Open Archive Toulouse Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

Any correspondence concerning this service should be sent to the repository administrator: staff-oatao@inp-toulouse.fr

Effect of cytostatic drug presence on extracellular polymeric substances formation in municipal wastewater treated by membrane bioreactor

A.C. Avella [a,*], L.F. Delgado [b], T. Görner [a], C. Albasi [b], M. Galmiche [a], Ph. de Donato [a]

[a] *Laboratoire Environnement et Minéralurgie, Nancy-Université, CNRS, 15 avenue du Charmois, 54500 Vandoeuvre les Nancy, France*
[b] *Laboratoire de Génie Chimique, UMR CNRS 5503, 5 rue de Paulin Talabot, 31106 Toulouse, France*

ARTICLE INFO

ABSTRACT

The effect of the cyclophosphamide and its mean metabolites on extracellular polymeric substances (EPS) formation was investigated. Two lab-scale membrane bioreactors were followed in parallel (one with the cytostatic drugs, the second without). Chromatographic and spectroscopic studies (UV–Vis spectroscopy and IR spectroscopy) showed that the presence of CPs induced an increase in EPS concentration in the biological sludge, especially of soluble substances, mainly polysaccharides and proteins. Size exclusion chromatography analysis revealed that in the presence of CPs, macromolecular EPS were formed (polysaccharides corresponding to about 6 KDa and proteins to about 18 KDa). The formation of EPS seemed to be a protection mechanism. More important membrane fouling in reactor with CPs seemed to be related to the retention of an increased amount of soluble substances.

1. Introduction

Pharmaceuticals and other micropollutants in wastewater pose a new challenge to wastewater professionals and to the pharmaceutical industry. Pharmaceuticals are designed to exhibit biological activity in humans and may have negative effects on aquatic organisms. Compounds with a very potent mechanism of action such as cytostatic drugs, present a particular environmental concern though consumption rates and expected concentrations in the environment may be low (e.g. Kümmerer, 2001).

The alkylating antineoplastic drug cyclophosphamide (CP) is one of the oldest known cytostatics and is one of the most frequently used in cancer chemotherapy (Kümmerer, 2001). 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 presents teratogenic and mutagenic properties and is a known human carcinogen (Steger-Hartmann et al., 1997). It is transformed via hepatic and intracellular enzymes to active alkylating metabolites, 4-hydroxycyclophophosphamide, aldophosphamide, acrolein and phosphoramide mustard (Joqueviel et al., 1998).

The increasing use of anticancer drugs and their presence in wastewater is a relatively new issue and only few studies have been reported (Kümmerer, 2001; Lenz et al., 2007; Steger-Hartmann et al., 1997). When the micropollutant-contaminated wastewaters reach biological treatment systems, they may negatively affect the performance of the treatment plant, due to the possible inhibition of biological activity. Although much research is being directed towards the study of the removal mechanisms of micropollutants (Urase and Kikuta, 2005), little attention is being given to the effect of micropollutants on the plant performance. The biological degradation in municipal wastewater treatment contributes only to a limited extent to the overall load reduction of different pharmaceutical compounds (Joss et al., 2006).

Some studies have indicated a significantly better removal of persistent pharmaceuticals by membrane bioreactor (MBR) technology (Bernhard et al., 2006; Weiss and Reemtsma, 2008). Nevertheless, the main problem in membrane application is a rapid decline of permeation flux due to the membrane fouling which requires a frequent membrane cleaning/replacement thus increasing the operation costs (Judd, 2008). Considerable investigations have been conducted over the last years in order to better understand the complex fouling mechanisms and recent reviews have been published (Le-Clech et al., 2006; Meng et al., 2009). There is a general consensus that the soluble EPS in sludge supernatant contribute significantly to the total specific cake resistance (Nuengjamnong et al., 2005; Rosenberger et al., 2006). Many studies of membrane foulant species have found different and often conflicting trends in fouling, so a comprehensive investigation of foulants remains a topic of interest in research and the application of MBRs (Judd, 2008; Meng et al., 2009). Two types of studies dominate the MBR research referring to: (i) the phenomenon of foulant demonstrates *in situ* (i.e. decrease in permeability) and (ii) physical and/or chemical characterization of the foulant species. This study refers to the second category: it was focused on EPS investigation for a better understanding of biomass behaviour in the presence of anticancer drug.

* Corresponding author. Tel.: +33 383596266; fax: +33 383596285.
  E-mail address: catalina.avella@ensg.inpl-nancy.fr (A.C. Avella).

The EPS play an important role in several stages of the activated sludge wastewater treatment process: from sludge flocculation and settling to sludge dewatering. Their role in the sludge was often studied, but not yet clearly established and studies are sometimes controversial (review of Liu and Fang, 2003). The EPS are issued from microbial metabolism and from cell lysis during the biological wastewater treatment; they are a main sludge component keeping the microbial aggregates together in a three dimensional matrix which allows micro-organisms to live at high cell densities in stable mixed population communities. Moreover, the cells produce EPS for their survival and in response to environmental stress (Wingender et al., 1999). The EPS form a complex polymeric network with a large surface area adsorbing pollutants, nutrients and minerals (Finlayson et al., 1998). They are involved in the adhesion of biofilm onto solid surfaces by electrostatic or by polymeric interactions (Tsuneda et al., 2003). Several propositions were made in recent years to describe the relationship between biomass and the EPS located at or outside the cell surface and their soluble part also called soluble microbial products (SMP). Laspidou and Rittmann, (2002) proposed a unified theory for EPS, SMP and active and inert biomass. In this approach, the soluble EPS are actually SMP, active biomass includes bound EPS and inert biomass comprises some bound EPS and the residual dead cells.

The EPS are composed of up to 75–90% of polysaccharides and proteins and small amounts of lipids, DNA and RNA (Wingender et al., 1999). They can be present as pure or mixed components with various functional groups and their molecular size can range from a few hundred to several hundreds of thousands of Daltons (Garnier et al., 2005).

In this study, we focused on EPS production in presence of cyclophosphamide and its mean metabolites (all called later CPs) treated by MBR. The results presented here were obtained with reactors running under steady-state conditions for 4 weeks, after 132 days of continuous operation. The EPS were examined by UV–Vis and infrared spectroscopy for protein and polysaccharide content. They were more deeply investigated by size exclusion chromatography and infrared spectroscopy to obtain information about macromolecular size distribution and possible chemical changes, respectively. Their size and presence were then related to the functioning of the MBRs and especially to transmembrane pressure (TMP) and EPS retention/permeation.

