CYTOXICITY AND CYTOSTATIC DRUG REMOVAL IN A MEMBRANE BIOREACTOR FROM WASTEWATER

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Abstract. The growing use of antineoplastic drugs in cancer therapy is an emerging issue in environmental research. The presence of the anticancer drug cyclophosphamide (CP) in municipal wastewater raises several environmental problems. Besides its cytotoxic effects, CP possesses teratogenic and mutagenic properties and is a known human carcinogen. The application of membrane bioreactor (MBR) technology was investigated with the aim of evaluating its potential for cytostatic drug bioremoval. The toxicity removal was assessed from biomarkers tests and related to the choice of the reactor operating conditions. The influence of sludge retention times (SRT) on CP removal was suited but not significant effects were found for variation of SRT from 50 days to 70 days. CP removal up to 80% was achieved under studied operating conditions. In front of such pollution, evidence has been made about the use of MBR. Our study proofed that advances wastewater treatment using a MBR provides a suitable process for lowering CP concentrations before discharge into the aqueous environment. However, a tertiary treatment is necessary for the complete elimination of toxicity.

Key-words. Membrane bioreactor, wastewater, cyclophosphamide, micropollutants.

INTRODUCTION

Pharmaceuticals are designed to exhibit biological activity in humans and may basically have adverse effects on aquatic organisms. Pharmaceuticals and other micropolluants in wastewater pose a new challenge to wastewater professionals as well as to the pharmaceutical industry [1]. Compounds with a very potent mechanism of action such as cytostatic drugs are of particular environmental concern, even though consumption rates and expected concentrations in the environment may be comparatively low [2, 3].

The increase of the demand for chemotherapy treatment in developed countries goes on at around 10% more per year. Further, cancer incidence is increasing and this is not simply due to a greater proportion of elderly people in the population [4]. The alkylating antineoplastic drug Cyclophosphamide (CP) is one of the oldest known cytostatics and is one of the most frequently used agents in cancer chemotherapy [5]. After application to patients the agent is renally excreted, whereby up to 20% of the dose may leave the body unmetabolized. Besides its cytotoxic effects, CP possesses teratogenic and mutagenic properties and is a known human carcinogen [6]. CP is a prodrug that requires biotransformation to become cytotoxic [7, 8]. It is transformed via hepatic and intracellular enzymes to active alkylating metabolites, 4-hydroxycyclophophosphamide, aldophosphamide, acrolein and phosphoramide mustard [9].

CP could be detected in concentrations ranging from 20ng/L to 4.5 µg/L in hospital sewage [6]. The occurrence of the agent could also be proved in samples from the influent and the effluent of the communal sewage treatment plant into which the hospital's sewage water is discharged. Concentrations ranged from 7 to 143 ng/L [6]. CP has been detected in surface waters in Switzerland, concentrations ranged from 50 to 170 pg/L and were thus several orders of magnitude lower than the levels at which acute ecotoxicological effects have been reported in the literature (mg/L range). However, due to a lack of

studies on chronic effects on aquatic organisms and data on occurrence and effects of metabolites, a final risk assessment cannot be made [3].

Membrane bioreactors (MBR) constitute a promising technology in industrial and urban wastewater treatment. The MBR incorporates a membrane for liquid-solid separation. Theoretically, several operational conditions exist in MBRs, which are in favour of enhanced biotransformation and mineralization of micropollutants [10, 11].

The application of membrane bioreactor (MBR) technology is investigated here with the aim of evaluating its potential for cytostatic drug bioremoval. Toxicity and CP removal in a MBR were investigated.

MATERIAL AND METHODS

The schematic diagram of crossflow MBR pilot system is shown in fig 1. The membrane modules were ceramic tubular Membralox® (MF) with 0.0055 m^2 of surface area and pore size 0.2 µm (Pall Exekia, France). In order to keep a complete mixing in the bioreactor a Ruston turbine was installed. Dissolved oxygen and pH in the bioreactor were monitored. The lab-scale crossflow MBR was used in the continous experiments. Cyclophosphamide (5µg/L) and its principal metabolites (Acrolein 2250 ng/l, Phosphoramide Mustard 8880 ng/L, 4-ketocyclophosphamide (Keto CP) 580 ng/L, Nitrogen Mustard 517 ng/L) were continuously added to the pilot. The MBR pilot was inoculated with activated sludge from a municipal sewage treatment plant (dry weight, 3g/L). Raw water was composed of domestic water and completed with Viandox® so as to reach the required Chemical oxygen demand (COD) amount. Treatment was operated in aerobic/anoxic conditions. The operating conditions of the MBR during the experimentation are given in Table 1. Two campaigns were run to investigate the sludge retention time, respectively campaign I for 50 days, campaign II for 70 days.

