Effect of cytostatic drugs on the sludge and on the mixed liquor characteristics of a cross-flow membrane bioreactor: Consequence on the process

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A B S T R A C T

The influence of cyclophosphamide and its principal metabolites (CPs) on the physicochemical properties of the mixed liquor of a cross-flow membrane bioreactor and the consequences for membrane fouling were investigated. The influence of CPs was determined by comparing the performance of two bioreactors running in parallel, MBR-CPs (with CPs) and MBR-control (without CPs). The physicochemical properties of the mixed liquor were characterized by soluble extracellular polymeric substances (soluble EPS content), particle size distribution and specific cake resistance. Results suggested that the CP toxicity altered the characteristics of the biological matrix of the activated sludge. Micro-organisms exposed to CPs showed higher endogenous respiration rates than MBR-control micro-organisms. The accumulation of soluble EPS and the formation of small particles (in MBR-CPs after cross-flow velocity was raised) increased the resistance to filtration. The fouling potential of the supernatant seemed to be linked more closely to the concentration of polysaccharides than of proteins and humic substances. Modifications of the membrane performance were observed. Under operating conditions, membrane fouling was faster in MBR-CPs than MBR-control. Moreover, the membrane played an important role in the permeate quality and the overall performance of the process, making possible the biological treatment of such an effluent.

1. Introduction

The increasing use of anticancer drugs and their presence in wastewater is a relatively new issue and few studies have been published [1–7]. Cytostatic drugs (among the most toxic pharmaceuticals in common use) are of particular environmental concern even though consumption rates and expected concentrations in the environment may be comparatively low [1,3]. Cyclophosphamide has been detected in surface waters in Switzerland, where 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 the chronic effects on aquatic organisms and data on the occurrence and effects of metabolites, a final risk assessment cannot be made [1].

The cytotstatic drug cyclophosphamide (CP) is one of the oldest known cytostatics and is one of the most frequently used in cancer chemotherapy [8]. CP is a prodrug that requires biotransformation to become cytotoxic [9,10]. It is transformed by hepatic and intracellular enzymes to active alkylating metabolites [11]. Besides its cytotoxic effects, CP possesses teratogenic and mutagenic properties and is a known human carcinogen [1,7]. Such drugs, partially transformed or even unchanged, usually enter hospital effluents via the urine and faeces of patients under medical treatment. Therefore, they are assumed to be environmentally relevant compounds. As hospital effluents generally reach the municipal sewage network without any preliminary treatment, hospitals are an undeniable release source of anticancer agents [6]. The compounds finally reach the aquatic environment via hospital or domestic wastewater and wastewater treatment plants (WWTPs) [1].

Theoretically, there are several operational conditions in membrane bioreactors (MBRs) that favour the enhanced biotransformation and mineralization of pharmaceutically active compounds (PhACs) [12,13]. Membrane bioreactors usually operate at high sludge retention times and high concentrations of biomass, allowing an intensification of biological processes by the implementation of resistant and low-growth biomass [13]. All these elements may increase the elimination of contaminants with special characteristics, such as low bio-degradability and low concentration, like PhACs. In some cases, MBRs have shown

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significantly better removal of persistent pharmaceuticals than conventional activated sludge treatment (CAS) [14–16]. Nevertheless, the main problem in membrane application is a rapid decline in the permeation flux due to membrane fouling, which requires frequent membrane cleaning/replacement, thus increasing the running costs [17]. This last point still needs investigation in the case of the presence of toxic compounds, especially on the understanding of the sludge development and subsequent characteristics.

The retention of soluble extracellular polymeric substances (soluble EPS) is important for MBR performance levels for both effluent quality and membrane fouling. Although there is no clear consensus on the exact nature of the phenomena that occur at the interface of the membrane during filtration, many studies indicate that the soluble EPS play a major role in fouling [18–23]. More specifically, Rosenberger et al. [24] suggest that, during filtration, the soluble microbial products (or soluble EPS) can adsorb on the membrane surface and block membrane pores as well as forming a gel structure on the membrane surface where they provide a possible nutrient for biofilm formation and a hydraulic resistance to permeate flow. It is often accepted that soluble EPS are principally soluble polysaccharides, proteins and soluble humic substances. So far, fouling of each component is still a controversial research topic. Some authors attribute it primarily to fouling proteins [19,25]. A larger number of recent publications indicate that soluble polysaccharide is one of the main parameters affecting MBR fouling [22–24,26–31].

Although much research is currently directed toward the study of micropollutant (i.e. cytostatic drug) removal mechanisms [4,5,32–34], little attention is being paid to the effect of micropollutants on the performance of treatment plants. The application of MBR technology in wastewater treatment for the removal of cyclophosphamide (CP) has been studied previously by Delgado et al. [35]. Cyclophosphamide and 4-ketocyclophosphamide (a CP's metabolite) removals of 80% were achieved under a hydraulic retention time of 48 h, a solid retention time of 50 days and a mixed liquor suspended solids concentration of 8.89 g/L. Thus CP concentration in MBR effluent was about 1 μg/L. Both adsorption and degradation affect the overall removal. COD and total nitrogen removal efficiency were not altered by anticancer drug toxicity. Removal rates observed for COD and TN were above 90% and 93% respectively. Nonetheless, the presence of CPs induced a modification in the biological suspended solids. The modifications in the biomass and in the bulk solution proved to be reflected in the membrane performance. The aim of this paper is to evaluate the influence cyclophosphamide and its principal metabolites (all called CPs in the following) on the physicochemical properties of the mixed liquor and the consequence for membrane fouling in a cross-flow membrane bioreactor (MBR). Two laboratory-scale membrane bioreactors (MBR) were run in parallel, one with the cytostatic drugs (MBR-CPs), and one without (MBR-control). A comparison between the two reactors was made for soluble EPS concentration, specific cake resistance of the mixed liquor, Membrane Fouling Index (MFI) of the supernatant and particle size distribution. This comparison was intended to check whether the addition of cyclophosphamide and its principal metabolites could affect the physicochemical properties of the mixed liquor and its fouling potential.