2. Methods

2.1. Reactors and operating conditions

The schematic diagram of cross-flow MBR is shown in Fig. 1. Two identical lab-scale cross-flow MBR were run in parallel. Each reactor was inoculated with activated sludge from a municipal sewage treatment plant (dry weight, 3 g/L). The raw water used (identical for both reactors) was composed of domestic water (9.75 L/day with an average COD of 550 mg/L) and completed with Viandox® (0.25 L/day) to reach the required chemical oxygen demand (COD) of around 2300 mg/L. Treatment was operated in aerobic/anoxic conditions. Mixed liquor suspended solids (MLSS) were measured according to the analytical methods described in the standard methods 2540 G (APHA et al., 2005).

Operating conditions of MBRs during the experimentation were as following: working volume: 20 L; temperature: 25–32 °C; pH: 7–8; flux: 75 L m^{-2} h^{-1}; inlet COD: 2300 mg/L; organic loading rate: 1.15 kg COD m^{-3} d^{-1}; sludge retention time (SRT): 50 days; hydraulic retention time (HRT): 48 h; MLSS (at steady-state) in R1: 8.89 g/L and in R2: 10.84 g/L; F/M (food/microorganism, at steady-state) in R1: 0.13 kg COD/kg MLSS/d and in R2: 0.11 kg
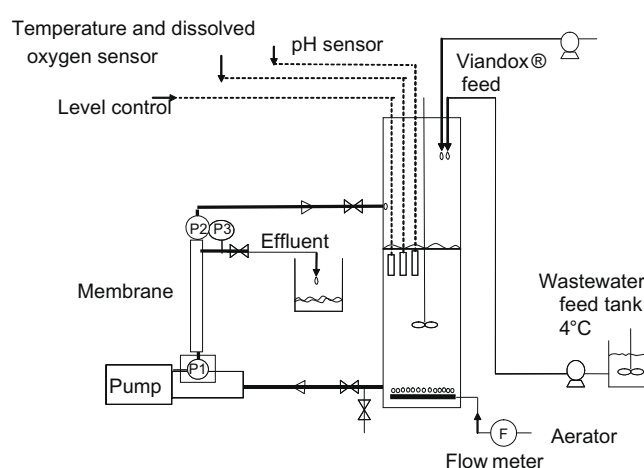

Fig. 1. Schematic diagram of lab-scale MBR. Pressure gauge (P) at the membrane entrance (P1), at the membrane outlet (P2) and at the permeate outlet (P3).

COD/kg MLSS/d; aeration cycle: 2 min aeration/23 min without aeration; dissolved oxygen: 0–4.5 mg/L.

The membrane modules were ceramic tubular Membralox® (MF) with 0.0055 m^2 of surface area and pore size of 0.1 μm (Pall Exekia, France). The transmembrane pressure (TMP) which indicates the extent of membrane fouling was regularly monitored.

The cyclophosphamide (5 μg/L) and its main metabolites (acrolein 2.25 μg/L, phosphoramide mustard 8.88 μg/L, 4-ketocyclophosphamide 0.58 μg/L, nitrogen mustard 0.517 μg/L) were continuously added to reactor R1, whereas R2 served as a control. Chemicals were supplied by NIOMECH, part of IIT GmbH (University of Bielefeld, Universitätsstr. 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.

2.2. EPS analysis

2.2.1. Sampling and samples preparation

Three kinds of samples were analysed: (i) the supernatant of the mixed liquor, (ii) the membrane permeate and (iii) the sludge biomass (settled pellets). The mixed liquor sampled from reactors was centrifuged (20 min at 4200 g) to separate the biomass (settled pellets) from the supernatant. The soluble matter present in the supernatants (S) and in the permeates (P) were directly analysed by methods described later. The settled biomass, before further analysis, was submitted to EPS extraction according to Frolund et al. (1996) with modification: extraction time of 4 h instead of 1 h; these samples will be called B.

2.2.2. Total protein and total polysaccharides analysis

For a total protein (PR) and total polysaccharides (PS) content, the classical UV–Vis methods were applied. Proteins determination was done with the total proteins kit; Micro Lowry® (Lowry et al., 1951) with Peterson's modification (Sigma–Aldrich, France) using bovine serum albumin as a standard for calibration from 20 to 200 mg/L, absorbance was measured at 750 nm. Polysaccharides were determined according to the classical Dubois protocol (Dubois et al., 1956) at 485 nm, calibration was done with glucose in the concentrations from 10 to 100 mg/L. All samples were measured in duplicate.

2.2.3. High pressure size exclusion chromatography

The EPS were analysed by high pressure size exclusion chromatography (HPSEC) to estimate molecular sizes of present chemical species (Garnier et al., 2005). The separation of EPS samples was

carried out on a Hewlett Packard 1100 series chromatograph. A Zorbax Bio series column (GF-250, 25 cm × 9.4 mm, Agilent Technologies, France) thermostated at 25 °C was used. The mobile phase (flow rate 1 mL/min) was 0.2 M NaCl. The detection was carried out at 25 °C with a diode array UV detector at 215 nm. All samples were filtered with 0.2 μm (Chromafil®) filters prior to injection. The column elution volumes V_e were calibrated by standard polysaccharides (180, 738, 3700, 5900, 11 800, 22 800, 47 300, 112 000, 212 000, 404 000 and 788 000 Da; Polymers Laboratories, France) and standard proteins (bovine insulin 5730 Da, ribonuclease A from bovine 13 700 Da, myoglobulin 17 600 Da, chicken albumin 44 300 Da, β-amylase 56 000 Da, bovine serum albumin 66 000 Da, apo-transferrin 78 000 Da, IgG from human serum 160 000 Da, thyroglobulin 670 000 Da; Sigma–Aldrich, France).

2.2.4. Fourier transform infrared spectroscopy (FT–IR)

Spectral analysis was performed on FT–IR spectrometer (Bruker IFS 55 with DTGS detector). All measurements were made in transmission mode in the 4000–600 cm^{-1} range (64 points scans, resolution ± 4 cm^{-1}). Each sample was deposed as droplets 3 × 100 μL in the centre of a ZnSe slide, the material was dried under low vacuum until was formed a solid film. The diameter of the IR spot was 5 mm. The spectra were treated using OPUS software.