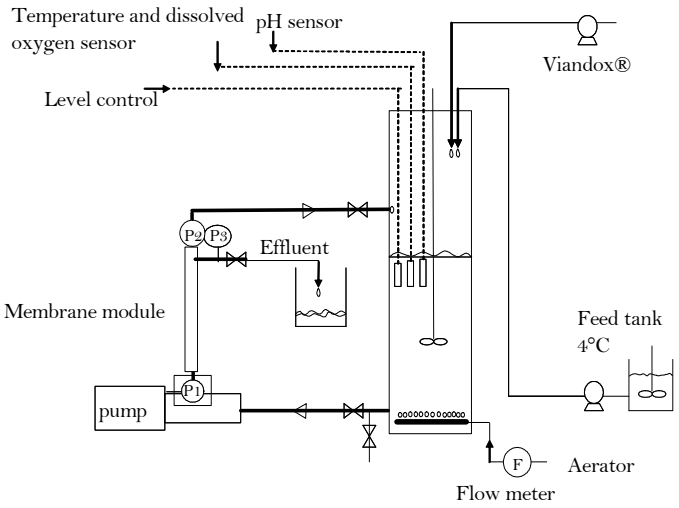

Fig. 1 Schematic diagram of MBR pilot

Table 1. Operating conditions of the membrane bioreactor (MBR) during the experimentation

Parameter	experimental campaign I	experimental campaign II
working volume (L)	20	20
Temperature (°C)	25-32	25-32
pH	7-8	7-8
flux (at 20°C, $L.m^{-2}h^{-1}$)	55.47 ± 8.37	83.87 ± 6.01
Inlet COD (mg DCO/L)	2609 ± 512	1774.7 ± 629.6
Organic loading rate (Kg $COD.m^{-3}.d^{-1}$)	1.02 ± 0.12	1.26 ± 0.42
F/M (Kg CODinlet/KgMLSS/d) at steady-state.	0.11	0.11
Solids retention time (SRT) (d)	50	70
Hydraulic retention time (HRT) (h)	48	32
aeration cycle	2 minutes aeration/23 without aeration	2 minutes aeration/17 without aeration
Crossflow velocity (m/s)	4-5	4-5

The chemical composition of soluble extracellular polymeric substances (EPS), was analyzed for proteins, humic substances and polysaccharides. Proteins and humic substances were measured by the modified Lowry method with Bovine serum Albumin and humic acid as standard [12]. Polysaccharides were

determined according to the anthrone method with glucose as standard [13]. The transmembrane pressure (TMP) which indicates the extent of membrane fouling was time to time regularly monitored. The analysis of CP was performed by HPLC/MS/MS after lyophilisation and extraction with dichloromethane.

RESULTS

The experiments were performed during day 160in the first experimental campaign and day 223 in the second one. Day 21 was the first day addition of cyclophosphamide and its principal metabolites to MBR pilot during the first experimental campaign and day 108 during the second one.

Cyclophosphamide removal efficiency in MBR

To compare the performance of MBR pilot in both campaigns, Figure 2 illustrates the CP removal efficiencies. The axis of time is recalculated, the day 0 being the first day of the cocktail addition. After 70 days continuous addition of the cocktail (CP and its principal metabolites), the CP removal efficiencies is about 80% during the two campaigns (Figure 2). According to these results, it appears that a sludge age of 50 days is sufficient to achieve removals efficiency of 80%. The increase in sludge age to 70 days does not improve significantly the removal efficiency. However, the question about the removal of CP at ages sludge less than 50 days is still open.

