



**HAL**  
open science

# Study of the effects of pharmaceutical micropollutants on the fouling of MBR used for municipal wastewater treatment : case of carbamazepine

Chengcheng Li

► **To cite this version:**

Chengcheng Li. Study of the effects of pharmaceutical micropollutants on the fouling of MBR used for municipal wastewater treatment : case of carbamazepine. Environmental Engineering. INSA de Toulouse, 2014. English. NNT : 2014ISAT0024 . tel-01124372

**HAL Id: tel-01124372**

**<https://theses.hal.science/tel-01124372>**

Submitted on 6 Mar 2015

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Université Fédérale



Toulouse Midi-Pyrénées

# THÈSE

**En vue de l'obtention du  
DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE**

**Délivré par :**

Institut National des Sciences Appliquées de Toulouse (INSA de Toulouse)

**Discipline ou spécialité :**

Génie des procédés et de l'Environnement

---

**Présentée et soutenue par :**

Chengcheng LI

**Le** lundi 26 mai 2014

**Titre :**

Etude de l'impact de micropolluants pharmaceutiques sur le colmatage  
des BAM utilisés en traitement des eaux usées urbaines:  
cas de la carbamazépine

---

**JURY**

Pr. Christelle WISNIEWSKI (Université Montpellier 1)  
Pr. Carme GÜELL (Universitat Rovira i Virgili)  
Dr. Anthony MASSE (Université de Nantes)  
Pr. Corinne CABASSUD (INSA de Toulouse)  
Pr. Christelle GUIGUI (INSA de Toulouse)

---

**Ecole doctorale :**

Mécanique, Energétique, Génie civil et Procédés (MEGeP)

**Unité de recherche :**

Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP)

**Directeur(s) de Thèse :**

*Corinne CABASSUD et Christelle GUIGUI*

**Rapporteurs :**

*Christelle WISNIEWSKI et Carme GÜELL*



# THESE

En vue de l'obtention du

**Doctorat de l'Université de Toulouse**

**Département :** INSA de Toulouse

**Spécialité :** Génie des procédés et de l'Environnement

**Présentée et soutenue par :**

Chengcheng LI

Le 26 mai 2014

Titre: Etude de l'impact de micropolluants pharmaceutiques sur le colmatage des BAM utilisés en traitement des eaux usées urbaines: cas de la carbamazépine

## **JURY :**

Pr. Christelle WISNIEWSKI (Université Montpellier 1)

Pr. Carme GÜELL (Universitat Rovira i Virgili)

Dr. Anthony MASSE (Université de Nantes)

Pr. Corinne CABASSUD (INSA de Toulouse)

Pr. Christelle GUIGUI (INSA de Toulouse)

**Ecole doctorale :** Ecole doctorale MEGEP

**Unité de recherche :** LISBP

**Directeur de thèse :** Corinne CABASSUD et Christelle GUIGUI

**Rapporteurs :** Christelle WISNIEWSKI et Carme GÜELL



## Résumé

Le colmatage des membranes reste la principale limitation pour le développement du bioréacteur à membrane (BAM). Dans cette thèse, l'objectif principal se concentre sur les effets des micropolluants pharmaceutiques qui se retrouvent dans les eaux usées domestiques sur le colmatage de la membrane du BAM. Carbamazépine (CBZ), un médicament antiépileptique, a été choisi. Les effets de la CBZ sur le colmatage du BAM ont été étudiés de deux manières: un pic de pollution pour étudier les effets des pics de CBZ à court terme sur le pouvoir colmatant et une pollution continue pour examiner les effets de CBZ à long terme sur le colmatage du BAM. Les résultats ont montré que, pendant 3 heures contact avec CBZ de  $100 \mu\text{g L}^{-1}$ , le pouvoir colmatant des boues activées a augmenté en raison de l'augmentation des protéines de 100-1000 kDa dans le surnageant, ce qui pourrait être complètement retenu par la membrane du BAM et les membranes utilisées dans les essais de filtrabilité. L'augmentation des protéines de 100-1000 kDa dans le surnageant peut probablement être causée par la décomposition bactérienne face aux médicaments. L'effet réduit a été observé pour les boues provenant du BAM fonctionné sous la charge organique plus élevée. Pendant le contact continu, la vitesse du colmatage plus élevée a été observée après l'addition en continu de CBZ dans le BAM ( $90 \mu\text{g L}^{-1}$  dans l'alimentation), qui pourrait être liée à l'augmentation importante des protéines de 10-100 kDa dans le surnageant. Des protéines de 10-100 kDa s'accumulent dans le dépôt de la membrane du BAM, modifient de la structure de dépôt et changent les caractéristiques de rétention de BAM. L'augmentation des protéines de 10-100 kDa a probablement été causée par la réaction de défense des bactéries face en permanence des médicaments. Légère inhibition de l'activité microbienne a été trouvée plusieurs jours après l'addition de la CBZ dans le BAM, puis il a été stabilisé à un certain niveau en raison de l'acclimatation des boues au stress pharmaceutique. Similaire, l'augmentation significative de la concentration en protéine a été observée au début plusieurs jours après l'addition de la CBZ dans le BAM, puis retourne à son niveau initial. Aucun changement significatif de la taille des flocons des boues et de la concentration en polysaccharides dans le surnageant n'a été constaté au cours de la période de contact continu à long terme. Cette étude pourrait contribuer à améliorer la compréhension des interactions complexes entre les micropolluants pharmaceutiques, boues activées et le colmatage du BAM.

**Mots clés:** BAM, micropolluants pharmaceutiques, colmatage, protéines



## Abstract

Membrane fouling still remains the main limitation for the development of membrane bioreactor (MBR). In this thesis, the main objective focuses on the effects of pharmaceutical micropollutants which are frequently found in domestic wastewater on MBR fouling. Carbamazepine (CBZ), an anti-epileptic drug, was chosen in this study due to its occurrence in domestic wastewater and persistency in MBR process. The effects of CBZ on MBR fouling were investigated in two different ways of contact, i.e. short-term peak contact and long-term continuous contact. The results showed that during only 3 hours contact with  $100 \mu\text{g L}^{-1}$  CBZ, the fouling propensity of the sludge increased due to the increase in 100-1000 kDa protein-like substances in the supernatant, which could be completely retained by the MBR membrane and the membranes used in the filterability tests. The increase of 100-1000 kDa protein-like compounds in the supernatant may probably be caused by the bacterial decay when facing the pharmaceutical stress. Besides, the reduced effect was observed for sludge obtained from MBR operated under higher organic loading rate. During the long-term continuous contact, significantly higher MBR fouling rate was observed after the continuous addition of CBZ in the MBR via the feed ( $90 \mu\text{g L}^{-1}$  CBZ in the feed), which could be related to the significant increase of 10-100 kDa protein-like compounds in the supernatant after addition of CBZ. The 10-100 kDa protein-like compounds could accumulate in the biocake, which was formed on MBR membrane surface, modify the biocake structure and change the retention characteristics of MBR. The increase of 10-100 kDa protein-like compounds was probably caused by the defensive response of bacteria when continuously facing the pharmaceutical stress. Slight inhibition of microbial activity was found several days after addition of CBZ in MBR, and then it was stabilized to some constant level due to the acclimation of sludge to the pharmaceutical stress. Similar, significant increase of protein concentration was observed at the beginning several days after addition of CBZ in MBR, then returned to the initial level. No significant change in sludge floc size and polysaccharide concentration in supernatant was found during the long-term continuous contact period. This study could help to enhance the understanding of complex interactions among pharmaceutical micropollutants, activated sludge and MBR fouling.

**Key words:** MBR, pharmaceutical micropollutants, fouling, protein-like substances





## Acknowledgements

This work was carried out at the Laboratory of Biological systems and Process Engineering, INSA Toulouse.

Foremost, I would like to express my sincere gratitude to my supervisors Corinne Cabassud and Christelle Guigui. Their insightful advices, encouragement and supports accompanied me throughout the whole thesis. Each discussion with them showed me the way how research was performed. Thanks to their supports, I could have the opportunities to attend many conferences and participate in the project with international cooperation, which also greatly broadened my view in terms of both academic and personal aspects.

I would like to thank Christelle Wisniewski, Carme Güell and Anthony Massé for accepting to be the defense committee members and for their pertinent comments on the thesis manuscript and during the defense discussion.

I would like to thank all my colleagues in the laboratory. Specially, I would like to thank Bernard for the technical maintenance of the experimental pilot and also for the transportation of feed wastewater from the sewage treatment plant to the laboratory for this study. Also, I would like to thank Aurore for the analytical techniques like HPLC-SEC and Frédéric for the administrative issues. I express my many thanks to Aurélien, Patrick, Duong, Wael, Manel, Mathias, Claire, Marie etc. All of them helped me a lot and made the working ambience in the laboratory comfortable for me. I would like to express my gratitude to the master student Luc á for her experimental works done in the framework of my thesis.

I would like to thank all my Chinese friends in Toulouse: Susu He, Yanping Liu, Dongdong Yang, Haojun Zhang, Liang Tian, Xinwei Zhang, Haoran Pang, Lijian Wang, Tao Hu,

Linqing Gui, Juan Lu, Hong Liu, Jin Yan, Xu Deng, Hang Ma, Tiantian Zhang, Wenjun Zhu, Ming Zhang, Jian Liu, Ran Liu, Jian Wei etc.

Great thanks are given to my parents and my sister for their unceasing supports. Finally, I would like to express my deepest gratitude to my wife and best friend, Yuanyuan, for her trust, encouragement and love. Without her support, I could not finish this thesis.

## Table of Contents

Thesis Overview.....	1
Publication 1. Evaluation of membrane bioreactor on removal of pharmaceutical micropollutants: a review .....	53
Publication 2. Effects of carbamazepine in peak injection on fouling propensity of activated sludge from a MBR treating municipal wastewater .....	89
Publication 3. Effects of pharmaceutical micropollutants on the membrane fouling of a submerged MBR treating municipal wastewater: case of continuous pollution by carbamazepine .....	121
Publication 4. HPLC-SEC with fluorescence detection as a tool for estimating fouling of MBRs used for municipal wastewater treatment.....	157
Résumé long en français .....	189



# Thesis Overview

---

---



This thesis, entitled “Study of the effects of pharmaceutical micropollutants on the fouling of MBR used for municipal wastewater treatment: case of carbamazepine”, consists of a thesis overview (52 pages) and 4 publications (3 accepted papers, 1 draft). The thesis overview introduces the background, motivations of the research, organization of the work, synthesizes and links the major results that are detailed in the publications, and gives the conclusions and perspectives for the work.

List of publications:

#### Paper 1

C. Li, C. Cabassud, C. Guigui, Evaluation of membrane bioreactor on removal of pharmaceutical micropollutants: a review, accepted for publication in *Desalination and Water Treatment*.

#### Paper 2

C. Li, C. Cabassud, C. Guigui, Effects of carbamazepine on fouling propensity of activated sludge from a MBR treating municipal wastewater, accepted for publication in *Journal of Membrane Science*.

#### Paper 3

C. Li, C. Cabassud, B. Reboul, C. Guigui, Effects of pharmaceutical micropollutants on the membrane fouling of a submerged MBR treating municipal wastewater: case of continuous pollution by carbamazepine, accepted for publication in *Water Research*.

#### Draft paper 4

C. Li, C. Cabassud, A. Didi, C. Guigui, HPLC-SEC with fluorescence detection as a tool for estimating fouling of MBRs used for municipal wastewater treatment, not submitted yet



## **1. Background**

Nowadays, water scarcity is still a big challenge facing humanity in many places around the world. To solve this problem, municipal wastewater has been considered to be an alternative water source for various applications after proper treatment. Among all kinds of treatment processes, membrane bioreactor (MBR) has been increasingly used in municipal wastewater reuse and reclamation applications due to its high effluent quality. MBR process is a hybrid process that couples the activated sludge process with membrane separation, i.e. microfiltration (MF) or ultrafiltration (UF). The coupling of membrane separation with the biological process enables the MBR to possess many advantages, which may include high effluent quality (especially the high removal of pathogenic microorganisms), small footprint, less sludge production, and flexible process design (independent control of solids and hydraulic retention time). However, the membrane fouling remains the major limitation for the development of MBR.

### **1.1 Membrane fouling in MBRs**

Membrane fouling means the decline of permeability during MBR operation. For MBRs operated at constant flux, it also means the increase of transmembrane pressure (TMP) during MBR operation. Membrane fouling was caused by the biocake, which is formed on membrane surface due to the convective flow of activated sludge and microbial growth, by the pore blocking due to small particles, colloids and solute adsorption [1-3]. Normally, three steps (see Fig. 1) of fouling are proposed for MBRs when they are operated at constant flux operation: conditioning fouling (step 1) caused by initial pore blocking and adsorption of solutes, steady fouling (step 2) occurring due to biofilm formation and further pore blocking, and the TMP jump (step 3) occurring due to the following different mechanisms or a

combination of them: (i) due to a progressive closure of pores, local flux starts to exceed the critical value and particles begin to deposit at an ever increasing rate; (ii) coagulation occurs in the first layers at a critical pressure; (iii) percolating colloids inside the cake reduce the size of the cake voids until connections are lost [2].

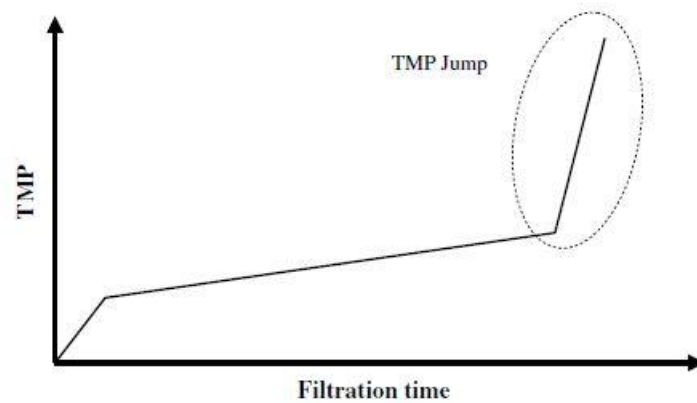


Fig. 1. Schematic illustration of the long-term TMP profile (adopted from Meng et al. [4] )

The supernatant of activated sludge, which contains colloids and solutes, was shown to play a more important role in membrane fouling of MBR than the biological flocs in many studies [5-6]. Due to the gas–liquid two-phase cross flow generated by aeration in submerged MBRs or liquid cross flow generated by a recirculation pump in side-stream MBRs, the low flux operation and the relatively easy removal of biomass deposit by the shear force, the biomass deposit is of minor importance on MBR fouling in long-term operation. Thus, soluble microbial products (SMPs), the major organic constituents of sludge supernatants, have been acknowledged as the major foulants that lead to membrane fouling in MBRs [7-8]. SMPs are identified as soluble extracellular polymeric substances (EPS) and are composed of proteins, polysaccharides, humic substances etc. [9-11]. Besides, SMPs could be further divided into two categories: utilization-associated products (UAPs), which are produced during substrate metabolism, and biomass-associated products (BAPs), which are formed from decay of biomass. Factors affecting the SMP production may include operating conditions such as

organic loading rate [12-13], sludge retention time (SRT) [14-15], hydraulic conditions like aeration and mechanical stirring [16-17], and the presence of environmental stress (for bacteria) in the wastewater such as heavy metals [18-20], osmotic pressure (salts) [21-22], and industrial compounds like pharmaceuticals [23-24]. However, simply correlating the MBR fouling with protein-like or polysaccharide-like SMP concentrations in the supernatant or with their retention in MBRs could not explain the contribution of each component in membrane fouling, since some of the SMPs may be retained by the cake layer and may not be attached on membrane surface or in membrane pores [25]. Thus, in MBRs, fouling is also affected by the molecular weight (MW) distribution of SMPs [26-27]. Besides, the physiochemical properties like relative hydrophobicity of SMP components may also significantly affect the MBR fouling [28]. Proteins were reported to dominate in foulants desorbed from the fouled membranes of MBRs due to its relatively high hydrophobicity [29].

Fouling propensity of biological fluids is currently estimated by a calculation of fouling resistance, permeability or fouling rate through an analysis of data (permeate flux, TMP) that are obtained (i) during operation of the MBR [30-31]; (ii) from batch stirred dead-end filtration cells [32-34]; (iii) from batch tangential modules (ex situ) operated under standardized operating conditions [35-36]; and (iv) from some submerged modules placed directly inside the MBR (in-situ) and using aeration to generate cross-flow conditions [37-38], which could avoid the problems like the change of sludge quality during storage period. In the case of a fouling occurring as a biocake deposit, some recent works have also proposed in-situ fouling characterization methods thanks to some filtration apparatus equipped with acoustic or optical sensors [39-42]. Still, there is a great interest in the industry to find some simple and cheap methods that could allow estimating the fouling propensity of biological fluids such as MBR supernatants. Thus, many analytical tools, including three-dimensional

excitation–emission matrix (EEM) fluorescence spectroscopy and high performance liquid chromatography-size exclusion chromatography (HPLC-SEC) equipped with different detectors (UV, refractive index, fluorescence and organic carbon), have been proposed to characterize SMPs in supernatants [43-44]. Among these techniques, HPLC-SEC-fluorescence, which integrates the advantages of EEM fluorescence spectroscopy with HPLC-SEC analysis, could achieve an accurate detection of protein-like substances in different molecular weight ranges, especially for the compounds in biopolymer range (MW bigger than 10 kDa) [44-46], which could help to improve understanding the effects of protein-like substances and their molecular weight distribution on membrane fouling.

## **1.2 Problematics of pharmaceutical micropollutants in MBRs**

### **1.2.1 Removal of pharmaceutical micropollutants in MBRs**

In the last decade, great attention has been paid to some emerging trace organic contaminants, also called micropollutants, such as endocrine-disrupting compounds, pharmaceuticals, personal care products etc., found in aquatic environment [47-48]. Among these micropollutants, pharmaceutical micropollutants got concerns since the late 1990s [49-51], as pharmaceuticals are designed to have some biological effects on living organisms and may have potential adverse effects on human health or aquatic organisms [52]. Also, concerns have been raised regarding that continuous discharge of antibiotics to aquatic environment may facilitate the development or proliferation of resistant strains of bacteria [53]. Moreover, chronic toxicity effects have been reported for aquatic organisms exposed to human pharmaceuticals at trace concentration [54-56]. So far, there is evidence of occurrence of some 160 different drugs in the effluents of wastewater treatment plants (WWTPs), and also of plants for the production of potable water from surface waters and groundwaters [57-58]. Pharmaceuticals found in water resources are classified into different therapeutic categories:

analgesics and anti-inflammatory drugs, lipid regulators, antiepileptic drugs, beta-blockers, antibiotics, cytostatic drugs etc. Many pharmaceutical micropollutants, such as analgesics like ibuprofen, diclofenac, naproxen and ketoprofen, lipid regulators like bezafibrate and gemfibrozil, the antiepileptic drug carbamazepine, and antibiotics such as trimethoprim and sulfamethoxazole, have been frequently detected at concentration up to or higher than 1.0 µg/L in effluents of WWTPs around the world [59]. Pharmaceutical residues are discharged to sewers through a mixture of urine and faeces as the initial molecules or their metabolites after absorption by human bodies. Thus, pharmaceuticals mainly enter WWTPs via sewer networks after human release. Due to the incomplete removal or degradation of pharmaceutical micropollutants in conventional WWTPs, effluents discharged from WWTPs become a major source of pharmaceutical micropollutants entering into the water surface or underground resources [60-61].

Extensive studies have been carried out to investigate the removal efficiency and removal mechanisms of pharmaceutical micropollutants in MBR process, due to its potential advantages (such as higher SRT, accumulation of enzymatic biopolymers in the biological reactor) on removal of persistent organic pollutants as compared to the activated sludge process employed in conventional WWTPs. MBR was found to significantly enhance the removal of some partial degradable pharmaceutical micropollutants such as diclofenac, mefenamic acid, ketoprofen, clofibric acid, gemfibrozil etc. as compared to conventional activated sludge process (CAS) [62-64]. According to their removal efficiency in MBRs reported in the literature, pharmaceutical micropollutants can be classified into different groups of molecules [59], depending on their propensity to be removed in MBRs: (i) easily, with an average removal efficiency higher than 90 %, such as ibuprofen and bezafibrate; (ii) moderately, with an average removal efficiency of 50%-80%, such as naproxen, ketoprofen,

gemfibrozil, trimethoprim, and sulfamethoxazole; (iii) poorly, with an average removal efficiency lower than 40%, such as diclofenac and carbamazepine. Thus, most pharmaceutical micropollutants are moderately removed in MBRs. In particular, carbamazepine is removed with an average removal efficiency of only 7% in MBRs [59].

The mechanisms involved for the removal of pharmaceutical micropollutant in MBRs may include physical retention by the membrane, sorption onto the activated sludge and biotransformation. Since the molecular size of most pharmaceuticals (molecular weight between 100 and 1000 g mol<sup>-1</sup>), is at least 100 times smaller than the pore size of the membranes that are used in MBRs, no direct physical retention of the compounds by a MBR membrane itself can be expected [65]. However, Urase et al. [66] suggested that the deposit formed on the membrane surface might act as an additional barrier for the micropollutants and contribute to an enhanced removal of pharmaceutical micropollutants in an MBR. Further studies need to be performed to investigate the role of deposits or preferentially of biofilm on the removal of pharmaceutical micropollutants. Since the value of sorption coefficient  $K_d$  (L kg<sup>-1</sup>), defined as the partition of a molecule between the activated sludge and the water phase, of many pharmaceutical micropollutants, is very low (less than 500 L kg<sup>-1</sup>) [67], as compared with that of some other micropollutants such as the fragrance tonalide (18000 L kg<sup>-1</sup>) and the steroid hormone 17 $\beta$ -estradiol (35000 L kg<sup>-1</sup>) [68], the removal of pharmaceutical micropollutants via sorption to the activated sludge in an MBR is often of minor importance [69]. Some studies identified the  $K_d$  value of 500 L kg<sup>-1</sup> to be the critical value, below which sorption is not relevant for the removal of organic micropollutants in biological wastewater treatment processes including MBRs [67, 70]. In particular, carbamazepine has a  $K_d$  value of only 1.2 L kg<sup>-1</sup> [70], indicating that it is not significantly sorbed into the activated sludge. However, attention should be paid to some antibiotics like ciprofloxacin and norfloxacin. As

the major mechanism relevant for their removal is sorption into the activated sludge, due to their high  $K_d$  value (higher than  $15000 \text{ L kg}^{-1}$ ) [71]. Taking into account the difficulty in the precise quantification of pharmaceuticals in the sludge phase and also the different ways used to determine the  $K_d$  value (for example, sludge samples could be sampled from batch experiments or sampled from full-scale WWTPs), the role of sorption on removal of pharmaceutical micropollutants in MBR may be underestimated. Thus, in the literature it is often considered that the main mechanism involved in the removal of most pharmaceutical micropollutants in MBR is biodegradation [72].

All above discussion concerned MBRs that were used for treating domestic wastewaters, which will be the focus of our study. For other kinds of waste waters, for example, effluents of hospitals or drug production facilities, since they may contain very high concentration of some pharmaceuticals, especially the antibiotics [73-75], a specific adapted biomass may be required for the treatment of these very specific effluents.

### **1.2.2 The effects of pharmaceutical micropollutants on MBR process**

Only a few studies examined the effects of the presence of pharmaceutical micropollutants on the MBR process, the majority of which focused on the effects on sludge microbial activity or the evolution of microbial community in the sludge. In batch experiments that used the activated sludge sampled from an MBR, Aubenneau et al. [76] examined the effect of trace concentration of carbamazepine on the mixed microbial communities and observed an inhibition of the exogenous respiration rate, a higher endogenous respiration rate, and a smaller floc size in presence of  $1 \mu\text{g L}^{-1}$  carbamazepine in the sludge. Delgado et al. [77] reported that the continuous introduction of  $5 \mu\text{g L}^{-1}$  of the cytostatic drug cyclophosphamide and its principal metabolites (CPs) in a MBR caused the inhibition of the exogenous

respiration rate and increased the mean floc size of the activated sludge. Through the denaturing gradient gel electrophoresis (DGGE) analyses of the sludge community, Lay et al. [23] observed shifts in microbial community and a reduced microbial diversity in the activated sludge several days after adding a mixture of carbamazepine, diclofenac, ibuprofen and naproxen in the feed tank (20–25  $\mu\text{g L}^{-1}$  of each pharmaceutical in the feed) of an Osmotic Membrane Bioreactor (OMBR). Through the terminal restriction fragment length polymorphism (T-RFLP) analyses of the bacterial 16S rRNA genes, Kraigher et al. [78] observed shifts in the bacterial community structure in the activated sludge of a bioreactor continuously fed with a synthetic wastewater containing pharmaceuticals (ibuprofen, naproxen, ketoprofen, diclofenac and clofibrac acid) at a concentration of 50  $\mu\text{g L}^{-1}$ , as compared to a control reactor, which was operated without addition of pharmaceuticals.

It is known that a chemical stress can induce the activated sludge to produce extracellular polymeric substances (EPSs) and soluble microbial products (SMPs), which are often identified as the main responsible for MBR fouling. The release of EPSs or SMPs may result from (i) the defensive response of bacteria to form a protective layer or (ii) decay (lysis) of bacteria and release of EPSs or SMPs inside the bacterial cells [79-80]. Still, very few studies reported the effects of pharmaceutical micropollutants on SMP production. In the literature, the effects of chemical stress on SMP production were examined in two ways:

(i) Long-term continuous contact. Lesage et al. [81] reported that after continuous addition of a toxic molecule, 2,4 dimethylphenol (DMP) in an MBR feed (90 mg/L in the feed), a significant increase in both protein and humic concentrations was observed. In contrast, the authors found no significant effect on polysaccharide concentration. Zhou et al. [20] reported that an MBR fed with a municipal wastewater containing 0.4 mg  $\text{L}^{-1}$  Cr (VI) had a higher concentration of SMPs in the supernatant, especially in proteins compared with



the MBR fed with the same municipal wastewater without addition of Cr (VI). Lay et al. [23] observed a significant increase in the protein and polysaccharide proportions for both soluble and bound EPS in the activated sludge after adding carbamazepine, diclofenac, ibuprofen and naproxen in the feed tank (20–25  $\mu\text{g L}^{-1}$  of each pharmaceutical in the feed) of an Osmotic Membrane Bioreactor (OMBR).

(ii) Short-term discontinuous contact. Wang et al. [22] reported that a high osmotic pressure (5% NaCl) induces the production of SMPs with a relative strong hydrophilicity and protein-like characteristics in batch experiments. Sheng et al. [19] reported that in the presence of toxic chemicals, such as 30  $\text{mg l}^{-1}$  Cu (II), 40  $\text{mg l}^{-1}$  Cr (VI), 5  $\text{mg l}^{-1}$  Cd (II) for a suspension of the bacteria strains *Rhodopseudomonas acidophila*, the EPS content increased by 5.5, 2.5, and 4.0 times, respectively. Moreover, the authors reported that under these toxic conditions, the increase in the protein content far exceeded the one of the other EPS constituents.

## **2 The objectives of this thesis**

From the literature, most studies focused on the removal of pharmaceutical micropollutants in MBRs, and only a few studies investigated the effects of pharmaceutical micropollutants on MBR process, most of which dealt with the effects of pharmaceutical micropollutants on microbial activities or microbial community structure of activated sludge in MBRs. Taking into account the effects of pharmaceutical micropollutants on the SMP production in the MBR supernatant and the role of SMPs in the fouling, there is a lack of knowledge on the effects of pharmaceutical micropollutants on MBR fouling, which remains a key issue.

**The main objective in this thesis was to examine the effects of pharmaceutical micropollutants, which are frequently found in domestic wastewaters, on the MBR**

**fouling.** Carbamazepine (CBZ), an anti-epileptic drug, was selected as a representative of pharmaceutical micropollutants in this study. It was chosen because it has been frequently detected in influent as well as effluents of domestic WWTPs [82] and it is almost not biodegraded in biological wastewater treatment processes including MBRs, which makes it become a possible anthropogenic marker in the aquatic environment [83]. In addition, CBZ was found to have some inhibition effects on sludge microbial activity [76, 84].

After a bibliographic review on the removal of pharmaceutical micropollutants in MBRs, including the effects of pharmaceutical micropollutants on MBR process (see paper 1), the thesis proposes to investigate the effects of CBZ on MBR fouling in two ways:

- (i) a discontinuous contact (see paper 2), where CBZ was added in a batch reactor that uses the sludge from a lab-scale MBR to study the **short term peak effects** of CBZ on the fouling propensity of both activated sludge and its supernatant;
- (ii) a continuous contact (see paper 3), where CBZ was added continuously in a lab scale MBR via the feed to examine the **long term effects** of CBZ on MBR fouling.

The objectives are to be able to relate the short-term as well as long-term effects of CBZ on sludge fouling propensity or MBR fouling with the change in the characteristics of the sludge and supernatant after adding CBZ in batch reactors or MBR to understand the mechanisms of interactions among the CBZ, activated sludge and fouling at different durations of interaction. Fouling propensity of the sludge and supernatants (sampled both in the MBR and in the batch reactors) will be characterized using some standardized filterability tests performed with a stirred dead-end filtration cell. Fouling will also be considered at the scale of the MBR for the mixture of flocs and supernatant during MBR operation and characterized by the transmembrane pressure (TMP) time-variation. In order to explain the effects of CBZ on the

fouling compartments, it is necessary to also obtain for the two ways (continuous/discontinuous) some information:

(i) on the biological activity: Respirometric experiments were performed to examine the effects of CBZ on sludge microbial activities during the short term period as well as long term period of contact with CBZ.

(ii) on the physico-chemical properties of the sludge (particle size distribution, soluble proteins and polysaccharides, molecular weight distribution of SMPs in the supernatant): Floc size distribution analysis was performed on the activated sludge to study the effects of CBZ on physico-chemical characteristics of biomass. HPLC-SEC-fluorescence was used to characterize protein-like SMPs in supernatant and permeate of filterability tests or MBR, with the information on the fluorescence intensity in different molecular size ranges. Concentrations of proteins and polysaccharides were analyzed in supernatant and MBR permeate to study the effects of CBZ on the change of SMP composition.

Finally, the possibility of using HPLC-SEC-fluorescence as a tool for rapid estimation of the modification of fouling in MBRs, when some variations in fouling conditions happen, was proposed (see draft paper 4). In real full scale applications, the concentrations of some chemicals, including pharmaceutical micropollutants, in raw waters and the operating conditions such as organic loading rates may vary a lot, which may affect the characteristics of MBR sludge and supernatant. In both situations, the fouling in MBRs may be unsteady. Thus, there is a great interest to find some simple and cheap methods that could allow estimating the fouling propensity of MBR supernatants under unsteady conditions. The organization of this thesis is shown in Fig. 2.

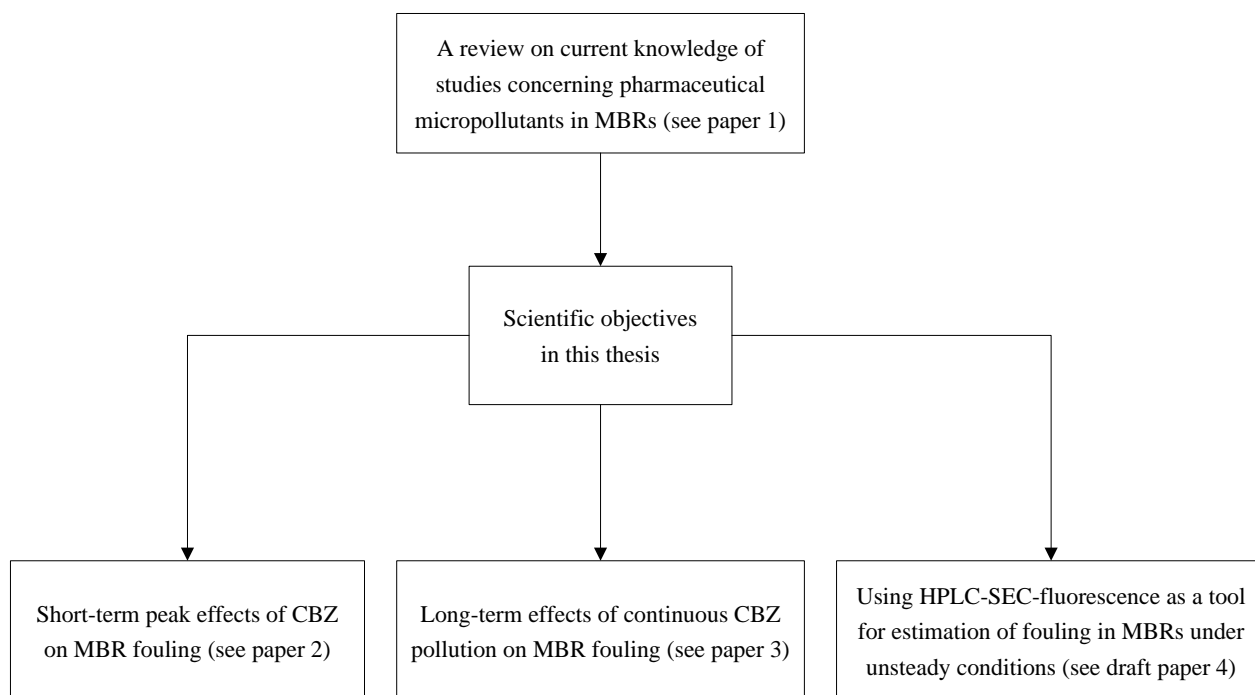


Fig. 2. Schematic diagram of the organization of this thesis

### 3. Materials and methods

This part gives the main details on materials and methods used in the following papers.

#### 3.1 Membrane bioreactor set-up

A lab-scale submerged MBR set-up consisting of an anoxic reactor (5.4 L) and an aerobic reactor (12.6 L) was used in this study (see Fig. 3). A flat sheet microfiltration membrane ( $L_{p0} = 1110 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ , Kubota, Japan) was submerged inside the aerobic reactor. It has an effective filtration area of  $0.1 \text{ m}^2$ , with an average pore size of  $0.2 \text{ }\mu\text{m}$ . Two aeration systems were used in the aerobic reactor. An aeration system with big air bubble was installed below the membrane to reduce fouling and it was operated continuously at flow rate of  $1.5 \text{ L/min}$ , i.e.  $\text{SAD}_m$  of  $0.9 \text{ m}^3 \text{ air/h/m}^2$  and  $\text{SAD}_p$  of  $90 \text{ m}^3 \text{ air/m}^3$  permeate. Besides, another aeration system with fine air bubbles was used to maintain the dissolved oxygen concentration in the sludge at about  $2\text{-}3 \text{ mg/L}$  and automatically controlled by an oxygen

sensor. The sludge recirculation ratio was fixed at 4. The MBR was fed with a municipal wastewater taken from a wastewater treatment plant close to Toulouse, France, after a primary physical treatment.

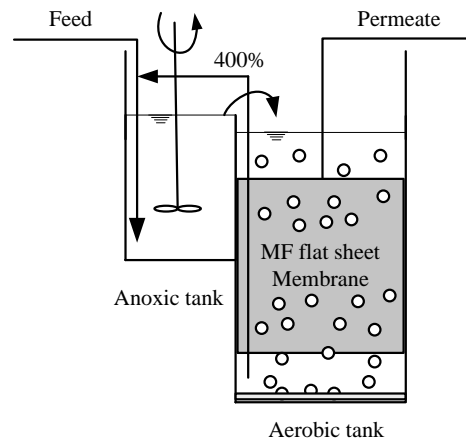


Fig. 3. Schematic diagram of the membrane bioreactor set-up

The MBR was operated in two steps (see table 1). In the first step, the MBR was operated at a sludge retention time (SRT) of 40 days, with a sludge concentration in the aerobic reactor at 4-6 g/L. In the second step, the MBR was operated at a 30 days SRT, with a sludge concentration in aerobic reactor at 6-8 g/L. The MBR reached high removal of COD, more than 95%, during both two steps. Whereas, a limited removal of TN, of about 40%, was observed during step 1 when the MBR was operated at an organic loading rate ( $C_m$ ) of about 0.1 kg COD/kg MLSS/d, due to the insufficiency of the organic source for heterotrophic denitrification [85].

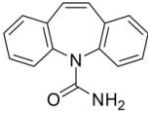
Table 1. Experimental conditions and MBR performances in this study

Step	organic loading rate (kg COD/kg MLSS/d)	Aerobic MLSS (g/L)	SRT (days)	Filtration/relaxation cycles (min/min)	Net flux (L/h/m <sup>2</sup> )	Average COD removal (%)	Average TN removal (%)
1	0.1	4-6	40	8/2	10	96.7 ± 2.2	41.4 ± 8.4
2	0.2	6-8	30	8/4	10	97.1 ± 1.6	82.5 ± 6.2

### 3.2 Physico-chemical properties of selected pharmaceutical micropollutant

Table 2 introduces the physicochemical properties of carbamazepine. During short-term batch experiments, a concentrated mother solution was prepared by dissolving carbamazepine (from Sigma-Aldrich) in pure ethanol at a concentration of 1 g/L, stored at 4 °C and used within a week. During long-term experiments, a concentrated mother solution was prepared by dissolving carbamazepine in pure ethanol at a high concentration of 10 g/L to rule out the effect of ethanol on the organic content in the feed. Then, quantities of this concentrated mother solution sufficient to yield about 90 µg L<sup>-1</sup> final concentrations of CBZ were added in the feed tank of the MBR in step 2 after the steady state of the MBR reached, i.e. in 84<sup>th</sup> day, which is higher than 2 SRTs (30 days). The final concentration of ethanol in the feed was less than 0.001% (v/v).

Table 2. Physico-chemical properties of carbamazepine [70, 82, 86]

Structure	
Formula	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O
CAS No.	298-46-4
Molecular weight (g/mol)	236
Usage	Anti-epileptic
Water solubility (mg/L at 25°C)	17.7
LogKow	2.45
Charge at pH 7	Neutral
sorption constant, K <sub>d</sub> (L/kgss)	1.2
degradation constant k <sub>biol</sub> (L/gss/d)	<0.01

### 3.3 Batch reactors

Batch reactors were used to characterize the short-term effects of CBZ on MBR sludge. Figure 4 shows the protocol used for batch experiments. Firstly, quantities of the concentrated mother solution sufficient to yield 100 µg L<sup>-1</sup> final concentrations of CBZ were added in one of the 2 batch reactors (reactor-with CBZ). As comparison, the same quantity of pure ethanol was added in the other batch reactor (control-reactor). Then the ethanol in the two batch reactors was blown off using a gentle stream of air, leaving only the CBZ in the reactors to rule out the impact of ethanol [87]. Finally, a same volume of sludge samples taken from the MBR aerobic tank was added in the two batch reactors and the mixed liquids were stirred using the aeration in the bottom of each reactor for 3 hours to dissolve CBZ and to make the sludge contact with CBZ in aerobic condition. Then the sludges in both reactors were sampled and the two corresponding supernatants were prepared by centrifugation of the sludge

samples at 4000 g during 10 minutes at room temperature at the same time. 3 hours of contact time was chosen since it takes about 3 hours before that all the readily degradable substances are consumed by the activated sludge [77]. Besides, no significant difference was found concerning the effects of CBZ on characteristics of sludge and supernatant between contact time of 3 hours and 5 hours. Too long time, for example 24 hours, for sludge in batch reactors without exogenous substrate would cause the decay of bacteria and impact the quality of activated sludge. Also, no significant difference was found concerning the effects of CBZ on characteristics of sludge and supernatant when the peak concentration of CBZ was modified to  $500 \mu\text{g L}^{-1}$ , as compared to the effects of CBZ at concentration of  $100 \mu\text{g L}^{-1}$ . Besides, no significant difference was observed between the sludge obtained from control-reactor and blank reactor where no ethanol was added (see Fig. 4) in terms of biomass and supernatant quality, which confirms that the ethanol did not affect the quality of sludge after being evaporated by the gentle stream of air.