3. Results

3.1. Steady-state conditions

The cumulative sludge production, SP$_{cum}$ [kg], for the total reactor operation time was calculated from the Eq. (1) and is shown in Fig. 2a. Sampling days are indicated by arrows.

$$SP_{cum} = \sum SP_i \cdot \Delta t = \frac{\sum_{i=1}^{i=160} Q_{ES} \cdot MLSS_i \cdot (t_i - t_{i-1})}{1000} \quad (1)$$

SPi is the quantity of sludge leaving the bioreactor per time unit [g/d], Q_{ES} is the excess sludge volume withdrawal rate [L/d], MLSS is the mixed liquor suspended solids concentration in the reactor [g/L] and t is the sampling day [d]. In control reactor R2 the MLSS values were always higher than in reactor R1 (with CPs).

The study presented here was focused over the period of the last 4 weeks of the total reactors' operation time in both reactors; R1 (with CPs) and R2 (control). In this period, the reactors have reached a steady-state as shown in Fig. 2a by the constant slopes of the tendency line of both reactors. It corresponds to 50 days of sludge retention time. Thus, it may be assumed that the biomass was completely adapted to the imposed operating conditions. Therefore, possible differences between the two MBR were only due to the presence of CPs.

3.2. Total protein and total polysaccharide analysis

Study of bioreactor liquor supernatants: to comply PR and PS content with MLSS evolution, all PR and PS values were normalized per g of MLSS. The normalized values are presented in Fig. 3. It can be seen that in supernatants, in the presence of CPs (Fig. 3a) the normalized PR and PS concentrations were significantly higher than in control reactor (Fig. 3b) where they remained fairly constant. So it seems that it was CPs presence which induced the increase of soluble PR and PS.

Study of permeates: the permeates analysed for PR and PS content are presented in Fig. 3c and d. Only slight concentrations of these two main EPS components persist in permeates, after membrane filtration.
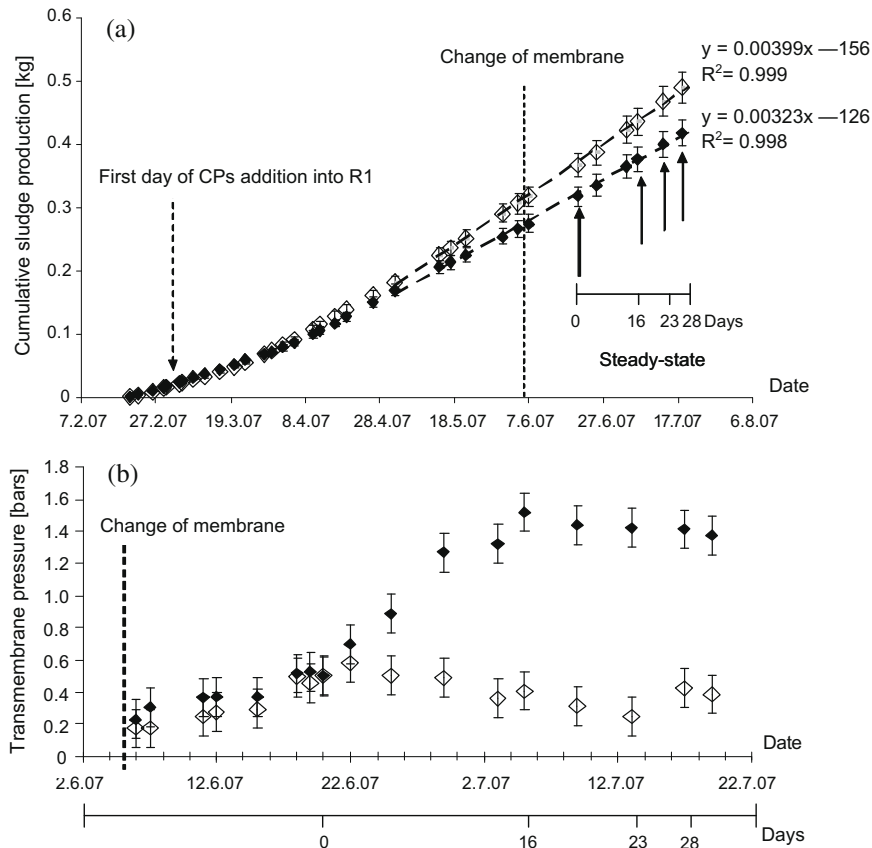
Fig. 2. (a) Evolution of cumulative sludge production against total reactor operation time. (b) Evolution of transmembrane pressure over the steady-state. ♦, R1 (with CPs) and ◊, R2 (control).
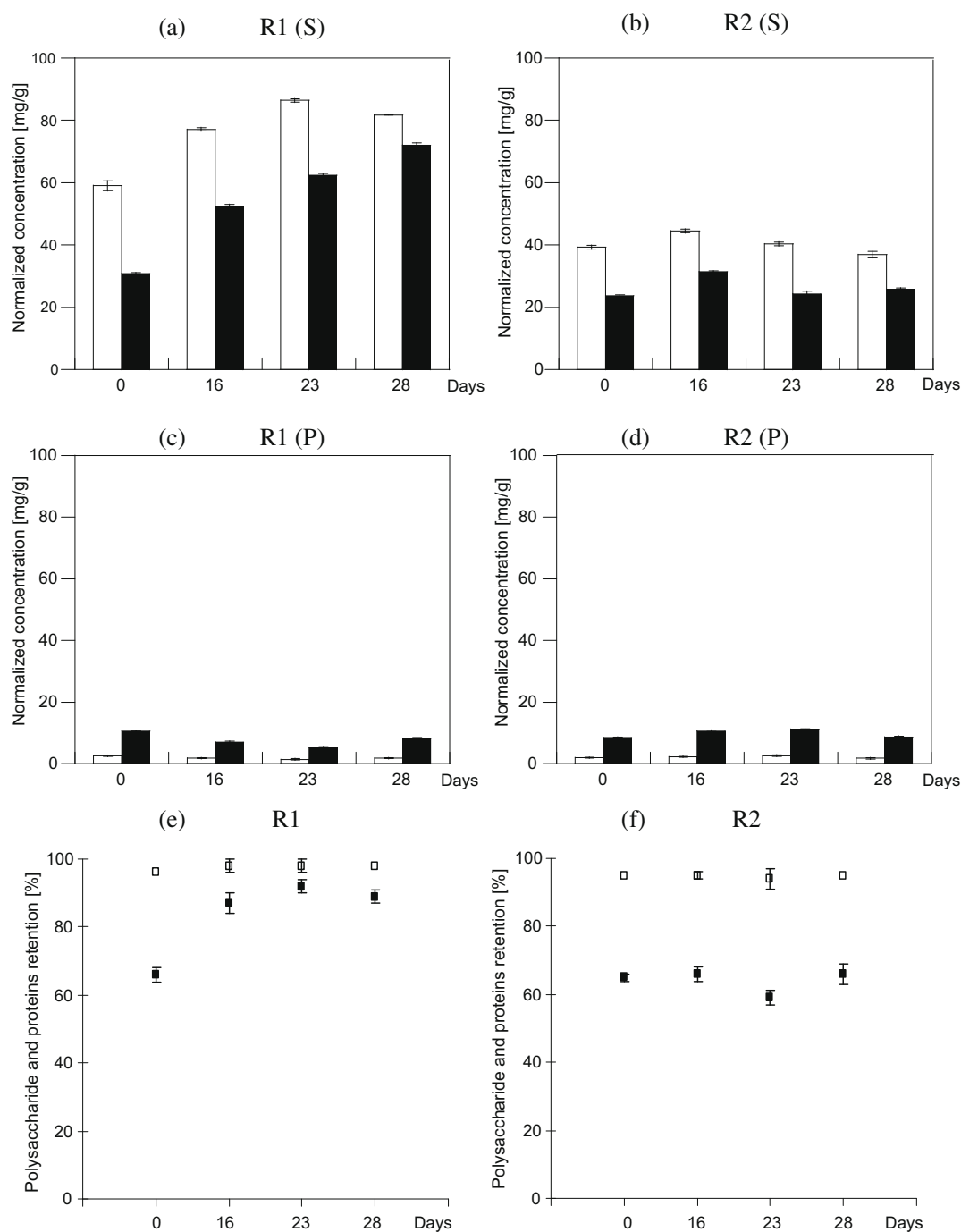
Fig. 3. Evolution over the steady-state running time of: normalized concentrations of polysaccharides (□) and proteins (■) in supernatants (a and b) and in permeates (c and d) in reactors R1 and R2, respectively. Percentage of polysaccharides (□) and of proteins (■) retention by membrane in reactors R1 and R2 (e and f).