2. Materials and methods

2.1. Reactors and operating conditions

The reactor consisted of a membrane bioreactor with a working volume of 20 L and a membrane module in an external circulation loop. The membrane module was a ceramic tubular Membralox® (MF) membrane with 0.0055 m^2 of surface area and pore size of 0.1 μm (Pall Exekia, France). A Ruston turbine (260 rpm) was installed to keep the bioreactor completely mixed.

Two identical lab-scale cross-flow MBRs were run in parallel. Each reactor was inoculated with the same activated sludge from a municipal wastewater treatment plant (dry weight, 3 g/L). Raw water was composed of domestic water (average flux 9.75 L/day, from the same wastewater treatment plant in Brax, France, 2000 person-equivalent) pre-screened to 200 μm and complemented with Viandox® (average flux 0.25 L/day, commercial product, soya bean extract) so as to reach the chemical oxygen demand (COD) required to achieve the high volumetric loading rate of 1.1 kg COD m^{-3} day^{-1} (average inlet COD, 2300 mg/L; average inlet TN soluble, 175 mg/L). One of the MBRs was used as a control (MBR2-control), while cyclophosphamide (5 μg/L) and its principal metabolites (acrolein 2.25 μg/L, phosphoramide mustard 8.88 μg/L, 4-ketocyclophosphamide 0.58 μg/L, and nitrogen mustard 0.517 μg/L) were continuously added to the other (MBR1-CPs).

Chemicals were supplied by NIOMECH, part of IIT GmbH (University of Bielefeld, Universitäts str. 25, DE-33615 Bielefeld): D-18845—4-keto-cyclophosphamide; D-18846—phosphoramide mustard; D-19990—nitrogen mustard hydrochloride, and by SIGMA (St Quentin Fallavier, France): 01680 Acrolein; C0768 cyclophosphamide.

The hydraulic retention time (HRT) was 48 h, temperature was 25–32 °C and pH was 7–8. The sludge retention time (SRT) was around 50 days, which led to a low food to micro-organisms (F/M) ratio. The F/M in MBR1-CP was 0.14 (kg COD/kg MLSS day) and 0.11 in the MBR2-control at steady-state. The resulting biomass concentrations were 9 in MBR1-CP and 11 in MBR2-control. Treatment was operated in aerobic/anoxic conditions to allow nitrification and denitrification of the influent. Dissolved oxygen levels were maintained between 0 and 4.5 mg O_2/L. The aeration cycle was 2 min aeration/23 min without aeration. Pressures were measured at the inlet (P1), outlet (P2), and permeate side of the membrane (P3) in order to determine the transmembrane pressure (TMP). At constant permeate flux, TMP indicates the extent of membrane fouling and it was calculated as follows:

$$\mathrm{TMP} = \left(\frac{\mathrm{P1} + \mathrm{P2}}{2} - \mathrm{P3}\right) \quad (1)$$

2.2. Analytical methods

Mixed liquor suspended solids (MLSS) were measured according to standard methods (APHA, 2005). Chemical oxygen demand (COD) and total nitrogen (TN) were measured by spectrometric methods with reagent kits (HACH). Particle size distributions of the activated sludge were measured by the light scattering method (Malvern MasterSizer/E, UK). The detection limit was between 0.2 and 2000 μm. The transmembrane pressure, which indicates the extent of membrane fouling, was monitored regularly.

2.2.1. EPS analysis

2.2.1.1. Sampling and sample preparation. Two kinds of liquid samples were analyzed: (i) the supernatant of the mixed liquor, dissolved fraction and (ii) the permeate. The suspension was centrifuged (4200 × *g*, 20 min) to separate the microbial cells from the supernatant and the supernatant was then filtered through a 0.45 μm pore size membrane to determine the dissolved fraction.

2.2.1.2. Analysis of total protein, humic substances and polysaccharides. The chemical composition of soluble extracellular polymeric substances (EPS) was analyzed for proteins, humic substances and carbohydrates. Proteins and humic substances were measured by the modified Lowry method [36] with bovine serum albumin (BSA,

SIGMA A7906) and humic acid (Fluka 53680) as the standard for calibration from 20 to 200 mg/L. Carbohydrates were determined according to the modified anthrone method described by Raunkjer et al. [37] with D-glucose (Prolabo) as the standard for calibration from 10 to 100 mg/L. All samples were measured in duplicate.

The concentrations of EPS in the inlet water were respectively 527 ± 100 mg/L for polysaccharides, 186 ± 72 mg/L for proteins and 417 ± 157 mg/L for humic acids.

2.2.2. Resistance analysis

The specific cake resistance of the mixed liquor and the membrane fouling index (MFI) of the supernatant were estimated by dead-end filtration in a Sartorius filtration module (diameter, 47 mm) with a flat-sheet, cellulose acetate, 0.2 μm membrane under constant trans-membrane pressure of 0.5 bar. The set-up consisted of a filtration cell, a compressed air cylinder, an electronic balance, a personal computer for data logging and their accessories. The acrylic filtration cell had a volume of 60 mL and effective filtration area 0.17 cm^2. No stirring was imposed in any of the filtration experiments. Prior to each of the filtration runs, the membrane was "wetted" by filtering Milli-Q water through the membrane in its unfouled state at the applied pressure desired for the experiment.