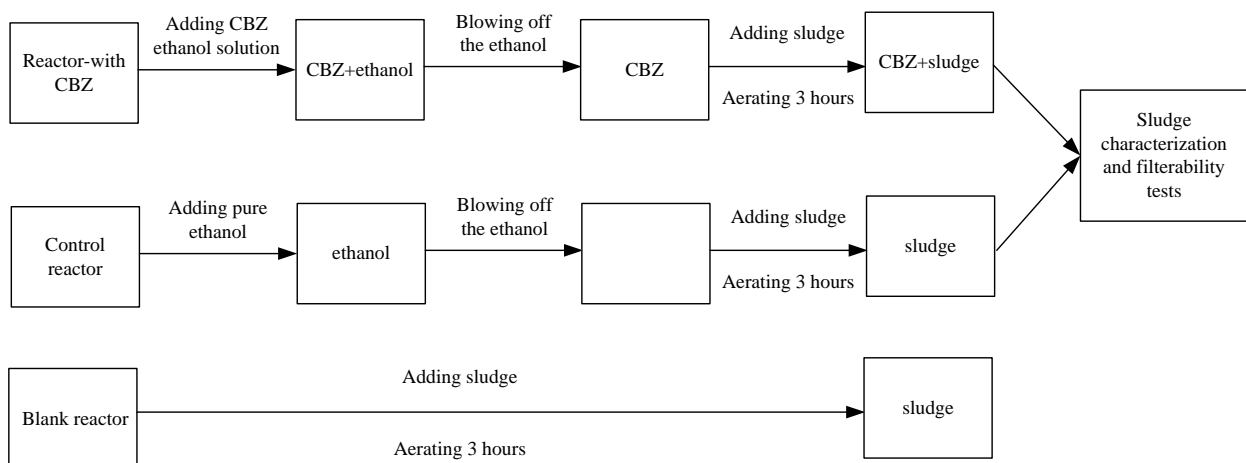


Fig. 4. Schema of batch experiments



### 3.4 Filterability tests

Fouling propensity of sludge and supernatant sampled from batch reactors (reactor-with CBZ and control-reactor) was characterized by filterability test performed with a stirred dead-end filtration cell (Amicon 8050, Millipore). Fig. 5 shows the schematic diagram of the filterability test. In the study of short-term CBZ effect, microfiltration membranes (from Alfa Laval, FR), made of polysulphone (PS), with a pore size of  $0.2 \mu\text{m}$  ( $L_{p0} = 200 \text{ l h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ ), were used for the filterability tests of sludge, because a pore size of  $0.2 \mu\text{m}$  is in the range of pore size of microfiltration membranes used in the flat sheet MBRs. Whereas, ultrafiltration membranes (from Orelis, FR), made of polyethersulfone (PES), with a nominal pore size of  $0.01 \mu\text{m}$  ( $L_{p0} = 50\text{-}80 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ ) were used for the filterability tests of supernatant to retain biopolymer components in the MBR supernatant, behaving like the second layer on MBR membrane surface. A new membrane sample was used for each filtration test. All membranes were soaked in ultra pure water over night to maintain pores wetted. Then they were rinsed by filtration of ultra pure water at 1 bar during 15 min prior to filtration test. All filtration tests were carried out in an Amicon cell (Millipore), with an effective membrane filtration surface of about  $13.4 \text{ cm}^2$ . The filtration tests were performed at three constant transmembrane pressures (TMP): 0.5 bar, 0.8 bar, and 1.0 bar. For each filtration test, sludge or supernatant was filtered through selected membranes until 70 ml permeate was produced (corresponding to a filtered permeate volume of about  $50 \text{ L m}^{-2}$ ); temperature was measured and flux was normalized to the flux at  $20 \text{ }^\circ\text{C}$ . For supernatant filtration, no stirring was performed, and for sludge filtration, the sludge was stirred at 200 rpm. After filtration the membrane was turned face down and was backwashed with ultra pure water at 1 bar for 15 min, in order to investigate flux recovery by permeability measurement. In the study of long-term CBZ effect, ultrafiltration membranes (Microdyn-Nadir GmbH,

Germany), made of polyethersulfone (PES), with a molecular weight cutoff (MWCO) 150 kDa ( $L_{p0} = 400\text{-}600 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ ) were used for the filterability tests of both sludge and supernatant. For the 150 kDa PES membranes, the same protocol for the filterability test was used except that prior to filtration test, they were rinsed by filtration of ultra pure water at 0.7 bar during 15 min and all filtration tests were carried out only at constant TMP of 0.5 bar. Membrane fouling resistance was determined according to Eq. (1).

$$J = \frac{TMP}{\mu(R_m + R_f)} \quad (1)$$

In this equation, J is the permeate flux ( $\text{m}^3 \text{ s}^{-1} \text{ m}^{-2}$ ) at  $20 \text{ }^\circ\text{C}$ , with  $R_m$  the membrane resistance ( $\text{m}^{-1}$ ),  $R_f$  the fouling resistance ( $\text{m}^{-1}$ ), TMP the transmembrane pressure (Pa) and  $\mu$  is the dynamic viscosity at  $20 \text{ }^\circ\text{C}$  (Pa s).

In the dead-end filtration cell,  $\alpha C$  can be determined by cake deposit model as expressed in Eq. (2)

$$R_f = \alpha C \frac{V}{A} \quad (2)$$

In this equation,  $\alpha$  is the specific cake resistance ( $\text{m kg}^{-1}$ ), C is the deposited mass per filtered permeate volume ( $\text{kg m}^{-3}$ ), V is the cumulated permeate volume ( $\text{m}^3$ ) and A is the effective membrane area for filtration ( $\text{m}^2$ ). For supernatant filtration, C could be calculated by the retained DOC ( $\text{kg m}^{-3}$ ). For sludge filtration, C is assumed to be MLSS value of sludge.

### 3.5 Characterization of sludge microbial activity

Sludge microbial activity was characterized by the respiration rate, i.e. oxygen uptake rate (OUR). It was measured in respirometric batch experiments as defined by Sperandio and Paul

[88]. During study on short-term effect, sludge samples were collected from the aerobic tank of the MBR. Firstly, the collected sludge was aerated for at least 3 h to reach the endogenous condition. Because CBZ was prepared in pure ethanol, pure ethanol was used as exogenous substrate in the respirometric experiments. Then pure ethanol was added to respirometric reactor to test the biological activity of sludge before adding CBZ. Then quantities of the concentrated mother solution sufficient to yield  $100 \mu\text{g L}^{-1}$  final concentrations of CBZ were added in the respirometric reactor to test the inhibition effect of CBZ on the activated sludge. During the long-term effect study, the sludge samples were collected from the aerobic tank of the MBR before and after addition of CBZ in the MBR. Then, the collected sludge was aerated for at least 3 h to reach the endogenous condition. Then, sodium acetate (NaAC), a readily biodegradable compound, was used as exogenous substrate to measure the maximum respiration rate. Then the exogenous respiration rate (see Fig. 5) was calculated as the difference between the maximum respiration rate and the endogenous respiration rate (measured in the endogenous condition). The specific exogenous respiration rate is defined as the exogenous respiration rate divided by the concentration of mixed liquor suspended solids (MLSS).

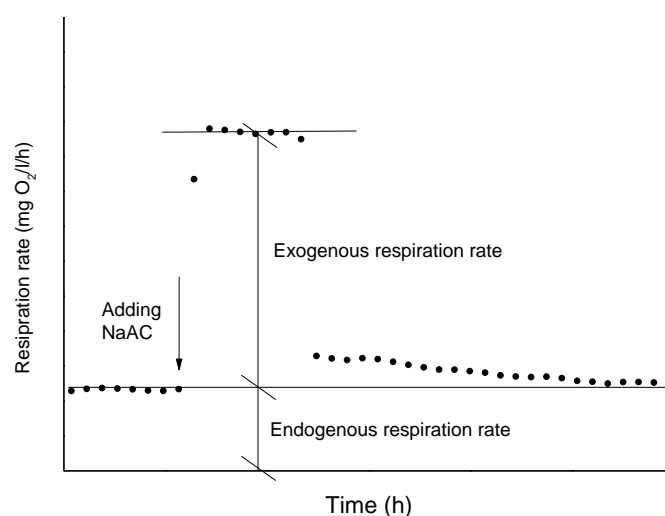


Fig. 5. Schematic diagram of respirometric analysis

### 3.6 Analytical methods

The concentration of mixed liquor suspended solids (MLSS) was measured according to the standard method AFNOR NFT 90-105. Chemical oxygen demand (COD) and total nitrogen (TN) were measured by spectrometry with reagent kits. Dissolved organic carbon (DOC) was measured by a Total Organic Carbon analyser (Shimadzu, FR). Prior to analysis of DOC, each sample was prefiltered on a 0.45 µm membrane. Floc size distribution was measured by Malvern Mastersizer 2000. Protein content in supernatant, expressed in mg equivalent of bovine serum albumin per litre, was measured by the acid bicinchoninic method [89]. Polysaccharide concentration in supernatant, expressed in mg equivalent of glucose per litre, was measured by the Anthrone method [90]. Carbamazepine concentration was analyzed using LC-MS/MS method by the Departmental Water Laboratory in Toulouse, France.

High performance liquid chromatography—size exclusion chromatography (HPLC—SEC) analysis was performed by an Akta Purifer (GE Healthcare, USA), equipped with a column Protein Kw804 (Shodex, Japan), a silica-based column with an exclusion limit of 1000 kDa, and a fluorescence detector (Varian, USA). The eluent solution for the mobile phase was prepared with 25 mM Na<sub>2</sub>SO<sub>4</sub> and a phosphate buffer (2.4 mM NaH<sub>2</sub>PO<sub>4</sub> and 1.6 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 6.8). The flow rate of eluent passing through the column was 1.0 mL/min. Excitation/emission wavelengths were set at 280/350 nm, which were confirmed as the required values by EEM fluorescence results [44, 46], to detect protein-like substances. The column was calibrated using standard proteins: thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa) from GE Healthcare. Prior to analysis of HPLC-SEC-fluorescence, each sample was prefiltered on a 0.45 µm membrane.

## 4 Main results of the work

### 4.1 Effects of CBZ on fouling

#### 4.1.1 Effect of CBZ on sludge fouling propensity

Fouling propensity is defined as the fouling resistance produced, which is measured by filterability test that uses a dead-end filtration cell, when a fixed volume of permeate was filtered. The short term effect of CBZ on sludge fouling propensity was examined by the filterability tests using sludges sampled from batch reactors (reactor-with CBZ and control-reactor), detailed in paper 2. Due to the significant differences in the SMP composition and microbial activity of activated sludge in MBRs operated under different organic loading rates, sludge from MBR operated under two different organic loading rates, i.e. 0.1 kg COD/kg MLSS/d and 0.2 kg COD/kg MLSS/d, respectively, were tested in batch reactors. Fig. 6 shows result of the filterability tests of sludges, sampled from batch reactors using sludge from the MBR operated under organic loading rate of 0.1 kg COD/kg MLSS/d, at 1.0 bar. The result showed that with addition of CBZ in the sludge the fouling propensity was significantly higher (32%). Reduced CBZ effect (12%) on fouling resistance was observed for sludge from MBR operated under organic loading rate of 0.2 kg COD/kg MLSS/d (see Fig. 7).

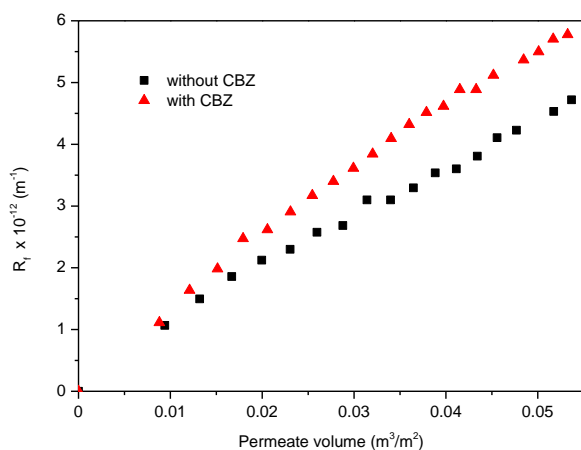


Fig. 6. Short-term effect of CBZ on sludge fouling propensity ( $C_m = 0.1$  kg/kg/d,  $CBZ = 100 \mu\text{g L}^{-1}$ )

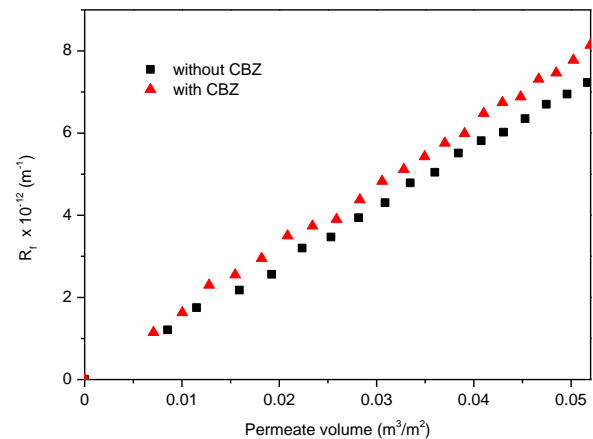


Fig. 7. Short-term effect of CBZ on sludge fouling propensity ( $C_m = 0.2$  kg/kg/d,  $CBZ = 100 \mu\text{g L}^{-1}$ )

Concerning the long term CBZ effect, sludge and supernatant filterability tests using sludge and supernatant sampled from MBR before and after addition of CBZ were performed at 0.5 bar, detailed in paper 3. Fig. 8 shows that the continuous addition of CBZ in MBR increased the fouling propensity of sludge. However, result from Fig. 9 shows that the addition of CBZ in MBR decreased the fouling propensity of supernatant.

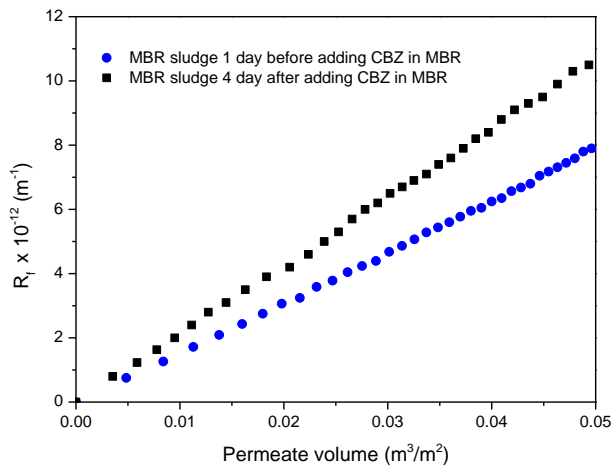


Fig. 8. Long-term CBZ effect on sludge fouling propensity ( $C_m = 0.2 \text{ kg/kg/d}$ ,  $\text{CBZ} = 90 \text{ } \mu\text{g L}^{-1}$ )

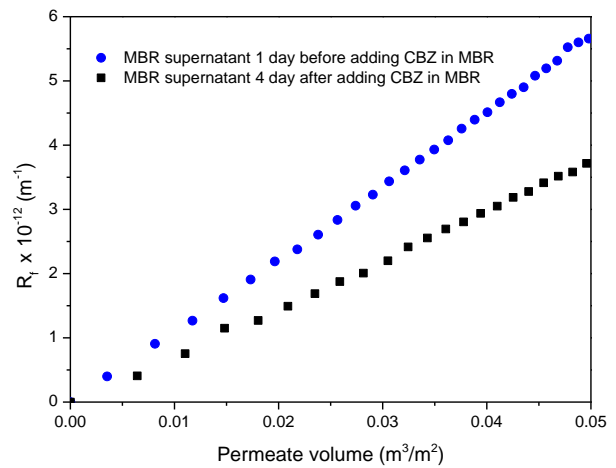


Fig. 9 Long-term CBZ effect on supernatant fouling propensity ( $C_m = 0.2 \text{ kg/kg/d}$ ,  $\text{CBZ} = 90 \text{ } \mu\text{g L}^{-1}$ )

#### 4.1.2 Effect of CBZ on MBR fouling

Effect of CBZ on MBR fouling during the long-term contact was characterized by the variation of TMP in MBR, detailed in paper 3. Fig. 10 presents the time-variation of TMP during operation of the MBR. Four different periods and compartments can be distinguished (no membrane cleaning was performed during the four periods):

- Before the addition of CBZ in the MBR quite steady fouling conditions were reached, where TMP increases very slightly.
- 1 day after addition of CBZ in MBR, a significant increase of TMP was observed. Thanks to the addition of CBZ the TMP jump was very rapidly created and it

clearly appears as an answer of the bioseparation process to the effect of a stress on the biomass.

- Between 1 day and 8 days after addition of CBZ, TMP increased gradually while still at a higher rate as compared to that during the steady fouling conditions before addition of CBZ.
- Starting from 8 days after addition of CBZ, a continuous TMP jump started to occur, where TMP increased continuously at a significantly higher rate.

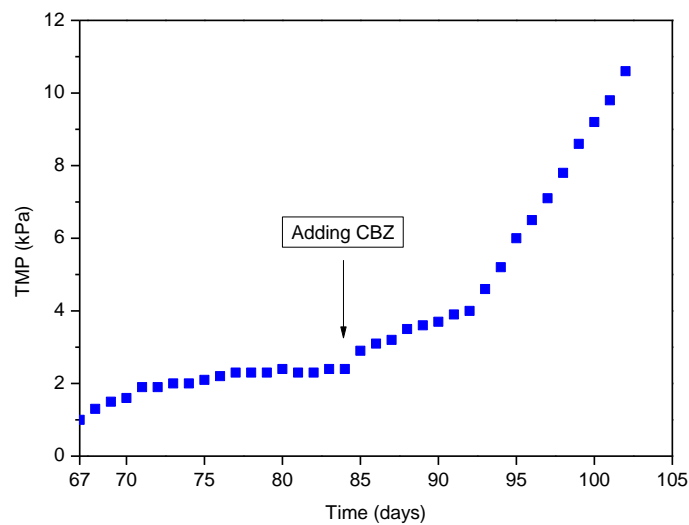


Fig. 10. Effect of CBZ on TMP of MBR

In conclusion, the contact of activated sludge with CBZ increased the sludge fouling propensity and MBR fouling. Three assumptions can be proposed to explain the increased sludge fouling propensity and MBR fouling after addition of CBZ: i) a change in microbial activity linked to a stress on the microorganisms ii) a change in biomass physico-chemical properties, like mean floc size distribution, or iii) a change in the supernatant quality. These three assumptions will be discussed in the following paragraphs that aim to evaluate the effects of CBZ on the supernatant and biomass properties.

## 4.2 Effects of CBZ on biomass characteristics

### 4.2.1 Effect of CBZ on sludge microbial activity

Effect of CBZ on sludge microbial activity was characterized by respirometric experiments. During the short-term contact in batch reactor, no inhibition of sludge microbial activity with presence of  $100 \mu\text{g L}^{-1}$  CBZ in the sludge was observed (see paper 2). Whereas, during the long term contact (see paper 3), the continuous addition of CBZ in the MBR via feed ( $90 \mu\text{g L}^{-1}$  in the feed) caused about 20% inhibition of the sludge microbial activity, expressed as specific exogenous respiration rate in the MBR, at the beginning several days, as shown in Fig. 11. No further inhibition was observed between 4 days and 11 days after addition of CBZ in the MBR, which indicates that some extent of adaption or acclimation of the activated sludge was reached from 4 days after addition of CBZ in the MBR. However, during the long-term contact, after addition of CBZ in MBR, no significant change of the COD and TN removal was observed, as compared to that before addition of CBZ. This suggests that the inhibition is also not significant in the long-term contact condition.

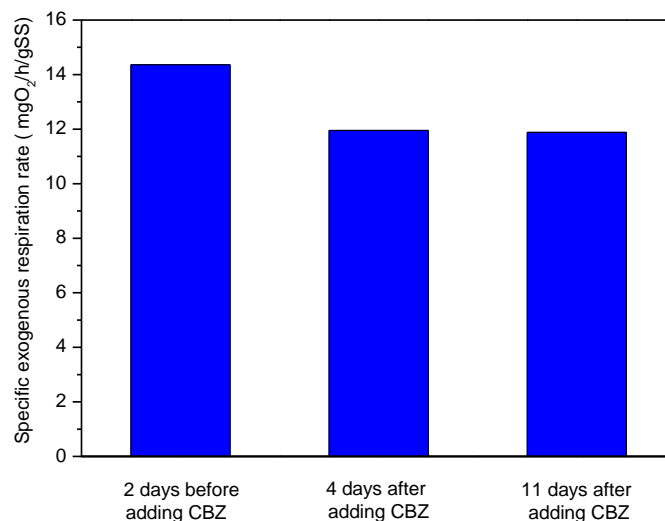


Fig. 11. Long-term effect of CBZ on sludge specific exogenous respiration rate



In conclusion, the effect of CBZ on sludge microbial activity is not significant in both short-term and long-term situations and the modification of sludge fouling propensity and MBR fouling could not be attributed to the modification in sludge microbial activity.

#### 4.2.2 Effect of CBZ on sludge floc size distribution

No significant effects of CBZ on sludge floc size distribution were observed during short-term peak contact as well as during the long-term continuous contact (see paper 2 and paper 3). This may indicate that no significant bacterial decay (lysis) occurred when facing CBZ stress in both situations. Thus, the modification of sludge fouling propensity and MBR fouling could not be attributed to the modification in sludge floc size distribution.

### **4.3 Effects of CBZ on supernatant characteristics**

#### 4.3.1 Effect of CBZ on SMP composition in supernatant

The short term effects of CBZ on supernatant composition were examined on supernatants sampled from the batch reactors (see paper 2). Fig. 12 shows the result of protein and polysaccharide concentrations in the supernatants from batch reactors using the sludges from the MBR operated under organic loading rate of 0.2 kg COD/kg MLSS/d. Slight increase in protein concentration (about 20%) was observed in supernatant from the reactor-with CBZ, as compared with that from the control-reactor. In contrast, no significant difference in polysaccharide concentration was found in supernatant from both reactors.

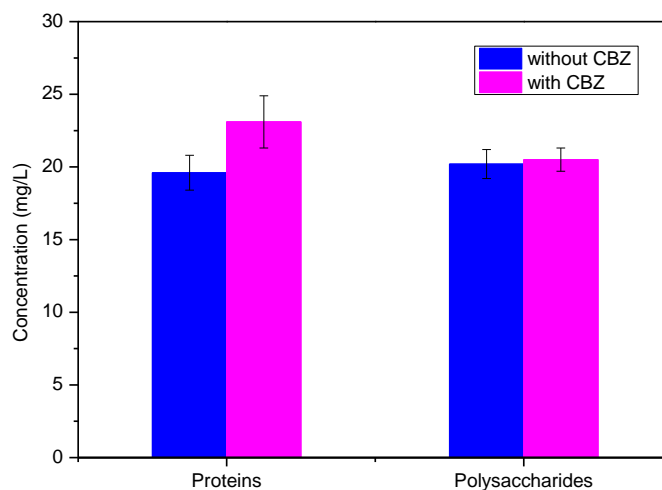


Fig. 12. Short-term effect of CBZ on SMP composition in supernatant

The long term effects of CBZ on supernatant composition were examined on supernatants sampled from MBR before and after addition of CBZ in MBR (see paper 3). Fig. 13 shows that a significant increase in the protein concentration in supernatant was observed just 1 day after addition of CBZ, then the protein concentration kept almost constant for several days, and then reduced effect on protein production was observed after about 7 days after the addition of CBZ in MBR. However, no significant increase in retention of protein in MBR was found, which indicates that the protein with a higher concentration were mainly relatively small molecules that could pass through the MBR membrane and biocake. In contrast, no significant change was observed for the polysaccharide concentration in supernatant after addition of CBZ in MBR.

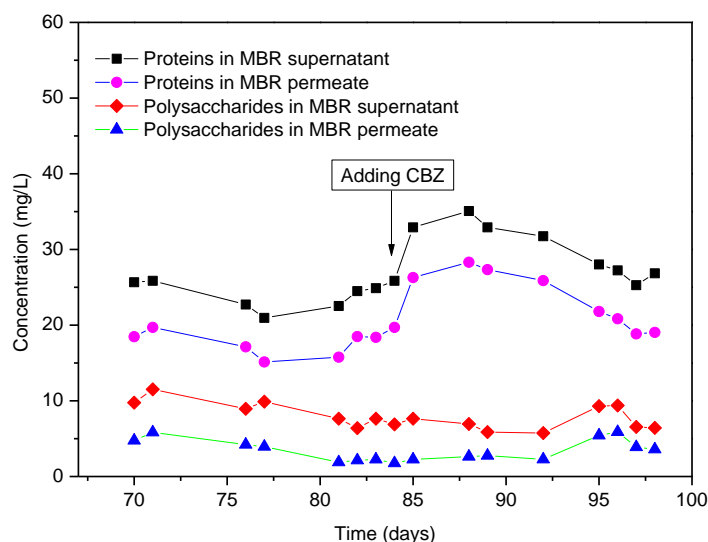


Fig. 13. Long-term effect of CBZ on SMP concentration in supernatant and permeate of MBR

In conclusion, consistent results were found concerning the effects of CBZ on SMP composition in supernatants in both short-term and long-term contact situations, i.e. an increase of protein SMPs and no significant change of polysaccharide SMPs after addition of CBZ in the sludge. The result found in this study indicates that protein-like SMPs seemed to play a more important role for microorganisms when facing a pharmaceutical stress. Thus, the increase in sludge fouling propensity and MBR fouling with addition of CBZ could be attributed to the modification of protein-like SMPs in supernatant, since no significant effect was observed in floc size distribution and microbial activity under short-term as well as long-term continuous contact conditions.

#### 4.3.2 Effect of CBZ on SMP molecular size distribution in supernatant

To further investigate the role of protein-like SMPs in fouling, the molecular size distribution of protein-like SMPs was characterized by HPLC-SEC-fluorescence with excitation/emission wavelengths set at 280/350 nm. In case of the short-term contact (see paper 2), HPLC-SEC analysis of supernatants prepared from the batch reactors using sludges that were taken from

the MBR operated under low organic loading rate (0.1 kg COD/kg MLSS/d) was shown in Fig. 14. The molecular weight of protein-like substances in MBR supernatants showed bimodal distribution. Indeed, two peaks were observed in the HPLC-SEC results which represent two groups of protein-like molecules, i.e. molecular size of 100-1000 kDa and 10-100 kDa, respectively. A significantly higher peak of protein-like substances corresponding to 100-1000 kDa molecules in the supernatant prepared from reactor-with CBZ was observed as compared to that in the supernatant prepared from the control-reactor. In contrast, no significant difference of detected peak heights of protein-like substances with a molecular size of 10-100 kDa between the two supernatants that were prepared from the reactor-with CBZ and the control-reactor was observed. Taking into account that the 100-1000 kDa protein-like SMPs were completely retained by 0.2  $\mu\text{m}$  PS membranes during the sludge filtration tests (see paper 2), the increased sludge fouling propensity with addition of CBZ in short-term contact condition (see Fig. 6) could be attributed to the increase of 100-1000 kDa protein-like SMPs in supernatant.

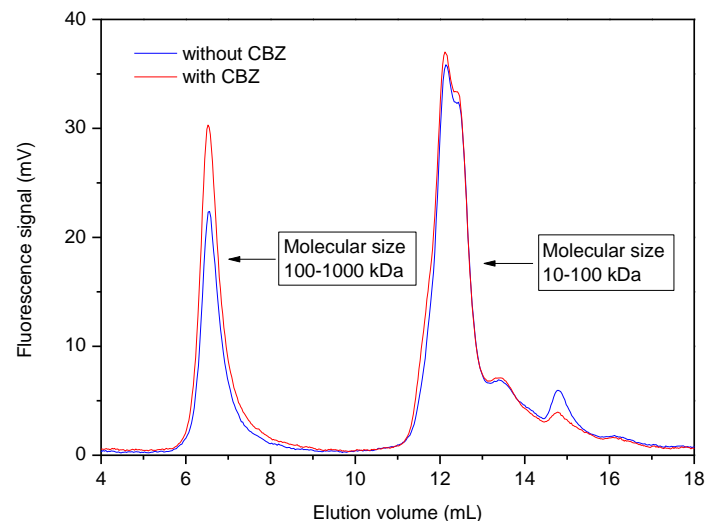


Fig. 14. Short-term effect of CBZ on protein-like SMPs in supernatant ( $C_m = 0.1$  kg COD/kg MLSS/d)

To investigate the impact of organic loading rate on CBZ effects, supernatants coming from batch reactors using sludges that were taken from the MBR operated under organic loading rate of 0.2 kg COD/kg MLSS/d were analyzed by HPLC-SEC, as shown in Fig. 15. Comparing with the HPLC-SEC result of supernatants from batch reactors that using sludges that were taken from MBR operated under low organic loading rate of 0.1 kg COD/kg MLSS/d (see Fig. 14), much less significant effect of CBZ on 100-1000 kDa protein-like SMPs was observed, which could explain the reduced effect of CBZ on fouling propensity of sludge that was from the MBR operated under organic loading rate of 0.2 kg COD/kg MLSS/d (see Fig. 7).

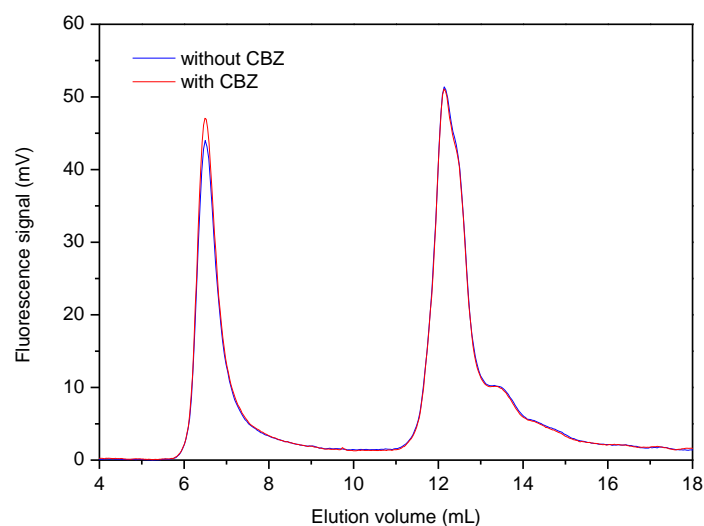


Fig. 15. Short-term effect of CBZ on protein-like SMPs in supernatant ( $C_m = 0.2$  kg COD/kg MLSS/d)

Concerning the long-term CBZ effect (see paper 3), MBR supernatants before and after adding CBZ in the MBR were analyzed by HPLC-SEC, as shown in Fig. 16. The result from Fig. 16 showed that the presence of  $90 \mu\text{g L}^{-1}$  CBZ in MBR during a long-term period could cause two measurable effects on protein-like SMPs:

- a decrease of the concentration of 100-1000 kDa protein-like SMPs. More specifically, 1 day after addition of CBZ in the MBR, a significant decrease (about 16%) of 100-1000 kDa protein-like substances, was observed in the MBR supernatant. Whereas, only a slight decrease of 100-1000 kDa protein-like SMP was observed between 1 day and 7 days after addition of CBZ in MBR.
- a more significant increase of 10-100 kDa protein concentration. More specifically, 1 day after addition of CBZ in the MBR, a quite significant increase of the 10-100 kDa protein-like substances, about 23%, was observed in the MBR supernatant. Whereas, only a slight increase of 10-100 kDa protein-like substances was observed in MBR supernatant between 1 day and 7 days after addition of CBZ.

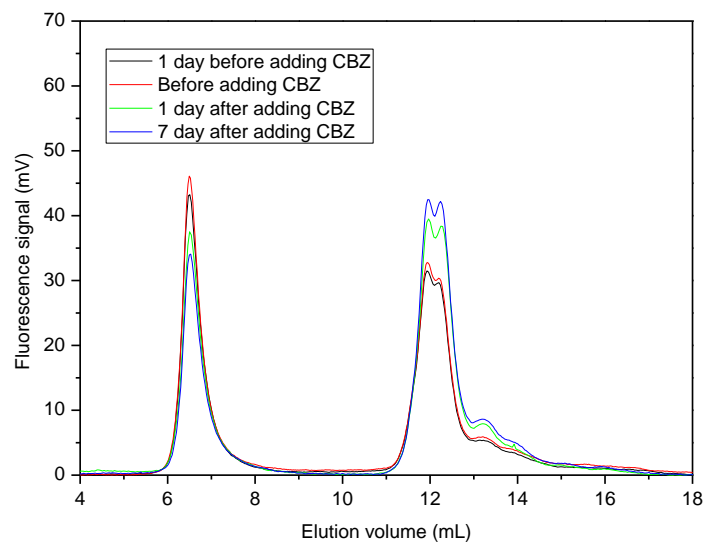


Fig. 16. Long-term effect of CBZ on protein-like SMPs in supernatant of MBR

To investigate the role of different molecular size protein-like SMPs on the sludge and supernatant fouling propensity, the retention of protein-like SMPs in the MBR supernatant during sludge and supernatant filtration tests was analyzed by HPLC-SEC, as shown in Fig. 17 and Fig. 18. The results showed that the 100-1000 kDa protein-like SMPs could be almost

completely retained by 150 kDa PES membranes during both sludge and supernatant filtration tests. However, concerning the retention of 10-100 kDa protein-like SMPs, different retention behavior was observed. No retention of 10-100 kDa protein-like SMPs was found during the supernatant filtration test as shown in Fig. 17, whereas partial retention of 10-100 kDa protein-like SMPs was observed during the sludge filtration test, which indicates that the fouling layer formed during the sludge filtration is more compact than that formed during the supernatant filtration. The results also suggests that the supernatant fouling propensity mainly depends on the 100-1000 kDa SMPs, whereas the both 10-100 kDa SMPs and 100-1000 kDa SMPs could affect the sludge fouling propensity.

Thus, the increase in sludge fouling propensity during long-term contact (see Fig. 8) could be attributed to the significant increase in 10-100 kDa protein-like SMPs in supernatant after addition of CBZ (see Fig. 16). Whereas, the decrease in supernatant fouling propensity (Fig. 9) could be attributed to the decrease in 100-1000 kDa protein-like after addition of CBZ in MBR (see Fig. 16). This further demonstrated that the 10-100 kDa protein-like SMPs in supernatant also played an important role in sludge fouling propensity. The difference between the effect of CBZ on sludge filterability and supernatant filterability could be attributed to the different deposits formed on the membrane surface during the filtration tests, since the same membranes were used for the sludge and supernatant filterability tests. Thus, the increased sludge fouling propensity could be attributed to the significant increase in 10-100 kDa protein-like SMPs in supernatant that may reduce the voids in the biocake when passing through the biocake and modify the structure of biocake formed on the membrane surface.

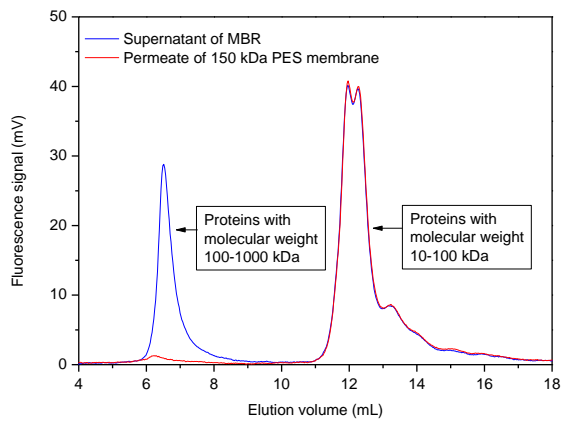


Fig. 17. HPLC-SEC analysis of supernatant and permeate in the supernatant filterability test by 150 kDa PES membrane

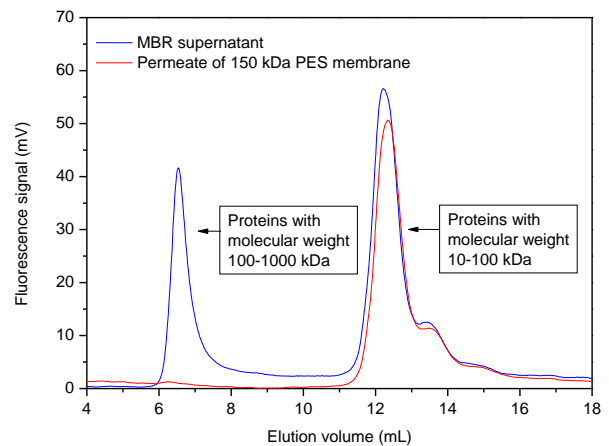


Fig. 18. HPLC-SEC analysis of supernatant and permeate in the sludge filterability test by 150 kDa PES membrane

In conclusion, the effect of CBZ on molecular weight distribution of protein-like SMPs in supernatant depends on the contact time. During short-term contact, the CBZ induced augmentation of 100-1000 kDa protein-like SMPs. In contrast, during long-term contact, the CBZ induced augmentation of 10-100 kDa protein-like SMPs and a decrease of 100-1000 kDa protein-like SMPs. Both 10-100 kDa and 100-1000 kDa protein-like SMPs in supernatant contribute to the increase in sludge fouling propensity. The 100-1000 kDa protein-like SMPs could be retained by the biocake on membrane surface and thus enhance formation of biofilm in the biocake, while the 10-100 kDa protein-like SMPs could reduce the voids in the biocake and modify the biocake structure.

#### 4.4 Effect of CBZ on characteristics of MBR biocake

The characteristics of MBR biocake could be examined by its retention capacity for SMPs in supernatant, detailed in paper 3. Fig. 19 shows the HPLC-SEC analysis of supernatant and permeate obtained in the MBR on the day just before adding CBZ. The result showed that



before adding CBZ, the MBR membrane retained almost all the 100-1000 kDa protein-like substances. In contrast, almost all the 10-100 kDa protein-like substances passed through the MBR membrane. Fig. 20 shows that the 10-100 kDa protein-like that were present in the MBR supernatant were partially retained by the MBR membrane 11 days after addition of CBZ. Taking into account the role of 10-100 kDa in modification of biocake structure, it could be assumed that the significant increase of 10-100 kDa protein-like SMPs in MBR supernatant after addition of CBZ induced an enhanced accumulation of these small biopolymers inside the biocake, which caused the loss of voids in the biocake, and finally led to a more “tight” retention characteristics of the MBR “membrane”.