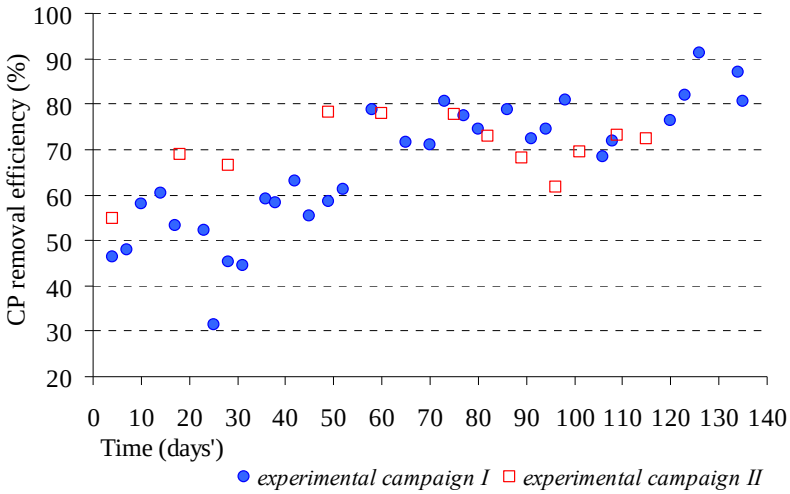

Figure 2. CP removal efficiencies

Cytotoxic removal efficiency in MBR

The evaluation of cytotoxicity was achieved by measuring the proliferation of treated cells compared to control (untreated cells). Proliferation was measured quantitatively by the colorimetric tetrazolium (MTT) and based on the metabolic activity of viable cells. Tests of cell proliferation were made on human liver cells (HepG2) because this cell line was most sensitive to the effects of the products tested [14].

In order to establish a link between the cytotoxicity (death or proliferation) observed in the supernatant and permeate of MBR pilot and bioreactor behaviour, we recalculate the toxicity measured as the absolute values of difference between the values of viability (death or proliferation) for cytotoxicity tests compared to 100% viability of the control. These values, in the supernatant and in the permeate, are represented in Figure 3(a) and (b), respectively for the first campaign. The evolution of transmembrane pressure during the experimental campaign I is also presented. It is interesting to note that during the first experimental campaign, Figure 3a: the increase in cytotoxicity in supernatant MBR pilot occurred around the days when transmembrane pressure increased, days 64, 108 and 145.
Figure 3b: for some days (63 and 116-131), when the transmembrane

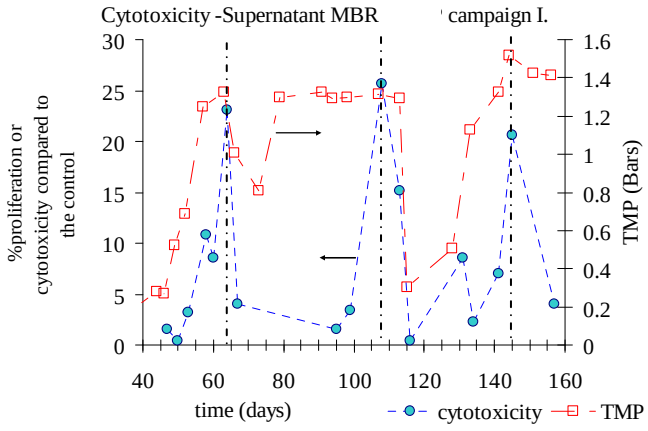

Figure 3(a). Supernatant MBR pilot cytotoxic and Transmembrane pressure during the experimental

pressure decreased widely, the permeate toxicity increased significantly (drastic changes of the parameter in concomitant).

Figure 4 shows supernatant and permeate MBR pilot cytotoxicity and the evolution of the humic acids and polysaccharides concentration in the time. Increased cytoxicity was observed in the supernatant and the permeate, on days when the concentration of humic acid also increased. Furthers research are needed to evaluate if this phenomena was due to the formation of new toxics cyclophosphamide metabolites. Anyway , these graphs strongly suggested that these toxic compounds are adsorbed on the colloidal particles and humic substances, which are themselves retained by the membrane.