The specific cake resistance was calculated to characterize the filterability of the mixed liquor of MBRs. For a given pressure ΔP, the specific cake resistance α, can be calculated using Eq. (2) [38,39].

$$\frac{t}{v} = \frac{\mu}{\Delta P \cdot \Omega}\left(\frac{\alpha C}{2} \cdot \frac{V}{\Omega} + R_{\text{ini}}\right) \quad (2)$$

where μ is the viscosity of the filtered solution (Pa s), C is the bulk concentration of particles (kg/m^3), Ω is the membrane surface area (m^2), V is the cumulative filtrate volume (m^3), ΔP is transmembrane pressure (Pa); R_{ini} is the membrane initial resistance (m^{-2}) and t is the time (s). Specific cake resistance is the cake resistance normalized by the mass of materials deposited per unit of membrane surface area and it is a unique property of particles consistent with their size and conformation [40].

The MFI of the supernatant was calculated from the filtration curves of the supernatant filtration. The raw experimental data (V and t) were plotted as t/V versus V to obtain the slope (s/L^2) representative of the MFI. The MFI is defined as the gradient of the linear region found in the plot of the well-known cake filtration equation at constant pressure [41].

2.3. Biological activity measurement

Respirometry analyses were conducted periodically for each of the bioreactors during the experimental campaign. In order to determine microbial activity when adapting to CPs, endogenous respiration rates were evaluated.

The endogenous respiration rate is defined as the oxygen consumption rate in the absence of exogenous substrate and includes consumption for the bacterial growth–decay cycle, maintenance energy production (such as the maintenance of required redox potential, cell motility) and protozoa respiration [42].

MBR sludge samples were placed in the respirometer immediately after sampling. The respirometer consisted of a bioreactor with a working volume of 1.5 L, with controlled aeration, agitation and temperature.

Prior to the experiment, the investigated sludge was aerated for 3 h so that all readily degradable substances were consumed. As the residual substrate was exhausted, the sludge reached a physiological state known as endogenous respiration. A solution of allylthiourea (ATU) at 10 g/L was added to the activated sludge as a selective inhibitor of nitrification. Thus, the heterotrophic endogenous respiration rate could be measured. ATU is a selective inhibitor of nitrosobacteria (Nitrosomonas), bacteria that convert ammonium to nitrite. Mixed liquor suspended solids (MLSS) were measured in [g/L] before the beginning of each experiment, i.e. after the permanent aeration of 3 h.

Then the reactor was aerated through a perforated tube placed below the Rushton turbine (300 rpm). The air flow was controlled to maintain a dissolved oxygen concentration between 2.7 and 4.2 mg/L. The temperature was controlled at 26 °C. The oxygen concentration was measured continuously by a probe (YSI 5739) connected to an oxymeter YSI MODEL 57. The oxygen concentration was continuously recorded. This device allowed the dissolved oxygen concentration to be used for continuous observation of the activity of the biological suspension.

3. Results

The experiments were performed for 160 days. The three major changes were: day 21, the first day of addition of CPs into MBR1-CP; day 65, an increase of cross-flow velocity from 4 to 5 m/s was applied to both reactors; day 114, membranes were replaced by two new membranes with similar initial permeability. The concentrations of EPS in the inlet water were respectively 527 ± 100 mg/l for polysaccharides, 186 ± 72 mg/l for proteins, 417 ± 157 mg/l for humic acids. It could be observed that concentration were almost identical in the influents of both MBRs since the influents were the same.

3.1. Influence of CPs on the concentration and nature of exopolymers in the supernatant of the membrane bioreactor

3.1.1. Protein

Protein concentrations were very low and below 50 mg/L. At this order of magnitude, the error of the method was rather high (more than 25% for protein concentration < 30 mg/L). Thus, making comparisons between the performance of the two reactors in relation to protein concentration became very inaccurate.

3.1.2. Humic substances and polysaccharides

Fig. 1(a) and (b) shows the variation in the concentrations of humic substances and polysaccharides, respectively. The differences in polysaccharide and humic substance supernatant concentrations between MBRs were significant after the increase of cross-flow velocity from 4 to 5 m/s. Supernatant concentrations of humic substances and polysaccharides were higher in MBR-CPs than in MBR-control. This difference was maintained throughout the experiment, including after the membrane replacement (day 114).

Humic substances are directly brought in by the influent. These compounds, considered as soluble EPS in this case, are not produced by micro-organisms. Therefore their concentration in the supernatant depends on their adsorption onto microbial flocs, their removal by sludge withdrawal and their passage through the membrane. Since MBRs were fed in the same way and operated in identical running conditions, the differences in the humic substance supernatant concentrations would appear to be influenced by membrane retention. Fig. 2(a) shows the retention of humic substances by the membrane. As shown in Fig. 2(a), after 65 days, the membrane retention of humic substances in MBR-CPs was higher than in MBR-control. It can thus be supposed that the higher concentration in humic substances in MBR-CPs compared to MBR-control was linked to the membrane + gel layer retention of these compounds being higher in MBR-CPs than MBR-control.