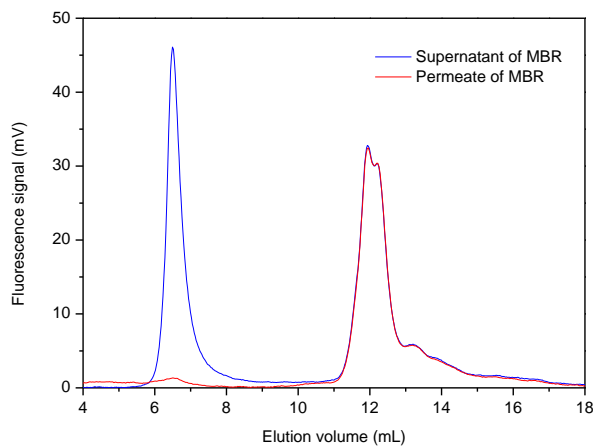


Fig. 19 HPLC-SEC analysis of MBR supernatant and permeate before adding CBZ in MBR

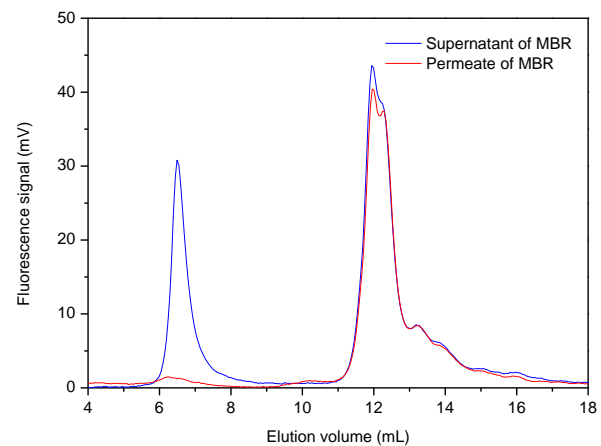


Fig. 20 HPLC-SEC analysis of MBR supernatant and permeate 11 days after adding CBZ in MBR

Thus, the TMP plateau before addition of CBZ in MBR could be attributed to the retention of 100-1000 kDa SMPs in supernatant and thus to the formation of the biofilm and fouling layer on the membrane surface by protein bridges and bindings, since no retention of 10-100 kDa protein-like substances in supernatant was found. The TMP jump that appeared 1 day after addition of CBZ may be caused by the significant increase of 10-100 kDa protein-like SMPs in the supernatant at that time. The sudden significant increase of 10-100 kDa protein-like

SMPs may cause a sudden loss of voids inside biocake, which increased the resistance of permeate passing through the MBR membrane. The gradual TMP increase, not the continuous TMP jump, between 1 day and 8 days after addition of CBZ could be attributed to the reduced effect of CBZ on protein-like SMP production (see Fig. 16). The higher increase rate in TMP during this period could be explained by the continued accumulation of 10-100 kDa protein-like SMPs inside biocake. The TMP jump that appeared after the gradual increase of TMP may be due to that the accumulation of 10-100 kDa protein-like SMPs, i.e. loss of voids inside biocake, reached a critical point, leading to that local flux starts to exceed the critical value. The result from Fig. 20 could support this assumption, where partial retention of 10-100 kDa protein-like substances was observed during this period.

As compressibility of fouling layer may also contribute to the MBR fouling, biocake compressibility test was performed by filtration of ultra-pure water through a formed biocake at different TMP in filtration cell, as shown in Fig. 21. Firstly, biocake was formed after filterability test of MBBR sludge (sampled at 11 days after addition of CBZ) at 0.5 bar. Then, Ultra-pure water was filtrated through the formed biocake at different TMP to examine the compressibility of biocake. Since no organic foulants were introduced into the formed biocake during the filtration of ultra-pure water, the increase in the fouling resistance could be attributed to the compressing effect of biocake. Moreover, slight higher fouling rate was observed at higher TMP, which further indicates that the fouling resistance increased more quickly with increasing TMP. Thus, the biocake formed by the MBR sludge is compressible and its compressibility may contribute to the continuous TMP jump, since the compressing effect could aggravate the MBR fouling when TMP increases significantly.

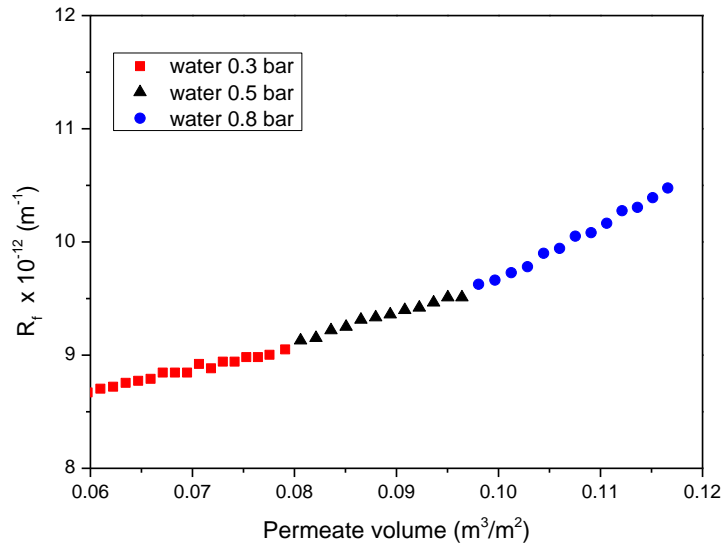


Fig. 21. Biocake compressibility test (MLSS=6.2 g/L)

In conclusion, consistent results concerning the CBZ effects on sludge fouling resistance were found in both short-term and long-term contact conditions, whereas caused by different fouling mechanisms due to the different effects of CBZ on molecular size distribution of protein-like SMPs between these two conditions. For filterability tests of sludges sampled from batch reactors during short term contact, the increase in fouling rate of sludge with addition of CBZ originated from the increased retention of 100-1000 kDa protein-like SMPs. Whereas for MBR fouling during long-term CBZ contact, the increase in MBR fouling rate, i.e. the TMP increase rate, after addition of CBZ was due to the increase of 10-100 kDa protein-like SMPs in the MBR supernatant, which accumulated inside the biocake that was formed on the MBR membrane, thus modified the biocake structure, and finally together with the compressing effect of biocake resulted in the TMP jump.

## 4. 5 HPLC-SEC-fluorescence for estimation of fouling in MBRs

### 4.5.1 In case of fouling situations modified by organic loading rates

In this case (see paper 4), supernatants sampled from MBR operated under different organic loading rates were analysed by HPLC-SEC-fluorescence. Then the modifications in HPLC-SEC-fluorescence results were compared with the membrane fouling rate in the MBR. Fig. 22 presents the time-variation of permeability during operation of the MBR with the numbers in the figure to indicate the sampling of supernatants. Result from Fig. 22 showed when the organic loading rate was increased from 0.1 kg COD/kg MLSS/d (set 1 and set 2) to 0.2 kg COD/kg MLSS/d (set 3), and to 0.4 kg COD/kg MLSS/d (set 4), increased average fouling rate, expressed as the decrease rate of permeability, in MBR was observed, i.e. from about 40 L/h/m<sup>2</sup>/bar/d (set 1 and set 2) to about 90 L/h/m<sup>2</sup>/bar/d (set 3) and to about 140 L/h/m<sup>2</sup>/bar/d (set 4).

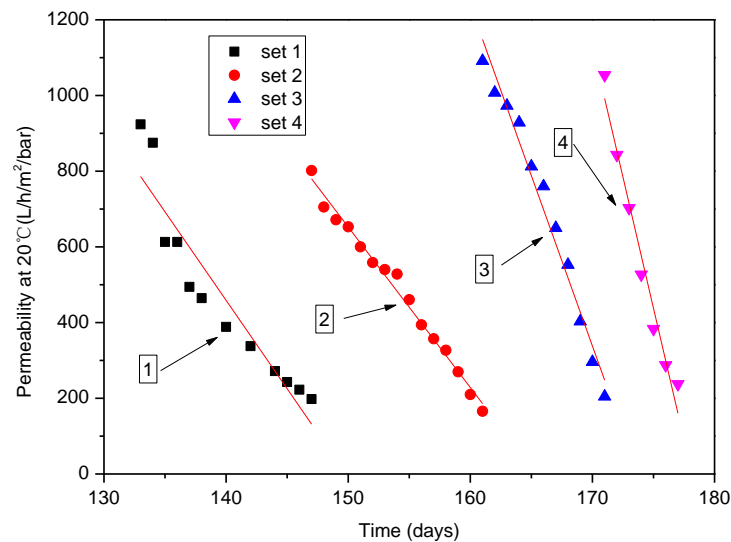


Fig. 22. Permeability of MBR operated under varied organic loading rates

Fig. 23 shows the results of HPLC-SEC-fluorescence analysis of supernatants sampled from MBR under different organic loading rates. As shown in Fig. 23, a significant increase of protein-like substances with molecular size of 100-1000 kDa was observed in MBR

supernatant, when the organic loading rate of MBR was increased, from 0.1 kg COD/kg MLSS/d (sample 1 and sample 2) to 0.2 kg COD/kg MLSS/d (sample 3), and to 0.4 kg COD/kg MLSS/d (sample 4). Whereas, no significant difference was observed concerning the content of 10-100 kDa protein-like SMPs when the organic loading rate of MBR was increased, from 0.1 kg COD/kg MLSS/d (sample 1 and sample 2) to 0.2 kg COD/kg MLSS/d (sample 3). Considering the complete retention of 100-1000 kDa protein-like SMPs by the MBR membrane (see Fig. 19), the significant increase in protein-like 100-1000 kDa SMPs when increasing the organic loading rate of MBR led to the higher fouling rate of MBR membrane, as indicted in Fig 22. The result indicates that HPLC-SEC-fluorescence could help to estimate the fouling in MBR that is caused by variation of 100-1000 kDa protein-like SMPs in MBR supernatant by characterizing the variation of 100-1000 kDa protein-like SMPs when the change in operating conditions of MBR occurs.

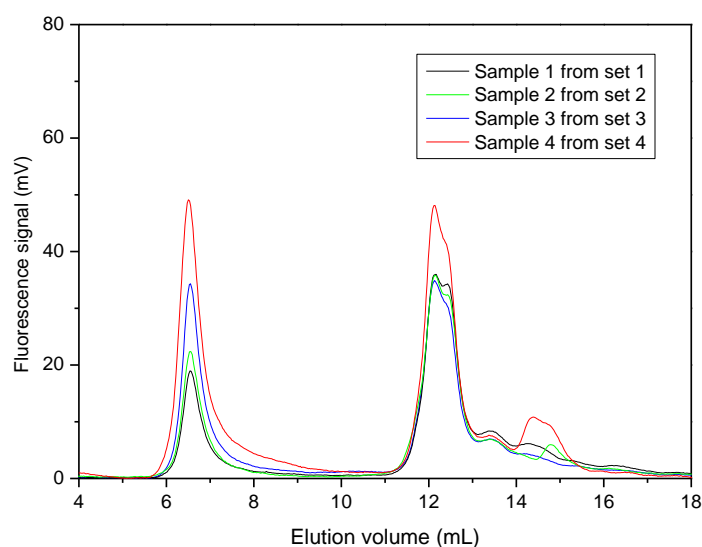


Fig. 23. HPLC-SEC analysis of supernatants of MBR under different organic loading rates

As the peak height is a simple and effective indicator to stand for the fluorescence intensity and to quantify the constituent content in the HPLC-SEC-fluorescence analysis [44] and almost complete retention of protein-like compounds with molecular size of 100-1000 kDa in

supernatant is obtained by MBR membrane (see Fig. 19), the fluorescence peak height of 100-1000 kDa protein-like compounds could be considered as fouling indicator to estimate fouling in MBRs. To relate the peak height of protein-like substances in 100-1000 kDa range with fouling propensity of supernatant samples (sample 1, 2, 3 and 4), the peak heights of 100-1000 kDa protein-like compounds in supernatants and calculated  $\alpha C$  values of supernatants obtained in filterability tests using 0.01  $\mu\text{m}$  PES membranes at three different TMP (0.5 bar, 0.8 bar, 1.0 bar) are plotted in Fig. 24. The result showed good linear relation between the peak height and  $\alpha C$  value at all three TMP for the selected samples, which further confirmed the feasibility of using HPLC-SEC-fluorescence as a tool to estimate fouling propensity of MBR supernatants.

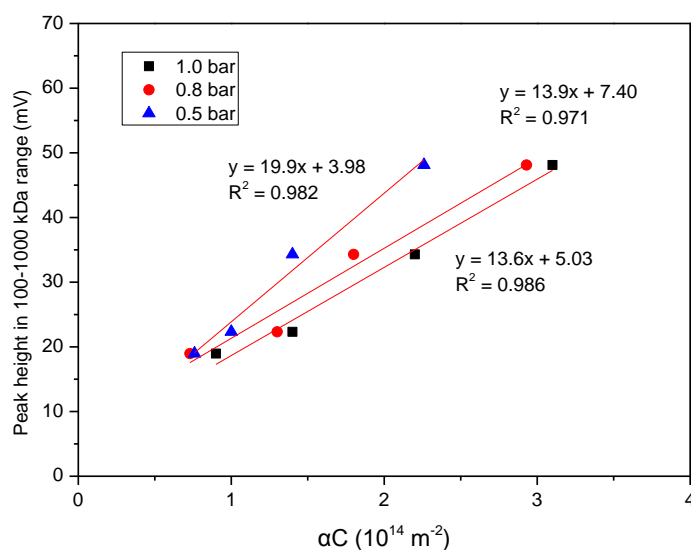


Fig. 24 Peak heights versus the  $\alpha C$  values of supernatants

#### 4.5.2 In case of fouling situations modified by continuous CBZ pollution

In this case, peak heights of protein-like substances in 100-1000 kDa range and calculated  $\alpha C$  values in filterability tests of 4 supernatants sampled from MBR before and after addition of CBZ in MBR, using 150 kDa PES membranes at TMP 0.5 bar, were plotted in Fig. 25. The

result also showed good linear relation between the peak height of 100-1000 kDa protein-like SMPs and  $\alpha C$  value. Remind that after addition of CBZ in MBR, a decrease of protein-like substances in 100-1000 kDa was observed (see Fig. 16) and a significant increase of TMP occurred in MBR (see Fig. 10). This means in case that fouling situations were modified by continuous CBZ pollution, the use of fluorescence peak height of 100-1000 kDa protein-like compounds as fouling indicator for MBR fouling is not sufficient, although peak height of 100-1000 kDa protein-like compounds is still a good fouling indicator for fouling propensity of MBR supernatants. Taking into account the role of 10-100 kDa protein-like SMPs in fouling of MBR contaminated by continuous pharmaceutical pollution (see paper 3), the capacity of HPLC-SEC-fluorescence to capture the change in 10-100 kDa protein-like SMPs in supernatant may help to estimate the modification of fouling trends of MBR under this situation.

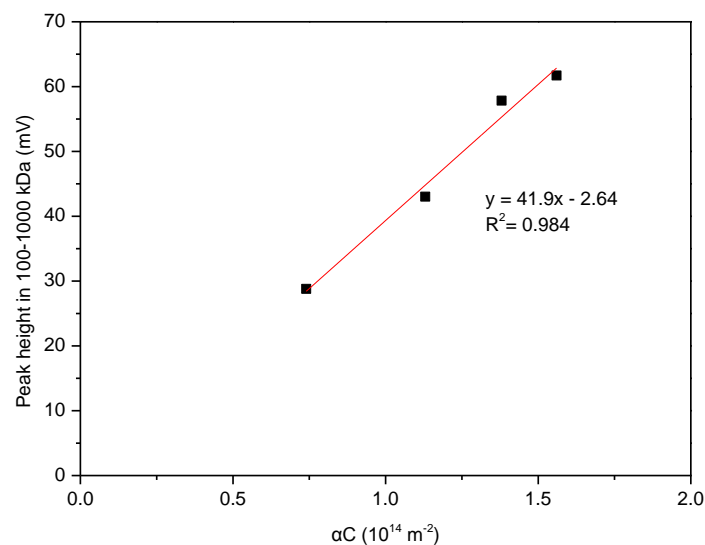


Fig. 25 Peak heights versus the  $\alpha C$  values of supernatants

In conclusion, the peak height of 100-1000 kDa protein-like compounds detected by the HPLC-SEC-fluorescence could be used as a fouling indicator in ultrafiltration of biological fluids like the secondary effluents from conventional activated sludge process and in MBRs

whatever the situations, as well as a fouling indicator in MBRs under different organic loading rates. However, concerning the fouling in MBR contaminated by continuous pharmaceutical pollution, the peak height of 10-100 kDa protein-like compounds detected by HPLC-SEC-fluorescence need to be used as a complementary fouling indicator to estimate the modification of fouling trends in this situation.

## **5. Conclusions and perspectives**

The presence of pharmaceutical micropollutants in domestic wastewater raised the questions like if the presence of pharmaceutical micropollutants may affect the MBR process, especially the fouling of MBR. This study investigated the effects of CBZ on sludge fouling propensity in batch experiments as well as on MBR fouling in a continuously operated lab-scale MBR. The effects of CBZ on activated sludge of MBR depend on contact time (continued or discontinued mode) due to the different microbial reactions in these two modes. The short term contact of CBZ with sludge led to an increase in 100-1000 kDa protein-like SMPs due to decay (lysis) of bacteria. In contrast, the continuous contact of CBZ with sludge in MBR induced the production of 10-100 kDa protein-like SMPs due to the defensive responses of bacteria facing CBZ stress. Table 3 summarized the effects of CBZ on characteristics of MBR sludge and supernatant in these two situations. Due to the different effects of CBZ on the characteristics of MBR sludge and supernatant in the two contact ways, the fouling mechanisms involved in these two situations are quite different. Both 100-1000 kDa and 10-100 kDa protein-like SMPs have potential to contribute to sludge fouling propensity or membrane fouling in MBR. The 100-1000 kDa SMPs could be completely retained by MF/UF membrane or MBR membrane during sludge filtration, leading to the formation of biofilm in biocake, which contributes to higher sludge fouling propensity and a steady increase of MBR fouling. The 10-100 kDa protein-like SMPs could be partially retained by MF/UF



membrane or MBR membrane during sludge filtration, leading to the reduction of the voids of biocake and result in change of biocake structure, which could lead to higher sludge fouling propensity and higher fouling rate and TMP jump in MBR.

Table 3. The comparison between the effects of CBZ on characteristics of MBR sludge

	Short-term peak effects	Long-term continuous effects
Sludge microbial activity	No significant effect	Slight inhibition
Mean floc size	No significant effect	Slight increase
Polysaccharide SMPs	No significant effect	No significant effect
Protein SMPs	Increase	Increase
100-1000 kDa protein-like SMPs	Increase	Decrease
10-100 kDa protein-like SMPs	No significant effect	Increase

This study paves ways to understand the complex interactions among pharmaceutical micropollutants, activated sludge and membrane fouling in different contact durations. The important role of the protein-like SMPs in these interactions has been demonstrated. The use of HPLC-SEC-fluorescence as a useful tool to study the phenomena involved in these interactions has also been demonstrated. However, many questions are remained unanswered. More study need to be performed to distinguish the two stress effects involved: the decay of cells and the defensive responses by the viable bacteria. Also, studies concerning the effects of pharmaceutical micropollutants on the change of microbial community structures and the corresponding effects on biofouling potential should be investigated.

To improve the capacity of MBR on removal of pharmaceutical micropollutants, more studies should be carried out to identify the bacterial species that favor removal of CBZ as well as other pharmaceuticals in MBR and determine the optimal operation conditions that help to enrich these bacterial species. In addition, the mass transfer and biodegradation of pharmaceuticals in sludge phase and also in the deposits of the membrane surface need to be examined to better understand the removal mechanisms of pharmaceutical micropollutants in MBR.

More tools such as LC-OCD (liquid chromatography with organic carbon detector), CLSM (confocal laser scanning microscopy), in-situ fouling layer analysis like LSGI (laser sheet at grazing incidence) etc. need to be employed to further analyze the biocake composition and structure to validate the hypotheses made in this study.

Moreover, future studies with the objectives to increase removal of pharmaceutical micropollutants and at the same time reduce fouling of MBR in presence of pharmaceutical stress need to be performed. For example, powdered activated carbon (PAC) could be added in MBR to increase removal of CBZ via adsorption and thus reduce the induced stress effects. Moreover, the process like integrated MBR-RO-Ozonation could be examined, which uses ozone for RO (reverse osmosis) concentrate treatment and recycles RO concentrate to MBR reactor after the treatment. The ozonation could reduce the toxic effects and also increase biodegradability of persistent substances in MBR.

In the last, more studies concerning the risk assessment of pharmaceutical micropollutants on human health and aquatic organisms should be conducted to evaluate the necessity of advanced treatment process like MBR to be employed in the wastewater treatment and reuse schemes.

## References

- [1]. P. Le-Clech, V. Chen, T.A. Fane, 2006. Fouling in membrane bioreactors used in wastewater treatment. *J. Membr. Sci.* 284, 17-53.
- [2]. A. Drews, 2010. Membrane fouling in membrane bioreactors—characterisation, contradictions, cause and cures. *J. Membr. Sci.* 363, 1-28.
- [3]. S. Ognier, C. Wisniewski, A. Grasmick, 2002. Influence of macromolecule adsorption during filtration of a membrane bioreactor mixed liquor suspension. *J. Membr. Sci.* 209, 27-37.
- [4]. F. Meng, S.-R. Chae, A. Drews, M. Kraume, H.-S. Shin, F. Yang, 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. *Water Res.* 43, 1489-1512.
- [5]. C. Wisniewski, A. Grasmick, 1998. Floc size distribution in a membrane bioreactor and consequences for membrane fouling. *Colloids Surf. Physicochem. Eng. Aspects* 138, 403-411.
- [6]. E.H. Bouhabila, R. Ben Aim, H. Buisson, 2001. Fouling characterisation in membrane bioreactors. *Separation and Purification Technology* 22, 123-132.
- [7]. S. Rosenberger, C. Laabs, B. Lesjean, R. Gnirss, G. Amy, M. Jekel, J.-C. Schrotter, 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. *Water Res.* 40, 710-720.
- [8]. M. Hernandez Rojas, R. Van Kaam, S. Schetrite, C. Albasi, 2005. Role and variations of supernatant compounds in submerged membrane bioreactor fouling. *Desalination* 179, 95-107.
- [9]. C.S. Laspidou, B.E. Rittmann, 2002. A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. *Water Res.* 36, 2711-2720.
- [10]. D.J. Barker, D.C. Stuckey, 1999. A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Res.* 33, 3063-3082.
- [11]. S. Liang, C. Liu, L. Song, 2007. Soluble microbial products in membrane bioreactor operation: behaviors, characteristics, and fouling potential. *Water Res.* 41, 95-101.
- [12]. R.S. Trussell, R.P. Merlo, S.W. Hermanowicz, D. Jenkins, 2006. The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. *Water Res.* 40, 2675-2683.
- [13]. K. Kimura, N. Yamato, H. Yamamura, Y. Watanabe, 2005. Membrane fouling in pilot-scale membrane bioreactors (MBRs) treating municipal wastewater. *Environ. Sci. Technol.* 39, 6293-6299.
- [14]. A. Masse, M. Spérandio, C. Cabassud, 2006. Comparison of sludge characteristics and performance of a submerged membrane bioreactor and an activated sludge process at high solids retention time. *Water Res.* 40, 2405-2415.

- [15]. D. Al-Halbouni, J. Traber, S. Lyko, T. Wintgens, T. Melin, D. Tacke, A. Janot, W. Dott, J. Hollender, 2008. Correlation of EPS content in activated sludge at different sludge retention times with membrane fouling phenomena. *Water Res.* 42, 1475-1488.
- [16]. M. Stricot, A. Filali, N. Lesage, M. Spírandio, C. Cabassud, 2010. Side-stream membrane bioreactors: Influence of stress generated by hydrodynamics on floc structure, supernatant quality and fouling propensity. *Water Res.* 44, 2113-2124.
- [17]. A. Menniti, S. Kang, M. Elimelech, E. Morgenroth, 2009. Influence of shear on the production of extracellular polymeric substances in membrane bioreactors. *Water Res.* 43, 4305-4315.
- [18]. S. Comte, G. Guibaud, M. Baudu, 2006. Biosorption properties of extracellular polymeric substances (EPS) resulting from activated sludge according to their type: soluble or bound. *Process Biochem.* 41, 815-823.
- [19]. G.-P. Sheng, H.-Q. Yu, Z.-B. Yue, 2005. Production of extracellular polymeric substances from *Rhodospseudomonas acidophila* in the presence of toxic substances. *Applied microbiology and biotechnology* 69, 216-222.
- [20]. L. Zhou, Z. Zhang, W. Jiang, W. Guo, H.-H. Ngo, X. Meng, J. Fan, J. Zhao, S. Xia, 2014. Effects of low-concentration Cr(VI) on the performance and the membrane fouling of a submerged membrane bioreactor in the treatment of municipal wastewater. *Biofouling* 30, 105-114.
- [21]. C. Sun, T. Leiknes, J. Weitzenböck, B. Thorstensen, 2010. Salinity effect on a biofilm-MBR process for shipboard wastewater treatment. *Separation and Purification Technology* 72, 380-387.
- [22]. Z.-P. Wang, T. Zhang, 2010. Characterization of soluble microbial products (SMP) under stressful conditions. *Water Research* 44, 5499-5509.
- [23]. W.C. Lay, Q. Zhang, J. Zhang, D. McDougald, C. Tang, R. Wang, Y. Liu, A.G. Fane, 2012. Effect of pharmaceuticals on the performance of a novel osmotic membrane bioreactor (OMBR). *Sep. Sci. Technol.* 47, 543-554.
- [24]. F. Meng, Z. Wang, Y. Li, 2012. Cure of filament-caused MBR fouling in the presence of antibiotics: taking ciprofloxacin exposure as an example. *Industrial & Engineering Chemistry Research* 51, 13784-13791.
- [25]. U. Metzger, P. Le-Clech, R.M. Stuetz, F.H. Frimmel, V. Chen, 2007. Characterisation of polymeric fouling in membrane bioreactors and the effect of different filtration modes. *J. Membr. Sci.* 301, 180-189.
- [26]. S. Arabi, G. Nakhla, 2010. Impact of molecular weight distribution of soluble microbial products on fouling in membrane bioreactors. *Separation and Purification Technology* 73, 391-396.
- [27]. T. Jiang, M.D. Kennedy, V.D. Schepper, S.-N. Nam, I. Nopens, P.A. Vanrolleghem, G. Amy, 2010. Characterization of soluble microbial products and their fouling impacts in membrane bioreactors. *Environ. Sci. Technol.* 44, 6642-6648.

- [28]. S. Arabi, G. Nakhla, 2008. Impact of protein/carbohydrate ratio in the feed wastewater on the membrane fouling in membrane bioreactors. *J. Membr. Sci.* 324, 142-150.
- [29]. N. Yamato, K. Kimura, T. Miyoshi, Y. Watanabe, 2006. Difference in membrane fouling in membrane bioreactors (MBRs) caused by membrane polymer materials. *J. Membr. Sci.* 280, 911-919.
- [30]. S. Rosenberger, H. Evenblij, S. Te Poele, T. Wintgens, C. Laabs, 2005. The importance of liquid phase analyses to understand fouling in membrane assisted activated sludge processes—six case studies of different European research groups. *J. Membr. Sci.* 263, 113-126.
- [31]. G. Ferrero, I. Rodríguez-Roda, J. Comas, 2012. Automatic control systems for submerged membrane bioreactors: A state-of-the-art review. *Water Res.* 46, 3421-3433.
- [32]. Y. Shen, W. Zhao, K. Xiao, X. Huang, 2010. A systematic insight into fouling propensity of soluble microbial products in membrane bioreactors based on hydrophobic interaction and size exclusion. *J. Membr. Sci.* 346, 187-193.
- [33]. J. Lee, W.-Y. Ahn, C.-H. Lee, 2001. Comparison of the filtration characteristics between attached and suspended growth microorganisms in submerged membrane bioreactor. *Water Res.* 35, 2435-2445.
- [34]. N. Lesage, M. Sperandio, C. Cabassud, 2008. Study of a hybrid process: Adsorption on activated carbon/membrane bioreactor for the treatment of an industrial wastewater. *Chemical Engineering and Processing: Process Intensification* 47, 303-307.
- [35]. J.H. Roorda, J.H. Van der Graaf, 2005. *SUR* test used for optimisation of membrane filtration plants treating wastewater effluents. *Desalination* 179, 131-150.
- [36]. H. Evenblij, S. Geilvoet, J. Van der Graaf, H. Van der Roest, 2005. Filtration characterisation for assessing MBR performance: three cases compared. *Desalination* 178, 115-124.
- [37]. T. de la Torre, M. Mottschall, B. Lesjean, A. Drews, A. Iheanaetu, M. Kraume, 2010. Filterability assessment in membrane bioreactors using an in-situ filtration test cell. *Water Sci. Technol.* 61.
- [38]. C. Huyskens, E. Brauns, E. Van Hoof, H. De Wever, 2008. A new method for the evaluation of the reversible and irreversible fouling propensity of MBR mixed liquor. *J. Membr. Sci.* 323, 185-192.
- [39]. J.-X. Li, R. Sanderson, G. Chai, 2006. A focused ultrasonic sensor for in situ detection of protein fouling on tubular ultrafiltration membranes. *Sensors Actuators B: Chem.* 114, 182-191.
- [40]. J. Mendret, C. Guigui, P. Schmitz, C. Cabassud, 2009. In situ dynamic characterisation of fouling under different pressure conditions during dead-end filtration: compressibility properties of particle cakes. *J. Membr. Sci.* 333, 20-29.
- [41]. P. Loulergue, C. André D. Laux, J.-Y. Ferrandis, C. Guigui, C. Cabassud, 2011. In-situ characterization of fouling layers: which tool for which measurement? *Desalination Water Treat.* 34, 156-162.
- [42]. P. Loulergue, M. Weckert, B. Reboul, C. Cabassud, W. Uhl, C. Guigui, 2014. Mechanisms of action of particles used for fouling mitigation in membrane bioreactors. *Water Res.* 66, 40-52.

- [43]. F. Meng, B. Liao, S. Liang, F. Yang, H. Zhang, L. Song, 2010. Morphological visualization, componential characterization and microbiological identification of membrane fouling in membrane bioreactors (MBRs). *J. Membr. Sci.* 361, 1-14.
- [44]. N. Her, G. Amy, D. McKnight, J. Sohn, Y. Yoon, 2003. Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection. *Water Research* 37, 4295-4303.
- [45]. H. Shon, S. Vigneswaran, I.S. Kim, J. Cho, H. Ngo, 2006. Fouling of ultrafiltration membrane by effluent organic matter: A detailed characterization using different organic fractions in wastewater. *J. Membr. Sci.* 278, 232-238.
- [46]. B. Teychene, C. Guigui, C. Cabassud, 2011. Engineering of an MBR supernatant fouling layer by fine particles addition: A possible way to control cake compressibility. *Water Res.* 45, 2060-2072.
- [47]. R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. Von Gunten, B. Wehrli, 2006. The challenge of micropollutants in aquatic systems. *Science* 313, 1072-1077.
- [48]. M. Auriol, Y. Filali-Meknassi, R.D. Tyagi, C.D. Adams, R.Y. Surampalli, 2006. Endocrine disrupting compounds removal from wastewater, a new challenge. *Process Biochem.* 41, 525-539.
- [49]. C.G. Daughton, T.A. Ternes, 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Perspect.* 107, 907.
- [50]. K. Kümmerer, 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, 957-969.
- [51]. D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environ. Sci. Technol.* 36, 1202-1211.
- [52]. B. Halling-Sørensen, S. Nors Nielsen, P. Lanzky, F. Ingerslev, H. Holten Lützhøft, S. Jørgensen, 1998. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. *Chemosphere* 36, 357-393.
- [53]. N. Le-Minh, S. Khan, J. Drewes, R. Stuetz, 2010. Fate of antibiotics during municipal water recycling treatment processes. *Water Res.* 44, 4295-4323.
- [54]. L.H.M.L.M. Santos, A.N. Araújo, A. Fachini, A. Pena, C. Delerue-Matos, M.C.B.S.M. Montenegro, 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *J. Hazard. Mater.* 175, 45-95.
- [55]. M. Crane, C. Watts, T. Boucard, 2006. Chronic aquatic environmental risks from exposure to human pharmaceuticals. *Sci. Total Environ.* 367, 23-41.
- [56]. K. Fent, A.A. Weston, D. Caminada, 2006. Ecotoxicology of human pharmaceuticals. *Aquat. Toxicol.* 76, 122-159.

- [57]. K. Kümmerer, 2009. The presence of pharmaceuticals in the environment due to human use—present knowledge and future challenges. *J. Environ. Manage.* 90, 2354-2366.
- [58]. O.A. Jones, J.N. Lester, N. Voulvoulis, 2005. Pharmaceuticals: a threat to drinking water? *Trends Biotechnol.* 23, 163-167.
- [59]. C. Li, C. Cabassud, C. Guigui, 2014. Evaluation of membrane bioreactor on removal of pharmaceutical micropollutants: a review. *Desalination and Water Treatment* In press.
- [60]. J.P. Bound, N. Voulvoulis, 2006. Predicted and measured concentrations for selected pharmaceuticals in UK rivers: implications for risk assessment. *Water Res.* 40, 2885-2892.
- [61]. T. Heberer, 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.* 131, 5-17.
- [62]. K. Kimura, H. Hara, Y. Watanabe, 2005. Removal of pharmaceutical compounds by submerged membrane bioreactors (MBRs). *Desalination* 178, 135-140.
- [63]. M. Bernhard, J. Müller, T.P. Knepper, 2006. Biodegradation of persistent polar pollutants in wastewater: comparison of an optimised lab-scale membrane bioreactor and activated sludge treatment. *Water Res.* 40, 3419-3428.
- [64]. J. Radjenović, M. Petrović, D. Barceló, 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water Res.* 43, 831-841.
- [65]. T.A. Larsen, J. Lienert, A. Joss, H. Siegrist, 2004. How to avoid pharmaceuticals in the aquatic environment. *J. Biotechnol.* 113, 295-304.
- [66]. T. Urase, C. Kagawa, T. Kikuta, 2005. Factors affecting removal of pharmaceutical substances and estrogens in membrane separation bioreactors. *Desalination* 178, 107-113.
- [67]. T.A. Ternes, N. Herrmann, M. Bonerz, T. Knacker, H. Siegrist, A. Joss, 2004. A rapid method to measure the solid–water distribution coefficient ( $K_d$ ) for pharmaceuticals and musk fragrances in sewage sludge. *Water Res.* 38, 4075-4084.
- [68]. M. Carballa, F. Omil, J.M. Lema, 2007. Calculation Methods to Perform Mass Balances of Micropollutants in Sewage Treatment Plants. Application to Pharmaceutical and Personal Care Products (PPCPs). *Environ. Sci. Technol.* 41, 884-890.
- [69]. J. Sipma, B. Osuna, N. Collado, H. Monclús, G. Ferrero, J. Comas, I. Rodriguez-Roda, 2010. Comparison of removal of pharmaceuticals in MBR and activated sludge systems. *Desalination* 250, 653-659.
- [70]. A. Joss, E. Keller, A.C. Alder, A. Göbel, C.S. Mc Ardell, T. Ternes, H. Siegrist, 2005. Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water Research* 39, 3139-3152.
- [71]. E.M. Golet, I. Xifra, H. Siegrist, A.C. Alder, W. Giger, 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environ. Sci. Technol.* 37, 3243-3249.

- [72]. A. Joss, S. Zabczynski, A. Göbel, B. Hoffmann, D. Löffler, C.S. McArdell, T.A. Ternes, A. Thomsen, H. Siegrist, 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. *Water Res.* 40, 1686-1696.
- [73]. P. Verlicchi, M. Al Aukidy, A. Galletti, M. Petrovic, D. Barceló, 2012. Hospital effluent: investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Science of the Total Environment* 430, 109-118.
- [74]. K.D. Brown, J. Kulis, B. Thomson, T.H. Chapman, D.B. Mawhinney, 2006. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. *Science of the Total Environment* 366, 772-783.
- [75]. D. Larsson, C. de Pedro, N. Paxeus, 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *Journal of hazardous materials* 148, 751-755.
- [76]. M. Aubenneau, A. Tahar, C. Casellas, C. Wisniewski, 2010. Membrane bioreactor for pharmaceutically active compounds removal: Effects of carbamazepine on mixed microbial communities implied in the treatment. *Process Biochemistry* 45, 1826-1831.
- [77]. L.F. Delgado, S. Schetrite, C. Gonzalez, C. Albasi, 2010. Effect of cytostatic drugs on microbial behaviour in membrane bioreactor system. *Bioresource technology* 101, 527-536.
- [78]. B. Kraigher, T. Kosjek, E. Heath, B. Kompare, I. Mandic-Mulec, 2008. Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors. *Water Research* 42, 4578-4588.
- [79]. L. Holakoo, G. Nakhla, E.K. Yanful, A.S. Bassi, 2006. Chelating properties and molecular weight distribution of soluble microbial products from an aerobic membrane bioreactor. *Water Res.* 40, 1531-1538.
- [80]. I.D. Henriques, N.G. Love, 2007. The role of extracellular polymeric substances in the toxicity response of activated sludge bacteria to chemical toxins. *Water Res.* 41, 4177-4185.
- [81]. N. Lesage, 2005, PhD thesis. Etude d'un procédé hybride Adsorption/Bioréacteur à membranes pour le traitement des effluents industriels.
- [82]. Y. Zhang, S.-U. Geißen, C. Gal, 2008. Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere* 73, 1151-1161.
- [83]. M. Clara, B. Strenn, N. Kreuzinger, 2004. Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration. *Water Res.* 38, 947-954.
- [84]. S. Wang, R.M. Holzem, C.K. Gunsch, 2008. Effects of pharmaceutically active compounds on a mixed microbial community originating from a municipal wastewater treatment plant. *Environ. Sci. Technol.* 42, 1091-1095.



- [85]. M. Kumar, P.-Y. Lee, T. Fukusihma, L.-M. Whang, J.-G. Lin, 2012. Effect of supplementary carbon addition in the treatment of low C/N high-technology industrial wastewater by MBR. *Bioresource technology* 113, 148-153.
- [86]. C. Abegglen, A. Joss, C.S. McArdell, G. Fink, M.P. Schlüsener, T.A. Ternes, H. Siegrist, 2009. The fate of selected micropollutants in a single-house MBR. *Water Research* 43, 2036-2046.
- [87]. S. Wang, C.K. Gunsch, 2011. Effects of selected pharmaceutically active compounds on treatment performance in sequencing batch reactors mimicking wastewater treatment plants operations. *Water Research* 45, 3398-3406.
- [88]. M. Spáandio, E. Paul, 2000. Estimation of wastewater biodegradable COD fractions by combining respirometric experiments in various  $S_o/X_o$  ratios. *Water Research* 34, 1233-1246.
- [89]. P. Smith, R.I. Krohn, G. Hermanson, A. Mallia, F. Gartner, M. Provenzano, E. Fujimoto, N. Goeke, B. Olson, D. Klenk, 1985. Measurement of protein using bicinchoninic acid. *Analytical biochemistry* 150, 76-85.
- [90]. R. Dreywood, 1946. Qualitative test for carbohydrate material. *Industrial & Engineering Chemistry Analytical Edition* 18, 499-499.

# Publication 1

---

---



# Evaluation of membrane bioreactor on removal of pharmaceutical micropollutants: a review

Accepted for publication in Desalination and Water Treatment

Chengcheng Li<sup>a,b,c,\*</sup>, Corinne Cabassud<sup>a,b,c</sup>, Christelle Guigui<sup>a,b,c</sup>

<sup>a</sup> Université de Toulouse, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, 31077 Toulouse, France

<sup>b</sup> INRA, UMR792 Ingénierie des Systèmes biologiques et des Procédés, 31400 Toulouse, France

<sup>c</sup> CNRS, UMR5504, 31400 Toulouse, France

\* Corresponding author at: INSA Toulouse, Hall GPE, 135 Avenue de Rangueil, 31077 Toulouse, France. Tel.: +33 05 61 55 97 91 ; Email : chengcheng.li@insa-toulouse.fr.

## Abstract

Municipal wastewater reclamation and reuse has become an important solution in many places around the world to deal with water scarcity problems. Among the available treatment approaches, membrane bioreactor (MBR) has a great potential to become a key element in municipal wastewater reclamation and reuse schemes due to its significantly higher treated effluent quality as compared to the conventional activated sludge process. As great concerns have been raised to some emerging trace pollutants found in aquatic environment in the last decade, notably the pharmaceuticals, removal of pharmaceutical micropollutants by MBR or MBR related processes should be evaluated to further understand the status of MBR in different wastewater treatment and reuse schemes. This paper gives an overview on removal of pharmaceutical micropollutants by MBR or MBR related processes, such as activated carbon assisted MBR, combined MBR-RO (membrane bioreactor and reverse osmosis) process etc.