3.3. High pressure size exclusion chromatography study

To provide more information about the EPS in both reactors and in all phases the HPSEC analysis was performed. Three types of samples were followed: the supernatants (S), the permeates (P), both directly injected onto the column, and the bound EPS (B) extracted from the sludge before analysis. To observe the influence of CPs on the EPS production by biomass, the chromatograms were normalized per gram of biomass (MLSS values as mentioned above). The area of peaks can be considered in approximation as representative of EPS concentration. This simplified approach (assuming all species having the same detector answer) is unavoid-

able because of the outstanding complexity of EPS which contain various polysaccharides and proteins, each with their own different chain lengths and moreover which are involved in molecule associations (Garnier et al., 2005). The chromatograms (Fig. 4) give following information:

(1) The main effect of the CPs presence on biomass was a significant increase of soluble EPS concentration in supernatants of the R1 reactor (Fig. 4a) while in the control reactor R2 (Fig. 4b), EPS concentration remained constant for the steady-state period. The EPS in supernatants had a wide range of molecular sizes with no predominant size family

(for PR: from 670 KDa to less than 0.9 KDa and for PS: from 404 KDa to less than 0.7 KDa). The addition of CPs in R1 induced an increased concentration of macromolecular compounds with 8 mL < V_e < 13 mL (peak maximum corresponding to PR of about 18 KDa and PS of about 6 KDa). The EPS concentration increased also in the sludge (bound fraction, Fig. 4e) with CPs presence but to a significantly smaller degree than in supernatants.

(2) All chromatograms of supernatants and bound EPS in both reactors (Fig. 4a, b, e and f) contained high macromolecular
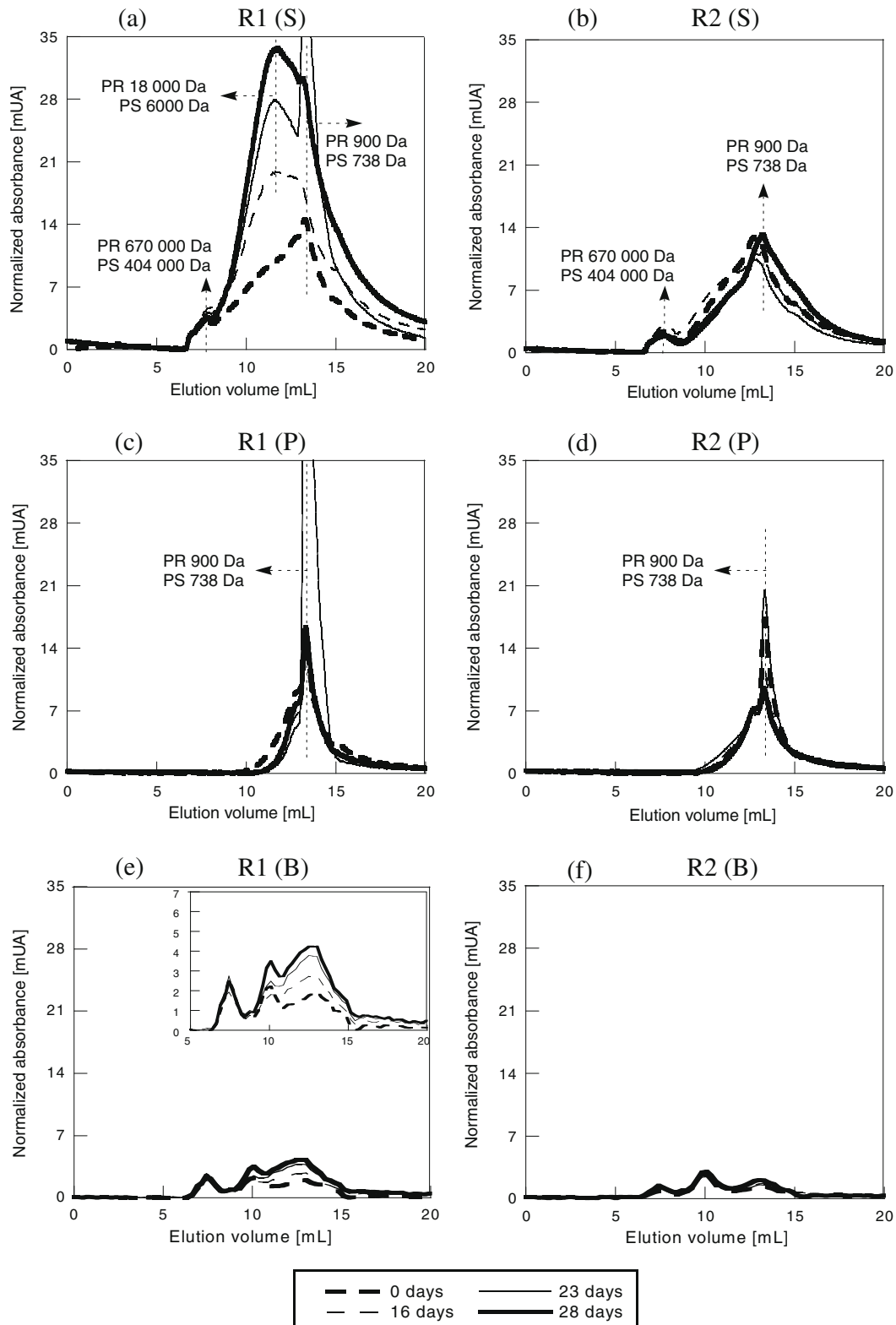