Regarding polysaccharide concentration in the supernatant, in addition to differences in the retention by the membrane + deposit, the concentration also depends on the production and/or assimilation of these compounds by micro-organisms. Fig. 2(b) shows the retention of polysaccharides by the membrane during the

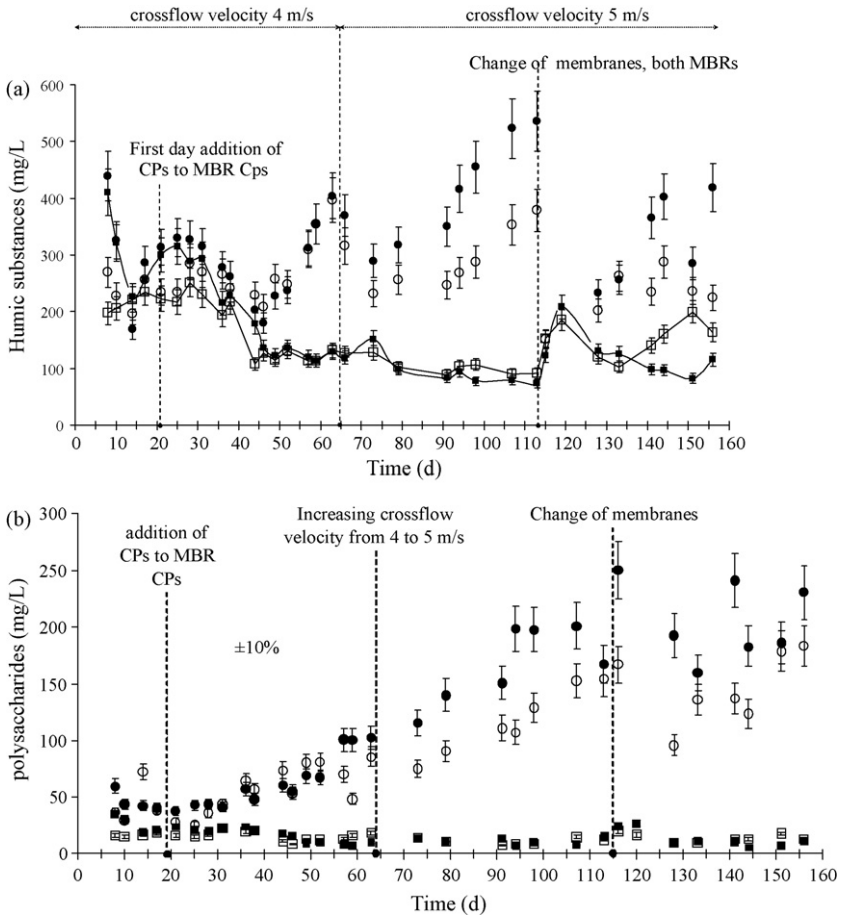

Fig. 1. EPS concentration variation in (a) humic substances and (b) polysaccharides. (●) Supernatant MBR-CPs; (○) supernatant MBR-control; (■) permeate MBR-CPs (□) permeate MBR-control.

experimental campaign. As shown in Fig. 2(b), after the increase of cross-flow velocity, the retention of polysaccharides by the membrane was very similar between the MBRs and was always greater than 90%. Therefore, differences between MBRs in the concentrations found for polysaccharides in the supernatant were rather associated with microbial activity (production) and/or to differences in the kinetics of degradation of these polymers by micro-organisms. A detailed study of the characterization of extracellular polymeric substance formed on cytostatic drug presence is the subject of a separate paper [43]. The results of this study show that the presence of CPs stimulates the survival mechanisms and production of EPS (a phenomenon observed in the bound EPS and soluble EPS) with a slightly higher production of polysaccharides than proteins. Molecules excreted due the presence of drugs are retained by the membrane (18,000 Da for proteins and 6000 Da for polysaccharides). It can thus be supposed that differences in the polysaccharide concentrations found in the supernatant between MBRs were rather associated with microbial activity (survival mechanisms and production as microbial responses to the presence of CPs in MBR-CPs).

3.2. Influence of CPs on the endogenous respiration rate

In order to confirm microbial activity when adapting to CPs, endogenous respiration rates were evaluated. Comparison of results for the reactor MBR-CPs with those of MBR-control indicated the influence of CPs on the biological activity. To facilitate comparisons between bioreactors, the responses corresponding to heterotrophic micro-organisms (the most numerous bacterial population) were expressed as specific values, i.e. normalized by MLSS concentration. Fig. 3 shows the variation of the ratio [heterotrophic specific endogenous respiration MBR-CPs/MBR-control]. After the addition of CPs, we observed an increase in the endogenous respiration of heterotrophic micro-organisms in bioreactor MBR-CPs compared to MBR-control, from day 37 to day 80. This suggested that, during this time, microbial activity when adapting to CPs were particularly higher than at other times, so specific analyses were performed on the samples corresponding to these days.

3.3. Influence of CPs on the floc size distribution

Fig. 4(a) shows the mean floc size variation for both MBRs. An increase in mean floc size was observed in MBR-CPs from day 45 (15 μm higher than MBR-control) to day 66. This difference became significantly smaller after the increase of cross-flow velocity. It could be assumed that the high mechanical stress imposed on microbial flocs led to considerable deflocculation, thus the average diameter of day 81, which was the same for both bioreactors, could be the consequence of the increase of the recirculation pump shear. Fig. 4(b) shows the particular size distributions of both MBRs corresponding to day 21 (before addition of CPs to MBR-CPs), and day 81 (after addition of CPs and after increasing cross-flow velocity). Particle size distributions of MBRs mixed liquor changed after the increase of cross-flow velocity. For both MBRs, the mean diameter of flocs was reduced and the quantity of small particles was increased. However, the fraction of smaller particles (0.6–3 μm) in MBR-CPs was relatively larger than in MBR-control on day 81.