**Key words:** pharmaceutical micropollutants; membrane bioreactor; activated carbon assisted MBR; MBR-RO; wastewater treatment and reuse

## 1. Introduction

Water scarcity is still a big challenge facing humanity in many places around the world. Thus, municipal wastewater has been considered to be an alternative water source for various applications after proper treatment [1-2]. Membrane bioreactor (MBR) which couples the activated sludge process and membrane separation, i.e. microfiltration (MF) or ultrafiltration (UF), has a significant potential to become a key element in municipal wastewater reclamation and reuse schemes worldwide, since it greatly improves the treated effluent quality as compared to conventional activated sludge (CAS) process, especially for removal of pathogenic microorganisms and micropollutants [3-4]. Besides, when higher effluent quality is required in some reuse applications, activated carbon could be added into MBR to further improve removal of organic contaminants [5]. Moreover, MBR could be employed as pre-treatment process for reverse osmosis (RO) which is served as a secondary barrier for removal of pathogenic bacteria, viruses and hazardous chemicals [6-7].

In the last decade, great attention has been paid to some emerging trace organic pollutants, also called micropollutants, such as endocrine-disrupting compounds, pharmaceuticals, personal care products etc., found in aquatic environment [8-9]. Among these micropollutants, pharmaceuticals got special concerns since late 1990s [10-13], as pharmaceuticals are designed to have some biological effect and to be persistent to avoid being metabolized before having a curing effect [14]. Besides, concerns have been raised regarding the possibility that continuous discharge of antibiotics to aquatic environment may facilitate the development or proliferation of resistant strains of bacteria [15]. Moreover, chronic toxicity effects have been reported for aquatic organisms exposed to human pharmaceuticals at trace concentration [16-18]. Thus, the existence of pharmaceutical micropollutants in aquatic environment may pose a potential danger on human health as well as aquatic organisms.

Therefore, extensive studies have been carried out to remove pharmaceutical micropollutants in municipal wastewater by all kinds of treatment approaches. In the literature, several reviews have been performed on removal of pharmaceutical micropollutants in municipal wastewater treatment [19-25], however, no review has been conducted specially on removal of pharmaceutical micropollutants by MBR or MBR related processes, such as activated carbon assisted MBR and combined MBR-RO process. This paper gives an overview on removal of pharmaceutical micropollutants by MBR or MBR related processes in order to evaluate the status of membrane bioreactor in wastewater treatment and reuse schemes.

## **2. Sources and occurrence of pharmaceutical micropollutants**

Nowadays, about 3000 substances are registered in the EU for pharmaceutical purposes alone [23, 26]. Also, there is evidence of occurrence of some 160 different drugs in effluent of wastewater treatment plants (WWTPs), surface water and groundwater [27]. Pharmaceuticals found in aquatic environment can be divided into different therapeutic categories: analgesics and anti-inflammatory drugs, lipid regulators, antiepileptic drugs, beta-blockers, antibiotics, cytostatic drugs etc. [28-29]. After consumed by human body, pharmaceutical residues are discharged to sewers through urine and faeces as unchanged compounds or metabolites. For unused or expired drugs, usually they are flushed down the drain or disposed of in the trash [27]. Thus, after domestic use pharmaceuticals mainly enter WWTPs. Concerning the contribution of hospitals to concentration of pharmaceuticals detected in WWTP influents, normally it is low for most pharmaceuticals except for some specific pharmaceuticals, for example, some antibiotics [30-32].

Due to incomplete removal of pharmaceuticals in conventional WWTPs, WWTP effluents become a major source of pharmaceutical micropollutants entering aquatic environment [29,

33]. Many pharmaceutical micropollutants have been detected at concentration up to  $\mu\text{g/L}$  level in conventional WWTP effluents around the world. Table 1 summarizes the occurrence of typical pharmaceutical micropollutants in conventional WWTP effluents at concentration higher than  $1.0 \mu\text{g L}^{-1}$ . Miège et al. [34] also confirmed these pharmaceutical micropollutants as among the most investigated pharmaceuticals in WWTPs.

Table 1. Occurrence of typical pharmaceutical micropollutants in conventional WWTP effluents at concentration higher than  $1.0 \mu\text{g L}^{-1}$

Compound	Concentration ( $\mu\text{g L}^{-1}$ )	Reference
<i>Analgesic</i>		
Ibuprofen	1.7-55.0	[28, 35-49]
Diclofenac	1.2-5.4	[28, 36, 38, 41, 43, 47, 50-53]
Naproxen	1.0-8.0	[35-40, 42, 48-49, 51, 54]
Ketoprofen	1.1-3.9	[36-37, 39-40, 49]
<i>Lipid regulator</i>		
Bezafibrate	1.0-4.8	[28, 36, 38, 47, 52]
Gemfibrozil	1.3-5.5	[28, 36, 38, 43, 48, 51, 55-56]
<i>Antiepileptic</i>		
Carbamazepine	1.1-6.3	[28, 36-37, 43, 47-50, 52-53, 57-60]
<i>Antibiotic</i>		
Erythromycin- $\text{H}_2\text{O}$	2.0-6.0	[57, 61-62]
Trimethoprim	1.2-3.0	[56-57, 63-65]
Sulfamethoxazole	1.5-2.0	[61, 65-66]

Since the pharmaceutical micropollutants listed in Table 1 have been frequently detected in WWTP effluents at relatively high concentration, they might be insufficiently removed in conventional WWTPs. Therefore, these pharmaceutical micropollutants are selected in this

review to examine the capacity of MBR or MBR related processes on removal of pharmaceutical micropollutants.

### **3. MBR process for removal of pharmaceutical micropollutants**

#### **3.1 Removal mechanisms of pharmaceutical micropollutants in MBR**

Table 2 lists the physico-chemical properties of selected pharmaceutical micropollutants that relate to their removal in MBR. The mechanisms involved in MBR for pharmaceutical micropollutant removal may include physical retention of membrane, biotransformation, air stripping, sorption and photo-transformation [19, 22, 67-70]. Since the molecular size of most pharmaceuticals (molecular weight between 100 and 1000 g mol<sup>-1</sup>), is at least 100 times smaller than the pore size of membrane used for MBR process, no direct physical retention of the compounds by MBR membrane can be expected [67]. However, Sahar et al. [71] and Urase et al. [72] suggested the deposits formed on the membrane surface might act as an additional barrier and thus contributed to an enhanced removal of pharmaceutical micropollutants in MBR. Further investigations should be carried out to confirm this assumption. As for removal by volatilization or stripping, the Henry coefficient (dimensionless,  $\mu\text{g L}^{-1}_{\text{air}} / \mu\text{g L}^{-1}_{\text{wastewater}}$ ) of a compound should be higher than 0.005 to have a significant removal (5%) as found in a EU project POSEIDON [26, 67]. Because most of pharmaceutical micropollutants have a Henry coefficient smaller than 10<sup>-5</sup> [73] (also see table 2), removal of pharmaceutical micropollutants via stripping is negligible. Since the turbidity of wastewater blocks most sunlight and no secondary clarifier is employed in MBR, the photo-degradation of pharmaceutical micropollutants in MBR is not significant. Thus, the main possible mechanisms for removal of pharmaceutical micropollutants in MBR are sorption and biodegradation.



Table 2. Physico-chemical properties of selected pharmaceutical micropollutants

Compound	MW	H <sup>a</sup>	pK <sub>a</sub>	LogK <sub>ow</sub>	K <sub>d</sub>	k <sub>biol</sub>
<i>Analgesics</i>						
Ibuprofen	206.3	$6.1 \times 10^{-6}$	4.91	3.79-3.97	7	9-22
Diclofenac	296.15	$1.9 \times 10^{-10}$	4.15	4.02-4.51	16	<0.1
Naproxen	230.3	$1.4 \times 10^{-8}$	4.15	3.10-3.18	13	0.4-0.8
Ketoprofen	254.3	$8.7 \times 10^{-10}$	4.45	3.0-3.12	16	0.68-1.59*
<i>Lipid regulator</i>						
Bezafibrate	361.8	$8.7 \times 10^{-14}$	3.61	4.25	20	3.4-4.5
Gemfibrozil	250.3	$4.9 \times 10^{-7}$	4.75	4.77	75	0.5-1.8
<i>Antiepileptic</i>						
Carbamazepine	236.27	$4.4 \times 10^{-9}$	13.9	2.25-2.45	1.2	<0.1
<i>Antibiotics</i>						
Erythromycin	734.0	$2.2 \times 10^{-27}$	8.88	2.48-3.06	165	<1.1
Trimethoprim	290.3	$9.8 \times 10^{-13}$	7.2	0.73-0.91	200	0.22
Sulfamethoxazole	253.3	$3.9 \times 10^{-11}$	5.6	0.89-0.91	260	0.2

MW, molecular weight (g/mol); H, Henry coefficient ( $\mu\text{g L}^{-1}_{\text{air}} / \mu\text{g L}^{-1}_{\text{wastewater}}$ ); pK<sub>a</sub>, dissociation constant; Log K<sub>ow</sub>, octanol-water partition coefficient; K<sub>d</sub>, sorption coefficient for activated sludge ( $\text{L kg}_{\text{SS}}^{-1}$ ); k<sub>biol</sub>, degradation rate constant in MBR ( $\text{L g}_{\text{SS}}^{-1}\text{d}^{-1}$ ); all data from [20, 22, 26, 68, 74-79]; \* data obtained from batch experiments using enriched nitrifier culture [79].

Sorption of organic micropollutants to activated sludge of MBR depends on two main mechanisms, absorption and adsorption: Absorption is the hydrophobic interactions of the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms or with the lipid fraction of the sludge; Adsorption is the electrostatic interactions of positively charged groups of a compound with the negatively charged surface of the microorganisms [23, 67]. Since sorption coefficient K<sub>d</sub> ( $\text{L kg}_{\text{SS}}^{-1}$ ) is defined as the

partition of a compound between the sludge and the water phase [23],  $K_d$  does not only depend on hydrophobicity of a compound, but also depends on the presence of positively charged groups (e.g., amino groups) in its structure. In addition, the relation between  $K_d$  and  $\text{Log}K_{ow}$  is not obvious for acidic pharmaceutical micropollutants ibuprofen, diclofenac, naproxen, ketoprofen and gemfibrozil as indicated in a study [80]. Because of the carboxyl functional group in their structures, these compounds are negatively charged (see their  $\text{p}K_a$  values in table 2) at neutral pH. Thus, they are hydrophilic in the ionic state at neutral pH with their low  $K_d$  values (less than  $100 \text{ L kg}_{SS}^{-1}$ ) even though their  $\text{Log} Kow$  values are high (see their  $K_d$  and  $\text{Log} Kow$  values in table 2). For compounds with  $K_d$  value less than  $500 \text{ L kg}_{SS}^{-1}$  their removal by sorption to activated sludge in biological process was found to be negligible [77, 81]. Since the  $K_d$  values of most pharmaceutical micropollutants, including all compounds listed in Table 2, are less than  $500 \text{ L kg}^{-1}$  and sludge production in MBR is generally smaller than conventional activated sludge process, the removal of pharmaceutical micropollutants via sorption in MBR is of minor importance. In particular, carbamazepine has a  $K_d$  value of  $1.2 \text{ L kg}^{-1}$ , far away from the critical value of  $500 \text{ L kg}^{-1}$ , indicating that it is not sorbed to activated sludge to a significant degree. For antibiotics trimethoprim and sulfamethoxazole, although their  $K_d$  values are relatively high (higher than  $200 \text{ L kg}^{-1}$ ), their sorption to activated sludge was not significant [63, 82]. However, attention should be paid for the antibiotics ciprofloxacin and norfloxacin, the major mechanism relevant for their removal is sorption to activated sludge, due to their high  $K_d$  values (higher than  $15000 \text{ L kg}^{-1}$ ) [83-84]. Ciprofloxacin and norfloxacin are not listed in table 1 due to their relatively low detected concentration in WWTP effluents.

Therefore, the main mechanism for removal of most pharmaceutical micropollutants in MBR is via biodegradation. Due to their trace level concentration in municipal wastewater, co-metabolism probably occurs for biological transformation or degradation of pharmaceutical micropollutants, in which case the bacteria accidentally break down or partially convert the micropollutant and do not use it as a carbon source for their growth [23]. In addition, the biodegradability of pharmaceutical micropollutants in MBR varies greatly in the range from zero to complete biotransformation [85]. Joss et al. [26] divided the pharmaceutical micropollutants into 3 different classes according to their degradation constant  $k_{\text{biol}}$  values in municipal wastewater treatment process: compounds with  $k_{\text{biol}} < 0.1 \text{ L g}_{\text{SS}}^{-1} \text{ d}^{-1}$  are not removed to a significant extent (<20%), compounds with  $k_{\text{biol}} > 10 \text{ L g}_{\text{SS}}^{-1} \text{ d}^{-1}$  are transformed by more than 90% and for compounds with  $k_{\text{biol}}$  in between, moderate removal efficiency is expected. Therefore, the remarkably poor removal of carbamazepine and diclofenac in biological wastewater treatment process is due to their low biodegradability with  $k_{\text{biol}}$  values less than  $0.1 \text{ L g}_{\text{SS}}^{-1} \text{ d}^{-1}$  as well as their low sorption potential to activated sludge with  $K_{\text{d}}$  values less than  $20 \text{ L kg}^{-1}$  [69]. Based on the degradation of a heterogeneous group of 35 compounds, Joss et al. [26] concluded that state of the art biological treatment schemes, including MBR, for municipal wastewater treatment are not efficient in degrading pharmaceuticals: only 4 out of 35 compounds are degraded by more than 90% while 17 compounds are removed by less than 50%.

### **3.2 Removal of typical pharmaceutical micropollutants in MBR**

Table 3 summarizes the removal efficiency of typical pharmaceutical micropollutants in aerobic MBR. The removal efficiency range as well as average removal efficiency together with standard deviations is presented. According to the data from Table 3, these typical pharmaceutical micropollutants could be classified into 4 groups: (1) compounds that are very

easily biodegraded in MBR with average removal efficiency higher than 95% (ibuprofen); (2) compounds easily biodegraded in MBR with average removal efficiency higher than 90% (bezafibrate); (3) compounds moderately biodegraded in MBR with average removal efficiency of 50%-80% (naproxen, ketoprofen, gemfibrozil, erythromycin, trimethoprim, and sulfamethoxazole); and (4) compounds poorly biodegraded in MBR with average removal efficiency less than 40% (diclofenac and carbamazepine). Notably, carbamazepine is removed with average removal efficiency of only 7% in MBR. Due to the persistency of carbamazepine in the biological treatment process and aquatic environment, Clara et al. [86] proposed carbamazepine as a possible anthropogenic marker in the aquatic environment. Moreover, it is interesting to note that negative removal efficiency of diclofenac and carbamazepine in MBR was observed. This could be attributed to enzymatic cleavage of the glucuronic conjugates of those pharmaceuticals and consequently the release of the parent compounds in the treated effluent [59, 69], or analytical uncertainty and sampling uncertainty occurred during the analysis process [87]. Similarly, Göbel et al. [88] attributed the observed high variability of sulfamethoxazole elimination to the possible transformation of N<sup>4</sup>-acetylsulfamethoxazole, main human metabolite of sulfamethoxazole, back to sulfamethoxazole and a simultaneous elimination of sulfamethoxazole itself during biological treatment.

Table 3. Removal of selected pharmaceutical micropollutants in MBR

Compound	Removal efficiency (%)	Average removal $\pm$ SD (%)	Reference
<i>Analgesics</i>			
Ibuprofen	90-100	96 $\pm$ 3	[47, 52, 77, 85, 89-103]
Diclofenac	-19-87	32 $\pm$ 27	[47, 52, 77, 85, 89-103]
Naproxen	16-99	69 $\pm$ 27	[77, 85, 89, 91-98, 100-104]
Ketoprofen	44-100	75 $\pm$ 20	[85, 91, 93-98, 100-101, 103-104]
<i>Lipid regulator</i>			
Bezafibrate	77-98	91 $\pm$ 6	[47, 52, 85, 91, 93-96, 98, 103]
Gemfibrozil	28-99	70 $\pm$ 29	[85, 93-95, 98, 100-103]
<i>Antiepileptic</i>			
Carbamazepine	-24-58	7 $\pm$ 18	[47, 52, 77, 85, 89-90, 92-95, 98-103, 105-106]
<i>Antibiotics</i>			
Erythromycin	25-91	60 $\pm$ 29	[71, 85, 88-89, 94, 105]
Trimethoprim	17-95	62 $\pm$ 30	[71, 85, 88-89, 101-102, 104, 107]
Sulfamethoxazole	24-92	63 $\pm$ 16	[47, 71, 85, 88-89, 92, 94, 101-102, 104-106, 108]

Thus, majority of pharmaceutical micropollutants are moderately biodegraded in MBR. In addition, degradation constant  $k_{\text{biol}}$  could be used as an effective parameter to predict the removal of pharmaceutical micropollutants in MBR. The  $k_{\text{biol}}$  values of the poorly removed compounds diclofenac and carbamazepine are less than  $0.1 \text{ L g}_{\text{SS}}^{-1}\text{d}^{-1}$ ; the  $k_{\text{biol}}$  values of moderately removed compounds (naproxen, ketoprofen, gemfibrozil, erythromycin, trimethoprim, and sulfamethoxazole) are between  $0.1 \text{ L g}_{\text{SS}}^{-1}\text{d}^{-1}$  and  $2 \text{ L g}_{\text{SS}}^{-1}\text{d}^{-1}$ ; the  $k_{\text{biol}}$  value of easily removed compound (bezafibrate) is in range from  $2 \text{ L g}_{\text{SS}}^{-1}\text{d}^{-1}$  to  $5 \text{ L g}_{\text{SS}}^{-1}\text{d}^{-1}$ . The  $k_{\text{biol}}$  value of very easily removed compound (ibuprofen) is higher than  $5 \text{ L g}_{\text{SS}}^{-1}\text{d}^{-1}$ .

### **3.3 Factors affecting removal of pharmaceutical micropollutants in MBR**

#### **3.3.1 SRT**

Solid retention time (SRT) seems to be the most important parameter affecting the removal of pharmaceutical micropollutants in MBR. Table 4 summarizes the effect of SRT on removal of selected pharmaceutical micropollutants in MBR.

Removal of diclofenac, ketoprofen, gemfibrozil, trimethoprim and erythromycin in MBR was found to be significantly affected by SRT [47, 88, 90, 97, 103-104]. Clara et al. [47] reported that no removal of diclofenac was observed with a SRT of approximate 10 days, while with increasing SRT a partial removal was observed. Kimura et al. [97] reported that the MBR operated with a longer SRT of 65 days significantly improved the elimination of ketoprofen and diclofenac as compared to the MBR with a shorter SRT of 15 days, from 82% to 98% and from 50% to 82%, respectively. Bernhard et al. [90] observed that the removal efficiency of diclofenac in a lab scale MBR was between 8% and 38% with an SRT increased from 20 to 48 days, and 59% with an SRT of 62 days. Maeng et al. [103] found that the removal efficiencies of gemfibrozil and ketoprofen were increased from 41% to 88% and from 64% to 90%, respectively, when SRT was increased from 20 days to 80 days. Göbel et al. [88] reported that for trimethoprim and erythromycin, a two to three times higher removal rate was seen for these compounds at a SRT of 60–80 days, up to 87—90% removal, as compared with the removal rate observed at SRT of  $16 \pm 2$  and  $33 \pm 3$  days.

No significant effect of SRT on removal of ibuprofen, bezafibrate, naproxen, carbamazepine and sulfamethoxazole was observed in MBR [23, 52, 88, 90, 97, 103-104]. Ibuprofen and bezafibrate were effectively removed when SRT is higher than a critical value, about 10 days [23, 52, 103]. While, carbamazepine was found to be recalcitrant to degradation in MBR

regardless of the change in SRTs and microbial activity [90, 103]. Concerning naproxen, Tambosi et al. [104] found that removal of naproxen in MBR was in the range of 85%-90% at SRT of 15 days and 30 days. Kimura et al. [97] observed removal efficiency of naproxen higher than 95% in MBR operated at SRT of 15 days and 65 days. Maeng et al. [103] found that the removal efficiency of about 25% for naproxen in MBR at SRT of 20 days and 80 days. As for sulfamethoxazole, Göbel et al. [88] reported removal efficiency of about 40% for sulfamethoxazole and about 80% for sulfamethoxazole together with its main human metabolite N<sup>4</sup>-acetylsulfamethoxazole in MBR, with no dependence on SRT from 16 days to 80 days.

High SRT allows the enrichment of slow-growing microorganisms (e.g. nitrifying bacteria) and consequently the establishment of a more diverse bacteria population, which favors removal of pharmaceutical micropollutants in MBR [52]. Thus, the effect of SRT on removal of some moderately removed pharmaceutical micropollutants is quite significant, for example, ketoprofen, gemfibrozil, trimethoprim and erythromycin. Terne et al. [23] concluded that the biological transformation of a pharmaceutical compound depended on the age of the activated sludge; Bezafibrate, sulfamethoxazole, and ibuprofen required a sludge age of 2–5 days for significant degradation; diclofenac needed 5–15 days; Carbamazepine was not degraded even at a sludge age >20 days. Similarly, Clara et al. [52] defined a critical SRT for effective removal of pharmaceutical micropollutants, e.g. amounting to about 5 days for ibuprofen and to about 10 days for bezafibrate. Thus, SRT of higher than about 15 days is recommended to significantly improve the removal of pharmaceutical micropollutants in municipal wastewater treatment system [23, 52, 76].

Table 4. Effect of SRT on removal of selected pharmaceutical micropollutants in MBR

Compound	Significantly affected by SRT	Not significantly affected by SRT	Reference
<i>Analgesics</i>			
Ibuprofen		×	[22-23, 52, 103]
Diclofenac	×		[47, 90, 97]
Naproxen		×	[97, 103-104]
Ketoprofen	×		[97, 103-104]
<i>Lipid regulator</i>			
Bezafibrate		×	[23, 52, 103]
Gemfibrozil	×		[103]
<i>Antiepileptic</i>			
Carbamazepine		×	[23, 90, 103]
<i>Antibiotics</i>			
Erythromycin	×		[88]
Trimethoprim	×		[88, 104]
Sulfamethoxazole		×	[23, 88, 104]

### 3.3.2 pH

Although sorption of most pharmaceutical micropollutants to activated sludge in MBR is negligible at neutral pH, enhanced sorption was observed for some acidic pharmaceuticals at lower pH. Urase et al. [72] reported that the removal rate of acidic pharmaceuticals gemfibrozil, ibuprofen, ketoprofen, and diclofenac in MBR was much higher at pH of 4.3-5.0 than that at pH of 6.8-7.6 and 7.5-8.0. On the other hand, the removal of neutral pharmaceutical carbamazepine was not significantly affected by pH. The authors explained that in the neutral pH condition, these acidic pharmaceuticals were ionized; however, in the acidic pH condition, these pharmaceuticals were not ions and their hydrophobicity was



increased, resulting in their sorption onto activated sludge. Moreover, it was found that the target substances attached to the sludge were not accumulated in the sludge phase, and they were biologically degraded. Tadkaew et al. [109] studied the removal of sulfamethoxazole, carbamazepine, diclofenac, ibuprofen and ketoprofen in a batch scale MBR at different pH (pH 5-9). The results showed that the influence of mixed liquor pH on the removal of pharmaceuticals was quite dramatic for all four ionisable compounds (sulfamethoxazole, diclofenac, ibuprofen and ketoprofen), with highest removal at pH 5; in contrast, no apparent variation in removal efficiency of the neutral compound carbamazepine was observed.

### 3.3.3 Redox conditions

Monsalvo et al. [110] observed a poor removal, lower than 15%, for diclofenac, carbamazepine, ibuprofen, gemfibrozil and ketoprofen, partial removal for trimethoprim and naproxen, i.e. 35.4% and 70.3%, respectively, and high removal of 95.2% for sulfamethoxazole in anaerobic MBR, suggesting the poor capacity of anaerobic MBR on removal of most pharmaceutical micropollutants. In contrast, Hai et al. [106] observed that during near-anoxic operation with dissolved oxygen concentration in the bioreactor about  $0.5 \text{ mg L}^{-1}$ , an exceptionally high removal ( $68 \pm 10\%$ ) of carbamazepine was achieved in MBR as compared to its low removal efficiency ( $12 \pm 11\%$ ) under aerobic conditions; On the other hand, an average removal efficiency of 65% for sulfamethoxazole was achieved irrespective of the DO concentrations.

### 3.3.4 Nitrifying biomass

The enrichment of nitrifying bacteria was reported to enhance removal of pharmaceutical micropollutants in biological wastewater treatment system [79, 103, 111-113]. Maeng et al. [103] found that the removal of gemfibrozil, diclofenac, bezafibrate, and ketoprofen was

enhanced by ammonia oxidizing bacteria in MBR. Suarez et al. [113] reported the removal efficiency of diclofenac, from zero to 74%, in the aerobic reactor was positively correlated with nitrifying biomass concentration rather than SRT. Tran et al. [79] reported that the enriched nitrifier culture enhanced degradation of gemfibrozil, ketoprofen, naproxen, diclofenac, and carbamazepine with their increased biodegradation constant  $k_{\text{biol}}$ , especially for carbamazepine and diclofenac. Besides, the authors found that removal efficiency of these compounds by enriched nitrifier culture increased with the increase of initial ammonium concentration in batch reactors. Similarly, Fernandez-Fontaina et al. [112] reported high biodegradation efficiencies of ibuprofen, naproxen, trimethoprim, and erythromycin were obtained with nitrifying activated sludge working at high nitrogen loading rates.

### 3.3.5 Molecular structures

Some authors related the removal of pharmaceutical micropollutants in MBR to their compound structures. Tadkaew et al. [101] reported that the removal of pharmaceuticals possessing only electron donating groups (like hydroxyl groups and primary amine groups), such as ibuprofen, ketoprofen, and sulfamethoxazole, was usually higher than 70%, while the removal of pharmaceuticals possessing only electron withdrawing groups (like a chlorine atom or amide group), such as carbamazepine and diclofenac, was usually lower than 20%. Kimura et al. [96] attributed the poor removal of diclofenac in MBRs to the presence of chlorine in their structures. Bouju et al. [114] attributed high removal of ibuprofen to its simple molecular structure.

## 3.4 Effects of pharmaceutical micropollutants on MBR process

The presence of pharmaceutical micropollutants in MBR may affect microbial activity and microbial community structure of microorganisms in activated sludge [115-117]. Besides, the

presence of pharmaceutical micropollutants in MBR may also induce protection mechanism of microorganisms to produce some extracellular polymeric substances (EPS) [118-119], which could affect membrane fouling of MBR [118].

#### 3.4.1 Effects on microbial activity and microbial community structure

Aubenneau et al. [115] examined the effect of trace concentration of carbamazepine on mixed microbial communities of activated sludge taken from MBR. The authors reported that in presence of  $1 \mu\text{g L}^{-1}$  carbamazepine, higher endogenous respiration rates, lower exogenous respiration rates, and smaller flocs size were observed. The authors explained that the increase in endogenous respiration rates suggested an increase in maintenance requirements of bacteria in order to manage the chemical stress induced by carbamazepine; and the decrease of exogenous respiration rates indicated a change in the metabolic pathways of the substrate or a change in the active bacterial species. Delgado et al. [118] also reported an increase in the endogenous respiration of heterotrophic micro-organisms after continuous addition of about  $5 \mu\text{g L}^{-1}$  cytostatic drug cyclophosphamide and its principal metabolites (CPs) in MBR. The authors attributed the high microbial activity in endogenous conditions to their adapting to the presence of CPs. Wang et al. [116] reported a 39%, 39%, and 19% decrease, respectively, in specific oxygen uptake rate (SOUR) when presence of  $10 \mu\text{M}$  naproxen, ketoprofen, and carbamazepine in activated sludge in batch experiments. The results indicated that the presence of some pharmaceuticals may inhibit microbial activity of some activated sludge microorganisms. The authors also found shifts in microbial community structure in the presence of ketoprofen and naproxen via DGGE analysis. Through T-RFLP analyses of the bacterial 16S rRNA genes, Kraigher et al. [120] observed a minor but consistent shift in the bacterial community structure in activated sludge of the bioreactor supplied with pharmaceuticals (ibuprofen, naproxen, ketoprofen, and diclofenac) at concentration of 50

$\mu\text{g L}^{-1}$ , compared to the control reactor operated without addition of pharmaceuticals; Moreover, a greater structural divergence was observed in the reactors operated with higher concentration of pharmaceuticals.

### 3.4.2 Effects on MBR performance and MBR fouling

Delgado et al. [118] studied the effects of continuous addition of  $5 \mu\text{g L}^{-1}$  CPs in MBR on characteristics of activated sludge and membrane fouling. In presence of CPs, formation of small particles and higher soluble EPS concentration in activated sludge were observed. Meanwhile, higher transmembrane pressure and higher specific cake resistance of activated sludge were observed, which indicated faster membrane fouling. However, no effect on removal of COD and total nitrogen by MBR was observed in presence of CPs. Lay et al. [121] observed increase in the protein and polysaccharide ratio for both soluble and bound EPS in the activated sludge after adding  $20\text{--}25 \mu\text{g L}^{-1}$  carbamazepine, diclofenac, ibuprofen and naproxen in the feed tank of a Novel Osmotic Membrane Bioreactor (OMBR). The authors attributed the increase in protein content to a natural microbial response or occurrence of cell lysis and release of intracellular polymers under the pharmaceutical stress. Avella et al. [119] reported that the continuous addition of trace concentration CPs in MBR 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 bound EPS in the sludge. The authors attributed the increase of these macromolecular species to a protection mechanism of microorganisms and attributed the more important membrane fouling to retention of these macromolecular species by the membrane. However, Jacob [122] observed stable operation of MBR using real municipal wastewater that contained pharmaceutical micropollutants (diclofenac  $0.7 \mu\text{g L}^{-1}$ , naproxen  $0.7 \mu\text{g L}^{-1}$ , ketoprofen  $1.6 \mu\text{g L}^{-1}$ , bezafibrate  $0.6 \mu\text{g L}^{-1}$  and carbamazepine  $0.5 \mu\text{g L}^{-1}$ ) during 164 days, which indicated that acclimation of microorganisms to pharmaceutical

stress may happen in this situation and no significantly additional fouling was induced by the presence of these pharmaceutical micropollutants in raw wastewater.

#### **4. MBR related processes for removal of pharmaceutical micropollutants**

Since MBR alone is not sufficient to completely eliminate all pharmaceutical micropollutants in municipal wastewater, hybrid or combined processes based on MBR get much attentions. Among MBR related processes, the capacity of activated carbon assisted MBR and combined MBR-RO process on removal of pharmaceutical micropollutants have been well investigated. Table 5 summarizes the removal efficiencies of selected pharmaceutical micropollutants in activated carbon assisted MBR and combined MBR-RO process. Other MBR related processes, such as MBR-Ozone [123], MBR-UV [124], MBR-TiO<sub>2</sub> [125] and OMBR [121, 126] were also reported to significantly enhanced the removal of pharmaceutical micropollutants, however, more studies are required to fully understand their capacity.

Activated carbon assisted MBR system can be further divided into two categories: MBR-PAC hybrid process and combined MBR-GAC process. In MBR-PAC hybrid process, powdered activated carbon (PAC) is added into MBR, while in combined MBR-GAC process, granular activated carbon column is used as post polishing unit after MBR. The coupling of activated carbon with MBR enables the activated carbon assisted MBR system to increase removal of some pharmaceutical micropollutants that are originally difficult to be removed in MBR, such as carbamazepine and diclofenac [95, 98, 127-129]. Serrano et al. [129] reported that after a single addition of 1 g L<sup>-1</sup> of powdered activated carbon (PAC) directly into the aeration tank of MBR an immediate and sharp removal increase was observed for the recalcitrant pharmaceutical micropollutants such as carbamazepine, diclofenac and trimethoprim, with removal efficiencies in the range of 93%-99%. Besides, the moderately degraded substance

erythromycin was completely removed after PAC addition (97%-99%). Moreover, microbial ecology present in the biomass showed a higher abundance of ammonium oxidizing bacteria after PAC addition, which may enhance degradation of pharmaceutical micropollutants in MBR. Nguyen et al. [128] reported that the removal of hydrophilic and biologically persistent pharmaceutical micropollutants (ketoprofen, naproxen, diclofenac and carbamazepine) was immediately improved to above 95%, after the addition of only 0.1 g/L PAC into MBR. While, the gradual decrease in removal underscored the requirement for the addition of fresh PAC during the continuous operation. Li et al. [127] reported that the removal efficiencies of sulfamethoxazole and carbamazepine increased to  $82 \pm 11\%$  and  $92 \pm 15\%$  from the levels of  $64 \pm 7\%$ , and negligible removal, respectively, when the PAC dosage was raised from  $0.1 \text{ g L}^{-1}$  to  $1.0 \text{ g L}^{-1}$ . Moreover, it is interesting to note that after the PAC addition the MBR membrane achieved significant additional removal of both micropollutants, especially for carbamazepine, as compared to the situation in MBR without addition of PAC. The authors attributed the enhanced removal to their sorption onto membrane cake layer. Nguyen et al. [98] reported that the granular activated carbon (GAC) post-treatment could significantly improve removal of the pharmaceutical micropollutants which were poorly removed by MBR. For example, the compounds, which were removed by MBR with efficiencies below 40% (ketoprofen, naproxen, diclofenac and carbamazepine), achieved overall removal efficiencies of 98% or above following GAC treatment. Since some pharmaceutical micropollutants could not be biodegraded in MBR, replacement or regeneration of activated carbon should be required to maintain high removal of pharmaceutical micropollutants during the long term operation for activated carbon assisted MBR systems.

The combined MBR-RO process is one of the dual membrane systems often used for production of reclaimed water besides the CAS-MF/UF-RO process [132]. Alturki et al. [74]

reported that the combination of MBR and a low pressure RO membrane resulted in more than 95% removal or removal to below the analytical detection limit of all 40 trace organic compounds including most typical pharmaceutical micropollutants. Dolar et al. [105] reported that the combination of MBR and RO treatment showed excellent overall removal of 20 detected pharmaceutical micropollutants in WWTPs with removal efficiencies above 99%. Cartagena et al. [99] also found that the post RO process can significantly improve the removal of pharmaceutical micropollutants like carbamazepine and diclofenac after MBR treatment in real municipal wastewater. However, the total removal of carbamazepine and diclofenac by MBR-RO process was not complete, 82.1–93.1% and 88.3–95.9%, respectively. Similarly, Joss et al. [130] also reported the incomplete removal of carbamazepine and diclofenac by MBR-RO process. Snyder et al. [102] reported almost complete removal of all detected pharmaceuticals in the tested MBR-RO pilots. While, Sahar et al. [131] reported that RO cannot serve as an absolute barrier for pharmaceutical removal in MBR-RO process since ibuprofen and diclofenac were detected in the effluent in the range of 28–223 ng L<sup>-1</sup>, although most pharmaceutical micropollutants investigated were removed in high efficiencies, higher than 93%, by MBR-RO process. Kimura et al. [133] reported that in the filtration tests with MBR effluent no significant variance of rejection of the pharmaceutical micropollutants was observed for RO membrane as compared with deionized pure water spiked with the pharmaceutical micropollutants, suggesting that the main mechanism for rejection of pharmaceutical micropollutants by RO membrane is size exclusion.

Table 5. Removal of selected pharmaceutical micropollutants in MBR related processes

Compound	Removal efficiency (%) in MBR-PAC <sup>a</sup>	Removal efficiency (%) in MBR-GAC <sup>b</sup>	Removal efficiency (%) in MBR-RO <sup>c</sup>
<i>Analgesics</i>			
Ibuprofen	>95	>98	>95
Diclofenac	>93	>98	>88
Naproxen	>95	>98	>95
Ketoprofen	>95	>98	>95
<i>Lipid regulator</i>			
Bezafibrate	n.a.	n.a.	
Gemfibrozil	>95	>98	>95
<i>Antiepileptic</i>			
Carbamazepine	>92*	>98	>82
<i>Antibiotics</i>			
Erythromycin	>97	n.a.	>95
Trimethoprim	>97	n.a.	>95
Sulfamethoxazole	82*	n.a.	>95

<sup>a</sup> [127-129]; <sup>b</sup> [98]; <sup>c</sup> [74, 92, 99, 102, 105, 124, 130-131]; \* The influent concentration in the experiment is 750  $\mu\text{g L}^{-1}$ ; n.a. = not available.

## 5. Conclusions

The presence of pharmaceutical micropollutants in the aquatic environment has become a serious problem for municipal wastewater reclamation and reuse around the world. MBR process with high SRT is a good choice to replace the conventional activated sludge process in water reuse projects due to its better removal of micropollutants. Although the MBR process alone could not assure the complete removal for the majority of pharmaceuticals, the possibility of MBR to be coupled with other processes, such as activated carbon adsorption



and RO membrane filtration, enables it to significantly improve removal of pharmaceutical micropollutants. Thus, MBR would play a more important role in wastewater treatment and reuse applications in the future.

It can be concluded that the main mechanism for removal of most pharmaceutical micropollutants in MBR is biodegradation; In terms of operation conditions, higher SRT, lower pH, higher nitrogen loading rate and anoxic condition favor removal of some pharmaceutical micropollutants in MBR; while, no evidence was found concerning the effect of MBR configuration (flat sheet or hollow fiber) on removal of pharmaceutical micropollutants in MBR; In addition, the presence of pharmaceutical micropollutants in MBR could affect the microbial activity and microbial community structures of microorganisms in activated sludge and cause more serious membrane fouling. However, the difficulty to compare data from different scales of MBR or MBR related processes (lab scale, pilot scale and full scale), the difficulty in quantifying sorped mass content of pharmaceutical micropollutants in sludge phase, the uncertainty in analysis of pharmaceutical micropollutants and their metabolites in liquid phase, and even sampling procedures make it hard to draw reliable conclusions from the literature.

Still, many questions are remained unanswered. Regarding the mechanisms for removal of pharmaceutical micropollutants, distinguish between sorption and biodegradation should be further investigated, taking into account the fact that the sorped pharmaceutical micropollutants in sludge phase may be further biodegraded by activated sludge and sorption may play an important role in biodegradation of pharmaceutical micropollutants in terms of mass transfer. Also, the function of deposits on membrane surface as an additional barrier for removal of pharmaceutical micropollutants should be further examined. In addition, more studies should be performed to identify the bacterial species that favor removal of

pharmaceutical micropollutants in MBR and determine the optimal operation conditions that help to enrich these bacterial species. Last but not least, it is recommended that more studies concerning the risk assessment of pharmaceutical micropollutants on human health and aquatic organisms be conducted to evaluate the necessity of advanced wastewater treatment processes, like MBR and MBR related processes, to be employed in the wastewater treatment and reuse schemes.

