP would vary in time and could be equal to zero for some periods.

3.5.2. Biodegradation process

Since the amounts of micropollutants seem too low to serve as growth substrate, co-metabolism is supposed to be the dominating biodegradation process [32]. Co-metabolism is defined as the transformation of a non-growth substrate in the obligatory presence of a growth substrate or another biodegradable compound [34]. So the biodegradation of a spike of CP by sludge taken from MBR during

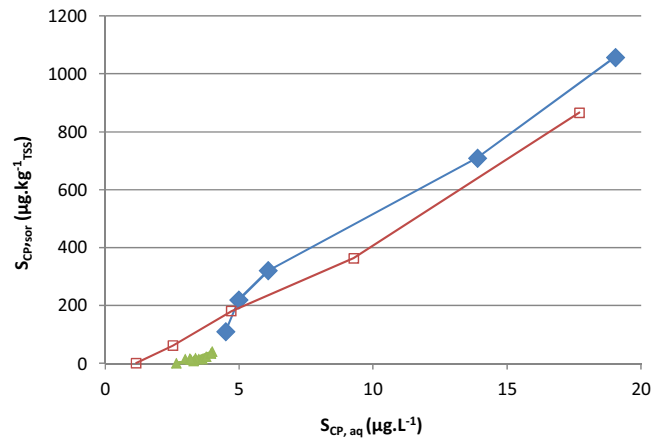


Fig. 7. Sorption isotherms from in situ measurement in continuous running MBR (\blacktriangle), and from batch assays for uncontaminated MBR sludge (\blacklozenge) and MBR acclimated sludge (\square).

contaminated phase (phase II) was investigated in the absence of growth substrate (without addition of carbon and nitrogen source). As can be seen in Fig. 8b, no decrease in CP concentration occurred whereas biodegradation was observed in the MBR. It seems there is a need of presence of carbon or nitrogen source to biodegrade CP.

Besides, during phase II of MBR experiment, specific consumption res of COD ($r_{\text{SCOD}}, \text{g}_{\text{COD}} \text{ g}_{\text{VSS}}^{-1} \text{ d}^{-1}$) and TN ($r_{\text{SN}}, \text{g}_{\text{N}} \text{ g}_{\text{VSS}}^{-1} \text{ d}^{-1}$) were calculated. And no obvious linear relation was found between $r_{\text{S,bio}}$ and r_{SCOD} ($r^2 = 0.7$) whereas a linear relation between $r_{\text{S,bio}}$ and r_{SN} ($r^2 = 0.91$) was found with:

$$r_{\text{S,bio}} = \beta \cdot r_{\text{SN}} \quad \text{with } \beta = 33.1 \mu\text{g}_{\text{CP}}/\text{g}_{\text{N}} \quad (9)$$

The biodegradation of cyclophosphamide could be linked with the degradation of ammonium. Similar results have been found for the biodegradation of 8 emerging organic micropollutants under nitrifying conditions [16]. Values of β between 35.4 and $107.3 \mu\text{g}_{\text{g}}^{-1} \text{ N}$ were found depending on the organic compound. Some works report the probable role of AMO enzyme from autotrophic ammonia oxidizers in the simultaneous degradation of ammonium and some micropollutants [11,30,33]. High biodegradation of some micropollutants was obtained under nitrification at high ammonia loading rate [30,37,38]. In the present study, the operating conditions of the MBR allowed high nitrogen elimination (removal rate $r_{\text{N}} = 1.3 \text{ g}_{\text{N}} \text{ d}^{-1}$ and specific removal rate $r_{\text{SN}} = 0.03 \text{ g}_{\text{N}} \text{ g}_{\text{VSS}}^{-1} \text{ d}^{-1}$), which could explain the rather high biodegradation rate in the MBR.

These results are consistent with the probable biodegradation of CP by co-metabolism mechanisms, but they must be confirmed by additional experiments.

3.5.3. Biodegradation kinetic modelling

3.5.3.1. First-order kinetic model. Pseudo-first order kinetic model was usually assumed for modelling of biodegradation rates [35]:

$$r_{\text{bio}} = k_{\text{biol}} \cdot X_{\text{VSS}} \cdot S_{\text{CP,aq}} \quad (10)$$

With biodegradation kinetic constant $k_{\text{biol}} (\text{L g}^{-1} \text{ VSS d}^{-1})$.

However, this model is not suitable to describe the observed increase with time of the specific CP removal rate due to change in metabolic activity or in active biomass fraction. As expected, if k_{biol} was calculated from Eq. (10), increased values from 0.06 to $0.28 \text{ L g}^{-1} \text{ VSS d}^{-1}$ were obtained. The mean value of k_{biol} was $0.16 \pm 0.06 \text{ L g}^{-1} \text{ VSS d}^{-1}$. From the classification of Joss et al. [36], cyclophosphamide seemed to be only slightly biodegradable (k_{biol} close to $0.1 \text{ L g}^{-1} \text{ VSS d}^{-1}$).