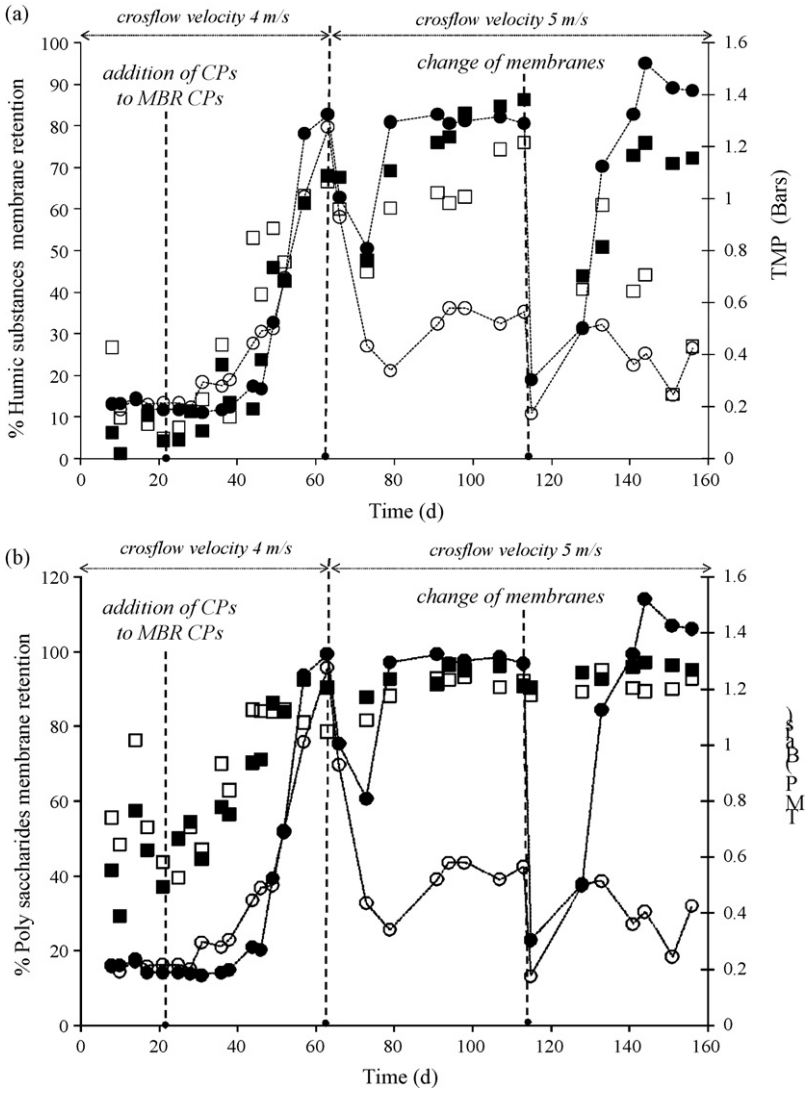

Fig. 2. (a) Humic substance and (b) polysaccharide membrane retention in (■) MBR-CPs, (□) MBR-control and transmembrane pressure variation in (●) MBR-CPs and (○) MBR-control. % EPS retention = (1 − (EPS$_{permeate}$ concentration/EPS$_{supernatant}$ concentration)) × 100.

3.4. Influence of CPs on the fouling potential of the activated sludge in the bulk phase

To evaluate the fouling potential of mixed liquor and supernatant, the specific cake resistance of the mixed liquor and the membrane fouling index (MFI) of the supernatant were estimated by dead-end filtration. Fig. 5(a) shows the specific cake resistance (α) of the mixed liquor and Fig. 5(b) the MFI of the supernatant, for both MBRs. As shown in Fig. 5(a) and (b), both mixed liquor and supernatant (consisting of soluble and colloidal substances) of MBR-CPs had a fouling potential higher than that of MBR-control.

3.4.1. Relationship between fouling potential and soluble EPS concentration

Fig. 6(a) shows the correlation between MFI values and concentration of soluble exopolymers for both bioreactors. According to these figures, MFI supernatant values were more closely related to polysaccharide concentration, as this was the only parameter for which a linear correlation could be found. In addition, this link was clearer in MBR-CPs than in MBR-control.

Fig. 6(b) shows the correlation between the supernatant polysaccharide concentration and α values during the first 80 days of operation. The linear correlation between these two parameters

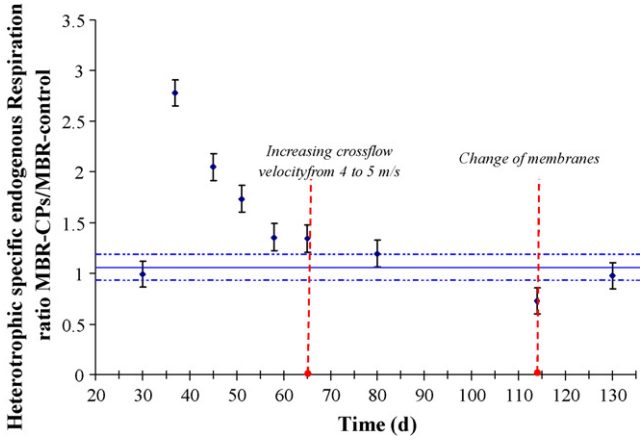

Fig. 3. Heterotrophic specific endogenous respiration ratio: MBR-CPs/MBR-control.