## References

- [1] A.D. Levine, T. Asano, 2004. Peer reviewed: Recovering sustainable water from wastewater. *Environmental science & technology* 38, 201A-208A.
- [2] D. Bixio, C. Thoeye, J. De Koning, D. Joksimovic, D. Savic, T. Wintgens, T. Melin, 2006. Wastewater reuse in Europe. *Desalination* 187, 89-101.
- [3] T. Melin, B. Jefferson, D. Bixio, C. Thoeye, W. De Wilde, J. De Koning, J. Van der Graaf, T. Wintgens, 2006. Membrane bioreactor technology for wastewater treatment and reuse. *Desalination* 187, 271-282.
- [4] S. Judd, 2008. The status of membrane bioreactor technology. *TRENDS in Biotechnology* 26, 109-116.
- [5] H. Lin, F. Wang, L. Ding, H. Hong, J. Chen, X. Lu, 2011. Enhanced performance of a submerged membrane bioreactor with powdered activated carbon addition for municipal secondary effluent treatment. *Journal of hazardous materials* 192, 1509-1514.
- [6] M.A. Shannon, P.W. Bohn, M. Elimelech, J.G. Georgiadis, B.J. Marinas, A.M. Mayes, 2008. Science and technology for water purification in the coming decades. *Nature* 452, 301-310.
- [7] E.L. Farias, K.J. Howe, B.M. Thomson, 2014. Effect of membrane bioreactor solids retention time on reverse osmosis membrane fouling for wastewater reuse. *Water Research* 49, 53-61.
- [8] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. Von Gunten, B. Wehrli, 2006. The challenge of micropollutants in aquatic systems. *Science* 313, 1072-1077.
- [9] M. Auriol, Y. Filali-Meknassi, R.D. Tyagi, C.D. Adams, R.Y. Surampalli, 2006. Endocrine disrupting compounds removal from wastewater, a new challenge. *Process Biochemistry* 41, 525-539.
- [10] C.G. Daughton, T.A. Ternes, 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives* 107, 907.
- [11] K. Kümmerer, 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, 957-969.

- [12] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environmental science & technology* 36, 1202-1211.
- [13] O.A. Jones, J.N. Lester, N. Voulvoulis, 2005. Pharmaceuticals: a threat to drinking water? *TRENDS in Biotechnology* 23, 163-167.
- [14] B. Halling-Sørensen, S. Nors Nielsen, P. Lanzky, F. Ingerslev, H. Holten Lützhøft, S. Jørgensen, 1998. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. *Chemosphere* 36, 357-393.
- [15] N. Le-Minh, S. Khan, J. Drewes, R. Stuetz, 2010. Fate of antibiotics during municipal water recycling treatment processes. *Water research* 44, 4295-4323.
- [16] L.H.M.L.M. Santos, A.N. Araújo, A. Fachini, A. Pena, C. Delerue-Matos, M.C.B.S.M. Montenegro, 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of hazardous materials* 175, 45-95.
- [17] M. Crane, C. Watts, T. Boucard, 2006. Chronic aquatic environmental risks from exposure to human pharmaceuticals. *Science of the Total Environment* 367, 23-41.
- [18] K. Fent, A.A. Weston, D. Caminada, 2006. Ecotoxicology of human pharmaceuticals. *Aquatic toxicology* 76, 122-159.
- [19] P. Verlicchi, M. Al Aukidy, E. Zambello, 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Science of the Total Environment* 429, 123-155.
- [20] B. Petrie, E.J. McAdam, M.D. Scrimshaw, J.N. Lester, E. Cartmell, 2013. Fate of drugs during wastewater treatment. *TrAC Trends in Analytical Chemistry* 49, 145-159.
- [21] I. Michael, L. Rizzo, C.S. McArdell, C.M. Manaia, C. Merlin, T. Schwartz, C. Dagot, D. Fatta-Kassinos, 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Research* 47, 957-995.
- [22] S. Suárez, M. Carballa, F. Omil, J.M. Lema, 2008. How are pharmaceutical and personal care products (PPCPs) removed from urban wastewaters? *Reviews in Environmental Science and Bio/Technology* 7, 125-138.
- [23] T.A. Ternes, A. Joss, H. Siegrist, 2004. Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment. *Environmental Science & Technology* 38, 392A-399A.
- [24] O.H. Jones, N. Voulvoulis, J. Lester, 2005. Human pharmaceuticals in wastewater treatment processes. *Critical Reviews in Environmental Science and Technology* 35, 401-427.
- [25] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment* 473–474, 619-641.

- [26] A. Joss, S. Zabczynski, A. Göbel, B. Hoffmann, D. Löffler, C.S. McArdell, T.A. Ternes, A. Thomsen, H. Siegrist, 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. *Water research* 40, 1686-1696.
- [27] K. Kümmerer, 2009. The presence of pharmaceuticals in the environment due to human use—present knowledge and future challenges. *Journal of Environmental Management* 90, 2354-2366.
- [28] T.A. Ternes, 1998. Occurrence of drugs in German sewage treatment plants and rivers. *Water Research* 32, 3245-3260.
- [29] T. Heberer, 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology letters* 131, 5-17.
- [30] T. Heberer, D. Feldmann, 2005. Contribution of effluents from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluents—modeling versus measurements. *Journal of Hazardous materials* 122, 211-218.
- [31] C. Ort, M.G. Lawrence, J. Reungoat, G. Eaglesham, S. Carter, J. Keller, 2010. Determining the fraction of pharmaceutical residues in wastewater originating from a hospital. *Water research* 44, 605-615.
- [32] P. Verlicchi, M. Al Aukidy, A. Galletti, M. Petrovic, D. Barceló, 2012. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Science of the Total Environment* 430, 109-118.
- [33] J.P. Bound, N. Voulvoulis, 2006. Predicted and measured concentrations for selected pharmaceuticals in UK rivers: implications for risk assessment. *Water Research* 40, 2885-2892.
- [34] C. Miège, J. Choubert, L. Ribeiro, M. Eusèbe, M. Coquery, 2009. Fate of pharmaceuticals and personal care products in wastewater treatment plants—Conception of a database and first results. *Environmental Pollution* 157, 1721-1726.
- [35] M. Carballa, F. Omil, J.M. Lema, M.a. Llompart, C. García-Jares, I. Rodríguez, M. Gomez, T. Ternes, 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Research* 38, 2918-2926.
- [36] R. Andreozzi, M. Raffaele, P. Nicklas, 2003. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. *Chemosphere* 50, 1319-1330.
- [37] J. Santos, I. Aparicio, E. Alonso, 2007. Occurrence and risk assessment of pharmaceutically active compounds in wastewater treatment plants. A case study: Seville city (Spain). *Environment International* 33, 596-601.
- [38] M. Stumpf, T.A. Ternes, R.-D. Wilken, S.V. Rodrigues, W. Baumann, 1999. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. *Science of the Total Environment* 225, 135-141.
- [39] N. Lindqvist, T. Tuhkanen, L. Kronberg, 2005. Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. *Water Research* 39, 2219-2228.

- [40] J.L. Santos, I. Aparicio, E. Alonso, M. Callejón, 2005. Simultaneous determination of pharmaceutically active compounds in wastewater samples by solid phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors. *Analytica Chimica Acta* 550, 116-122.
- [41] M.J. Gómez, M.J. Martínez Bueno, S. Lacorte, A.R. Fernández-Alba, A. Agüera, 2007. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. *Chemosphere* 66, 993-1002.
- [42] S.S. Verenitch, C.J. Lowe, A. Mazumder, 2006. Determination of acidic drugs and caffeine in municipal wastewaters and receiving waters by gas chromatography–ion trap tandem mass spectrometry. *Journal of Chromatography A* 1116, 193-203.
- [43] C.I. Kosma, D.A. Lambropoulou, T.A. Albanis, 2010. Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece. *Journal of hazardous materials* 179, 804-817.
- [44] P.H. Roberts, K.V. Thomas, 2006. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Science of the Total Environment* 356, 143-153.
- [45] A.Y.-C. Lin, T.-H. Yu, S.K. Lateef, 2009. Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan. *Journal of hazardous materials* 167, 1163-1169.
- [46] M.J. Hilton, K.V. Thomas, 2003. Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography A* 1015, 129-141.
- [47] M. Clara, B. Strenn, O. Gans, E. Martinez, N. Kreuzinger, H. Kroiss, 2005. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water research* 39, 4797-4807.
- [48] G.-G. Ying, R.S. Kookana, D.W. Kolpin, 2009. Occurrence and removal of pharmaceutically active compounds in sewage treatment plants with different technologies. *Journal of Environmental Monitoring* 11, 1498-1505.
- [49] J.L. Santos, I. Aparicio, M. Callejón, E. Alonso, 2009. Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). *Journal of hazardous materials* 164, 1509-1516.
- [50] T. Heberer, 2002. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. *Journal of Hydrology* 266, 175-189.
- [51] M.J.M. Bueno, A. Agüera, M.D. Hernando, M.J. Gómez, A.R. Fernández-Alba, 2009. Evaluation of various liquid chromatography–quadrupole–linear ion trap–mass spectrometry operation modes applied to the analysis of organic pollutants in wastewaters. *Journal of Chromatography A* 1216, 5995-6002.
- [52] M. Clara, N. Kreuzinger, B. Strenn, O. Gans, H. Kroiss, 2005. The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research* 39, 97-106.

- [53] T.A. Ternes, M. Bonerz, N. Herrmann, B. Teiser, H.R. Andersen, 2007. Irrigation of treated wastewater in Braunschweig, Germany: An option to remove pharmaceuticals and musk fragrances. *Chemosphere* 66, 894-904.
- [54] L. Lishman, S.A. Smyth, K. Sarafin, S. Kleywegt, J. Toito, T. Peart, B. Lee, M. Servos, M. Beland, P. Seto, 2006. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. *Science of the Total Environment* 367, 544-558.
- [55] R. Rosal, A. Rodríguez, J.A. Perdigón-Melón, A. Petre, E. García-Calvo, M.J. Gómez, A. Agüera, A.R. Fernández-Alba, 2010. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. *Water Research* 44, 578-588.
- [56] I. Muñoz, M.J. Gómez-Ramos, A. Agüera, A.R. Fernández-Alba, J.F. García-Reyes, A. Molina-Díaz, 2009. Chemical evaluation of contaminants in wastewater effluents and the environmental risk of reusing effluents in agriculture. *TrAC Trends in Analytical Chemistry* 28, 676-694.
- [57] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research* 43, 363-380.
- [58] C.M. Coetsier, S. Spinelli, L. Lin, B. Roig, E. Touraud, 2009. Discharge of pharmaceutical products (PPs) through a conventional biological sewage treatment plant: MECs vs PECs? *Environment International* 35, 787-792.
- [59] N. Vieno, T. Tuhkanen, L. Kronberg, 2007. Elimination of pharmaceuticals in sewage treatment plants in Finland. *Water Research* 41, 1001-1012.
- [60] A. Wick, G. Fink, A. Joss, H. Siegrist, T.A. Ternes, 2009. Fate of beta blockers and psycho-active drugs in conventional wastewater treatment. *Water Research* 43, 1060-1074.
- [61] R. Hirsch, T. Ternes, K. Haberer, K.-L. Kratz, 1999. Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment* 225, 109-118.
- [62] W. Xu, G. Zhang, X. Li, S. Zou, P. Li, Z. Hu, J. Li, 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. *Water Research* 41, 4526-4534.
- [63] A.L. Batt, S. Kim, D.S. Aga, 2007. Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. *Chemosphere* 68, 428-435.
- [64] R.H. Lindberg, P. Wennberg, M.I. Johansson, M. Tysklind, B.A. Andersson, 2005. Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. *Environmental science & technology* 39, 3421-3429.

- [65] J.E. Renew, C.-H. Huang, 2004. Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography A* 1042, 113-121.
- [66] C. Hartig, T. Storm, M. Jekel, 1999. Detection and identification of sulphonamide drugs in municipal waste water by liquid chromatography coupled with electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A* 854, 163-173.
- [67] T.A. Larsen, J. Lienert, A. Joss, H. Siegrist, 2004. How to avoid pharmaceuticals in the aquatic environment. *Journal of Biotechnology* 113, 295-304.
- [68] J. Sipma, B. Osuna, N. Collado, H. Monclús, G. Ferrero, J. Comas, I. Rodriguez-Roda, 2010. Comparison of removal of pharmaceuticals in MBR and activated sludge systems. *Desalination* 250, 653-659.
- [69] Y. Zhang, S.-U. Geißen, C. Gal, 2008. Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere* 73, 1151-1161.
- [70] M. Pomiès, J.-M. Choubert, C. Wisniewski, M. Coquery, 2013. Modelling of micropollutant removal in biological wastewater treatments: A review. *Science of the Total Environment* 443, 733-748.
- [71] E. Sahar, R. Messalem, H. Cikurel, A. Aharoni, A. Brenner, M. Godehardt, M. Jekel, M. Ernst, 2011. Fate of antibiotics in activated sludge followed by ultrafiltration (CAS-UF) and in a membrane bioreactor (MBR). *Water Research* 45, 4827-4836.
- [72] T. Urase, C. Kagawa, T. Kikuta, 2005. Factors affecting removal of pharmaceutical substances and estrogens in membrane separation bioreactors. *Desalination* 178, 107-113.
- [73] R.P. Schwarzenbach, P. Gschwend, D. Imboden, *Environmental Organic Chemistry*. 2003, Hoboken, New Jersey: Wiley-Interscience.
- [74] A.A. Alturki, N. Tadkaew, J.A. McDonald, S.J. Khan, W.E. Price, L.D. Nghiem, 2010. Combining MBR and NF/RO membrane filtration for the removal of trace organics in indirect potable water reuse applications. *Journal of Membrane Science* 365, 206-215.
- [75] M. Carballa, G. Fink, F. Omil, J.M. Lema, T. Ternes, 2008. Determination of the solid–water distribution coefficient ( $K_d$ ) for pharmaceuticals, estrogens and musk fragrances in digested sludge. *Water Research* 42, 287-295.
- [76] M. Cirja, P. Ivashechkin, A. Schäffer, P.F. Corvini, 2008. Factors affecting the removal of organic micropollutants from wastewater in conventional treatment plants (CTP) and membrane bioreactors (MBR). *Reviews in Environmental Science and Bio/Technology* 7, 61-78.
- [77] A. Joss, E. Keller, A.C. Alder, A. Göbel, C.S. McArdell, T. Ternes, H. Siegrist, 2005. Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water research* 39, 3139-3152.
- [78] C. Abegglen, A. Joss, C.S. McArdell, G. Fink, M.P. Schlüsener, T.A. Ternes, H. Siegrist, 2009. The fate of selected micropollutants in a single-house MBR. *Water research* 43, 2036-2046.

- [79] N.H. Tran, T. Urase, O. Kusakabe, 2009. The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds. *Journal of hazardous materials* 171, 1051-1057.
- [80] T. Urase, T. Kikuta, 2005. Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process. *Water Research* 39, 1289-1300.
- [81] T.A. Ternes, N. Herrmann, M. Bonerz, T. Knacker, H. Siegrist, A. Joss, 2004. A rapid method to measure the solid–water distribution coefficient ( $K_d$ ) for pharmaceuticals and musk fragrances in sewage sludge. *Water Research* 38, 4075-4084.
- [82] A. Göbel, A. Thomsen, C.S. McArdell, A. Joss, W. Giger, 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. *Environmental science & technology* 39, 3981-3989.
- [83] E.M. Golet, I. Xifra, H. Siegrist, A.C. Alder, W. Giger, 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environmental science & technology* 37, 3243-3249.
- [84] B. Li, T. Zhang, 2010. Biodegradation and Adsorption of Antibiotics in the Activated Sludge Process. *Environmental science & technology* 44, 3468-3473.
- [85] J. Radjenović, M. Petrović, D. Barceló, 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water research* 43, 831-841.
- [86] M. Clara, B. Strenn, N. Kreuzinger, 2004. Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration. *Water Research* 38, 947-954.
- [87] C. Ort, M.G. Lawrence, J. Rieckermann, A. Joss, 2010. Sampling for Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Systems: Are Your Conclusions Valid? A Critical Review. *Environmental science & technology* 44, 6024-6035.
- [88] A. Göbel, C.S. McArdell, A. Joss, H. Siegrist, W. Giger, 2007. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of the Total Environment* 372, 361-371.
- [89] R. Reif, S. Suárez, F. Omil, J. Lema, 2008. Fate of pharmaceuticals and cosmetic ingredients during the operation of a MBR treating sewage. *Desalination* 221, 511-517.
- [90] M. Bernhard, J. Müller, T.P. Knepper, 2006. Biodegradation of persistent polar pollutants in wastewater: comparison of an optimised lab-scale membrane bioreactor and activated sludge treatment. *Water research* 40, 3419-3428.



- [91] J.B. Quintana, S. Weiss, T. Reemtsma, 2005. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. *Water Research* 39, 2654-2664.
- [92] S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. *Water Research* 41, 1013-1021.
- [93] K.C. Wijekoon, F.I. Hai, J. Kang, W.E. Price, W. Guo, H.H. Ngo, L.D. Nghiem, 2013. The fate of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters and pesticides during MBR treatment. *Bioresource technology* 144, 247-254.
- [94] J. Radjenovic, M. Petrovic, D. Barceló, 2007. Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. *Analytical and Bioanalytical Chemistry* 387, 1365-1377.
- [95] L.N. Nguyen, F.I. Hai, J. Kang, W.E. Price, L.D. Nghiem, 2013. Coupling granular activated carbon adsorption with membrane bioreactor treatment for trace organic contaminant removal: Breakthrough behaviour of persistent and hydrophilic compounds. *Journal of environmental management* 119, 173-181.
- [96] K. Kimura, H. Hara, Y. Watanabe, 2005. Removal of pharmaceutical compounds by submerged membrane bioreactors (MBRs). *Desalination* 178, 135-140.
- [97] K. Kimura, H. Hara, Y. Watanabe, 2007. Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors. *Environmental science & technology* 41, 3708-3714.
- [98] L.N. Nguyen, F.I. Hai, J. Kang, W.E. Price, L.D. Nghiem, 2012. Removal of trace organic contaminants by a membrane bioreactor–granular activated carbon (MBR–GAC) system. *Bioresource technology* 113, 169-173.
- [99] P. Cartagena, M.E. Kaddouri, V. Cases, A. Trapote, D. Prats, 2013. Reduction of emerging micropollutants, organic matter, nutrients and salinity from real wastewater by combined MBR-NF/RO treatment. *Separation and Purification Technology*.
- [100] L.N. Nguyen, F.I. Hai, J. Kang, L.D. Nghiem, W.E. Price, W. Guo, H.H. Ngo, K.-L. Tung, 2013. Comparison between sequential and simultaneous application of activated carbon with membrane bioreactor for trace organic contaminant removal. *Bioresource technology* 130, 412-417.
- [101] N. Tadkaew, F.I. Hai, J.A. McDonald, S.J. Khan, L.D. Nghiem, 2011. Removal of trace organics by MBR treatment: the role of molecular properties. *Water research* 45, 2439-2451.
- [102] S.A. Snyder, S. Adham, A.M. Redding, F.S. Cannon, J. DeCarolis, J. Oppenheimer, E.C. Wert, Y. Yoon, 2007. Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. *Desalination* 202, 156-181.

- [103] S.K. Maeng, B.G. Choi, K.T. Lee, K.G. Song, 2013. Influences of solid retention time, nitrification and microbial activity on the attenuation of pharmaceuticals and estrogens in membrane bioreactors. *Water Research*.
- [104] J.L. Tambosi, R.F. de Sena, M. Favier, W. Gebhardt, H.J. José H.F. Schröder, R.d.F.P.M. Moreira, 2010. Removal of pharmaceutical compounds in membrane bioreactors (MBR) applying submerged membranes. *Desalination* 261, 148-156.
- [105] D. Dolar, M. Gros, S. Rodriguez-Mozaz, J. Moreno, J. Comas, I. Rodriguez-Roda, D. Barceló 2012. Removal of emerging contaminants from municipal wastewater with an integrated membrane system, MBR–RO. *Journal of hazardous materials* 239, 64-69.
- [106] F.I. Hai, X. Li, W.E. Price, L.D. Nghiem, 2011. Removal of carbamazepine and sulfamethoxazole by MBR under anoxic and aerobic conditions. *Bioresource technology* 102, 10386-10390.
- [107] T. Trinh, B. van den Akker, R. Stuetz, H. Coleman, P. Le-Clech, S. Khan, 2012. Removal of trace organic chemical contaminants by a membrane bioreactor. *Water Science & Technology* 66, 1856-1863.
- [108] L.D. Nghiem, N. Tadkaew, M. Sivakumar, 2009. Removal of trace organic contaminants by submerged membrane bioreactors. *Desalination* 236, 127-134.
- [109] N. Tadkaew, M. Sivakumar, S.J. Khan, J.A. McDonald, L.D. Nghiem, 2010. Effect of mixed liquor pH on the removal of trace organic contaminants in a membrane bioreactor. *Bioresource technology* 101, 1494-1500.
- [110] V.M. Monsalvo, J.A. McDonald, S.J. Khan, P. Le-Clech, 2014. Removal of trace organics by anaerobic membrane bioreactors. *Water Research* 49, 103-112.
- [111] N.H. Tran, T. Urase, H.H. Ngo, J. Hu, S.L. Ong, 2013. Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. *Bioresource technology* 146, 721-731.
- [112] E. Fernandez-Fontaina, F. Omil, J. Lema, M. Carballa, 2012. Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants. *Water Research*.
- [113] S. Suarez, J.M. Lema, F. Omil, 2010. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. *Water Research* 44, 3214-3224.
- [114] H. Bouju, G. Buttiglieri, F. Malpei, 2008. Perspectives of persistent organic pollutants (POPS) removal in an MBR pilot plant. *Desalination* 224, 1-6.
- [115] M. Aubenneau, A. Tahar, C. Casellas, C. Wisniewski, 2010. Membrane bioreactor for pharmaceutically active compounds removal: Effects of carbamazepine on mixed microbial communities implied in the treatment. *Process Biochemistry* 45, 1826-1831.
- [116] S. Wang, R.M. Holzem, C.K. Gunsch, 2008. Effects of pharmaceutically active compounds on a mixed microbial community originating from a municipal wastewater treatment plant. *Environmental science & technology* 42, 1091-1095.

- [117] L.F. Delgado, S. Schetrite, C. Gonzalez, C. Albasi, 2010. Effect of cytostatic drugs on microbial behaviour in membrane bioreactor system. *Bioresource technology* 101, 527-536.
- [118] L.F. Delgado, V. Faucet-Marquis, S. Schetrite, A. Pfohl-Leszkowicz, S. Paranthoen, C. Albasi, 2010. Effect of cytostatic drugs on the sludge and on the mixed liquor characteristics of a cross-flow membrane bioreactor: Consequence on the process. *Journal of Membrane Science* 347, 165-173.
- [119] A. Avella, L.F. Delgado, T. Görner, C. Albasi, M. Galmiche, P. De Donato, 2010. Effect of cytostatic drug presence on extracellular polymeric substances formation in municipal wastewater treated by membrane bioreactor. *Bioresource technology* 101, 518-526.
- [120] B. Kraigher, T. Kosjek, E. Heath, B. Kompare, I. Mandic-Mulec, 2008. Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors. *Water Research* 42, 4578-4588.
- [121] W.C. Lay, Q. Zhang, J. Zhang, D. McDougald, C. Tang, R. Wang, Y. Liu, A.G. Fane, 2012. Effect of pharmaceuticals on the performance of a novel osmotic membrane bioreactor (OMBR). *Separation Science and Technology* 47, 543-554.
- [122] M. Jacob, 2011. R utilisation des eaux usées épurées par association de procédés biologiques et membranaires. PhD Thesis.
- [123] M.M. Huber, A. Göbel, A. Joss, N. Hermann, D. Löffler, C.S. McArdell, A. Ried, H. Siegrist, T.A. Ternes, U. von Gunten, 2005. Oxidation of pharmaceuticals during ozonation of municipal wastewater effluents: a pilot study. *Environmental science & technology* 39, 4290-4299.
- [124] L.N. Nguyen, F.I. Hai, J. Kang, W.E. Price, L.D. Nghiem, 2013. Removal of emerging trace organic contaminants by MBR-based hybrid treatment processes. *International Biodeterioration & Biodegradation* 85, 474-482.
- [125] G. Laera, M.N. Chong, B. Jin, A. Lopez, 2011. An integrated MBR–TiO<sub>2</sub> photocatalysis process for the removal of Carbamazepine from simulated pharmaceutical industrial effluent. *Bioresource technology* 102, 7012-7015.
- [126] A. Alturki, J. McDonald, S.J. Khan, F.I. Hai, W.E. Price, L.D. Nghiem, 2012. Performance of a novel osmotic membrane bioreactor (OMBR) system: flux stability and removal of trace organics. *Bioresource technology* 113, 201-206.
- [127] X. Li, F.I. Hai, L.D. Nghiem, 2011. Simultaneous activated carbon adsorption within a membrane bioreactor for an enhanced micropollutant removal. *Bioresource technology* 102, 5319-5324.
- [128] L.N. Nguyen, F.I. Hai, L.D. Nghiem, J. Kang, W.E. Price, C. Park, K. Yamamoto, 2013. Enhancement of removal of trace organic contaminants by powdered activated carbon dosing into membrane bioreactors. *Journal of the Taiwan Institute of Chemical Engineers*.
- [129] D. Serrano, S. Suárez, J. Lema, F. Omil, 2011. Removal of persistent pharmaceutical micropollutants from sewage by addition of PAC in a sequential membrane bioreactor. *Water Research* 45, 5323-5333.

- [130] A. Joss, C. Baenninger, P. Foa, S. Koepke, M. Krauss, C.S. McArdell, K. Rottermann, Y. Wei, A. Zapata, H. Siegrist, 2011. Water reuse:> 90% water yield in MBR/RO through concentrate recycling and CO<sub>2</sub> addition as scaling control. *Water Research* 45, 6141-6151.
- [131] E. Sahar, I. David, Y. Gelman, H. Chikurel, A. Aharoni, R. Messalem, A. Brenner, 2011. The use of RO to remove emerging micropollutants following CAS/UF or MBR treatment of municipal wastewater. *Desalination* 273, 142-147.
- [132] L.S. Tam, T.W. Tang, G.N. Lau, K.R. Sharma, G.H. Chen, 2007. A pilot study for wastewater reclamation and reuse with MBR/RO and MF/RO systems. *Desalination* 202, 106-113.
- [133] K. Kimura, T. Iwase, S. Kita, Y. Watanabe, 2009. Influence of residual organic macromolecules produced in biological wastewater treatment processes on removal of pharmaceuticals by NF/RO membranes. *Water Research* 43, 3751-3758.



## Publication 2

---

---



# Effects of carbamazepine in peak injection on fouling propensity of activated sludge from a MBR treating municipal wastewater

Accepted for publication in Journal of Membrane Science

Chengcheng Li<sup>a,b,c,\*</sup>, Corinne Cabassud<sup>a,b,c</sup>, Christelle Guigui<sup>a,b,c</sup>

<sup>a</sup> Université de Toulouse, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, 31077 Toulouse, France

<sup>b</sup> INRA, UMR792 Ingénierie des Systèmes biologiques et des Procédés, 31400 Toulouse, France

<sup>c</sup> CNRS, UMR5504, 31400 Toulouse, France

\* Corresponding author at: INSA Toulouse, Hall GPE, 135 Avenue de Rangueil, 31077 Toulouse, France. Tel.: +33 05 61 55 97 91; Email: lichengcheng\_1002@hotmail.com, chengcheng.li@insa-toulouse.fr

Email addresses of other authors: corinne.cabassud@insa-toulouse.fr, christelle.guigui@insa-toulouse.fr

## Abstract

Membrane fouling remains the major obstacle for development of membrane bioreactors (MBRs). This paper aimed at investigating the effects of carbamazepine (CBZ) present in a discontinuous way at high concentration (peak injection) on fouling propensity of activated sludge from a MBR that treats domestic wastewater. Batch experiments with peak injection of CBZ (100  $\mu\text{g L}^{-1}$  in sludge for 3 hours) were performed for sludges sampled from a MBR operated under different organic loading conditions. HPLC-SEC analysis with fluorescence detector was employed to study the effects of CBZ on protein-like compounds in supernatant. With addition of CBZ in sludge, a significant increase of sludge fouling propensity was observed when using sludge sampled from the MBR operated under low organic loading rate (0.1 kg COD/kg MLSS/d), which could be attributed to the increase in the quantity of protein-like substances with a 100-1000 kDa molecular size in supernatant. Whereas reduced CBZ effect was found for sludge sampled from the MBR operated under higher organic loading rate (0.2 kg COD/kg MLSS/d). Whatever the sludge, the injection of CBZ induced no significant change of the floc size distribution and of the polysaccharide concentration in supernatant. Moreover, it did not affect the sludge microbial activity.

**Key words:** membrane bioreactor, carbamazepine, peak injection, fouling propensity, activated sludge



## 1. Introduction

The presence of micropollutants, notably the pharmaceuticals, in aquatic environment has been paid great attention since the late 1990s due to their potential adverse effects on human health [1-4]. Since then, pharmaceutical micropollutants have been frequently detected in domestic wastewater treatment plants (WWTPs) and also in the plants that produce potable water from surface waters or ground waters [5-7]. Many pharmaceutical micropollutants, including analgesics like ibuprofen, diclofenac, naproxen and ketoprofen, lipid regulators like bezafibrate and gemfibrozil, the antiepileptic drug carbamazepine, and antibiotics such as trimethoprim and sulfamethoxazole, have been frequently detected at concentration higher than 1.0 µg/L in WWTP influents around the world [8-12].

As membrane bioreactors (MBRs) get increasing importance in the field of municipal wastewater treatment and reuse [13-15], the removal of pharmaceutical micropollutants in MBRs has been thoroughly investigated [16-17]. The removal efficiency of pharmaceutical micropollutants in MBR varies greatly due to the difference in their biodegradability. For example, ibuprofen and bezafibrate are easily biodegraded with more than 90% elimination efficiency, carbamazepine is hardly biodegraded with an elimination efficiency lower than 10%, and the majority of pharmaceutical micropollutants (such as ketoprofen, gemfibrozil, trimethoprim, sulfamethoxazole etc.) are moderately biodegraded with elimination efficiency varying between 50% and 80% [18]. Thus, sludge retention time (SRT) is believed to be capable of affecting the biodegradation of pharmaceutical micropollutants in MBRs. High SRT allows the enrichment of slow-growing microorganisms (e.g. nitrifying bacteria) in the sludge and consequently the establishment of a more diverse bacteria population, which may favor removal of pharmaceutical micropollutants in MBR [19]. In particular, removal of diclofenac, ketoprofen, gemfibrozil, trimethoprim and erythromycin in MBR was found to be

significantly affected by SRT [20-22]. For other kinds of wastewaters, for example, effluents of hospitals or drug production facilities, a specific acclimated biomass may be required for MBRs treating these kinds of effluents, since they may contain very high concentration of pharmaceuticals, especially the antibiotics [23-25].

Taking into account that the concentration of pharmaceutical micropollutants in influents of domestic WWTPs varies greatly due to their therapeutic consumptions for treatment of seasonal diseases [26-29] or inappropriate disposal of unused pharmaceuticals [30], it is possible that peak concentration of some pharmaceutical micropollutants occur in domestic wastewaters during short periods, which may affect the performance of biological process such as MBRs. For example, ibuprofen was detected at concentration of 319  $\mu\text{g/L}$  in the influent of a Spanish WWTP [31]. Also, naproxen was detected at concentration of 611  $\mu\text{g/L}$  in the influent of a Canadian WWTP [32]. In the literature, a few studies have been carried out to investigate the effects of peak chemical stress, including pharmaceutical micropollutants, on the microbial activity of MBR sludges. Henriques et al. [33] observed that a cadmium shock load (20 mg/L) caused about 50% inhibition of heterotrophic respiration (through measurement of specific oxygen uptake rates) on MBR sludge. Lesage et al. [34] found that a peak concentration (130 mg/L) of a toxic compound, 2,4 dimethylphenol (DMP), could cause 50% inhibition of respiration on MBR sludge. Aubenneau et al. [35] examined the effect of trace concentration of carbamazepine on mixed microbial communities of activated sludge taken from MBR. The authors reported inhibition of exogenous respiration rate of activated sludge in presence of 1  $\mu\text{g L}^{-1}$  carbamazepine. Wang et al. [36] reported a 39%, 39%, and 19% decrease, respectively, in specific oxygen uptake rate (SOUR), defined as sludge oxygen uptake rate divided by the concentration of volatile suspended solid in the

sludge, when presence of 2.3 mg L<sup>-1</sup> naproxen, 2.5 mg L<sup>-1</sup>ketoprofen, and 2.4 mg L<sup>-1</sup> carbamazepine in the same activated sludge in batch experiments.

However, to our knowledge the bibliography do not report the peak effect of domestic pharmaceutical micropollutants on MBR fouling. It is known that a chemical stress can induce the production of soluble microbial products (SMP) or extracellular polymeric substances (EPS), which are identified as main foulants for MBR fouling [37-39]. For example, Reid et al. [40] reported that a salt shock, introduced by dosing directly 30 g L<sup>-1</sup> NaCl solution during 10 min in MBR, resulted in significant increase of SMP and EPS concentrations in MBR. Similar results have been recently observed by Wang et al. [41], who reported that a high osmotic pressure (5% NaCl) induces the production of SMPs with a relative strong hydrophilicity and protein-like characteristics in batch experiments. Sheng et al. [42] reported that in presence of toxic chemicals, such as 30 mg l<sup>-1</sup> Cu(II), 40 mg l<sup>-1</sup> Cr(VI), 5 mg l<sup>-1</sup> Cd(II), in the bacteria strain *Rhodospseudomonas acidophila*, the EPS content increased by 5.5, 2.5, and 4.0 times, respectively. Moreover, the authors reported that under these toxic conditions, the increase in the protein content far exceeded than that of other constituents in EPS. Lay et al. [43] observed a significant increase in the protein and polysaccharide ratio for both soluble and bound EPS in the activated sludge after adding carbamazepine, diclofenac, ibuprofen and naproxen in the feed tank (20–25 µg L<sup>-1</sup> of each pharmaceutical in the feed) of an Osmotic Membrane Bioreactor (OMBR).

The objective of this study is to investigate the peak effects of pharmaceutical micropollutants that are currently found in domestic wastewater on fouling propensity and physico-chemical characteristics of the activated sludge from a MBR treating domestic wastewater. Carbamazepine (CBZ), an anti-epileptic drug, was selected as the representative of pharmaceutical micropollutants found in domestic wastewater because of the following

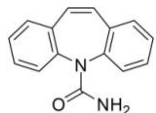
reasons: i) it has been frequently detected in influents as well as effluents of WWTPs in concentration up to or higher than  $1.0 \mu\text{g L}^{-1}$  [44]; ii) it is almost not biodegradable and its sorption to activated sludges is negligible in biological activated sludge processes [45-46]; iii) it was found to have some inhibition effects on biological activity of microorganisms in activated sludge [35-36].

## 2. Material and Methods

### 2.1 Peak effects of carbamazepine on MBR sludge

Table 1 introduces the physicochemical properties of carbamazepine. A concentrated mother solution was prepared by dissolving carbamazepine (from Sigma-Aldrich) in pure ethanol (from VWR International) at a concentration of 1 g/L, stored at 4 °C and used within a week.

Table 1 Physico-chemical properties of carbamazepine [44, 47-48]

Structure	
Formula	$\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}$
CAS No.	298-46-4
Molecular weight (g/mol)	236
Usage	Anti-epileptic
Water solubility (mg/L at 25°C)	17.7
LogKow	2.45
Charge at pH 7	Neutral
sorption constant, $K_d$ (L/kgss)	1.2
degradation constant $k_{\text{biol}}$ (L/gss/d)	<0.01

The peak effects of CBZ on sludge were characterized in batch reactors with sludge taken from the aerobic tank of a lab-scale MBR due to the easier manipulation for repeatability examination, as compared with the tests conducted in MBR reactor. Sludges from MBR operated under different organic loading rates were examined, since the SMP composition and microbial activity of MBR sludge was significantly affected by the organic loading rates [49-50]. Two batch reactors were operated in parallel in presence of CBZ (at concentration of  $100 \mu\text{g L}^{-1}$  in the same sludge) or without CBZ for 3 hours. Fig. 1 shows the protocol used for batch experiments. Firstly, quantities of the concentrated mother solution sufficient to yield  $100 \mu\text{g L}^{-1}$  final concentrations of CBZ were added in the reactor-with CBZ. As comparison, the same quantity of pure ethanol was added in the control-reactor to rule out the impact of ethanol. Then the ethanol in the two batch reactors was blown off using a gentle stream of air, leaving only the CBZ in the reactor-with CBZ [51]. Finally, a same volume of sludge samples taken from MBR aerobic tank was added in the reactor-with CBZ and control-reactor, and the mixed liquids were stirred using the aeration in the bottom of each reactor for 3 hours to dissolve CBZ and to make the sludge contact with CBZ in aerobic condition. Then the sludges in both reactors were sampled and the two corresponding supernatants were prepared by centrifugation of the sludge samples at 4000 g during 10 minutes at room temperature at the same time. 3 hours of contact time was chosen since it takes about 3 hours before all the readily degradable substances are consumed by the activated sludge [52]. Indeed, no significant difference was found concerning the effects of CBZ on characteristics of sludge and supernatant between contact time of 3 hours and 5 hours. Too long time, for example 24 hours, for sludge in batch reactors without exogenous substrate, would cause the decay of bacteria and impact the quality of activated sludge. And no significant difference was found concerning the effects of CBZ on characteristics of sludge and supernatant when the peak concentration of CBZ was modified to  $500 \mu\text{g L}^{-1}$ , as compared to the effects of CBZ at

concentration of  $100 \mu\text{g L}^{-1}$ . Moreover, no significant difference was observed between the sludge obtained from control-reactor and blank reactor where no ethanol was added (see Fig. 1) in terms of biomass and supernatant quality, which confirms that the ethanol did not affect the quality of sludge after evaporated by the gentle stream of air.