Fig. 4. Normalized chromatograms of soluble EPS present in supernatants (a and b) and in permeates (c and d) and of bound EPS (e and f) in reactors R1 and R2 respectively.

compounds with V_e about 7.5 mL (corresponding to PR of about 670 KDa and PS of 404 KDa). The concentrations of these species remained constant over time regardless of the reactor: this indicates that they were not influenced by the presence of CPs. (A lonely peak in the supernatant R1 (Fig. 4a) on the 23rd day's sampling is an outlier, it was also observed in corresponding permeate Fig. 4c).

(3) More simple chromatographic profiles were obtained from permeates issued from R1 and R2 (Fig. 4c and d). According to the column calibration, only oligomers molecules smaller than 0.9 KDa for proteins and smaller than about 0.7 KDa for polysaccharides were present in permeates. This shows clearly that the membrane filtration eliminated an important part of the soluble macromolecules in reactor's bulk solutions.

3.4. Fourier transform infrared spectroscopy (FT-IR)

To obtain more chemical information about the EPS present in both reactors, samples were submitted to FTIR analysis. Different components can be identified from their specific infrared profiles and the bands intensity can be related to the amount of compounds.

The most representative samples (according to HPSEC results, Fig. 4) were chosen for FTIR analysis. The corresponding FTIR spectra are presented in Fig. 5.

The supernatant samples from R1 and R2 (Fig. 5a and b), have IR spectra displaying similar features with six characteristic bands at 835, 1067, 1370, 1616, 2934 and 3370 cm^{-1}. The samples from the control reactor R2 do not exhibit any significant variation over time, whereas in the samples from R1, the intensity of the IR bands
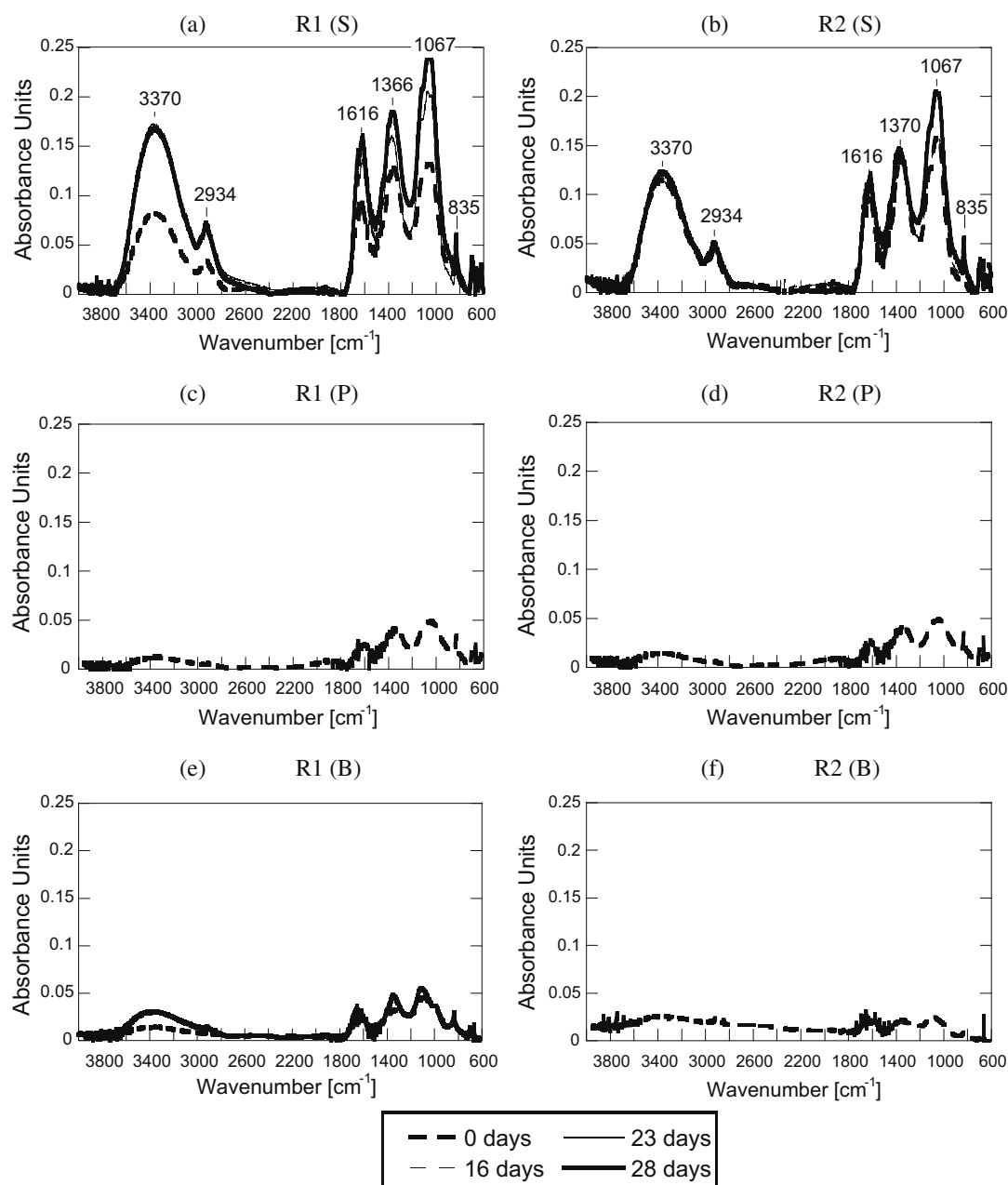