(α, polysaccharide concentration) was more obvious in MBR-CPs (R^2 = 0.8681) than MBR-control (R^2 = 0.6464).

3.5. Transmembrane pressure

Membrane performance was tested by measuring transmembrane pressure. Fig. 7(a) shows the variation of transmembrane pressure for both bioreactors.

TMP showed the same behaviour in both bioreactors until day 65, even after the addition of Cps. The increase in TMP from day 45 to day 65 was similar for both reactors, indicating that membrane fouling was rather governed by operating conditions. To reduce membrane fouling, on day 65, the cross-flow velocity was increased from 4 to 5 m/s. In MBR2-control, this increase resulted in a reduction of membrane fouling (the pressure stabilized around 0.60 bar). In MBR1-CPs, TMP decreased from day 66 to day 75, and then TMP returned to the value (1.3 bar) it had before the increase in cross-flow velocity. On day 114, we changed the membranes of both MBRs for two new membranes with the same initial permeability. This was done not only to reduce the transmembrane pressure but also to determine whether the increase in TMP in MBR1-CPs was related to irreversible membrane fouling or to the physicochemical properties of the mixed liquor. After this change, the TMP of MBR2-control increased, reaching the same value as before the change of membrane (0.6 bar) and then TMP decreased to 0.4 bar. Regarding MBR1-CPs, TMP increased significantly up to 1.5 bar (higher than

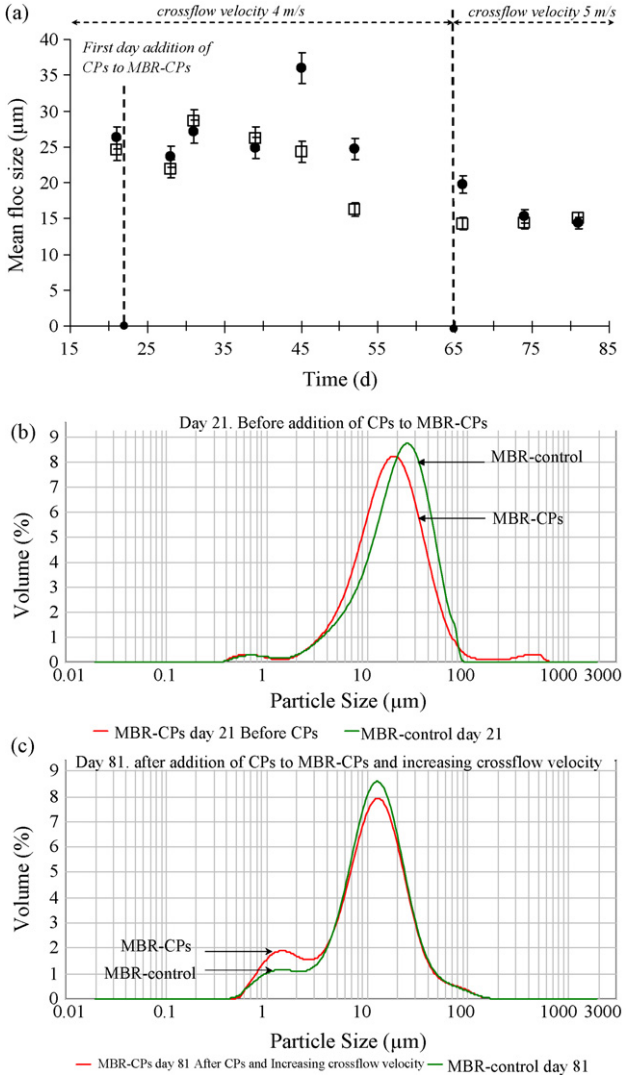

Fig. 4. (a) Variation of mean floc sizes. (●) MBR-CPs, (□) MBR-control. (b) Particle size distribution day 21, before the addition of CPs to MBR-CPs. (c) Particle size distribution day 81, after addition of CPs and after increasing cross-flow velocity to 5 m/s.

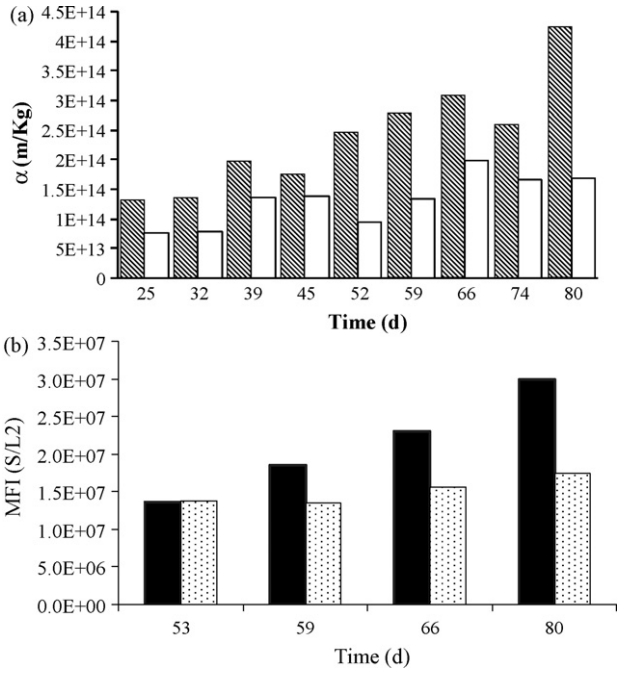

Fig. 5. (a) MBRs Specific cake resistances. Samples corresponding to 25–80 days' operating time. (b) Supernatant MFI variation. (■) MBR-CPS and (□) MBR-control.