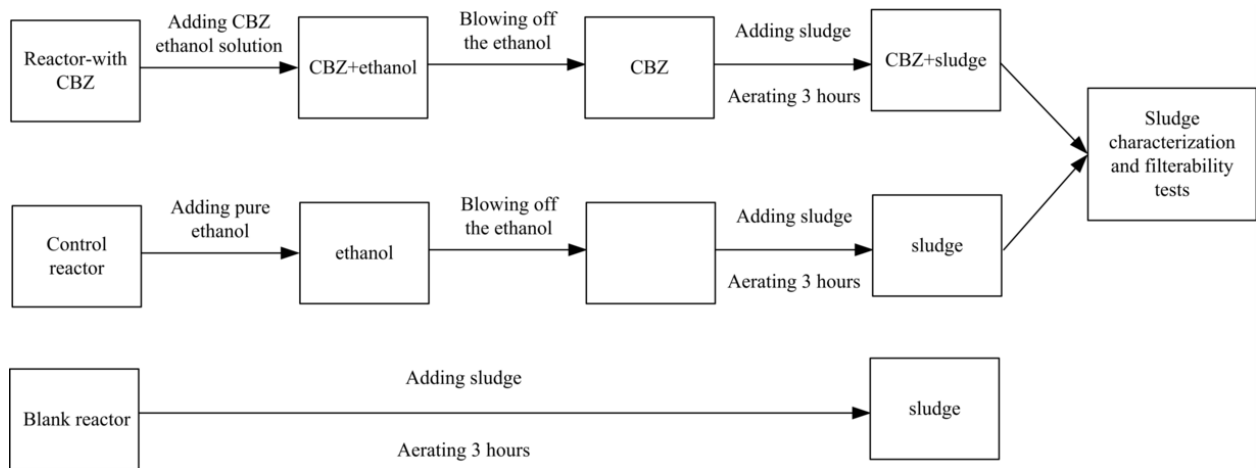


Fig. 1. Schema of batch experiments

## 2.2 Characterization of fouling propensity

Fouling propensity of sludges and supernatants sampled from batch reactors (reactor-with CBZ and control-reactor) was characterized by filterability test performed with a stirred dead-end filtration cell (Amicon 8050, Millipore) in order to determine the fouling resistance during filtration of sludges and supernatants under different transmembrane pressures (TMP). Microfiltration (MF) membranes (from Alfa Laval, FR), made of polysulphone (PS), with a pore size of  $0.2 \mu\text{m}$  ( $L_{p0} = 200 \text{ l h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ ), were used for sludge filterability tests, because a pore size of  $0.2 \mu\text{m}$  is in the range of pore size of microfiltration membranes used in the flat sheet MBRs. Whereas, ultrafiltration (UF) membranes (from Orelis, FR), made of polyethersulfone (PES), with a nominal pore size of  $0.01 \mu\text{m}$  ( $L_{p0} = 50\text{-}80 \text{ l h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ ), were used for supernatant filterability tests to retain biopolymer components in the

MBR supernatant, behaving like the second layer on MBR membrane surface. A new membrane sample was used for each filtration test. All membranes were soaked in ultra pure water over night to maintain pores wetted. Then they were rinsed by filtration of ultra pure water at 1 bar during 15 min prior to filtration test. The filtration tests were performed under three constant transmembrane pressures (TMP): 0.5 bar, 0.8 bar, and 1.0 bar, respectively. For each filtration test, sludge or supernatant was filtered through selected membranes until 70 ml permeate was produced (corresponding to a filtered permeate volume of about 50 L m<sup>-2</sup>). Temperature was measured and flux was normalized to the flux at 20 °C. For supernatant filtration, no stirring was performed, while for sludge filtration, the sludge was stirred at 200 rpm. After filtration the membrane was turned face down and was backwashed with ultra pure water at 1 bar for 15 min in order to investigate flux recovery by permeability measurement. Membrane fouling resistance was determined according to Eq. (1).

$$J = \frac{TMP}{\mu(R_m + R_f)} \quad (1)$$

In this equation, J is the permeate flux (m<sup>3</sup>/s/m<sup>2</sup>) at 20 °C, with R<sub>m</sub> the membrane resistance (m<sup>-1</sup>), R<sub>f</sub> the fouling resistance (m<sup>-1</sup>), TMP the transmembrane pressure (Pa) and μ the dynamic viscosity at 20 °C (Pa s).

Then in the dead-end filtration cell, αC can be determined by cake deposit model as expressed in Eq. (2).

$$R_f = \alpha C \frac{V}{A} \quad (2)$$

In this equation, α is the specific cake resistance (m kg<sup>-1</sup>), with C the deposited mass per filtered permeate volume (kg m<sup>-3</sup>), V the cumulated permeate volume (m<sup>3</sup>) and A the effective

membrane area for filtration ( $\text{m}^2$ ). For supernatant filtration, C could be calculated by the retained DOC ( $\text{kg m}^{-3}$ ). For sludge filtration, C is assumed to be MLSS value of sludge.

### **2.3 Characterization of sludge microbial activity**

Sludge microbial activity was characterized by the microbial respiration rate. It was measured in respirometric batch experiments defined by Sp érandio and Paul [53]. Sludge samples were collected from the aerobic tank of the MBR. Firstly, the collected sludge was aerated for at least 3 h to reach the endogenous condition. Then pure ethanol was added to respirometric reactor to test the sludge microbial activity before addition of CBZ. Pure ethanol was used as exogenous substrate in the respirometric experiments, because CBZ was prepared in pure ethanol. Then, quantities of the concentrated mother solution sufficient to yield  $100 \mu\text{g L}^{-1}$  final concentration of CBZ were added in the respirometric reactor to test the effect of CBZ on the sludge microbial activity.

### **2.4 Sludge**

Sludge for batch experiments was sampled from the aerobic tank of a lab-scale submerged MBR set-up consisting of an anoxic reactor (5.4 L) and an aerobic reactor (12.6 L). A flat sheet microfiltration membrane ( $L_{p0} = 1110 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ , Kubota, Japan) was submerged inside the aerobic reactor. It has an effective filtration area of  $0.1 \text{ m}^2$ , with an average pore size of  $0.2 \mu\text{m}$ . Two aeration systems were used in the aerobic reactor. An aeration system with coarse air bubble was installed below the membrane to reduce fouling and it was operated continuously at flow rate of  $1.5 \text{ L/min}$ , i.e.  $\text{SAD}_p$  of  $90 \text{ m}^3 \text{ air/m}^3$  permeate. Besides, another aeration system with fine air bubbles was used to maintain the dissolved oxygen concentration in the sludge at about  $2\text{-}3 \text{ mg/L}$  and automatically controlled by an oxygen sensor. The sludge recirculation ratio was fixed at 4. The MBR was fed with a



municipal wastewater taken from a wastewater treatment plant close to Toulouse, France, after a primary physical treatment. The MBR was operated in two steps (see Table 2). In the first step, the MBR was operated at a SRT of 40 days, with a sludge concentration in the aerobic reactor at 4-6 g/L. In the second step, the MBR was operated at a 30 days SRT, with a sludge concentration in aerobic reactor at 6-8 g/L. The MBR reached high removal of COD, more than 95%, during both two steps. Whereas a limited removal of TN, about 40%, was observed during step 1 when the MBR was operated at an organic loading rate ( $C_m$ ) of about 0.1 kg COD/kg MLSS/d.

Table 2. Experimental conditions and MBR performances in this study

Step	Experiment duration (days)	organic loading rate (kg COD/kg MLSS/d)	Aerobic MLSS (g/L)	SRT (days)	Filtration/relaxation cycles (min/min)	Net flux (L/h/m <sup>2</sup> )	Average COD removal (%)	Average TN removal (%)
1	161	0.1	4-6	40	8/2	10	96.7 ± 2.2	41.4 ± 8.4
2	102	0.2	6-8	30	8/4	10	97.1 ± 1.6	82.5 ± 6.2

## 2.5 Analytical methods

The concentration of mixed liquor suspended solids (MLSS) was measured according to the standard method AFNOR NFT 90-105. Chemical oxygen demand (COD) and total nitrogen (TN) were measured by spectrometry with reagent kits. Dissolved organic carbon (DOC) was measured by a Total Organic Carbon analyzer (Shimadzu, FR). Prior to DOC analysis, each sample was pre-filtered on a 0.45 µm membrane. Floc size distribution was measured by Malvern Mastersizer 2000. Quantification of proteins and polysaccharides was performed on the supernatants. Protein content, expressed in mg equivalent of bovine serum albumin per

liter, was measured by the acid bicinchoninic method [54]. Polysaccharide concentration, expressed in mg equivalent of glucose per liter, was measured by the Anthrone method [55].

High performance liquid chromatography—size exclusion chromatography (HPLC—SEC) analysis was performed by an Akta Purifier (GE Healthcare, USA) equipped with a column Protein Kw804 (Shodex, Japan), a silica-based column with an exclusion limit of 1000 kDa, and a fluorescence detector (Varian, USA). The eluent solution for the mobile phase was prepared with 25 mM Na<sub>2</sub>SO<sub>4</sub> and a phosphate buffer (2.4 mM NaH<sub>2</sub>PO<sub>4</sub> and 1.6 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 6.8). The flow rate of eluent passing through the column was 1.0 mL/min. The excitation/emission wavelengths were set at 280/350 nm, which were confirmed as the required values by EEM fluorescence results [56-57], to detect protein-like substances. The column was calibrated using standard proteins: thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa) from GE Healthcare. Prior to analysis of HPLC-SEC-fluorescence, each sample was pre-filtered on a 0.45 µm membrane.

### **3. Results and Discussion**

#### **3.1. Effect of CBZ on sludge fouling propensity**

Sludge fouling propensity was characterized by filterability test. Fig. 2 shows the result of filterability tests of sludges sampled from the batch reactors (reactor-with CBZ and control-reactor), using sludges taken from the MBR operated under low organic loading rate (0.1 kg COD/kg MLSS/d), at 1.0 bar. As shown in Fig. 2, for the sludge from the reactor-with CBZ, the fouling propensity, expressed as the fouling resistance produced when a fixed volume of permeate was filtered, was higher as compared with the sludge from the control-reactor.

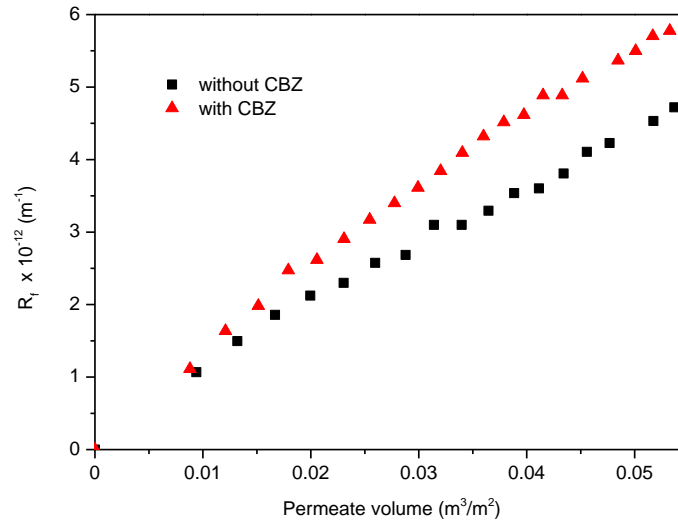


Fig. 2. Effect of CBZ on sludge fouling propensity ( $C_m = 0.1$  kg COD/kg MLSS/d)

Three assumptions can be proposed to explain the increased sludge fouling propensity after addition of CBZ: i) a change in microbial activity linked to a stress on the microorganisms ii) a change in biomass physico-chemical properties, like mean floc size distribution, or iii) a change in the supernatant quality. These three assumptions will be discussed in the following paragraphs that aim to evaluate the effects of CBZ on the supernatant and biomass properties.

### 3.1.1 Effect of CBZ on sludge microbial activity

The effect of CBZ on sludge microbial activity was investigated in a respirometric reactor. The sludge microbial activity was characterized by the microbial respiration rate. Fig. 3 shows the result of respirometric tests for the sludge sampled from aerobic tank of the MBR operated under organic loading rate of 0.1 kg COD/kg MLSS/d. As shown in Fig. 3, the activated sludge was in the endogenous condition before adding ethanol in the respirometric reactor. Then an immediately significant increase of respiration rate was observed after addition of pure ethanol in the respirometer due to the degradation of ethanol. Then the respiration rate kept constant for a short period, during which time the bacteria may adapt to the presence of ethanol. After this period, increased respiration rate was observed until the exhaustion of

ethanol, which indicates that the microbial growth occurred in this period. Then after the activated sludge reached endogenous condition in absence of exogenous substrate, quantities of the concentrated CBZ solution sufficient to yield  $100 \mu\text{g L}^{-1}$  final concentrations of CBZ in sludge were added in the respirometric reactor to test the effect of CBZ on the sludge microbial activity. The result showed no inhibition of sludge microbial activity with presence of  $100 \mu\text{g L}^{-1}$  CBZ in the sludge, since after addition of CBZ ethanol solution in the respirometric reactor the respiration rate was immediately increased to the same level that was reached before exhaustion of ethanol when pure ethanol was added in the respirometric reactor. Moreover, respiration rate continued to increase without adaptation period to the chemical stress induced by CBZ until the exhaustion of ethanol, which again indicated the microbial growth without inhibition in presence of exogenous substrate. This result indicates that the presence of  $100 \mu\text{g L}^{-1}$  CBZ in the sludge during a short period contact time did not change the degradation and growth behavior of bacteria in activated sludge or the growth of bacteria offset the small inhibition effect induced by CBZ in presence of exogenous substrate. This result is consistent with the result of Delgado et al. [52], who found no inhibition on microbial activity of activated sludge taken from MBR in presence of pulse of cytostatic compounds (about  $200 \mu\text{g L}^{-1}$  cyclophosphamide and its principal metabolites) during the short contact time in the respirometric reactor. No inhibition effect of CBZ on microbial activity was also found for the sludge sampled from the MBR operated under organic loading rate of  $0.2 \text{ kg COD/kg MLSS/d}$ . Thus, the modification of sludge fouling propensity could not be attributed to the modification in sludge microbial activity.

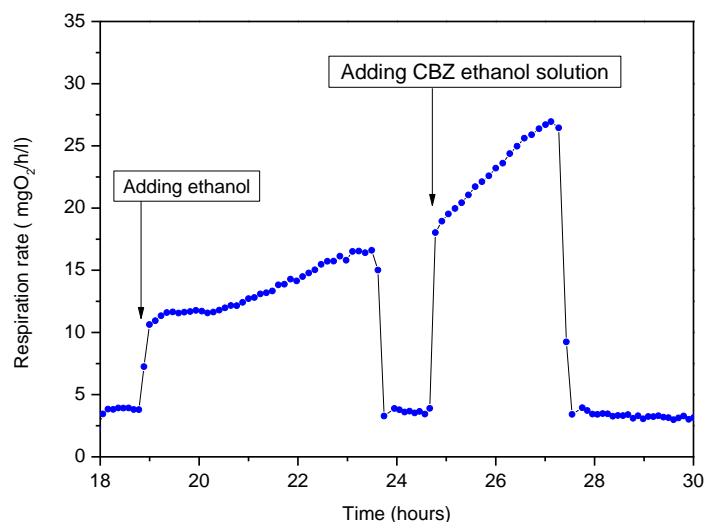


Fig. 3. Effect of CBZ on sludge microbial activity

### 3.1.2 Effect of CBZ on sludge floc size distribution

Fig. 4 shows the result of floc size distribution analysis of activated sludges sampled from the batch reactors that use sludges taken from the MBR operated under organic loading rate of 0.1 kg COD/kg MLSS/d. No significant change in floc size distribution was observed. The result indicates that no significant decay or reconstruction of sludge occurred in presence of 100  $\mu\text{g L}^{-1}$  CBZ during short contact time (3h). Besides, no significant effect of CBZ on floc size distribution of activated sludge taken from the MBR operated under organic loading rate of 0.2 kg COD/kg MLSS/d was also observed. Thus, the modification of sludge fouling propensity could not be originated from the modification in sludge floc size distribution.

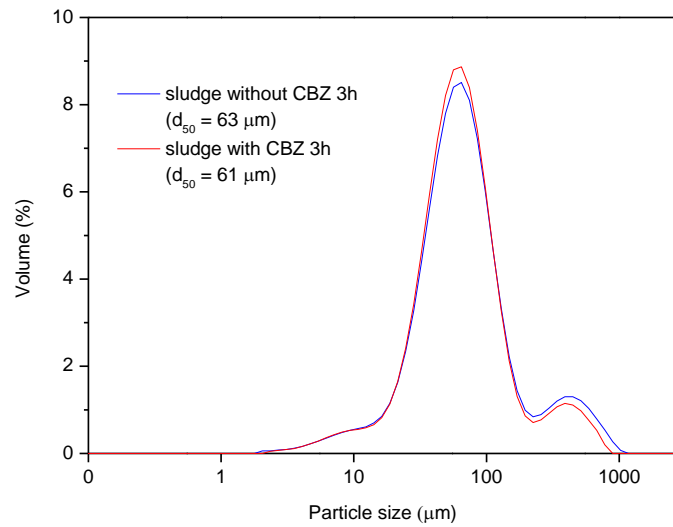


Fig. 4. Effect of CBZ on sludge floc size distribution

### 3.1.3 Effect of CBZ on supernatant composition

Fig. 5 shows the result of protein and polysaccharide concentration in the supernatants of sludges sampled from the batch reactors. Slight higher protein concentration (about 20%) was observed in supernatant from the reactor-with CBZ, as compared with that from the control-reactor. However, no significant difference in polysaccharide concentration was found. This result suggests that for sludge under CBZ stress the released SMPs are mainly proteins, or the released polysaccharides were easily biodegraded.

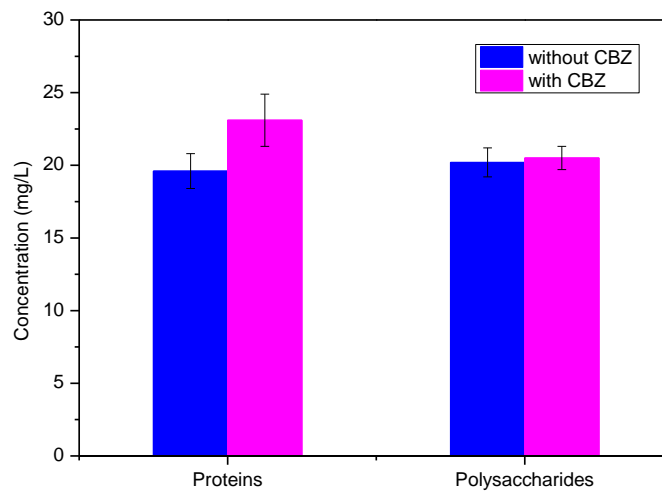


Fig. 5. Effect of CBZ on supernatant composition

Thus, the proteins may play a more important role in the CBZ effect. Then, the effect of CBZ on production of protein-like SMPs was further examined by HPLC-SEC that uses fluorescence detector with excitation/emission wavelengths set at 280/350 nm to obtain the information on the molecular weight distribution, which is important to understand fouling propensity of sludge. Fig. 6 shows the result of HPLC-SEC analysis of supernatants prepared from the batch reactors using sludges that were taken from the MBR operated under low organic loading rate (0.1 kg COD/kg MLSS/d). The molecular weight of protein-like substances in MBR supernatants showed bimodal distribution. Indeed, two peaks were observed in the HPLC-SEC results which represent two groups of protein-like molecules, i.e. molecular size of 100-1000 kDa and 10-100 kDa, respectively. As shown in Fig. 6, a significantly higher peak of protein-like substances corresponding to 100-1000 kDa molecules in the supernatant prepared from the reactor-with CBZ was observed, as compared to that in the supernatant prepared from the control-reactor. This result indicates that only 3 hours contact of  $100 \mu\text{g L}^{-1}$  CBZ with activated sludge could cause the sludge to release some protein-like SMPs, which could be attributed to the defensive response of bacteria when facing chemical stress [58] or to the decay (lysis) of some bacteria and release of SMPs inside the bacterial cells. The latter assumption is consistent with the results of Ni et al. [59-60] who found that the molecular size of biomass associated products (BAPs), which is associated with biomass decay, is bigger than 100 kDa. In contrast, Fig. 6 showed no significant difference of detected peak heights of protein-like substances with a molecular size of 10-100 kDa between the two supernatants prepared from reactor-with CBZ and the control-reactor.

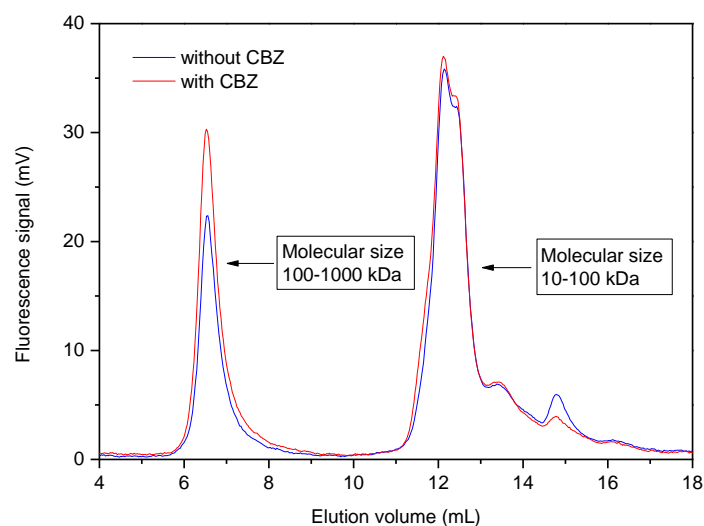


Fig. 6. Effect of CBZ on protein-like SMPs in supernatant ( $C_m = 0.1$  kg COD/kg MLSS/d)

In conclusion, the change in fouling propensity of sludge could be mainly attributed to the increase in the quantity of protein-like substances with a molecular size of 100-1000 kDa in the supernatant, since no significant change of floc size distribution and microbial activity was observed after addition of CBZ in the sludge.

### 3.2. Effect of CBZ on fouling layer characteristics

The fouling layer of a membrane developed during sludge filtration is related to the retained components in the sludge, thus it includes biomass cake layer and biofilm layer. Fig. 7 shows one example of HPLC-SEC analysis of a MBR supernatant (prepared from the MBR sludge) and the corresponding permeate obtained on a  $0.2 \mu\text{m}$  PS membrane. As shown in Fig.7, the 100-1000 kDa protein-like SMPs in supernatant could be almost completely retained by the  $0.2 \mu\text{m}$  PS membrane, indicating that the fouling layer formed on the  $0.2 \mu\text{m}$  PS membrane surface behaves as an additional barrier and made the effective pore size of the fouled MF membrane be more in the UF range. Moreover, the  $0.2 \mu\text{m}$  PS membrane could partially retain 10-100 kDa protein-like SMPs in the supernatant during the filtration of activated sludge, which indicates that some small flocs may cause pore blocking of the MF membrane, thus



reducing the pore size and allowing the partial retention of small molecules in the 10-100 kDa range.

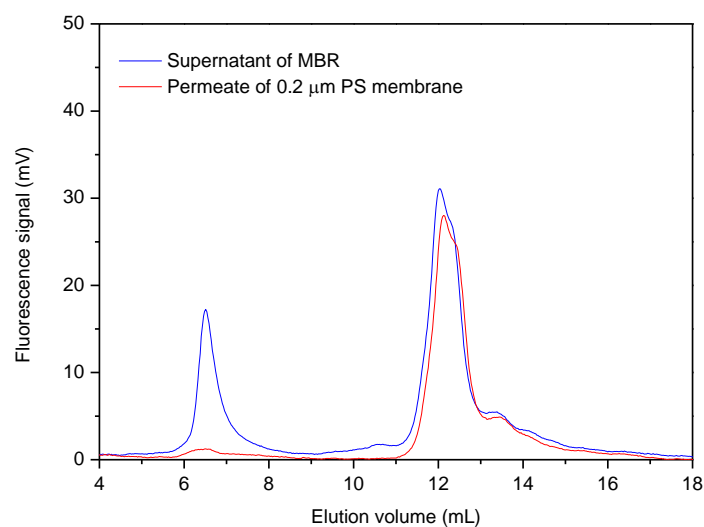


Fig. 7. HPLC-SEC analysis of sludge supernatant and permeate from a sludge filterability test

Fouling layer property was characterized by specific resistance ( $\alpha$  value) obtained from the filterability test. Table 3 shows the calculated results of  $\alpha$  values in filterability tests of sludges sampled from batch reactors using sludges taken from the MBR operated under low organic loading rate (0.1 kg COD/kg MLSS/d). As shown in table 3, with addition of CBZ in the sludge, the  $\alpha$  value increased at each different TMP (0.5 bar, 0.8 bar and 1.0 bar). Particularly, with addition of CBZ, the  $\alpha$  value increased more significantly at higher TMP. These results may suggest that the increase of 100-1000 kDa protein-like SMPs in supernatant with addition of CBZ (see Fig.6) could modify the structure of fouling layer formed during the sludge filtration and the effect of CBZ was more significant for the fouling layer formed at higher TMP (1.0 bar). In addition, higher flux recovery by backwash was observed in filtration tests that were performed using the sludge with addition of CBZ at each TMP (see table 3), which suggests that the more compact fouling layer formed on the 0.2  $\mu$ m PS membrane during filtration of the sludge with addition of CBZ could reduce the irreversible fouling caused by adsorption of small molecules or pore blocking due to colloids, and also

suggests that the increased fouling resistance during filtration of the sludge with addition of CBZ may be caused by the modification of fouling layer on the membrane surface instead of the modification of fouling inside the membrane pores.

Table 3. Effect of CBZ on fouling propensity of sludges ( $C_m = 0.1$  kg COD/kg MLSS/d)

	$\Delta P$ (bar)	Flux recovery (%)	$\alpha C \times 10^{-13}$ (m <sup>-2</sup> )	$\alpha \times 10^{-12}$ (m kg <sup>-1</sup> )
Sludge without CBZ	0.5 bar	79 ± 6	4.8 ± 0.36	8.1 ± 0.61
	0.8 bar	82 ± 3	5.0 ± 0.41	9.6 ± 0.79
	1.0 bar	71 ± 4	8.2 ± 0.62	15.2 ± 1.15
Sludge with CBZ	0.5 bar	94 ± 2	5.4 ± 0.45	9.2 ± 0.77
	0.8 bar	86 ± 4	6.2 ± 0.48	11.9 ± 0.92
	1.0 bar	90 ± 5	10.8 ± 0.91	20.0 ± 1.68

The fouling layer of a membrane developed during filtration of a supernatant, i.e. the biofilm, is related to the retained components in the supernatant. Fig. 8 shows one example of HPLC-SEC result for a MBR supernatant (used as feed for supernatant filtration) and its corresponding permeate obtained on a 0.01  $\mu\text{m}$  PES membrane. The result showed that the 0.01  $\mu\text{m}$  PES membrane retained almost all 100-1000 kDa protein-like SMPs and let pass almost all the 10-100 kDa protein-like SMPs. This could be explained by the fact that a 0.01  $\mu\text{m}$  pore size is often considered as corresponding to the 100 kDa molecular weight cut-off (MWCO) for ultrafiltration membranes. This result also emphasizes the importance of protein-like compounds in 100-1000 kDa range to the supernatant fouling propensity and indicates that the biofilm formed could not retain the 10-100 kDa SMPs.

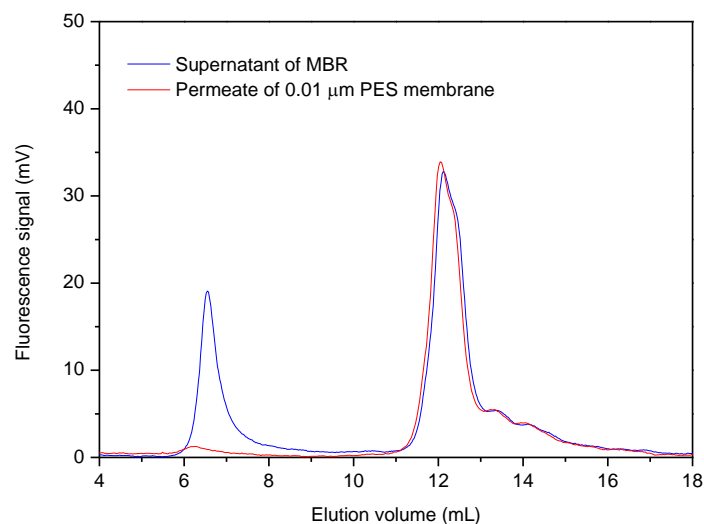


Fig. 8 HPLC-SEC analysis of supernatant and permeate from a supernatant filterability test

Table 4 shows the calculated results of  $\alpha$  values obtained from filterability tests of supernatants coming from the batch reactors using sludges that were taken from the MBR operated under low organic loading rate (0.1 kg COD/kg MLSS/d). Similar to the case of sludge filtration, table 4 indicates that the increase of 100-1000 kDa protein-like SMPs in supernatant with addition of CBZ (see Fig.6) could modify the structure of fouling layer formed during the supernatant filtration and the effect of CBZ was more significant for the fouling layer formed at higher TMP (0.8 bar or 1.0 bar) as compared to that formed at lower TMP (0.5 bar). Table 4 also showed slight higher flux recovery after backwash for filtration of supernatant with addition of CBZ, compared with that without addition of CBZ, indicating fouling layer formed on the 0.01  $\mu\text{m}$  PES membrane during filtration of the supernatant with addition of CBZ could slightly reduce the irreversible fouling caused by adsorption of small molecules or pore blocking due to colloids.

Table 4. Effect of CBZ on fouling propensity of supernatants ( $C_m = 0.1$  kg COD/kg MLSS/d)

	$\Delta P$ (bar)	Flux recovery (%)	$\alpha C \times 10^{-14}$ (m <sup>2</sup> )	$\alpha \times 10^{-16}$ (m kg <sup>-1</sup> )
Supernatant without CBZ	0.5 bar	81 ± 5	0.91 ± 0.07	2.6 ± 0.20
	0.8 bar	84 ± 4	1.2 ± 0.10	3.5 ± 0.29
	1.0 bar	85 ± 6	1.3 ± 0.12	3.8 ± 0.35
Supernatant with CBZ	0.5 bar	86 ± 4	1.3 ± 0.09	3.1 ± 0.21
	0.8 bar	87 ± 5	2.0 ± 0.16	4.7 ± 0.38
	1.0 bar	87 ± 3	2.1 ± 0.19	5.0 ± 0.45

### 3.3. Effect of MBR organic loading rate on CBZ effects

The effects of CBZ was examined for sludges sampled from MBR operated under two different organic loading rates, i.e. 0.1 kg COD/kg MLSS/d and 0.2 kg COD/kg MLSS/d, due to the significant differences in the SMP composition and microbial activity of MBR sludge under different organic loading rates. Fig.9 shows result of the filterability tests of sludges, sampled from batch reactors using sludges taken from the MBR operated under organic loading rate of 0.2 kg COD/kg MLSS/d at 1.0 bar. The result showed that fouling resistance was slightly higher during filtration of the sludge with presence of 100  $\mu\text{g L}^{-1}$  CBZ. Compared to the filterability test result of sludges sampled from batch reactors using sludges taken from the MBR operated under organic loading rate of 0.1 kg COD/kg MLSS/d (see Fig.2), a significantly reduced effect of CBZ on sludge fouling propensity was observed.

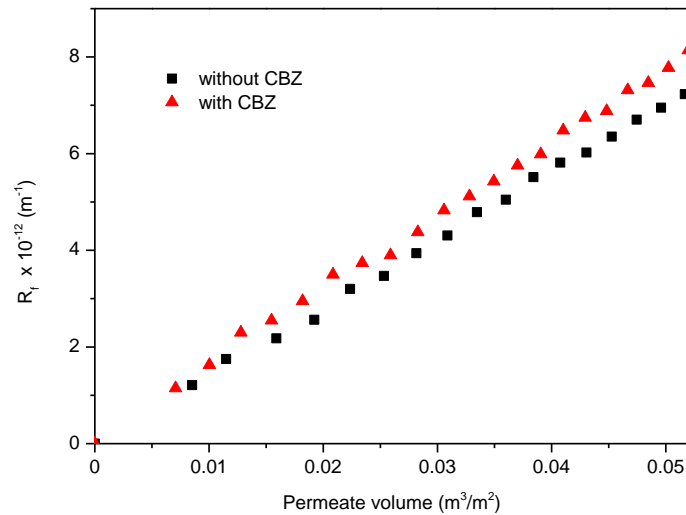


Fig. 9. Effect of CBZ on sludge fouling propensity ( $C_m = 0.2$  kg COD/kg MLSS/d)

To elucidate the difference of CBZ effects on sludge fouling propensity, the HPLC-SEC analysis of supernatants coming from batch reactors that used sludges taken from the MBR operated under organic loading rate of 0.2 kg COD/kg MLSS/d was performed, as shown in Fig. 10. Compared with the result obtained using sludges from MBR operated under organic loading rate of 0.1 kg COD/kg MLSS/d (see Fig. 6), much less significant effect of CBZ on 100-1000 kDa protein-like SMPs in supernatant was observed, which suggests that under higher organic loading rate condition, the bacteria in the activated sludge may more easily adapt to the pharmaceutical stress. Thus, the higher organic loading rate condition may protect the bacteria from decay (lysis) when facing pharmaceutical stress. Or this effect may be caused by the difference in bacterial species that were developed in MBRs operated under different organic loading rates. However, further studies need to be performed to verify these assumptions.

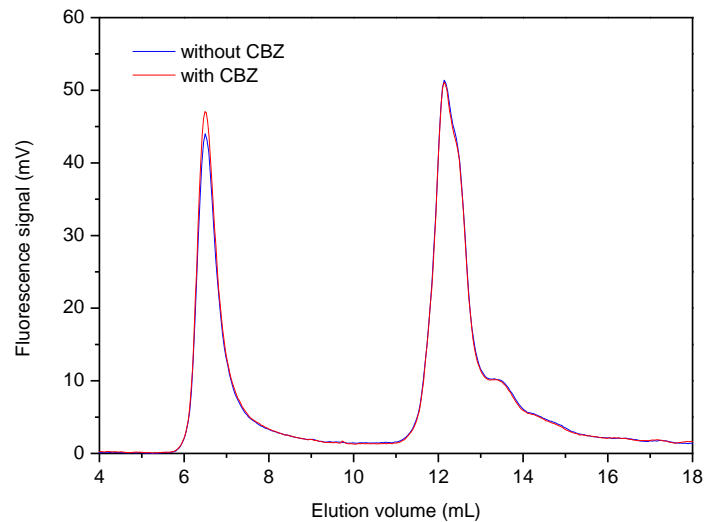


Fig. 10. Effect of CBZ on protein-like SMPs in supernatant ( $C_m = 0.2$  kg COD/kg MLSS/d)

Thus, the reduced effect of CBZ on sludge fouling propensity for sludge from MBR operated under higher organic loading rate could be attributed to the reduced effect of CBZ on the increase of 100-1000 kDa protein-like SMPs in supernatant.

#### 4. Conclusions

To study the interactions between peak concentration of pharmaceutical micropollutants and fouling in MBRs, this study investigated the effects of presence of peak concentration carbamazepine in sludge on the fouling propensity and physico-chemical characteristics of activated sludge from a submerged MBR.

The results showed that the presence of  $100 \mu\text{g L}^{-1}$  CBZ in the sludge caused no inhibition on the sludge microbial activity, no significant change of floc size distribution and no significant change of polysaccharide SMPs in supernatant. However, a significant increase of protein-like substances with a molecular size of 100-1000 kDa in supernatant was observed for the sludge sampled from the MBR operated under organic loading rate of  $0.1$  kg COD/kg MLSS/d, which led to higher fouling propensity during filtration of sludge and supernatant.

The increased fouling resistance of sludge and supernatant mainly originated from the increased retention of 100-1000 kDa protein-like SMPs, which led to modification of fouling layer on membrane surface. In addition, higher flux recovery by backwash was observed in the filtration tests of sludge sampled from batch reactor with addition of peak CBZ, which indicates that the “CBZ-modified” fouling layer could reduce the irreversible fouling that is caused by the adsorption of soluble organics or pore blocking due to colloids. Moreover, the CBZ effects are reduced for sludge from MBR operated under higher organic loading rate (0.2 kg COD/kg MLSS/d), which suggests that for MBR under higher organic loading rate condition, the bacteria in the activated sludge may more easily adapt to the environmental stress and under this condition less decay (lysis) of bacteria may happen.

This study sheds some light on fouling mechanisms of MBR when facing some accidental pharmaceutical stress and helps to understand the effect of organic loading rate on performance of MBR under pharmaceutical stress. Long-term study concerning effects of pharmaceutical stress on MBR performance needs to be carried out by addition of CBZ directly in MBR to better understand the interaction between pharmaceutical micropollutants and MBR process and to also examine the possibility of using MBR as pre-treatment process of reverse osmosis (RO) when RO concentrate was recycled to the MBR, in which case the accumulation of pharmaceutical micropollutants occurs in the biological reactor.

## References

- [1] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. Von Gunten, B. Wehrli, The challenge of micropollutants in aquatic systems, *Science*, 313 (2006) 1072-1077.
- [2] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, *Sci. Total Environ.*, 473–474 (2014) 619-641.

- [3] M. Jacob, C. Li, C. Guigui, C. Cabassud, G. Lavison, L. Moulin, Performance of NF/RO process for indirect potable reuse: interactions between micropollutants, micro-organisms and real MBR permeate, *Desalination Water Treat.*, 46 (2012) 75-86.
- [4] Z. Yu, S. Peldszus, P.M. Huck, Adsorption characteristics of selected pharmaceuticals and an endocrine disrupting compound—Naproxen, carbamazepine and nonylphenol—on activated carbon, *Water Res.*, 42 (2008) 2873-2882.
- [5] P. Verlicchi, M. Al Aukidy, E. Zambello, Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review, *Sci. Total Environ.*, 429 (2012) 123-155.
- [6] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Occurrence and removal of pharmaceuticals and hormones through drinking water treatment, *Water Res.*, 45 (2011) 1432-1442.
- [7] O.A. Jones, J.N. Lester, N. Voulvoulis, Pharmaceuticals: a threat to drinking water?, *Trends Biotechnol.*, 23 (2005) 163-167.
- [8] T. Heberer, Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data, *Toxicol. Lett.*, 131 (2002) 5-17.
- [9] B. Petrie, E.J. McAdam, M.D. Scrimshaw, J.N. Lester, E. Cartmell, Fate of drugs during wastewater treatment, *TrAC Trends in Analytical Chemistry*, 49 (2013) 145-159.
- [10] K. Fent, A.A. Weston, D. Caminada, Ecotoxicology of human pharmaceuticals, *Aquat. Toxicol.*, 76 (2006) 122-159.
- [11] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters, *Water Res.*, 43 (2009) 363-380.
- [12] J. Radjenović, M. Petrović, D. Barceló, Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment, *Water Res.*, 43 (2009) 831-841.
- [13] T. Melin, B. Jefferson, D. Bixio, C. Thoeye, W. De Wilde, J. De Koning, J. Van der Graaf, T. Wintgens, Membrane bioreactor technology for wastewater treatment and reuse, *Desalination*, 187 (2006) 271-282.
- [14] S. Judd, *The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment*, Elsevier, 2010.
- [15] B. Lesjean, E.H. Huisjes, Survey of the European MBR market: trends and perspectives, *Desalination*, 231 (2008) 71-81.
- [16] J. Sipma, B. Osuna, N. Collado, H. Monclús, G. Ferrero, J. Comas, I. Rodríguez-Roda, Comparison of removal of pharmaceuticals in MBR and activated sludge systems, *Desalination*, 250 (2010) 653-659.



- [17] E. Sahar, R. Messalem, H. Cikurel, A. Aharoni, A. Brenner, M. Godehardt, M. Jekel, M. Ernst, Fate of antibiotics in activated sludge followed by ultrafiltration (CAS-UF) and in a membrane bioreactor (MBR), *Water Res.*, 45 (2011) 4827-4836.
- [18] C. Li, C. Cabassud, C. Guigui, Evaluation of membrane bioreactor on removal of pharmaceutical micropollutants: a review, *Desalination Water Treat.*, (2014) In press.
- [19] M. Clara, N. Kreuzinger, B. Strenn, O. Gans, H. Kroiss, The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants, *Water Res.*, 39 (2005) 97-106.
- [20] A. Göbel, C.S. McArdell, A. Joss, H. Siegrist, W. Giger, Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies, *Sci. Total Environ.*, 372 (2007) 361-371.
- [21] K. Kimura, H. Hara, Y. Watanabe, Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors, *Environ. Sci. Technol.*, 41 (2007) 3708-3714.
- [22] S.K. Maeng, B.G. Choi, K.T. Lee, K.G. Song, Influences of solid retention time, nitrification and microbial activity on the attenuation of pharmaceuticals and estrogens in membrane bioreactors, *Water Res.*, (2013).
- [23] P. Verlicchi, M. Al Aukidy, A. Galletti, M. Petrovic, D. Barceló, Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment, *Sci. Total Environ.*, 430 (2012) 109-118.
- [24] K.D. Brown, J. Kulis, B. Thomson, T.H. Chapman, D.B. Mawhinney, Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico, *Sci. Total Environ.*, 366 (2006) 772-783.
- [25] D.G.J. Larsson, C. de Pedro, N. Paxeus, Effluent from drug manufactures contains extremely high levels of pharmaceuticals, *J. Hazard. Mater.*, 148 (2007) 751-755.
- [26] N.M. Vieno, T. Tuhkanen, L. Kronberg, Seasonal Variation in the Occurrence of Pharmaceuticals in Effluents from a Sewage Treatment Plant and in the Recipient Water, *Environ. Sci. Technol.*, 39 (2005) 8220-8226.
- [27] K. Choi, Y. Kim, J. Park, C.K. Park, M. Kim, H.S. Kim, P. Kim, Seasonal variations of several pharmaceutical residues in surface water and sewage treatment plants of Han River, Korea, *Sci. Total Environ.*, 405 (2008) 120-128.
- [28] S. Coutu, V. Wyrsh, H.K. Wynn, L. Rossi, D.A. Barry, Temporal dynamics of antibiotics in wastewater treatment plant influent, *Sci. Total Environ.*, 458-460 (2013) 20-26.
- [29] Q. Sui, J. Huang, S. Deng, W. Chen, G. Yu, Seasonal Variation in the Occurrence and Removal of Pharmaceuticals and Personal Care Products in Different Biological Wastewater Treatment Processes, *Environ. Sci. Technol.*, 45 (2011) 3341-3348.