Fig. 5. Evolution of IR spectra of supernatants (a and b), permeates (c and d) and bound EPS (e and f) over time in the reactors R1 and R2 respectively.

increased however with no variation in band positions. The IR spectra revealed that the presence of anticancer drug induced an increase in the concentration of various chemical species however their chemical nature remained unchanged. Spectra corresponding to EPS in permeates and in bound EPS (Fig. 5c–f) display broad infrared bands with a relatively low intensity, therefore they were not submitted to further examination.

The IR profile of supernatants samples reveals the presence of various chemical species. In order to determine which band combinations correspond to a given species, we have analysed the evolution of the band's intensity ratio and the band's area ratio against time. As both methods provided similar results, only the intensity analysis will be presented in this paper. The approach used here was based on the two following assumptions: (i) constant band ratios refer to the same chemical species, (ii) variable ratios refer to bands that do not belong to the same species. This approach can only be applied when a significant evolution in the global infrared

response against time is observed, as was the case for the R1 supernatant samples. Therefore the data from these samples (Fig. 5a) will be treated: Fig. 6 (I) shows, on the left side, the evolution of the intensity ratio of various band combinations against time. Among the six different investigated bands, two groups of bands were identified: the first group is formed by the combination of bands at 3370, 2934 and 1067 cm^{-1} (Fig. 6a), the second group is composed of bands at 1370 and 1616 cm^{-1} (Fig. 6b). These band combinations allow us to build corresponding independent IR profiles (Fig. 6 (I), on the right side) which are representative to the main chemical groups present in the EPS mixture: the first band combination is typical of IR spectra of polysaccharide species (Fig. 6a), the second combination indicates the presence of an amine or amide functions and is likely indicative of protein-type species (Fig. 6b). However, it does not allow the complete identification of the protein-type family, which should exhibit additional IR signals at around 3300 cm^{-1} and at around 1000 cm^{-1}, these
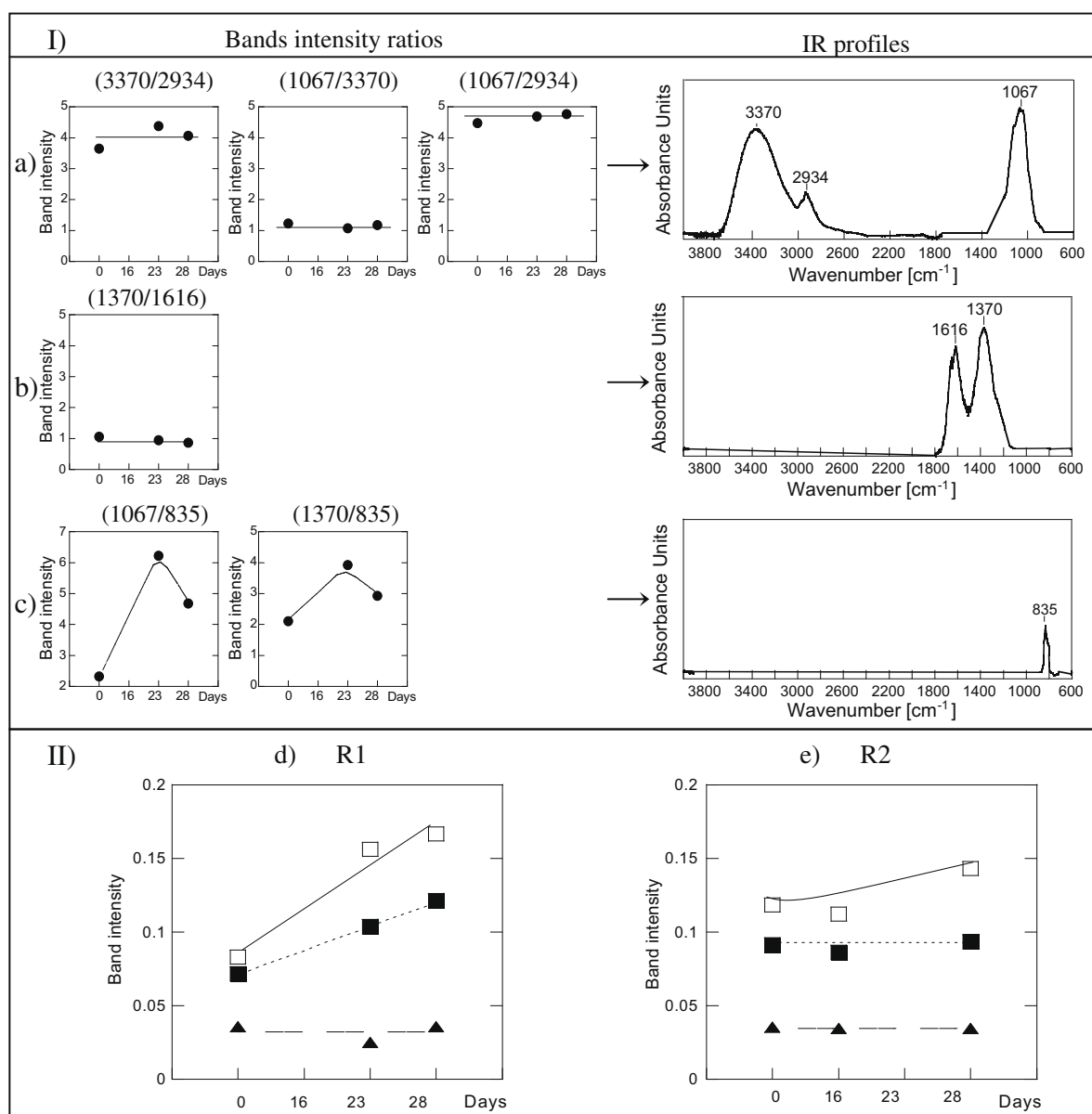
Fig. 6. (I) Determination of the IR profiles of major chemical species present in the R1 supernatant. Band combination and typical IR profile of: (a) polysaccharide, (b) protein-type moieties and (c) inorganic nitrates. (II) Evolution over time of IR bands of polysaccharide (□), amine/amide (■) and nitrate (▲) species in the supernatant of reactor R1 and R2 (d and e).

signals are masked in our samples by dominant polysaccharides. Indeed, band intensity ratio analysis is not able to assign the minority contributions to a given IR profile. The band at 835 cm^{-1} was not involved in any combination, it characterizes inorganic nitrate groups (Fig. 6c).