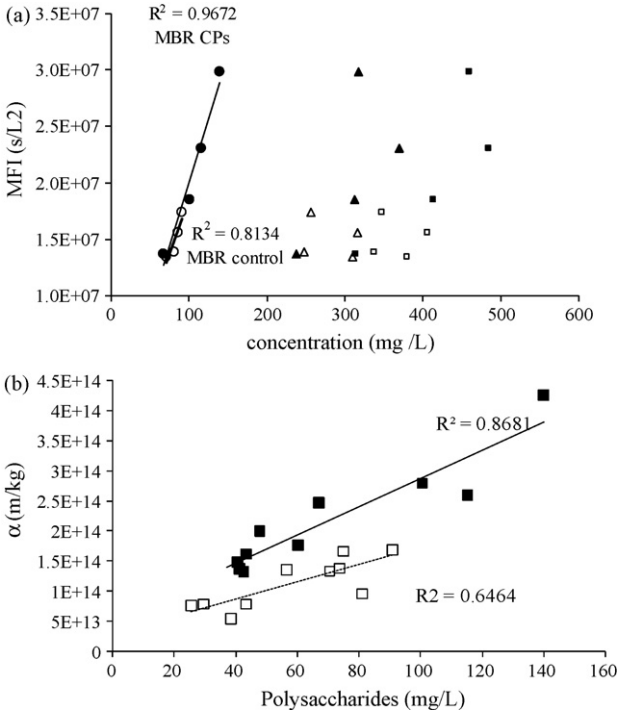

Fig. 6. (a) Correlation between supernatant MFI values and EPS soluble supernatant concentration. (●) Polysaccharides MBR-CPs, (○) polysaccharides MBR-control, (▲) humic substances MBR-CPs, (△) humic substances MBR-control, (■) soluble EPS (polysaccharides + proteins + humic substances) MBR-CPs, (□) soluble EPS (polysaccharides + proteins + humic substances) MBR-control. (b) Correlation between specific cake resistance and polysaccharide concentration in supernatant in (■) MBR-CPs and (□) MBR-control.

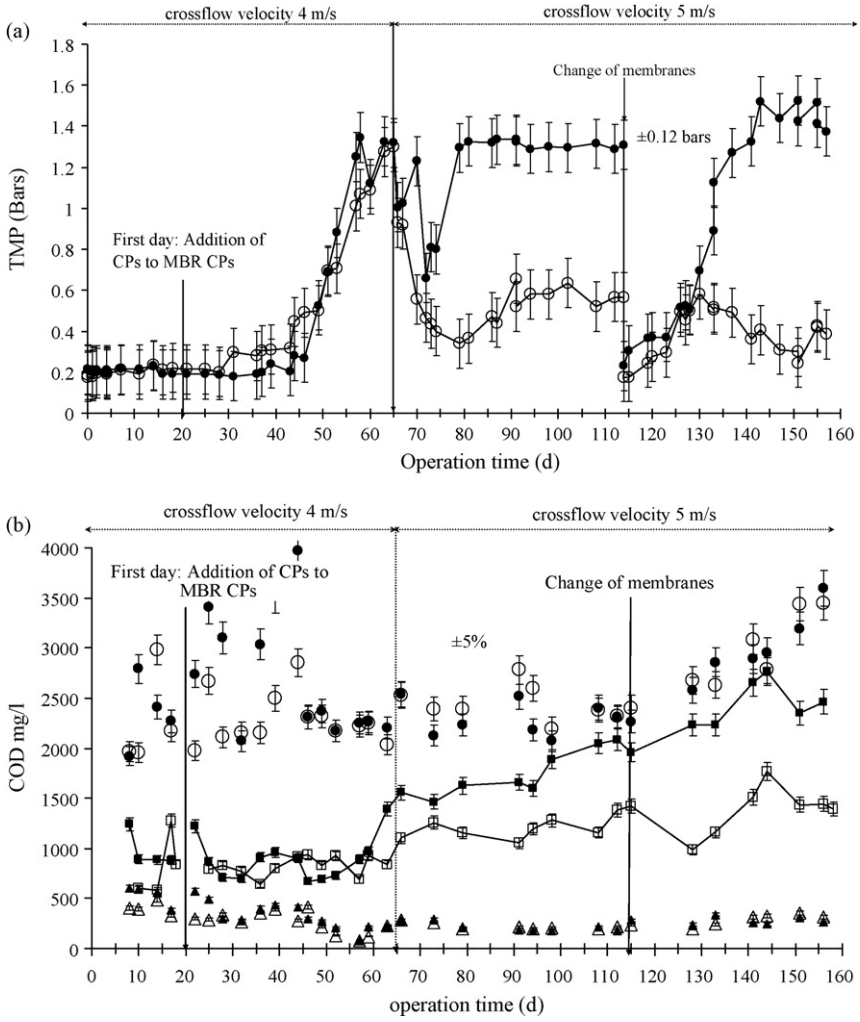

Fig. 7. (a) Transmembrane pressure variation (●) MBR-CPs, (○) MBR-control. (b) COD concentration in MBR-influent (●) MBR-CPs, (○) MBR-control, MBR-supernatant, (■) MB-Cps, (□) MBR-control and MBR-permeate, (▲) MBR-CPs, (△) MBR-control.

before the change, 1.3 bar). The change of membrane (day 114) did not change anything in the TMP variation. Thus, the increase in TMP in MBR1-CPs was rather related to the physicochemical properties of the mixed liquor.