- [30] S.T. Glassmeyer, E.K. Hinchey, S.E. Boehme, C.G. Daughton, I.S. Ruhoy, O. Conerly, R.L. Daniels, L. Lauer, M. McCarthy, T.G. Nettesheim, Disposal practices for unwanted residential medications in the United States, *Environ. Int.*, 35 (2009) 566-572.
- [31] J.L. Santos, I. Aparicio, M. Callejón, E. Alonso, Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain), *J. Hazard. Mater.*, 164 (2009) 1509-1516.
- [32] C.D. Metcalfe, B.G. Koenig, D.T. Bennie, M. Servos, T.A. Ternes, R. Hirsch, Occurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants, *Environ. Toxicol. Chem.*, 22 (2003) 2872-2880.
- [33] I.D.S. Henriques, R.D. Holbrook, R.T. Kelly, N.G. Love, The impact of floc size on respiration inhibition by soluble toxicants—a comparative investigation, *Water Res.*, 39 (2005) 2559-2568.
- [34] N. Lesage, M. Sbrana, C. Cabassud, Performances of a hybrid adsorption/submerged membrane biological process for toxic waste removal, *Water Sci. Technol.*, 51 (2005) 173-180.
- [35] M. Aubenneau, A. Tahar, C. Casellas, C. Wisniewski, Membrane bioreactor for pharmaceutically active compounds removal: Effects of carbamazepine on mixed microbial communities implied in the treatment, *Process Biochem.*, 45 (2010) 1826-1831.
- [36] S. Wang, R.M. Holzem, C.K. Gunsch, Effects of pharmaceutically active compounds on a mixed microbial community originating from a municipal wastewater treatment plant, *Environ. Sci. Technol.*, 42 (2008) 1091-1095.
- [37] P. Le-Clech, V. Chen, T.A. Fane, Fouling in membrane bioreactors used in wastewater treatment, *J. Membr. Sci.*, 284 (2006) 17-53.
- [38] H. Lin, M. Zhang, F. Wang, F. Meng, B.-Q. Liao, H. Hong, J. Chen, W. Gao, A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: Characteristics, roles in membrane fouling and control strategies, *J. Membr. Sci.*, 460 (2014) 110-125.
- [39] F. Meng, S.-R. Chae, A. Drews, M. Kraume, H.-S. Shin, F. Yang, Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material, *Water Res.*, 43 (2009) 1489-1512.
- [40] E. Reid, X. Liu, S.J. Judd, Effect of high salinity on activated sludge characteristics and membrane permeability in an immersed membrane bioreactor, *J. Membr. Sci.*, 283 (2006) 164-171.
- [41] Z.-P. Wang, T. Zhang, Characterization of soluble microbial products (SMP) under stressful conditions, *Water Res.*, 44 (2010) 5499-5509.
- [42] G.-P. Sheng, H.-Q. Yu, Z.-B. Yue, Production of extracellular polymeric substances from *Rhodospirillum rubrum* in the presence of toxic substances, *Appl. Microbiol. Biotechnol.*, 69 (2005) 216-222.

- [43] W.C. Lay, Q. Zhang, J. Zhang, D. McDougald, C. Tang, R. Wang, Y. Liu, A.G. Fane, Effect of pharmaceuticals on the performance of a novel osmotic membrane bioreactor (OMBR), *Sep. Sci. Technol.*, 47 (2012) 543-554.
- [44] Y. Zhang, S.-U. Geiß, C. Gal, Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies, *Chemosphere*, 73 (2008) 1151-1161.
- [45] M. Clara, B. Strenn, N. Kreuzinger, Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration, *Water Res.*, 38 (2004) 947-954.
- [46] N. Tadkaew, F.I. Hai, J.A. McDonald, S.J. Khan, L.D. Nghiem, Removal of trace organics by MBR treatment: the role of molecular properties, *Water Res.*, 45 (2011) 2439-2451.
- [47] A. Joss, E. Keller, A.C. Alder, A. Göbel, C.S. McArdell, T. Ternes, H. Siegrist, Removal of pharmaceuticals and fragrances in biological wastewater treatment, *Water Res.*, 39 (2005) 3139-3152.
- [48] C. Abegglen, A. Joss, C.S. McArdell, G. Fink, M.P. Schlüsener, T.A. Ternes, H. Siegrist, The fate of selected micropollutants in a single-house MBR, *Water Res.*, 43 (2009) 2036-2046.
- [49] R.S. Trussell, R.P. Merlo, S.W. Hermanowicz, D. Jenkins, The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater, *Water Res.*, 40 (2006) 2675-2683.
- [50] A. Masse, M. Spáandio, C. Cabassud, Comparison of sludge characteristics and performance of a submerged membrane bioreactor and an activated sludge process at high solids retention time, *Water Res.*, 40 (2006) 2405-2415.
- [51] S. Wang, C.K. Gunsch, Effects of selected pharmaceutically active compounds on treatment performance in sequencing batch reactors mimicking wastewater treatment plants operations, *Water Res.*, 45 (2011) 3398-3406.
- [52] L.F. Delgado, S. Schetrite, C. Gonzalez, C. Albasi, Effect of cytostatic drugs on microbial behaviour in membrane bioreactor system, *Bioresour. Technol.*, 101 (2010) 527-536.
- [53] M. Spáandio, E. Paul, Estimation of wastewater biodegradable COD fractions by combining respirometric experiments in various  $S_0/X_0$  ratios, *Water Res.*, 34 (2000) 1233-1246.
- [54] P. Smith, R.I. Krohn, G. Hermanson, A. Mallia, F. Gartner, M. Provenzano, E. Fujimoto, N. Goeke, B. Olson, D. Klenk, Measurement of protein using bicinchoninic acid, *Anal. Biochem.*, 150 (1985) 76-85.
- [55] R. Dreywood, Qualitative test for carbohydrate material, *I&EC Analytical Edition*, 18 (1946) 499-499.
- [56] N. Her, G. Amy, D. McKnight, J. Sohn, Y. Yoon, Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection, *Water Res.*, 37 (2003) 4295-4303.
- [57] B. Teychene, C. Guigui, C. Cabassud, Engineering of an MBR supernatant fouling layer by fine particles addition: A possible way to control cake compressibility, *Water Res.*, 45 (2011) 2060-2072.

- [58] I.D. Henriques, N.G. Love, The role of extracellular polymeric substances in the toxicity response of activated sludge bacteria to chemical toxins, *Water Res.*, 41 (2007) 4177-4185.
- [59] B.-J. Ni, R.J. Zeng, F. Fang, W.-M. Xie, G.-P. Sheng, H.-Q. Yu, Fractionating soluble microbial products in the activated sludge process, *Water Res.*, 44 (2010) 2292-2302.
- [60] B.-J. Ni, B.E. Rittmann, H.-Q. Yu, Soluble microbial products and their implications in mixed culture biotechnology, *Trends Biotechnol.*, 29 (2011) 454-463.



## Publication 3

---

---



# Effects of pharmaceutical micropollutants on the membrane fouling of a submerged MBR treating municipal wastewater: case of continuous pollution by carbamazepine

Accepted for publication in Water Research

Chengcheng Li<sup>a,b,c,\*</sup>, Corinne Cabassud<sup>a,b,c</sup>, Bernard Reboul<sup>a,b,c</sup>, Christelle Guigui<sup>a,b,c</sup>

<sup>a</sup> Université de Toulouse, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, 31077 Toulouse, France

<sup>b</sup> INRA, UMR792 Ingénierie des Systèmes biologiques et des Procédés, 31400 Toulouse, France

<sup>c</sup> CNRS, UMR5504, 31400 Toulouse, France

\* Corresponding author at: INSA Toulouse, Hall GPE, 135 Avenue de Rangueil, 31077 Toulouse, France. Tel.: +33 (0)5 61 55 97 91; Email: lichengcheng\_1002@hotmail.com, chengcheng.li@insa-toulouse.fr

Email addresses of other authors: corinne.cabassud@insa-toulouse.fr, bernard.reboul@insa-toulouse.fr, christelle.guigui@insa-toulouse.fr



## Abstract

Membrane bioreactor (MBR) is increasingly used for municipal wastewater treatment and reuse and great concerns have been raised to some emerging trace pollutants found in aquatic environment in the last decade, notably the pharmaceuticals. As a consequence the removal of pharmaceutical micropollutants by MBRs has been extensively investigated. But there is still a lack of knowledge on the effects of the current presence of pharmaceutical micropollutants in domestic wastewaters on MBR fouling. Among the different pharmaceuticals, it was decided to focus on carbamazepine (CBZ), an anti-epileptic drug, because of its occurrence in domestic wastewaters and persistency in biological processes including MBRs. This paper focuses on the effects of continuous carbamazepine pollution on MBR fouling. A continuous introduction of CBZ into the MBR via the feed (about  $90 \mu\text{g L}^{-1}$  CBZ in the feed) provoked a TMP jump. It occurred just 1 day after the addition of CBZ in MBR and a significantly higher increase rate of TMP was also observed after 1 day after addition of CBZ in MBR, as compared to that before addition of CBZ. This indicates that the pharmaceutical stress induced by CBZ causes more severe membrane fouling. Addition of CBZ was shown to induce a significant increase of the concentration of proteins in the supernatant at the beginning several days then stabilized to original level whereas no significant change was found for polysaccharides. HPLC-SEC analysis showed that addition of CBZ induced a decrease of 100-1000 kDa protein-like SMPs and a more significant increase of 10-100 kDa protein-like SMPs in the supernatant. Moreover it was found that addition of CBZ in the MBR affected the sludge microbial activities, as a slight inhibition (about 20%) of the exogenous respiration rate was observed. The increased membrane fouling could be related to the change in biomass characteristics and supernatant quality after addition of CBZ in MBR. This study allows also suggesting that 10-100kDa protein-like SMPs might accumulate inside the biocake that was formed on the membrane surface during MBR operation and play an important role in the TMP jump phenomenon.

**Key words:** membrane bioreactor, pharmaceutical micropollutants, carbamazepine, fouling, protein-like SMPs

## 1. Introduction

Membrane bioreactors (MBRs), which couple the activated sludge process with membrane separation, have been increasingly used in wastewater treatment and reuse due to their high effluent quality. However, membrane fouling remains a major limitation for the development of MBR process. Membrane fouling in MBRs involves i) biocake fouling on the membrane surface due to the deposit of suspended solids in relation with the convective flow of biological fluids and to the microbial growth and to the excretion of biopolymers, ii) pore blocking by particles, colloids and iii) adsorption of solutes onto the membrane material (Ognier et al., 2002; Le-Clech et al., 2006; Meng et al., 2009). Very often, three stages of fouling are proposed for MBRs when they are operated under constant flux condition: conditioning fouling caused by initial pore blocking and adsorption of solutes, steady fouling occurring due to biocake formation and further pore blocking, and TMP jump. This last phenomenon is observed in MBRs and the mechanisms involved in its occurrence are not completely elucidated. Different possible causes for TMP jump have been suggested:

- Due to a successive closure of pores, local flux starts to exceed the critical value and particles begin to deposit (Ognier et al., 2004);
- Coagulation occurs in the first layers of the gel layer at a critical pressure (Chang et al., 2006);
- Percolating colloids reduce the size of the biocake voids until connections are lost (Drews, 2010);
- Biofouling is compressible and this effect is measurable until a limit pressure (Mass é 2004).

Many studies showed that the supernatant of activated sludges, which contains colloids and solutes, plays a more important role in membrane fouling of MBR than the biological flocs (Wisniewski and Grasmick, 1998; Bouhabila et al., 2001). Thus, soluble microbial products (SMPs), the major organic constituents of sludge supernatants, have been acknowledged as major foulants that lead to membrane fouling in MBRs (Hernandez Rojas et al., 2005; Rosenberger et al., 2006). Recently, SMPs were identified as soluble extracellular polymeric substances (EPS) and are mainly composed of proteins, polysaccharides and humic substances (Liang et al., 2007). Among these components, humic SMPs are generally believed to be not relevant to MBR fouling due to their small molecular size as compared with that of proteins and polysaccharides. In addition, SMPs could be further divided into two categories: utilization-associated products (UAPs), which are produced during substrate metabolism, and biomass-associated products (BAP), which are produced from decay of biomass. However, simply correlating the MBR fouling with protein or polysaccharide concentration in the supernatant or even their retention in MBR could not explain the contribution of each component in MBR fouling, since some SMP components may be retained by the cake layer and may not be attached on membrane surface or in membrane pores (Metzger et al., 2007). Thus, MBR fouling is also affected by molecular weight (MW) distribution of SMPs (Arabi and Nakhla, 2010; Jiang et al., 2010). Besides, the physiochemical properties like relative hydrophobicity of SMP components may also significantly affect the MBR fouling. For example, proteins were reported to dominate in foulants desorbed from the fouled membranes of MBRs due to their hydrophobic properties (Yamato et al., 2006). In the literature, factors affecting the SMP production in MBR supernatant may include operating conditions such as organic loading rate (Kimura et al., 2005; Trussell et al., 2006), sludge retention time (SRT) (Masse et al., 2006; Al-Halbouni et al., 2008) and hydraulic conditions like aeration and mechanical stirring (Menniti et al., 2009;

Stricot et al., 2010), and the presence of environmental stress in the wastewater such as heavy metals (Zhou et al., 2014), osmotic pressure (salts) (Sun et al., 2010), and industrial compounds like pharmaceuticals (Lay et al., 2012), due to the defensive response or decay (lysis) of bacteria when facing chemical stress (Holakoo et al., 2006; Henriques and Love, 2007).

In the last decade, great attention has been paid to the presence of pharmaceutical micropollutants in the domestic wastewater due to their potential adverse effects on human health (Verlicchi et al., 2012). Many pharmaceutical micropollutants, such as analgesics like ibuprofen, diclofenac, naproxen and ketoprofen, lipid regulators like bezafibrate and gemfibrozil, and the antiepileptic drug carbamazepine, have been frequently detected in domestic wastewater at concentration higher than 1.0 µg/L and their removal in MBR process has been extensively investigated (Li et al., 2014). However, few studies were carried out to examine the effects of pharmaceutical micropollutants on MBR fouling. Nevertheless, some studies reported that the presence of pharmaceutical micropollutants could induce some microbial effects on activated sludge. Lay et al. (2012) observed a significant increase in the protein and polysaccharide ratio in supernatant after addition of carbamazepine, diclofenac, ibuprofen and naproxen in the feed of an Osmotic Membrane Bioreactor (20–25 µg L<sup>-1</sup> of each pharmaceutical in the feed). Delgado et al. (2010) reported the continuous introduction of 5 µg L<sup>-1</sup> cytostatic drug cyclophosphamide and its principal metabolites (CPs) in MBR caused the inhibition in exogenous respiration rate of activated sludge and increased its mean floc size. Through the terminal restriction fragment length polymorphism (T-RFLP) analysis of the bacterial 16S rRNA genes, Kraigher et al. (2008) observed shifts in the bacterial community structure in activated sludge of a bioreactor continuously fed with synthetic wastewater containing pharmaceuticals (ibuprofen, naproxen, ketoprofen, diclofenac and

clofibrinic acid) at a concentration of  $50 \mu\text{g L}^{-1}$ , as compared to the control reactor, which was operated without addition of pharmaceuticals.

The main objective of this study was to examine the effects of one pharmaceutical micropollutant, which is frequently found in domestic wastewaters, on the MBR fouling. Carbamazepine (CBZ), an anti-epileptic drug, was selected in this study. Because it has been frequently detected in domestic wastewater (Zhang et al., 2008) and it is almost not biodegraded in biological wastewater treatment processes including MBRs, which makes it become a possible anthropogenic marker in the aquatic environment (Clara et al., 2004). In addition, CBZ was found to have some inhibition effects on sludge microbial activity (Wang et al., 2008; Aubenneau et al., 2010). In this study, CBZ was added in the feed of MBR to a relatively high concentration of  $90 \mu\text{g L}^{-1}$  to have an intensified effect to represent the seasonal or accidental variations in concentration of mixtures of pharmaceutical micropollutants present in real municipal wastewater and continuously introduced to MBR via the feed after a steady operation of MBR was achieved. Thus, the objectives are to be able to relate the effects of CBZ on MBR fouling with the change in the characteristics of the biomass and supernatant after adding CBZ in MBR in order to understand the mechanisms of interactions among the CBZ, activated sludge and MBR fouling. Fouling propensity of the sludge and supernatant sampled from the MBR was characterized using some standardized filterability tests performed with a stirred dead-end filtration cell. Fouling was also considered at the scale of the MBR for the mixture of flocs and supernatant during MBR operation and characterized by the time-variation of transmembrane pressure (TMP). In order to explain the effects of CBZ on the fouling compartments, it was thus necessary to obtain some information:

- (i) on the biological activity: Respirometric experiments were performed to examine the effects of CBZ on sludge microbial activity;

(ii) on the physico-chemical properties of the sludge (particle size distribution, soluble proteins and polysaccharides, molecular weight distribution of SMPs in the supernatant): Floc size distribution analysis was performed on the activated sludge to study the effects of CBZ on physico-chemical characteristics of biomass. Concentrations of proteins and polysaccharides were analyzed in supernatant and MBR permeate to study the effects of CBZ on the change of SMP compositions in supernatant and their retention in MBR. HPLC-SEC-fluorescence was used to characterize protein-like SMPs in supernatant and permeate of filterability tests or the MBR, with the information on molecular size distribution.

## **2. Material and Methods**

### **2.1 Membrane bioreactor set-up**

A lab-scale submerged MBR set-up consisting of an anoxic reactor (5.4 L) and an aerobic reactor (12.6 L) was used in this study (see Fig. 1). A flat sheet microfiltration membrane ( $L_{p0} = 1110 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ , Kubota, Japan), made of chlorinated polyethylene, was submerged inside the aerobic reactor. It has an effective filtration area of  $0.1 \text{ m}^2$ , with an average pore size of  $0.2 \text{ }\mu\text{m}$ . This MBR was operated at the high SRT of 30 days, with a sludge concentration in the aerobic reactor between 6-8 g/L and an organic loading rate at about  $0.2 \text{ kg COD/kg MLSS/d}$ . The instantaneous permeate flux was fixed at  $15 \text{ L/h/m}^2$  with filtration/relaxation cycles of 8 min/4 min, leading to a net permeate flux of  $10 \text{ L/h/m}^2$ . Two aeration systems were used in the aerobic reactor. An aeration system with coarse air bubbles was installed below the membrane to reduce fouling and it was operated continuously at flow rate of  $1.5 \text{ L/min}$ , i.e.  $\text{SAD}_p$  of  $90 \text{ m}^3 \text{ air/m}^3 \text{ permeate}$ . Besides, another aeration system with fine air bubbles was used to maintain the dissolved oxygen concentration in the sludge at about  $2\text{-}3 \text{ mg/L}$  and automatically controlled by an oxygen sensor. The sludge recirculation

ratio was fixed at 4. Municipal wastewater was taken from a wastewater treatment plant close to Toulouse, France, after primary physical treatment.

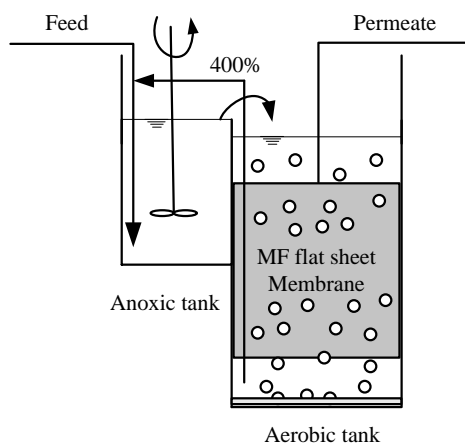
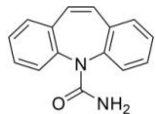


Fig. 1. MBR set-up used in this study

## 2.2 Selected pharmaceutical micropollutant

Table 1 introduces the physicochemical properties of carbamazepine. A concentrated mother solution was prepared by dissolving carbamazepine (from Sigma-Aldrich) in pure ethanol (VWR International) at a high concentration of 10 g/L to rule out the effect of ethanol on the organic content in the feed. Then, quantities of this concentrated mother solution sufficient to yield about  $90 \mu\text{g L}^{-1}$  final concentrations of CBZ were added in the feed tank after the steady state of the MBR reached, i.e. on 84<sup>th</sup> day, which is higher than 2 times of SRTs (30 days).

Table 1 Physico-chemical properties of carbamazepine (Joss et al., 2005; Zhang et al., 2008; Abegglen et al., 2009)

Structure	
Formula	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O
CAS No.	298-46-4
Molecular weight (g/mol)	236
Usage	Anti-epileptic
Water solubility (mg/L at 25°C)	17.7
LogKow	2.45
Charge at pH 7	Neutral
sorption constant, K <sub>d</sub> (L/kgss)	1.2
degradation constant k <sub>biol</sub> (L/gss/d)	<0.01

### 2.3 Characterization of sludge microbial activity

Sludge microbial activity was characterized by the specific exogenous respiration rate. The respiration rate, i.e. oxygen uptake rate (OUR), was determined in respirometric batch experiments, defined by Spérandio and Paul (2000), by measuring the dissolved oxygen concentration in activated sludge in an air-tight vessel. The sludge samples were collected from the aerobic tank of the MBR before and after addition of CBZ in MBR. Firstly, the collected sludge was added in the respirometric vessel and aerated for at least 3 h to reach the endogenous condition. Then, sodium acetate (NaAC), a readily biodegradable compound, was used as exogenous substrate, and added in the respirometric vessel to measure the maximum respiration rate. Then the exogenous respiration rate was calculated as the difference between the maximum respiration rate and the endogenous respiration rate (see Fig. 2). The specific



exogenous respiration rate is defined as the exogenous respiration rate divided by the concentration of mixed liquor suspended solids (MLSS).

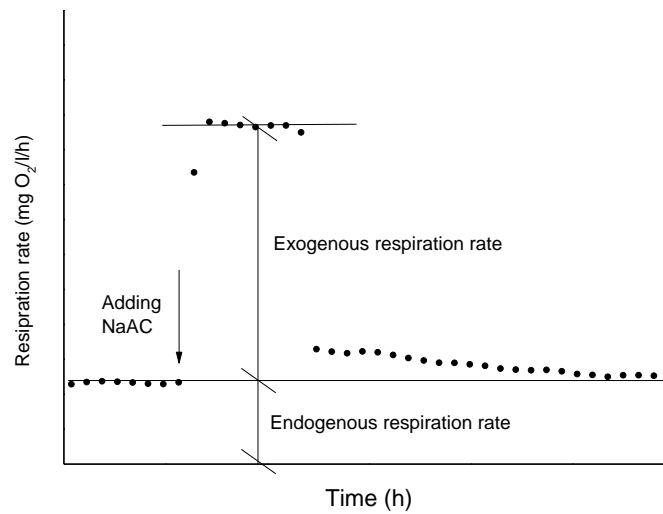


Fig. 2. The principle of respirometric experiments

## 2.4 Filterability tests

Filterability tests of sludges and supernatants sampled from MBR were performed by a stirred dead-end filtration cell (Amicon 8050, Millipore). Ultrafiltration membranes (Microdyn-Nadir GmbH, Germany), made of polyethersulfone (PES), with a molecular weight cut-off (MWCO) 150 kDa ( $L_{p0} = 400\text{-}600 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ ), were used for the filterability tests because in MBRs that employ microfiltration membranes, the deposits formed on the membrane surface and inside the pores constitute an additional barrier and make the apparent pore size of the fouled microfiltration membrane be in the ultrafiltration (UF) range (Judd, 2010). This 150 kDa PES membrane was chosen also because its permeability is comparable to that of MBR membrane used in this study and its membrane surface is permanently hydrophilic like the MBR membrane, i.e., chlorinated polyethylene, used in this study (Hai et al., 2013). A new membrane sample was used for each filtration test. All membranes were soaked in ultra pure water over night to maintain pores wetted. Then they were rinsed by

filtration of ultra pure water at 0.7 bar during 15 min prior to filtration test. All filtration tests were carried out at constant TMP of 0.5 bar. For each filtration test, the sludge or supernatant was filtered through the selected membrane until 70 ml permeate was produced (corresponding to a filtered permeate volume of about 50 L m<sup>-2</sup>); temperature was measured and flux was normalized to the flux at 20 °C. During sludge filtration, the sludge was stirred at 200 rpm. For supernatant filtration, no stirring was applied. Membrane fouling resistance was determined according to Eq. (1).

$$J = \frac{TMP}{\mu(R_m + R_f)} \quad (1)$$

In this equation, J is the permeate flux (m<sup>3</sup>/s/m<sup>2</sup>) at 20 °C, with R<sub>m</sub> the membrane resistance (m<sup>-1</sup>), R<sub>f</sub> the fouling resistance (m<sup>-1</sup>), TMP the transmembrane pressure (Pa) and μ the dynamic viscosity at 20 °C (Pa s).

## 2.5 Analytical methods

The concentration of mixed liquor suspended solids (MLSS) was measured according to the standard method AFNOR NFT 90-105. Supernatants were prepared by centrifugation of the sludge samples at 4000 g during 10 minutes at room temperature. Chemical oxygen demand (COD) and total nitrogen (TN) were measured by spectrometry with reagent kits. Floc size distribution was measured by Malvern Mastersizer 2000. Carbamazepine concentration was analyzed using LC-MS/MS method by the Departmental Water Laboratory in Toulouse, France. Quantification of proteins and polysaccharides was performed on the supernatants. Protein content, expressed in mg equivalent of bovine serum albumin per liter, was measured by the acid bicinchoninic method (Smith et al., 1985). Polysaccharide concentration,

expressed in mg equivalent of glucose per liter, was measured by the Anthrone method (Dreywood, 1946).

High performance liquid chromatography—size exclusion chromatography (HPLC—SEC) analysis was performed by an Akta Purifier (GE Healthcare, USA), equipped with a column Protein Kw804 (Shodex, Japan), a silica-based column with an exclusion limit of 1000 kDa, and a fluorescence detector (Varian, USA). The eluent solution for the mobile phase was prepared with 25 mM Na<sub>2</sub>SO<sub>4</sub> and a phosphate buffer (2.4 mM NaH<sub>2</sub>PO<sub>4</sub> and 1.6 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 6.8). The flow rate of eluent passing through the column was 1.0 mL/min. Excitation/emission wavelengths were set at 280/350 nm, which were confirmed as the required values by EEM fluorescence results (Her et al., 2003; Teychene et al., 2011), to detect protein-like substances. The column was calibrated using standard proteins: thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa) from GE Healthcare. Prior to analysis of HPLC-SEC-fluorescence, each sample was pre-filtered on a 0.45 µm membrane.

### **3. Results and Discussion**

#### **3.1 CBZ removal in MBR**

As the MBR was fed with a real domestic wastewater, CBZ may be present in the raw wastewater. Actually, the average concentration of CBZ in the raw domestic wastewater was about 0.2-0.3 µg L<sup>-1</sup>, which is negligible as compared to the concentration of about 90 µg L<sup>-1</sup> in the feed after adding CBZ, as shown in Fig. 3. No removal of CBZ was achieved in the MBR before and after addition of extra CBZ in the feed, which is consistent with the literature (Radjenović et al., 2009). This result also suggests that CBZ itself does not participate to

membrane fouling via adsorption onto membrane material. The low removal of the added CBZ could be attributed to its low sorption and biodegradation capacities. Indeed:

- i) Its sorption constant  $K_d$ , defined as the partition of a compound between the sludge and the water phase, is 1.2 L/kg<sub>ss</sub> (see table 1), much lower than the critical value 500 L/kg<sub>ss</sub> identified for a significant removal by sorption (Ternes et al., 2004; Joss et al., 2005). Thus the removal of CBZ via sorption to the activated sludge is expected to be of minor importance.
- ii) The biodegradability of CBZ is also very low since its degradation constant  $k_{biol}$ , defined as a pseudo first-order reaction constant, is less than 0.01 L/g<sub>ss</sub>/d (see table 1). Based on the measurement of  $k_{biol}$  in batch experiments, Joss et al. (2006) concluded that the pharmaceutical micropollutants with  $k_{biol} < 0.1$  L/g<sub>ss</sub>/d are not removed to a significant extent (<20%).

Instead, higher average concentration of CBZ was found in the MBR permeate than that in the feed before addition of extra CBZ. According to Vieno et al. (2007), who also reported that carbamazepine was constantly found at higher concentrations in the treated water than in the raw water of WWTPs, the elevated concentration in the permeate could be attributed to an enzymatic cleavage of the carbamazepine glucuronides and thus to a release of carbamazepine in the treatment process. However, only a few studies reported the  $k_{biol}$  value of CBZ. Thus, the biodegradation of CBZ in MBR remains to be further investigated. For example, Hai et al. (2011) observed that during near-anoxic operation with a dissolved oxygen concentration in the bioreactor of about 0.5 mg L<sup>-1</sup>, an exceptionally high removal (68 ± 10%) of carbamazepine was achieved in the MBR as compared to its low removal efficiency (12 ± 11%) under aerobic conditions.

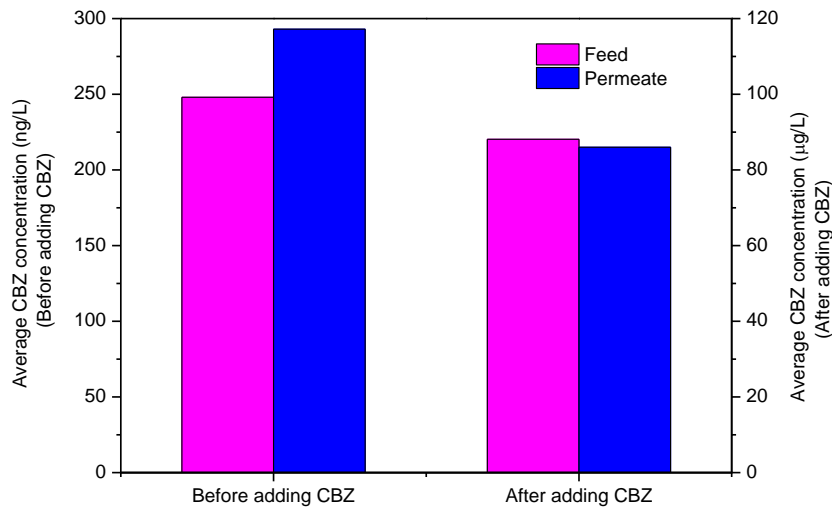


Fig. 3. The CBZ concentration in the feed and permeate of MBR before and after addition of extra CBZ

### 3.2 Effects of CBZ on MBR fouling

Membrane fouling in MBR was characterized by the change of TMP in MBR. Fig. 4 presents the time-variation of TMP during operation of the MBR before and after addition of CBZ in MBR. Three different periods and compartments can be distinguished:

- Before the addition of CBZ in the MBR, quite steady fouling condition (a TMP plateau) was reached, where TMP increases very slightly.
- Between the day when CBZ was added and 8 days after addition of CBZ: 1 day after addition of CBZ in MBR, a significant increase of TMP was observed. Thanks to the addition of CBZ, the TMP jump was very rapidly created and it clearly appears as a response of the bioseparation process to the effect of a chemical stress on the biomass. Between 1 day and 8 days after addition of CBZ, TMP increased gradually while at a higher rate as compared to that during the steady fouling condition, i.e. before addition of CBZ.

- Starting from 8 days after addition of CBZ, a continuous TMP jump started to occur, where TMP increased continuously at a significantly higher rate as compared to that during the period between 1 day and 8 days after addition of CBZ.

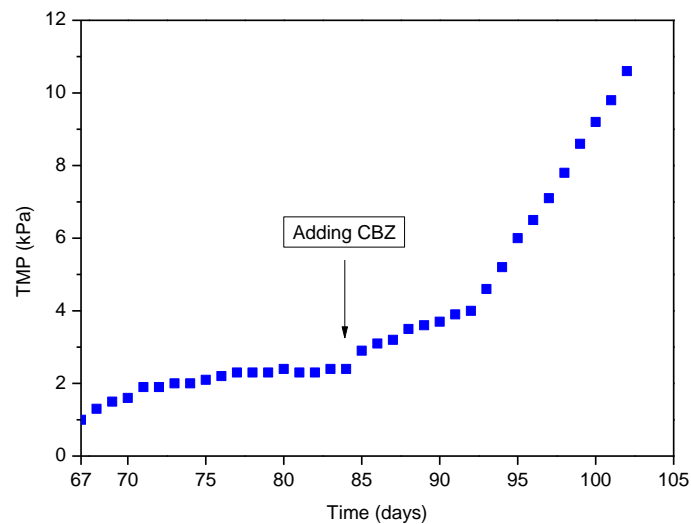


Fig. 4. Effect of CBZ on TMP of MBR

The steady fouling before addition of CBZ could be related to the gel layer formation due to the EPS deposition after the initial rapid fouling, since during this phase particles are thought to deposit by random motion rather than convective flow (Le-Clech et al., 2006; Drews, 2010). Thus, the HPLC-SEC analysis of supernatant and permeate sampled from the MBR on the day (84<sup>th</sup> day) just before adding CBZ was examined, as shown in Fig. 5. The molecular weight of protein-like substances in MBR supernatants showed bimodal distribution. Indeed, two peaks were observed in the HPLC-SEC results which represent two groups of protein-like molecules, i.e. molecular size of 100-1000 kDa and 10-100 kDa, respectively. The results showed that before adding CBZ, the MBR membrane could retain almost all the 100-1000 kDa protein-like substances. Taking into account that the average pore size of the membrane used in the MBR is 0.2  $\mu\text{m}$  (corresponding to the retention of molecules bigger than 1000 kDa), the 100-1000 kDa molecules may be retained by the deposits formed on the membrane

surface and inside the pores. In contrast, almost all the 10-100 kDa protein-like substances passed through the MBR membrane. Therefore, the TMP plateau before addition of CBZ (see Fig. 4) was attributed to the retention of 100-1000 kDa SMPs in MBR and thus to the formation of the gel layer on the membrane surface by protein bridges and bindings, as earlier proposed by Masse et al. (2006), since no retention of 10-100 kDa protein-like substances in supernatant was found.

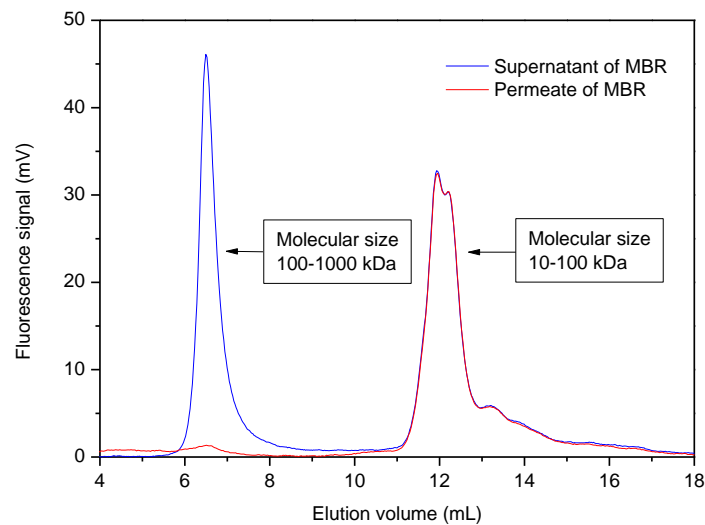


Fig. 5. HPLC-SEC analysis of supernatant and permeate of MBR before adding CBZ in MBR

Concerning to the TMP jump and subsequent increased fouling rate that appeared in MBR after addition of CBZ, three assumptions could be proposed: i) a change in biological activity linked to a stress on the microorganisms ii) a change in biomass physico-chemical properties, like mean floc size distribution, or iii) a change in the supernatant quality. These three assumptions will be discussed in the following paragraphs that aim to evaluate the effects of extra CBZ on the supernatant and biomass properties.

### 3.2.1 Dissertation on Assumption 1 to explain CBZ enhanced fouling: change in microbial activity

Sludge microbial activity was characterized in a respirometric batch reactor. Fig. 6 shows that the continuous addition of CBZ in the MBR caused about 20% inhibition of the sludge microbial activity, which is expressed as specific exogenous respiration rate, at the beginning several days. The exogenous respiration rate means the degradation rate of exogenous substrate. Thus, the inhibition of exogenous respiration rate caused by CBZ indicates that the presence of  $90 \mu\text{g L}^{-1}$  CBZ in the MBR changed degradation behavior of bacteria or modified the community structure of bacteria in activated sludge. Whereas, no further inhibition was observed between 4 days and 11 days after addition of CBZ in the MBR, which indicates that some extent of adaption or acclimation of the activated sludge to CBZ stress was reached 4 days after addition of CBZ in MBR. The results indicate that the presence of CBZ caused some stress on the bacteria in the sludge, thus bacterial lysis or bacterial protective responses, which can lead to release of EPS (as shown later in Fig. 8), may occur during the bacterial acclimation to CBZ stress. In this case, deterioration in sludge filterability might happen.

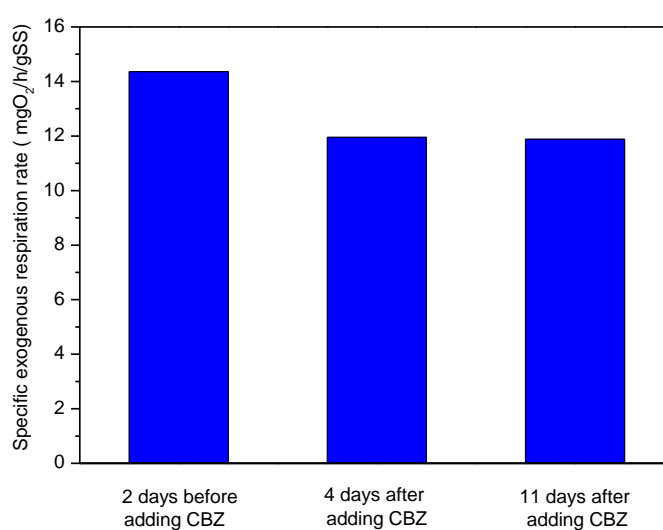


Fig. 6. Effect of CBZ on sludge exogenous respiration rate



The inhibition of sludge microbial activity induced by CBZ give rise to the question like if the inhibition would affect the removal of COD and TN in the MBR. No significant variation was observed for COD and TN removal before and after addition of CBZ in MBR, which suggests that the presence of  $90 \mu\text{g L}^{-1}$  CBZ in MBR did not significantly affect the biodegradation, nitrification and denitrification processes for an MBR operated at the organic loading rate of  $0.2 \text{ kg COD/kg MLSS/d}$ , in spite of a slight inhibition of microbial activity in the sludge.