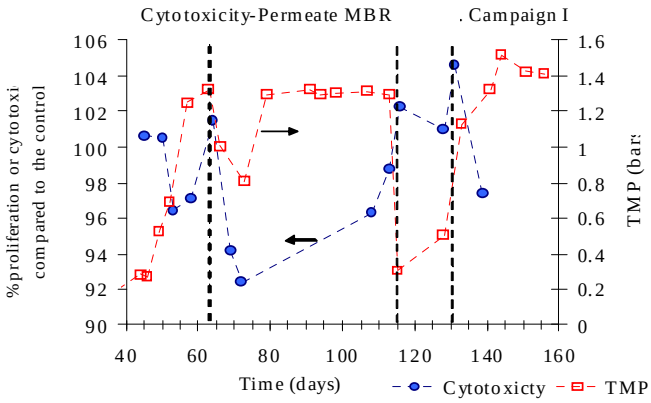

Figure 3(b). Permeate MBR pilot cytotoxic and Transmembrane pressure during the experimental campaign I.

Discussion

The issue concerning the presence and the potential risks associated with micropollutants in the environment has become a highlighted topic. Today, the wastewater treatment plants are not able to adequately remove this new type of pollution. Therefore, it is necessary to consider a more effective treatment to eliminate these compounds upstream sewage treatment plants.

Preliminary investigations in a simplified test system indicated a low degradability of CP [3, 15]. Based on the degradation studies in laboratory scale sewage treatment plant and the analytical findings in sewage water, Steger-Hartmann et al, 1997 concluded that excreted CP is only poorly degraded during its passage through the sewage treatment plant [6]. Even though other studies indicated, that CP is not or poorly biodegradable, we observed a removal of this molecule in our study. Removal of CP started from the beginning of experiment (fig. 2). At steady-state conditions, the pharmaceuticals removal efficiencies remained quite stable, up to 80% for CP, and proved the ability of the MBR for the cytotoxic compounds partial removal. In another previous study, it was documented that both adsorption and degradation affect the overall removal [16]. Furthermore, sorption may also influence the rate of other processes such as

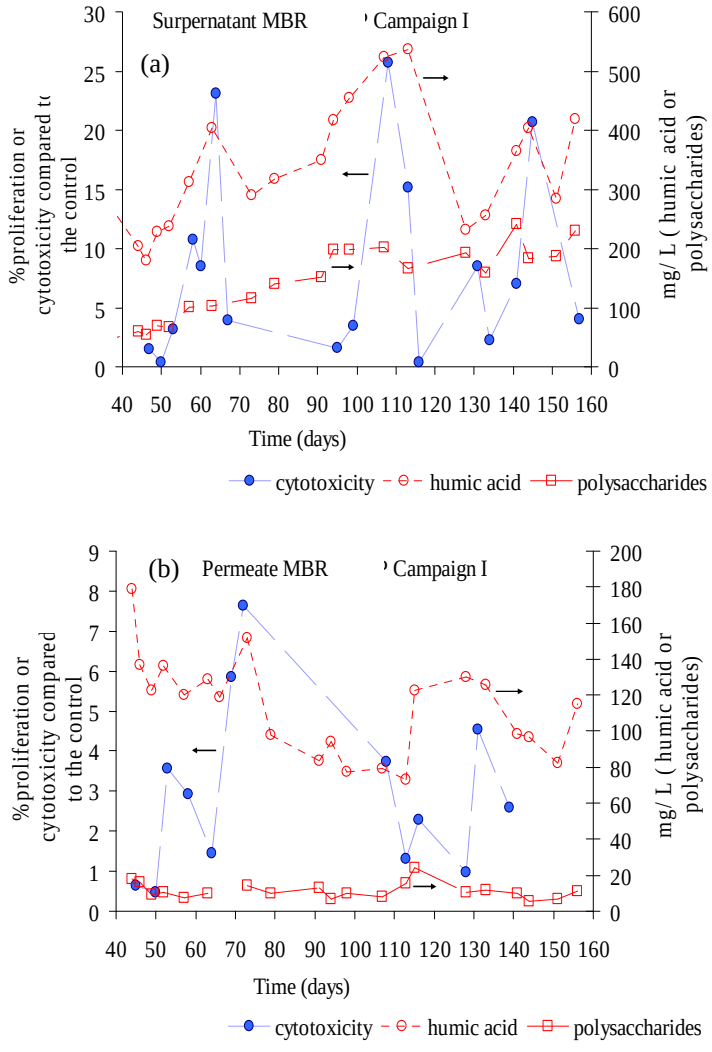

Figure 4. Supernatant (a) and permeate (b) MBR pilot cytotoxic and variation of humic acid and polysaccharides in the MBR during the experimental campaign I.

biodegradation [17]. As CP was present at low concentrations, CP could not be used as the primary source of energy/carbon, so it could be suggested that CP was cometabolically degraded. In previous studies, some authors indicate that cometabolic transformation may be the major removal mechanism of some PhAC compounds in activated sludge treatment of municipal wastewater [18, 19].