Once the various species were identified, it was possible to follow their evolution over time referring to their characteristic bands. Polysaccharides were followed by the intensity of their signal at 1067 cm^{-1}, proteins at 1616 cm^{-1} and nitrate evolution was related to the band at 835 cm^{-1}. In the control reactor (Fig. 6e), PR band intensity remained constant over time and only a slight increase in polysaccharide intensity was observed. In contrast, PR and PS species significantly increased in the reactor with CPs (Fig. 6d) and PS increment seems to be more important. In both reactors the nitrate band intensity was constant.

The FTIR approach confirmed the significant increase of PR and PS species in bulk solution in the reactor with CPs presence as previously seen by UV–Vis analysis. Moreover, it showed that the CPs presence did not seem to induce any chemical changes in soluble species.

4. Discussion

The chromatographic and UV–Vis analysis showed that CPs presence induced an increased formation especially of soluble macromolecular EPS with their main components PR and PS. It is known that biomass generates microbial intra or extra cellular components that participate in the formation of aggregates, and EPS in the bulk solution due to cell renewal, cell lysis or deliberate excretion for some purposes (Laspidou and Rittmann, 2002). Aquino and Stuckey (2004) observed an increased concentration of soluble EPS with a high molecular size in anaerobic chemostat in the presence of toxicants (chloroform or chromium). Hydrolysis did not seem to contribute to EPS accumulation and they suggested that EPS were produced by bacteria to cope with stress. In our case all observations argue for similar phenomenon. HPSEC showed that high macromolecular PR and PS (M.W.: 670 KDa and 404 KDa, respectively) were almost identical in both reactors; they were present in supernatants and in bound fraction. Their concentration did not change over reactor running time. Moreover, no significant increase in oligomers compounds appeared in CPs reactor. This suggests that EPS hydrolysis cannot be the principal factor responsible for the increase of macromolecular PR and PS species of 18 KDa and 6 KDa, respectively, in bulk solution. It was reported (Bott and Love, 2002) that bacterial stress response mechanism called K$^+$ efflux is a significant contribution to the deflocculation. In our case the floc volumetric diameters were not at all diminished (Delgado, 2009) in reactor with pharmaceuticals, they were identical or sometimes higher than in control reactor. Thus in reactor with pharmaceutical, the increased amount of soluble EPS, in relation to control MBR, can not be attributed to the floc disintegration and to the subsequent EPS material release. As both reactors had identical operating conditions except the presence of CPs, it was likely that bacteria produced EPS for better survival in hostile environment. Henriques and Love (2007) found that the EPS matrix inside sludge flocs was a protective barrier for bacteria exposed to chemicals toxins octanol and cadmium.

Our results corroborate the Aquino and Stuckey (2004) study on soluble microbial products (SMP) in bioreactor spiked with chloroform or chromium: they observed enhanced soluble microbial production, composed mainly of PR and PS and no change in SMP composition in toxin's presence. They suggested that some SMP might be deliberately excreted by micro-organisms in cell to cell communication (quorum sensing). It is now established that the quorum sensing influences the biofilm development or aggregates dispersion (Parsek and Greenberg, 2005) regulating the excretion of PS or PR for biomass survival. It was found that bacteria are a thousand times more resistant to antibiotics in a biofilm than in liquid suspension (Everst, 2006).

The application of MBR technology in wastewater treatment for removal of CPs was previously studied by Delgado (2009). Under steady-state conditions, CPs removal remained quite stable (75–80%) and COD and total nitrogen removal efficiency were not altered by the toxicity of the anticancer drug. However, CPs presence induced a modification of biological suspended solids. The modifications in the biomass and in the bulk solution appeared to influence the membrane performance. In our study, membrane performance was followed by measuring transmembrane pressure (TMP) illustrated in Fig. 2b. It was observed that TMP in R1 (with CPs), increased 3 times more than in the control reactor and the fouling dynamics were faster.

Previous studies have identified the extracellular polymeric substances (EPS) or soluble microbial products (SMP) as one of the most significant factors responsible for membrane fouling (Drews et al., 2006; Janga et al., 2007; Judd, 2008; Le-Clech et al., 2006; Meng et al., 2009) and for membrane deterioration (Tansel et al., 2006). Furthermore, many investigations have been done about the role of PR or PS in membrane fouling mechanisms. However in some cases, the cake resistance was found to be strongly related to PR content in the supernatant (Meng et al., 2006), or to PS having inherent fouling potential (Drews et al., 2006; Rosenberger et al., 2006) or to PR/PS ratio (Arabi and Nakhla, 2008). Indeed, it seems that PR have an important role in synergistic effects PR–PS and the trend towards fouling (Susanto et al., 2008). In our study, membrane filtration efficiently eliminated PS and PR: in control reactor R2 up to 95% of PS and up to 68% of PR (Fig. 3f). In R1 (with CPs), where an increased amount of EPS was presented, PS were retained up to 98% and PR up to 92%, as shown in Fig. 3e. Indeed, the accumulation of EPS in the MBR sludge mixture would facilitate the formation of an EPS fouling gel layer on the membrane surface and eventually pore narrowing/blocking, thus potentially causing a serious fouling problem, as observed in reactor with CPs.

5. Conclusion

Our experiments showed that cyclophosphamide and its mean metabolites in the studied concentrations range influenced the biomass exopolymer production. All methods applied for EPS studies (HPSEC, FTIR and UV–Vis) showed clearly that cyclophosphamide presence induced an increase in soluble EPS (mainly proteins of about 18 KDa and polysaccharides of about 6 KDa) in bulk solution and to a much lower degree in EPS bound in the sludge. The increase of these macromolecular species may be attributed to a protection mechanism. Their retention by the membrane was very efficient and was most likely related to the more important membrane fouling of reactor with cyclophosphamide.

References

APHA – American Public Health Association, AWWA – American Water Works Association, WEF – Water Environment Federation, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. Washington, DC, USA.