4. Discussion

The influence of hydrodynamic conditions in a cross-flow membrane bioreactor on the mixed liquor and the membrane fouling has been studied previously. It was demonstrated that an increase in the speed of recirculation of the activated sludge in a cross-flow membrane bioreactor induced a decrease in the floc size distribution, leading to different intensities of fouling. The studies report that the presence of small particles and of various polymers, resulting from the floc breakage, could explain the fouling nature of the MBR suspension [44,45]. In our study, these phenomena were observed after cross-flow velocity was increased from 4 to 5 m/s. However, while the shear stresses imposed were similar in both bioreactors, the magnitude of these phenomena and the impact on the membrane fouling was not the same. The membrane fouling was greater in the membrane bioreactor in the presence of Cps (MBR-CPs) than in absence of CPs (MBR-control). This result shows that the response of activated sludge to imposed mechanical shear differed markedly according to the presence or absence of CPs.

On the other hand, it is often accepted that cells produce EPS for their survival and in response to environmental stress [46]. Henriques and Love [47] found that the EPS matrix inside sludge flocs acted as a protective barrier for bacteria exposed to the chemical toxins octanol and cadmium. In a previously studies (same MBR reactor, under a hydraulic retention time of 32 h, a solid retention time of 70 days) it was observed an increase in the mean floc size in the bioreactor MBR-CPs compared to MBR-control after the addition of CPs during more than 70 days [48]. Moreover, in our study, it was observed that the presence of pharmaceutical compounds (cyclophosphamide and metabolites) stimulated mechanisms of survival (endogenous respiration rate). It can thus be supposed that micro-organisms produce bound EPS as a protective barrier to improve their survival in presence of Cps, promoting the agglomeration of flocs and, in doing so, increasing the average floc size as seen in Fig. 4 (days 45 and 52) and as previously observed [48]. Thus, after the cross-flow velocity increases, more EPS and colloidal particles were released from the EPS floc-matrix into the bulk liquid in MBR-CPs than MBR-control. Then, the soluble EPS concentration was greater in the MBR-CP than in the MBR-control.

It could be also observed that the fouling potential of the mixed liquor (i.e. specific cake resistance) and of the supernatant (i.e. MFI) seem to be linked more closely to polysaccharides than other EPS, according to the other studies [23,24,29,49,50]. The fouling potential of polysaccharides seems to be related to their tendency to form a gel layer on the surface of the membrane filter [49,50]. Therefore, these compounds in the liquid phase are

critical in the sense of potential accumulation in the concentration boundary layer and consequently the formation of fouling layers [24].

On the other hand, the significance of soluble microbial products (SMP) retention for MBR operation in terms of membrane fouling has been studied by Wang and Waite [51]. Membrane fouling was closely related with SMP retention as a result of the formation of a gel layer. The framework of the layer was mainly formed from polysaccharides, which controlled its permeability. The SMP proteins appeared to be trapped in the gel layer by steric and/or adsorptive effects but had little structural importance. In addition, the gel layer was usually highly porous and compressible, which led to an accelerated TMP increase during filtration. The authors suggested that the elevation in TMP required to maintain constant flux during filtration (as in our study), caused by membrane fouling, would progressively compress the gel layer formed, resulting in a lowered channel size for water passage. With the decrease of the channel size, it would be expected that more and more colloidal particles, including proteins, may be unable to pass through the gel layer because of size exclusion. In accordance to this study, it could be assumed that humic substances (in our study) were adsorbed and/or retained by the gel layer of polysaccharides. This would explain the increase observed in the retention of humic substances by the membrane when the TMP increased, as shown in Fig. 2(a) in MBR-CPs.

Hence, according to the literature and our results, it can be suggested that the quality of sludge (floc size) and the overproduction of polysaccharides (induced by the presence of toxic CPs) governed the fouling phenomena in the MBR-CPs bioreactor. Thus, membrane fouling was greater in MBR-CPs than in MBR-control. Nevertheless, membrane filtration plays a crucial role for MBR1-CP performance (i.e. COD removal) by retention of soluble EPS and colloidal particles. Fig. 7(b) illustrates the COD concentrations in the influent, supernatant and permeates of both MBRs. After the increase in the cross-flow velocity, the supernatant COD content in MBR1-CP was higher than that in MBR2-control. However, the permeate COD concentrations were almost the same for both reactors. Considering the COD concentration in the supernatant (greater in MBR1-CP than in MBR2-control) highlights the role of the membrane in the permeate quality (high retention of COD supernatant).

5. Conclusion

This work has studied the influence of Cps on the physicochemical properties of the mixed liquor and the fouling properties of the activated sludge. The analyses performed on the supernatant and activated sludge bioreactors allow us to draw the following conclusions:

- Despite the low CPs concentration studied, the toxicity of the cocktail of pharmaceutical compounds (CPs) on activated sludge altered the characteristics of the biological matrix. The presence of CPs stimulated the mechanisms of survival (higher endogenous respiration rate in MBR-CPs than MBR-control) and production of EPS. Fouling potential seems to be linked more closely to polysaccharides than other EPS. The accumulation of EPS (polysaccharide production) and the formation of small particles after the raising of the cross-flow velocity increased the resistance to filtration of mixed liquor in MBR-CPs. Thus, under the operating conditions studied, membrane fouling was faster in MBR-CPS than in MBR-control.
- Finally, this study highlights the robustness of membrane bioreactors in the treatment of wastewater containing cytostatic compounds which, despite their low concentrations, modify the biological suspension behaviour. In spite of this, the membrane plays an important role in the permeate quality and the overall performance of the process.

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