In the same previous study [16], it was also demonstrated that CP and its metabolites toxicity do not alter COD and total nitrogen removal efficiency of MBRs. Removal rates observed for COD and TN were above 90% and 93% respectively. COD removal in the system was attributable to two factors, one was biological removal by micro-organisms and the other was physical retention by the membrane. Figure 5 illustrates the important role of membrane filtration in the performance of MBR. After increasing the crossflow velocity, the supernatant COD content in MBR increased considerably nevertheless, the permeate COD concentration was almost constant after increasing the crossflow velocity on day 65. It points out the role of membrane in the quality of permeate.

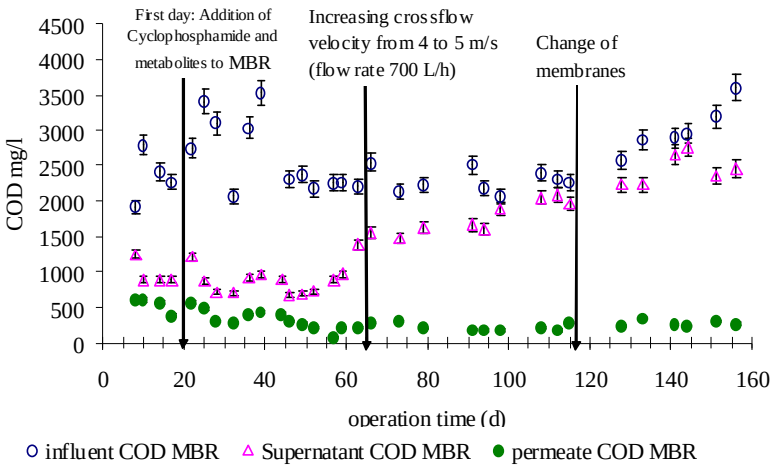

Figure 5. COD concentration MBR influent, MBR supernatan and MBR permeate during the experimental campaign I.

The evaluation of this technology (membrane bioreactors) in industrial scale on long-term hospital effluent, preferably at the exit of Oncology service, or on wastewater from the pharmaceutical industry will ultimately clarify the industrial-scale membrane bioreactor potential in the chemical removal of cytotoxic compounds and their associated toxicity. However, membrane bioreactors offer two important advantages for the elimination of pharmaceutical compounds from hospital wastewater:
1. The compact design allows implementation at the hospital site.
2. The possibility of operation at high sludge ages to adapt the biomass to the micropollutant. In these conditions the amount of sludge produced is reduced, and the simultaneous elimination of the COD and nitrogen can be conducted under operating conditions selected. In addition, if the sludge become toxic when treating hospital wastewater, treatment by incineration would become relevant. The decrease in the quantity of sludge to incinerate represents a reduction of operating costs.

CONCLUSION

The continuous release of Cytostatics via hospital wastewater is problematic. Cytostatics belong to the CMR (carcinogenic, mutagenic and reprotoxic) drugs. As hospital effluents reach the municipal sewage network generally without any preliminary treatment, their presence in the environment could affect animal and human health, but also the plants. In this work, MBR was operated in order to evaluate its potential for cytostatic drugs bioremoval. The alkylating antineoplastic drug Cyclophosphamide (CP) which is one of the oldest known cytostatics and is one of the most frequently used agents in cancer chemotherapy has been chosen. CP removal up to 80% was achieved under a hydraulic retention time of 48h, a solid retention time of 50 days. Variation of sludge age between 50 and 70 days showed no significant impact on the transformation efficiency of CP. The results of this study proved that advances wastewater treatment using a MBR provides a suitable process for lowering CP concentrations before discharge into the aqueous environment. Despite of this clear benefit of MBR, the removal is only partially achieved and a tertiary treatment is necessary for the complete elimination of cytostatic compounds toxicity.

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