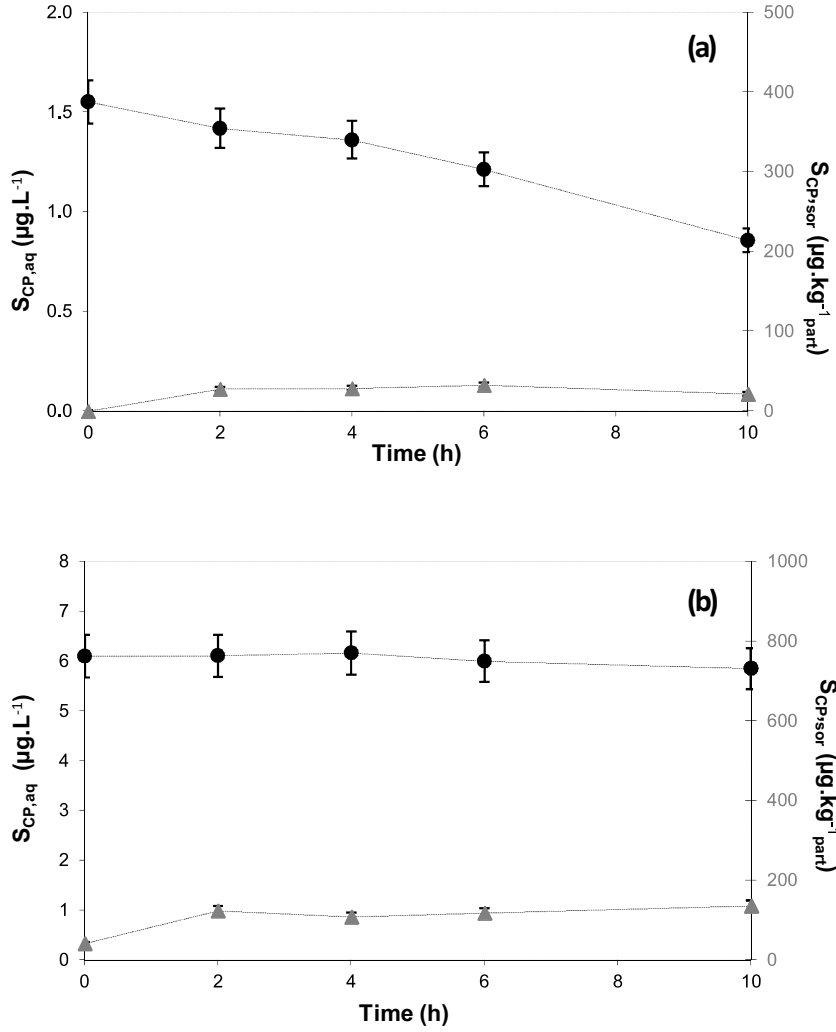


Fig. 8. Evolution of CP sorbed on sludge ($S_{CP,sor}$, ▲) and CP in aqueous phase ($S_{CP,aq}$, ●) during biodegradation test: (a) in the MBR with carbon and nitrogen sources, and (b) in batch without carbon or nitrogen sources.

3.5.3.2. *Kinetic model linked to nitrogen consumption.* With regard to the observed link between CP and ammonia biodegradation rates (Eq. (9)), a model adapted from the co-metabolic Monod-type kinetics model proposed by Fernandez-Fontana et al. [38] for the elimination of organic micropollutants in nitrifying reactors was tested:

$$r_{S,bio} = T_C \cdot r_{S_N} \cdot \frac{S_{CP,aq}}{K_C + S_{CP,aq}} \quad (11)$$

The micropollutant transformation capacity (T_C) is the maximum amount of micropollutant that can be biotransformed per gram of nitrogen consumed ($\mu\text{g g}_N^{-1}$). The micropollutant affinity (K_C) is the concentration of pollutant providing half of the maximum biotransformation rate ($\mu\text{g L}^{-1}$). With our operating conditions, the values of T_C and K_C were $37.8 \mu\text{g}_{CP} \text{g}_N^{-1}$ and $1.2 \mu\text{g L}^{-1}$, with a good fitting between predicted and experimental values (Fig. 9). The values of T_C and K_C were in accordance with the β value in Eq. (9) since the aqueous concentration varied little during the experimental period, so $T_C \approx \beta$ and K_C was lower than the mean values of $S_{CP,aq}$.

However, this model needs further validation under different operating conditions.

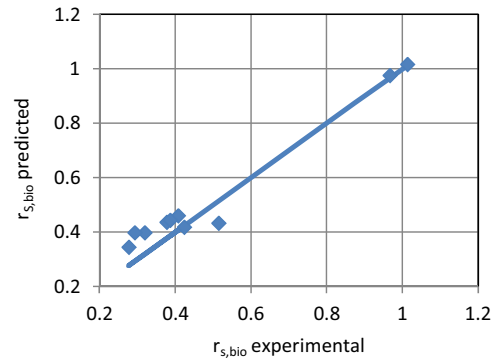


Fig. 9. Comparison of specific biodegradation rate measured in the 20L-MBR ($r_{S,bio}$ experimental) and predicted ($r_{S,bio}$ predicted) by the model proposed by Fernandez-Fontana et al. [38] (Eq. (11)).

4. Conclusion

A high cyclophosphamide removal efficiency was demonstrated in an MBR with SRT = 20 d whatever the variations in COD, N or CP concentrations in the inlet flow. Removal by sludge sorption can be neglected and biodegradation was the predominant mechanism. It was assumed that co-metabolism mechanisms by ammonia oxi-

dizers were involved in cyclophosphamide elimination. However, a better understanding of the mechanisms and factors controlling biodegradation rates is still needed to enhance biodegradation of such compounds.

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