Aquino, S.F., Stuckey, D.C., 2004. Soluble microbial products formation in anaerobic chemostats in the presence of toxic compounds. Water Research 38 (2), 255–266.

Arabi, S., Nakhla, G., 2008. Impact of protein/carbohydrate ratio in the feed wastewater on the membrane fouling in membrane bioreactors. Journal of Membrane Science 324 (1–2), 142–150.

Bernhard, M., Muller, J., Knepper, T.H., 2006. Biodegradation of persistent polar pollutants in wastewater: comparison of an optimised lab-scale membrane bioreactor and activated sludge treatment. Water Research 40 (18), 3419–3428.

Bott, C., Love, N., 2002. Investigating a mechanistic cause for activated sludge deflocculation in response to shock loads of toxic electrophilic chemicals. Water Environment Research 74 (3), 306–315.

Delgado, L.F., 2009. Bioréacteur à membrane externe pour le traitement d'effluents contenant des médicaments anticancéreux: élimination et influence du cyclophosphamide et de ses principaux métabolites sur le procédé. PhD thesis, INP Toulouse, France.

Drews, A., Vocks, M., Iversen, V., Lesjean, B., Kraume, M., 2006. Influence of unsteady membrane bioreactor operation on EPS formation and filtration resistance. Desalination 192 (1–3), 1–9.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28 (3), 350–356.

Everst, S., 2006. Bacterial Conversations. C&EN, Washington. October 23, pp. 17–26. <http://www.CEN-online.org>.

Finlayson, J.C., Liao, B., Droppo, T.G., Leppard, G.G., Liss, S.N., 1998. The relationship between the structure of activated sludge flocs and the sorption of hydrophobic pollutants. Water Science and Technology 37 (4–5), 353–357.

Frolund, B., Palmgren, R., Keiding, K., Nielsen, P.H., 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Research 30 (8), 1749–1758.

Garnier, Ch., Görner, T., Lartiges, B., Abdelouhab, S., de Donato, Ph., 2005. Characterization of activated sludge exopolymers from various origins: a combined size-exclusion chromatography and infrared microspectroscopy study. Water Research 39 (13), 3044–3054.

Henriques, I.D.S., Love, N.G., 2007. The role of extracellular polymeric substances in the toxicity response of activated sludge bacteria to chemical toxins. Water Research 41 (18), 4177–4185.

Janga, N., Ren, X., Kim, G., Ahn, Ch., Cho, J., Kim, I.S., 2007. Characteristics of soluble microbial products and extracellular polymeric substances in the membrane bioreactor for water reuse. Desalination 202 (1–3), 90–98.

Joqueviel, C., Martino, R., Gilard, V., Malet-Martino, M., Canal, P., Niemeyer, U., 1998. Urinary excretion of cyclophosphamide in humans, determined by phosphorus-31 nuclear magnetic resonant spectroscopy. Drug Metabolism and Disposition 26 (5), 418–428.

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing of classification scheme. Water Research 40 (8), 1686–1696.

Judd, S., 2008. The status of membrane bioreactor technology. Trends in Biotechnology 40 (2), 109–116.

Kümmerer, K., 2001. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. Chemosphere 45 (6–7), 957–969.

Laspidou, Ch.S., Rittmann, B.E., 2002. A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. Water Research 36 (11), 2711–2720.

Le-Clech, P., Chen, V., Fane, T.A.G., 2006. Fouling in membrane bioreactors used in wastewater treatment. Review. Journal of Membrane Science 284 (1–2), 17–53.

Lenz, K., Koellensperger, G., Hann, S., Weissenbacher, N., Mahnik, S.N., Fuerhacker, M., 2007. Fate of cancerostatic platinum compounds in biological wastewater treatment of hospital effluents. Chemosphere 69 (11), 1765–1774.

Liu, Yan, Fang, Herbert H.P., 2003. Influences of extracellular polymeric substances (EPS) on flocculation, settling and dewatering of activated sludge. Critical Reviews in Environmental Science and Technology 33 (3), 237–273.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin–Phenol reagents. Journal of Biological Chemistry 193, 265–275.

Meng, F., Zhang, H., Yang, F., Zhang, S., Li, Y., Zhang, X., 2006. Identification of activated sludge properties affecting membrane fouling in submerged membrane bioreactors. Separation and Purification Technology 51 (1), 95–103.

Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S., Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water Research 43 (6), 1489–1512.

Nuengjamnong, Ch., Kweon, J.H., Cho, J., Polprasert, Ch., Ahn, K.-H., 2005. Membrane fouling caused by extracellular polymeric substances during microfiltration processes. Desalination 179 (1–3), 117–124.

Parsek, M.R., Greenberg, E.P., 2005. Sociomicrobiology: the connections between quorum sensing and biofilms. Trends in Microbiology 13 (1), 27–33.

Rosenberger, S., Laabs, C., Lesjean, B., Gnirss, R., Amy, G., Jekel, M., Schrotter, J.C., 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. Water Research 40 (4), 710–720.

Steger-Hartmann, T., Kümmerer, K., Hartmann, A., 1997. Biological degradation of cyclophosphamide and its occurrence in sewage water. Ecotoxicology and Environmental Safety 36 (2), 174–179.

Susanto, H., Arafat, H., Janssen, E.M.L., Ulbricht, M., 2008. Ultrafiltration of polysaccharide-protein mixtures: elucidation of fouling mechanisms and fouling control by membrane surface modification. Separation and Purification Technology 63 (3), 558–565.

Tansel, B., Sager, J., Garland, J., Xu, S., Levine, L., Bisbee, P., 2006. Deposition of extracellular polymeric substances (EPS) and microtopographical changes on membrane surfaces during intermittent filtration conditions. Journal of Membrane Science 285 (1–2), 225–231.

Tsuneda, S., Aikawa, H., Hayashi, H., Yuasa, A., Hirata, A., 2003. Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. FEMS Microbiology Letters 223 (2), 287–292.

Urase, T., Kikuta, T., 2005. Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process. Water Research 39 (7), 1289–1300.

Weiss, S., Reemtsma, T., 2008. Membrane bioreactors for municipal wastewater treatment – a viable option to reduce the amount of polar pollutants discharged into surface waters? Water Research 42 (14), 3837–3847.

Wingender, J., Neu, T.R., Flemming, H.C. (Eds.), 1999. Microbial Extracellular Polymeric Substances. Springer, Heidelberg.