In conclusion, the addition of CBZ in MBR induced a slight inhibition of sludge microbial activity, which may indirectly cause the enhanced membrane fouling via modification of EPS concentration in sludge. However, taking into account that no significant change in COD biodegradation or in TN removal was observed after addition of CBZ in the MBR, the effect of CBZ on the sludge microbial activity was probably not significant.

### **3.2.2 Dissertation on Assumption 2 to explain CBZ enhanced fouling: change in biomass physico-chemical properties**

Sludge floc size distribution represents an important indicator of biomass characteristics and sludge filterability. Fig. 7 shows that after adding CBZ in the MBR, the mean floc size increased slightly from  $128 \mu\text{m}$  to  $161 \mu\text{m}$ . Similar result was observed by Delgado et al., (2010), who reported the continuous introduction of  $5 \mu\text{g L}^{-1}$  cytostatic drug cyclophosphamide in MBR caused increase in mean floc size of activated sludge. This means that the presence of CBZ caused flocculation of activated sludge flocs and produced larger flocs, which may be induced by the released EPS when facing the CBZ stress, as shown later in Fig. 8. EPS may behave as glue that binds cells, particles, colloids together to form sludge flocs. Henriques and Love (2007) also pointed out the released EPS when facing toxic chemicals could function as a protective barrier for the bacteria inside activated sludge flocs

to hinder access of the toxin to the bacterial cells. The increase of protein content in sludge (see Fig. 8) may increase the hydrophobicity of flocs and lead to deterioration in sludge filterability. In addition, the possibility of increase in small flocs after addition of CBZ could not be completely ruled out, since deflocculation may also happen for activated sludge exposed to toxic chemicals (Henriques et al., 2007), and some small flocs, for example, flocs at size of near micro or sub-micro level, are difficult to be detected. In this case, the deterioration in sludge filterability might also happen.

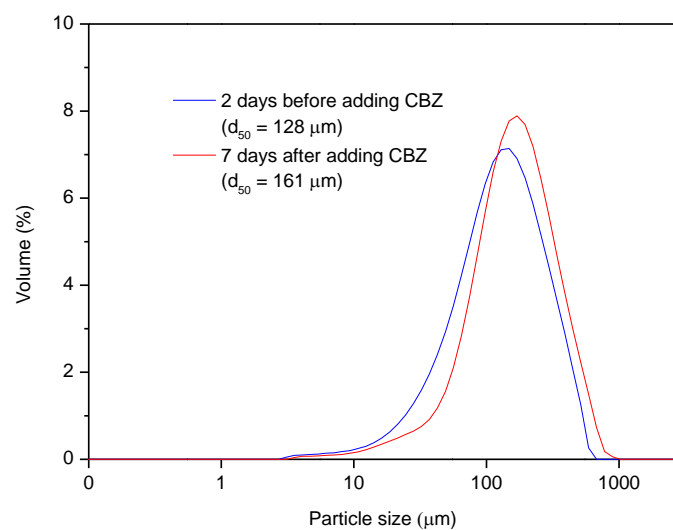


Fig. 7. Effect of CBZ on sludge floc size distribution

In conclusion, the addition of CBZ in MBR induced only a slight change in sludge floc size distribution. The modification of fouling compartment after addition of CBZ thus could probably not be attributed to a modification in the floc size distribution. Rather, the CBZ induced modification in EPS characteristics might play a more important role in the change of biomass physico-chemical properties and in the enhanced fouling.

### **3.2.3 Dissertation on Assumption 3 to explain CBZ enhanced fouling: change in supernatant characteristics**

The effects of CBZ on concentration of proteins and polysaccharides in the MBR supernatant were examined due to their potential fouling capacity. Fig. 8 shows that the addition of CBZ caused a significant increase in the protein concentration in supernatant at the beginning several days as compared to concentration level before addition of CBZ, and then reduced effect on protein production was observed after about 7 days after the addition of CBZ in MBR. In contrast, no significant change was observed for the polysaccharide concentration in supernatant. This result indicates that microorganisms mainly released protein-like SMPs when facing a pharmaceutical stress or the released polysaccharides were easily biodegraded. Similar results were observed concerning the effects of other chemical stress. Zhou et al. (2014) reported that the MBR fed with municipal wastewater containing  $0.4 \text{ mg L}^{-1}$  Cr (VI) had a higher concentration of SMPs in the supernatant, especially the protein content, as compared with the MBR (MBR-control) fed with municipal wastewater without addition of Cr (VI). The reduced protein production after about 7 days after the addition of CBZ may be caused by the acclimation of activated sludge to the pharmaceutical stress, as shown in Fig. 6. No significant increase in retention of protein in MBR was also observed after the significant increase of protein concentration in supernatant (see Fig. 8), which indicates that the increased protein were mainly relatively small molecules that could pass through the MBR membrane and biocake layer.

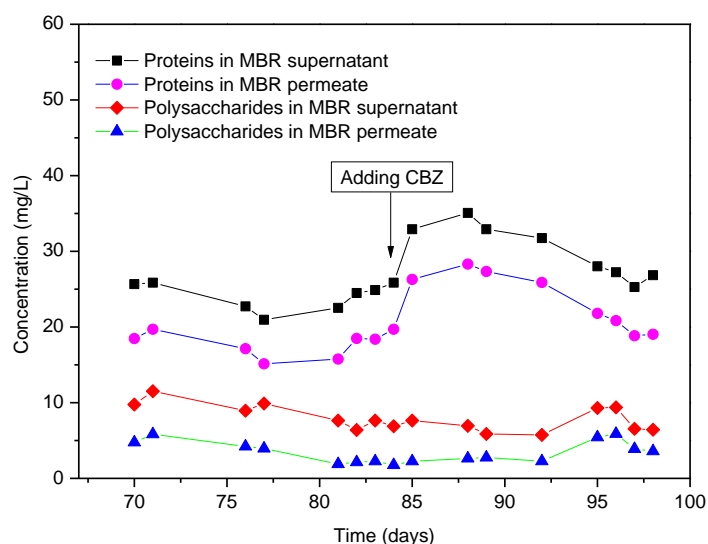


Fig. 8. Effects of CBZ on SMP concentration in supernatant and permeate of MBR

To further investigate the effects of CBZ on the release of protein-like SMPs, HPLC-SEC-fluorescence analysis of supernatants was performed. Fig. 9 shows the results of HPLC-SEC analysis of MBR supernatants 1 day (83<sup>th</sup> day) before, the day (84<sup>th</sup> day) just before and 1 day (85<sup>th</sup> day) after adding CBZ in the MBR. The results showed that the continuous presence of 90  $\mu\text{g L}^{-1}$  CBZ in MBR could cause two measurable effects on SMPs just 1 day after addition of CBZ:

- a decrease of the concentration in 100-1000 kDa protein-like SMPs. More specifically, 1 day after addition of CBZ in the MBR a significant decrease, about 16%, of 100-1000 kDa protein-like SMPs was observed in the MBR supernatant, as compared to that in the MBR supernatant that was obtained 1 day before addition of CBZ and the one obtained the day just before addition of CBZ in the MBR.
- a more significant increase in 10-100 kDa protein-like SMPs. More specifically, for the 10-100 kDa protein-like SMPs, a quite significant increase, about 23%, was observed in the MBR supernatant 1 day after addition of CBZ as compared to

that in the MBR supernatant that was obtained 1 day before addition of CBZ and the day just before addition of CBZ.

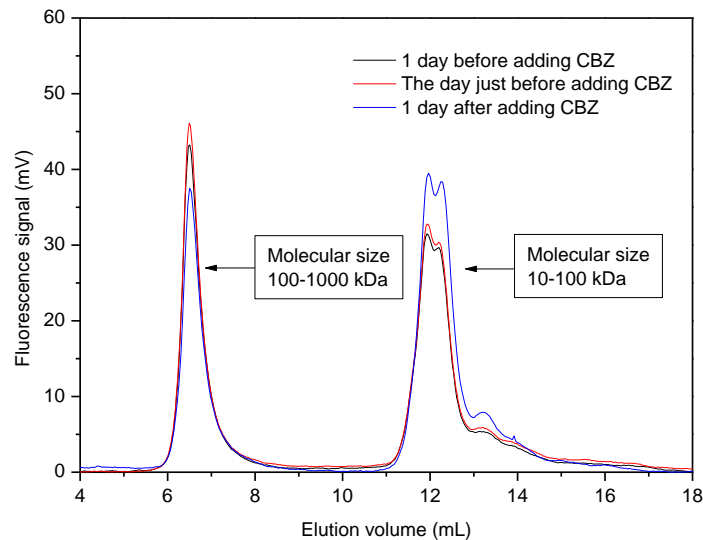


Fig. 9. HPLC-SEC analysis of MBR supernatants before and 1 day after addition of CBZ

Taking into account that inhibition of the microbial activity occurred after addition of CBZ in the MBR (see Fig. 6), the decrease of 100-1000 kDa protein-like substances in the MBR supernatant 1 day after addition of CBZ might be explained by an inhibition of the microbial activity. Ni et al. (2010) reported that BAP, which was produced from decay of bacteria, could be further classified into the growth-associated BAP (GBAP), which are produced in the microbial growth phase, and the endogeny-associated BAP (EBAP), which are generated in the endogenous phase. Therefore, the inhibition of microbial activity might reduce the production of growth-associated BAPs in the supernatant which is considered as having a molecular weight higher than 100 kDa. Moreover, Zhou et al. (2012) reported that the MBR sludge in the rapid growth phases with high microbial activities produced significantly higher BAPs than the MBR sludge in stationary growth phase and more large molecular weight compounds (higher than 100 kDa) were observed in MBR supernatant in the rapid growth phase. Whereas, the significant increase of 10-100 kDa protein-like substances in the MBR

supernatant 1 day after addition of CBZ may be caused by the defensive response of microorganisms to release SMPs when facing a chemical stress, since molecules of this size are not in the range of BAPs. Similarly, Avella et al. (2010) found that the continuous addition of  $5 \mu\text{g L}^{-1}$  cytostatic drug cyclophosphamide and its main metabolites in a MBR caused a significant increase of proteins in supernatant, with molecular weight of about 18 kDa.

Fig. 10 shows the results of HPLC-SEC analysis of MBR supernatants at 1 day and 7 days after adding CBZ in the MBR. Reduced CBZ effects on production of both 100-1000 kDa and 10-100 kDa protein-like SMPs in supernatant were observed between 1 day and 7 day after addition of CBZ in MBR, i.e. a slight decrease of 100-1000 kDa protein-like SMPs and a slight increase of 10-100 kDa protein-like SMPs in the MBR supernatants, as compared to that in the MBR supernatant 1 day after addition of CBZ (see Fig. 9). This also suggested the adaptation of MBR biomass to the pharmaceutical stress, as indicated in Fig. 6.

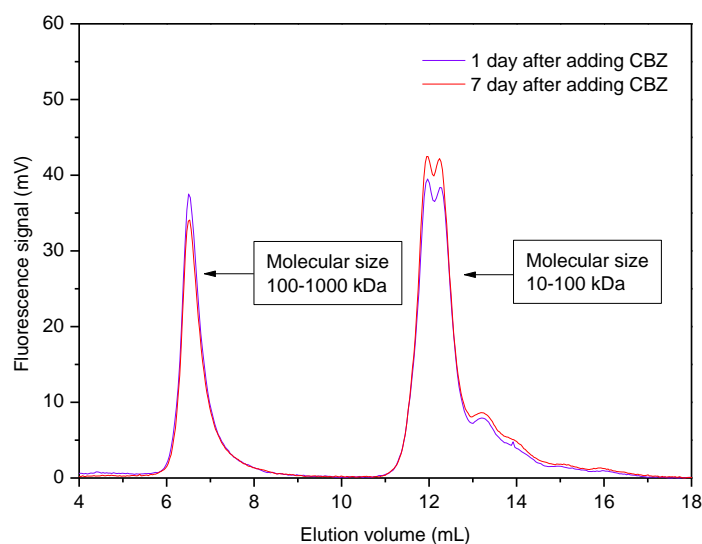


Fig. 10. HPLC-SEC analysis of MBR supernatants at 1 day and 7 days after addition of CBZ

In order to better understand change of 100-1000 kDa and 10-100 kDa protein-like SMPs in supernatant on membrane fouling, their retention by the MBR membrane after addition of CBZ was investigated. Fig. 11 showed that almost complete retention of 100-1000 kDa protein-like SMPs and almost negligible (very slight) retention of 10-100 kDa protein-like SMPs was obtained by the MBR membrane 1 day after addition of CBZ. Reminding that no retention of 10-100 kDa protein-like SMPs by the MBR membrane was observed on the day (84th day) just before addition of CBZ in MBR (see Fig. 5), this result indicated that the retention characteristics of MBR “membrane” did not change significantly just 1 day after addition of CBZ. However, Fig. 12 showed that enhanced retention of 10-100 kDa protein-like SMPs by the MBR membrane was obtained 11 days after addition of CBZ, as compared to that at 1 day after addition of CBZ. Thus, it could be hypothesized that the significant increase of 10-100 kDa protein-like SMPs in MBR supernatant after addition of CBZ induced an enhanced accumulation of these small biopolymers inside the biocake that was formed on MBR membrane surface, which caused the loss of voids in the biocake, and finally led to a more “tight” retention characteristics of the MBR “membrane” and enhanced membrane fouling.

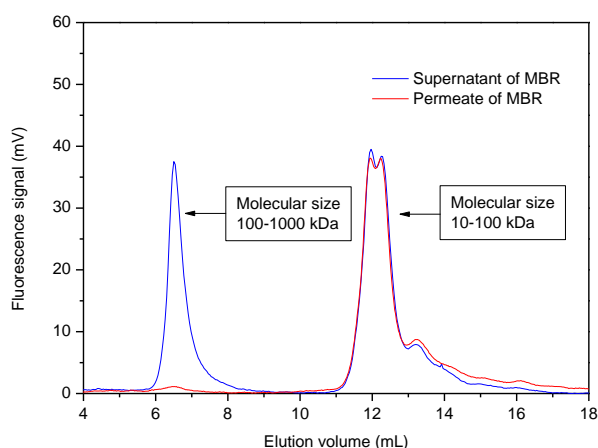


Fig. 11. HPLC-SEC analysis of supernatant and permeate of MBR 1 day after addition of CBZ

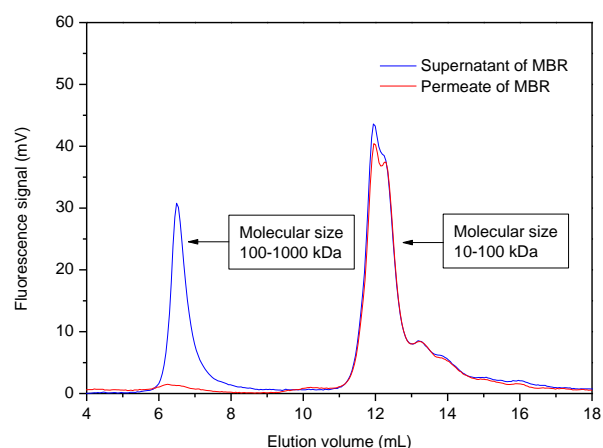


Fig.12. HPLC-SEC analysis of supernatant and permeate of MBR 11 days after addition of CBZ

In conclusion, the continuous introduction of CBZ in MBR caused significant variation in 10-100 protein-like SMPs, which could be related to the change in membrane fouling. The TMP jump occurring just 1 day after the addition of CBZ in MBR could be probably attributed to the significant increase of 10-100 kDa protein-like SMPs in the supernatant. And the higher increase rate of TMP, not the continuous TMP jump, after 1 day after addition of CBZ in MBR as compared to that before addition of CBZ could be probably attributed to the reduced increase in 10-100 kDa protein-like SMPs in the supernatant. The continuous TMP jump starting from 8 days after addition of CBZ might be due to the convective flow of biomass, which could be caused by the enhanced fouling that leads the local flux to exceed the critical value, and to the compressing effect of biocake at increasing local TMP.

### **3.3 Effects of CBZ on sludge and supernatant filterability**

In order to further understand the effect of CBZ on MBR fouling, the sludge and supernatant filterabilities before and after addition of CBZ in MBR were examined in filterability tests using a dead-end filtration cell. Fig. 13 shows that the fouling propensity of sludge, expressed as the fouling resistance produced when the fixed amount of permeate volume was filtered, increased after the continuous addition of CBZ in the MBR. This result further demonstrated that the modification in the biomass physico-chemical characteristics and supernatant quality mentioned in the above sections after addition of CBZ in sludge could lead to the deterioration in sludge filterability. Since after addition of CBZ in MBR, a decrease in 100-1000 kDa protein-like SMPs and an more significant increase in 10-100 kDa protein-like SMPs occurred at the same time (see Fig. 9), this results also suggests the important role of small biopolymer in the sludge fouling propensity.



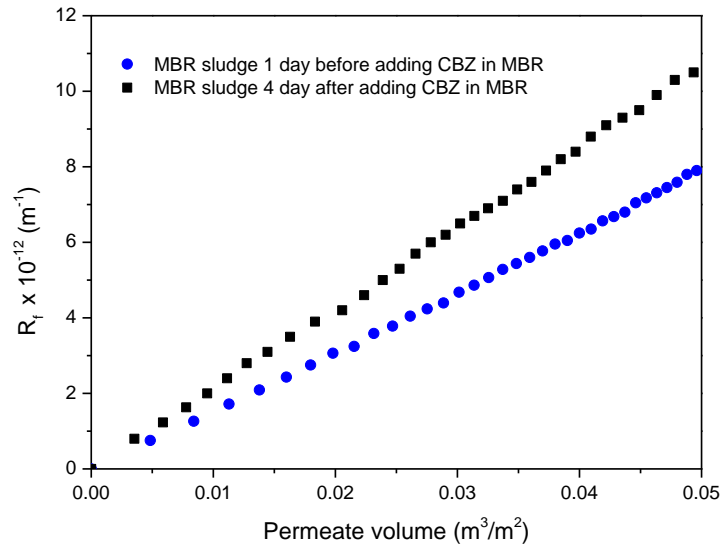


Fig. 13. Effect of CBZ on sludge filterability

Fig. 14 shows the change of supernatant filterability after addition of CBZ in MBR. The result showed that the fouling propensity of supernatant decreased after the continuous addition of CBZ in the MBR. This could be attributed to the decrease of 100-1000 kDa protein-like SMPs in the supernatant after addition of CBZ in MBR (see Fig. 9), since in supernatant filtration, the 10-100 kDa protein-like SMPs in supernatant could pass through the 150 kDa PES membrane. Thus, the fouling mainly originated from the retention of 100-1000 kDa SMPs in supernatant by the 150 kDa PES membranes and thus the formation of the gel layer on the membrane surface. This result further suggests that, when considering the change induced in supernatant quality after addition of CBZ, the increase in sludge fouling propensity was not originated from the change in 100-1000 kDa protein-like SMPs, but from the increase in 10-100 kDa protein-like SMPs.

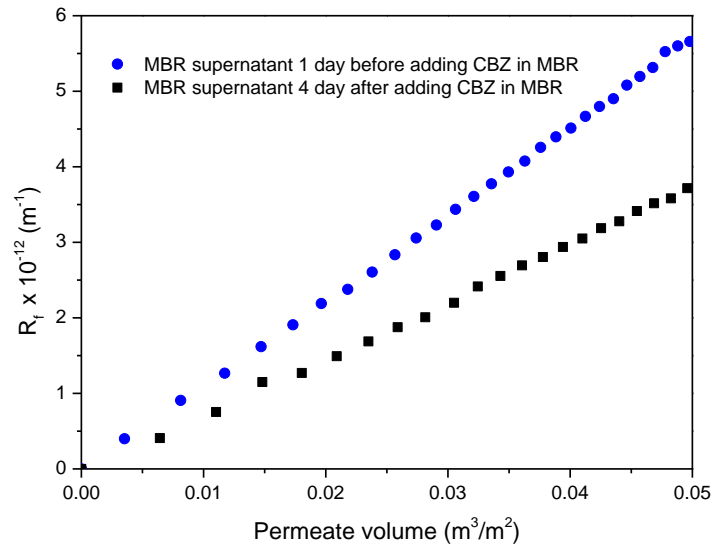


Fig. 14. Effect of CBZ on supernatant filterability

#### 4. Conclusions

Understanding fouling mechanisms of membranes in MBRs, and particularly in presence of domestic pharmaceutical micropollutants is still a key issue. In this study, the effects of a pharmaceutical micropollutant, carbamazepine, on membrane fouling of a submerged MBR treating domestic wastewater were investigated. CBZ was continuously added in the MBR via feed ( $90 \mu\text{g L}^{-1}$  in the feed). A TMP jump occurred just 1 day after the addition of CBZ in MBR and a significantly higher increase rate of TMP was also observed after 1 day after addition of CBZ in MBR, as compared to that before addition of CBZ. This indicates that the pharmaceutical stress induced by CBZ causes more severe membrane fouling, which could be related to the change in biomass and supernatant characteristics. The continuous introduction of CBZ in the MBR led to a significant increase of protein content in the MBR supernatant, whereas no significant difference of polysaccharide content in supernatant was observed. Besides, the increased protein-like compounds were mainly in the 10-100 kDa range, which

may be caused by the protection mechanisms of microorganisms when facing a pharmaceutical stress. In contrast, a decrease of 100-1000 kDa protein-like compounds in the supernatant was observed after addition of CBZ, which may be due to the reduced microbial activity. The increase of protein-like SMPs in 10-100 kDa range may cause the accumulation of 10-100 kDa protein-like compounds inside the biocake formed on MBR membrane surface. In addition, the continuous presence of CBZ in the activated sludge caused a slight inhibition in sludge exogenous respiration rate and a slight increase in mean sludge floc size. Thus, the modification of biomass biological and physico-chemical characteristics together with the change in supernatant quality could modify the composition and structure of biocake and lead to a significant increase of TMP in MBR.

This study may help to understand the effects of mixtures of pharmaceutical micropollutants on MBR fouling, since with mixtures of pharmaceutical micropollutants enhanced effects are expected on the sludge microbial activities, biomass physico-chemical characteristics and on the release of SMPs by the bacteria under stress, as compared to the effect of CBZ alone. More studies should also be carried out with the objectives to avoid the TMP-jump induced by pharmaceutical stress, for example by modifying operating operations to develop sludge that is less sensitive to the pharmaceutical stress. Or hybrid MBR processes, like MBR-PAC process where powdered activated carbon (PAC) is added in MBR to enhance removal of pharmaceutical micropollutants via adsorption, could be proposed to reduce the contact of activated sludge with pharmaceutical micropollutants, thus reducing the stress effect induced by pharmaceutical micropollutants.

## References

- Abegglen, C., Joss, A., Mc Ardell, C. S., Fink, G., Schlüsener, M. P., Ternes, T. A., Siegrist, H., 2009. The fate of selected micropollutants in a single-house MBR. *Water research* 43 (7), 2036-2046.
- Al-Halbouni, D., Traber, J., Lyko, S., Wintgens, T., Melin, T., Tacke, D., Janot, A., Dott, W., Hollender, J., 2008. Correlation of EPS content in activated sludge at different sludge retention times with membrane fouling phenomena. *Water Research* 42 (6), 1475-1488.
- Arabi, S., Nakhla, G., 2010. Impact of molecular weight distribution of soluble microbial products on fouling in membrane bioreactors. *Separation and Purification Technology* 73 (3), 391-396.
- Aubenneau, M., Tahar, A., Casellas, C., Wisniewski, C., 2010. Membrane bioreactor for pharmaceutically active compounds removal: Effects of carbamazepine on mixed microbial communities implied in the treatment. *Process Biochemistry* 45 (11), 1826-1831.
- Avella, A., Delgado, L. F., Görner, T., Albasi, C., Galmiche, M., De Donato, P., 2010. Effect of cytostatic drug presence on extracellular polymeric substances formation in municipal wastewater treated by membrane bioreactor. *Bioresource technology* 101 (2), 518-526.
- Bouhabila, E. H., Ben Aïm, R., Buisson, H., 2001. Fouling characterisation in membrane bioreactors. *Separation and Purification Technology* 22, 123-132.
- Chang, S., Fane, A. G., Waite, T. D., 2006. Analysis of constant permeate flow filtration using dead-end hollow fiber membranes. *Journal of Membrane Science* 268 (2), 132-141.
- Clara, M., Strenn, B., Kreuzinger, N., 2004. Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration. *Water Research* 38 (4), 947-954.
- Delgado, L. F., Schetrite, S., Gonzalez, C., Albasi, C., 2010. Effect of cytostatic drugs on microbial behaviour in membrane bioreactor system. *Bioresource technology* 101 (2), 527-536.
- Drews, A., 2010. Membrane fouling in membrane bioreactors—characterisation, contradictions, cause and cures. *Journal of Membrane Science* 363 (1), 1-28.

- Dreywood, R., 1946. Qualitative test for carbohydrate material. *Industrial & Engineering Chemistry Analytical* Edition 18 (8), 499-499.
- Hai, F. I., Li, X., Price, W. E., Nghiem, L. D., 2011. Removal of carbamazepine and sulfamethoxazole by MBR under anoxic and aerobic conditions. *Bioresource technology* 102 (22), 10386-10390.
- Hai, F. I., Yamamoto, K., Lee, C. H. (2013). Membrane Biological Reactors: Theory, Modeling, Design, Management and Applications to Wastewater Reuse, IWA Publishing.
- Henriques, I. D., 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.
- Henriques, I. D. S., Aga, D. S., Mendes, P., O'Connor, S. K., Love, N. G., 2007. Metabolic Footprinting: A New Approach to Identify Physiological Changes in Complex Microbial Communities upon Exposure to Toxic Chemicals. *Environmental Science & Technology* 41 (11), 3945-3951.
- Her, N., Amy, G., McKnight, D., Sohn, J., Yoon, Y., 2003. Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection. *Water Research* 37 (17), 4295-4303.
- Hernandez Rojas, M., Van Kaam, R., Schetrite, S., Albasi, C., 2005. Role and variations of supernatant compounds in submerged membrane bioreactor fouling. *Desalination* 179 (1), 95-107.
- Holakoo, L., Nakhla, G., Yanful, E. K., Bassi, A. S., 2006. Chelating properties and molecular weight distribution of soluble microbial products from an aerobic membrane bioreactor. *Water research* 40 (8), 1531-1538.
- Jiang, T., Kennedy, M. D., Schepper, V. D., Nam, S.-N., Nopens, I., Vanrolleghem, P. A., Amy, G., 2010. Characterization of soluble microbial products and their fouling impacts in membrane bioreactors. *Environmental science & technology* 44 (17), 6642-6648.
- Joss, A., Keller, E., Alder, A. C., Göbel, A., Mc Ardell, C. S., Ternes, T., Siegrist, H., 2005. Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water Research* 39 (14), 3139-3152.

- 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 a classification scheme. *Water research* 40 (8), 1686-1696.
- Judd, S. (2010). The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment, Elsevier.
- Kimura, K., Yamato, N., Yamamura, H., Watanabe, Y., 2005. Membrane fouling in pilot-scale membrane bioreactors (MBRs) treating municipal wastewater. *Environmental science & technology* 39 (16), 6293-6299.
- Kraigher, B., Kosjek, T., Heath, E., Kompare, B., Mandic-Mulec, I., 2008. Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors. *Water Research* 42 (17), 4578-4588.
- Lay, W. C., Zhang, Q., Zhang, J., McDougald, D., Tang, C., Wang, R., Liu, Y., Fane, A. G., 2012. Effect of pharmaceuticals on the performance of a novel osmotic membrane bioreactor (OMBR). *Separation Science and Technology* 47 (4), 543-554.
- Le-Clech, P., Chen, V., Fane, T. A., 2006. Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science* 284 (1), 17-53.
- Li, C., Cabassud, C., Guigui, C., 2014. Evaluation of membrane bioreactor on removal of pharmaceutical micropollutants: a review. *Desalination and Water Treatment*, In press.
- Liang, S., Liu, C., Song, L., 2007. Soluble microbial products in membrane bioreactor operation: behaviors, characteristics, and fouling potential. *Water Research* 41 (1), 95-101.
- Massé A., 2004. Bioréacteur à membranes immergées pour le traitement des eaux résiduaires urbaines: spécificités physico-chimiques du milieu biologique et colmatage.
- Masse, A., Spérandio, M., Cabassud, C., 2006. Comparison of sludge characteristics and performance of a submerged membrane bioreactor and an activated sludge process at high solids retention time. *Water Research* 40 (12), 2405-2415.

- 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.
- Menniti, A., Kang, S., Elimelech, M., Morgenroth, E., 2009. Influence of shear on the production of extracellular polymeric substances in membrane bioreactors. *Water Research* 43 (17), 4305-4315.
- Metzger, U., Le-Clech, P., Stuetz, R. M., Frimmel, F. H., Chen, V., 2007. Characterisation of polymeric fouling in membrane bioreactors and the effect of different filtration modes. *Journal of Membrane Science* 301 (1), 180-189.
- Ni, B.-J., Zeng, R. J., Fang, F., Xie, W.-M., Sheng, G.-P., Yu, H.-Q., 2010. Fractionating soluble microbial products in the activated sludge process. *Water Research* 44 (7), 2292-2302.
- Ognier, S., Wisniewski, C., Grasmick, A., 2002. Influence of macromolecule adsorption during filtration of a membrane bioreactor mixed liquor suspension. *Journal of Membrane Science* 209 (1), 27-37.
- Ognier, S., Wisniewski, C., Grasmick, A., 2004. Membrane bioreactor fouling in sub-critical filtration conditions: a local critical flux concept. *Journal of Membrane Science* 229 (1-2), 171-177.
- Radjenović, J., Petrović, M., Barceló, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water research* 43 (3), 831-841.
- 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.
- Smith, P., Krohn, R. I., Hermanson, G., Mallia, A., Gartner, F., Provenzano, M., Fujimoto, E., Goetze, N., Olson, B., Klenk, D., 1985. Measurement of protein using bicinchoninic acid. *Analytical biochemistry* 150 (1), 76-85.
- Spérandio, M., Paul, E., 2000. Estimation of wastewater biodegradable COD fractions by combining respirometric experiments in various  $S_0/X_0$  ratios. *Water Research* 34 (4), 1233-1246.

- Stricot, M., Filali, A., Lesage, N., Spárandio, M., Cabassud, C., 2010. Side-stream membrane bioreactors: Influence of stress generated by hydrodynamics on floc structure, supernatant quality and fouling propensity. *Water Research* 44 (7), 2113-2124.
- Sun, C., Leiknes, T., Weitzenböck, J., Thorstensen, B., 2010. Salinity effect on a biofilm-MBR process for shipboard wastewater treatment. *Separation and Purification Technology* 72 (3), 380-387.
- Ternes, T. A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid–water distribution coefficient (K<sub>d</sub>) for pharmaceuticals and musk fragrances in sewage sludge. *Water Research* 38 (19), 4075-4084.
- Teychene, B., Guigui, C., Cabassud, C., 2011. Engineering of an MBR supernatant fouling layer by fine particles addition: A possible way to control cake compressibility. *Water Research* 45 (5), 2060-2072.
- Trussell, R. S., Merlo, R. P., Hermanowicz, S. W., Jenkins, D., 2006. The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. *Water Research* 40 (14), 2675-2683.
- Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Science of the Total Environment* 429 (0), 123-155.
- Vieno, N., Tuhkanen, T., Kronberg, L., 2007. Elimination of pharmaceuticals in sewage treatment plants in Finland. *Water Research* 41 (5), 1001-1012.
- Wang, S., Holzem, R. M., Gunsch, C. K., 2008. Effects of pharmaceutically active compounds on a mixed microbial community originating from a municipal wastewater treatment plant. *Environmental science & technology* 42 (4), 1091-1095.
- Wisniewski, C., Grasmick, A., 1998. Floc size distribution in a membrane bioreactor and consequences for membrane fouling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 138 (2), 403-411.



- Yamato, N., Kimura, K., Miyoshi, T., Watanabe, Y., 2006. Difference in membrane fouling in membrane bioreactors (MBRs) caused by membrane polymer materials. *Journal of Membrane Science* 280 (1), 911-919.
- Zhang, Y., Geißén, S.-U., Gal, C., 2008. Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere* 73 (8), 1151-1161.
- Zhou, L., Zhang, Z., Jiang, W., Guo, W., Ngo, H.-H., Meng, X., Fan, J., Zhao, J., Xia, S., 2014. Effects of low-concentration Cr(VI) on the performance and the membrane fouling of a submerged membrane bioreactor in the treatment of municipal wastewater. *Biofouling* 30 (1), 105-114.
- Zhou, Z., Meng, F., Liang, S., Ni, B.-J., Jia, X., Li, S., Song, Y., Huang, G., 2012. Role of microorganism growth phase in the accumulation and characteristics of biomacromolecules (BMM) in a membrane bioreactor. *RSC Advances* 2 (2), 453-460.

# Publication 4

---

---



# HPLC-SEC with fluorescence detection as a tool for estimating fouling of MBRs used for municipal wastewater treatment

Chengcheng Li<sup>a,b,c,\*</sup>, Corinne Cabassud<sup>a,b,c</sup>, Aurore Didi<sup>a,b,c</sup>, Christelle Guigui<sup>a,b,c</sup>

<sup>a</sup> Université de Toulouse, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, 31077 Toulouse, France

<sup>b</sup> INRA, UMR792 Ingénierie des Systèmes biologiques et des Procédés, 31400 Toulouse, France

<sup>c</sup> CNRS, UMR5504, 31400 Toulouse, France

\* Corresponding author at: INSA Toulouse, Hall GPE, 135 Avenue de Rangueil, 31077 Toulouse, France. Tel.: +33 05 61 55 97 91 ; Email : chengcheng.li@insa-toulouse.fr.

## Abstract

Membrane bioreactor (MBR) has been increasingly used for municipal wastewater treatment and reuse due to its good effluent quality. However, membrane fouling remains the major limitation of MBR. Understanding fouling is still a key issue for a more sustainable operation of MBRs. Thus, there is a need for simple and cheap methods that could allow estimating the fouling propensity of biological fluids such as supernatants. Some authors have proposed to measure directly the filterability of the supernatant. The objective of our publication was to study if some analytical tools that can characterize the retention of some soluble molecules contained in the supernatant of an MBR could be used as an indicator of the fouling propensity of this supernatant and thus, in the future, to predict it. The analytical method that was chosen is High Performance Liquid Chromatography equipped with Size Exclusion Column and a Fluorescence detector (HPLC-SEC-fluorescence). Thanks to this method, it was found that for both operation of a MBR pilot plant fed with domestic waste waters, and for batch filterability tests performed with the supernatants sampled from the MBR, protein-like soluble microbial products (SMP) with molecular size in 100-1000 kDa range are the main responsible for membrane fouling. By characterization of protein-like SMPs in the MBR's supernatant, a linear relationship between the information obtained by HPLC-SEC-fluorescence and fouling propensity (expressed as  $\alpha C$ ) of supernatants was established. This is opening the door for a future use of this method as a tool for optimizing the operating conditions of MBR and in order to reduce fouling and to increase the productivity of MBRs.

**Key words:** MBR, HPLC-SEC-fluorescence, fouling, SMP, protein-like substances

## 1. Introduction

Membrane bioreactors, which couple the activated sludge process with membrane filtration, greatly increase the treated effluent quality as compared to conventional activated sludge process. Thus, they have been increasingly used for wastewater treatment and reuse applications [1-2]. The other advantages of MBRs include smaller footprint or less excess sludge production, depending on the operating conditions, and flexibility of process design, i.e. independent control of sludge retention time (SRT) and hydraulic retention time (HRT) [3]. However, membrane fouling is still a key scientific issue and a limitation for the development of the MBR process, because of the increase in operating costs and also the potential decrease in membrane lifetime.

The decline of permeability in MBR could be caused by clogging, which involves filling of the channels between the membranes with sludge solids, or membrane fouling [4-5]. Membrane fouling in MBR was caused by biomass deposit, biofilm and pore blocking due to particles, colloids and solute adsorption [6-8]. Due to gas-liquid two-phase cross flow generated by aeration in submerged MBRs or liquid cross flow induced by recirculation pump in side-stream MBRs, the low flux operation and the relatively easy removal of cake deposit, cake deposit formation is of minor importance on MBR fouling in long-term operation. Many studies showed that the supernatant of activated sludges (which contains colloids and solutes) plays a more important role in membrane fouling of MBR than biological flocs [9-10]. Soluble microbial products (SMP), the major organic constituents of sludge supernatants [11],

have been acknowledged as major foulants that lead to membrane fouling in MBRs [12-14]. SMP have been identified as soluble extracellular polymeric substances (EPS) which are composed of proteins, polysaccharides, humic substances, nucleic acids etc. [11, 15-16]. SMP could be divided into two categories: utilization-associated products (UAP), which are produced during substrate metabolism, and biomass-associated products (BAP) which are formed from decay of biomass [11, 17]. The composition and concentration of SMP is significantly affected by operating conditions, for example, organic loading rates [18-19], sludge age [20-22] or hydraulic conditions [23-24], and by the presence of chemicals in the feed that can affect the biological activity [25-27].

Conflicting observations were often reported in the literature concerning the relative importance of SMP components on fouling in MBRs. Some authors attributed protein-like substances to be the main foulant responsible for fouling [14, 22, 28-29]. By HPLC–SEC and fluorescence analysis, Teychene et al. [30] demonstrated foulant species of MBR to be protein-like substances. On the basis of statistical analysis of sample analytical results, protein-like substances were also found in a study to be highly correlated to the fouling potential of two MBR supernatants [31]. In contrast, some other authors related a preponderant MBR fouling with polysaccharide-like substances in supernatant [12, 32]. Besides, some studies showed that proteins were observed to dominate in foulants obtained from the fouled membranes of MBRs [33] and are preferentially attached to the membrane surface and membrane pores, whereas carbohydrates are enriched in the intermediate layer of cake deposit [34].

However, simply correlated the fouling with protein or polysaccharide concentration in the supernatant or their retention in MBR could not explain the contribution of each component in fouling, since some SMP components may be retained by the cake layer, and may not reach to the membrane surface or the membrane pores [34]. Thus, in MBRs, fouling is not only affected by the SMP concentration in the sludge supernatant, but is also affected by molecular weight (MW) distribution. Some studies found that the major responsible for fouling are the SMP compounds with a molecular size higher than 100 kDa [12, 35], whereas in another study the compounds with a molecular size 10-100 kDa were observed to cause the main contribution the fouling propensity [36]. Similarly, the UAP was reported to have higher specific cake resistance and pore blocking resistance than BAP, although their retention percentage was lower than BAP due to their higher percentage of low molecular weight molecules [37]. The common shared point is that the SMP compounds that cause the major fouling are in biopolymer range (MW higher than 10 kDa) and the fouling is caused by biofilm (gel) layer formation or pore blocking. The divergences concerning the contribution of biopolymers in different MW range to membrane fouling may be caused by the differences in the pore size of membranes used for the fouling propensity tests and the methods used for biopolymer MW distribution analysis.

Fouling propensity of biological fluids is currently estimated by a calculation of fouling resistance, permeability or fouling rate obtained from an analysis of operating data (permeate flux, TMP) [38-40]; by batch stirred dead-end filtration cells fed with samples of the sludge or supernatants [35, 41-43]; by batch tangential modules (*ex situ*) operated under standardized

operating conditions and continuously fed with wastewater effluents [44] or the sludge [45]; and by some submerged modules placed directly inside MBR (in-situ) and using aeration to generate cross-flow conditions [46-47], which could avoid the problems like the change of sludge filterability during store period. In the case of a fouling occurring as a cake deposit, some recent works have proposed in-situ fouling characterization filtration apparatus equipped with acoustic or optical sensors [48-51]. The later method was studied at lab scale with diluted sludges, but has not been yet applied to in-line detection of membrane fouling in MBR [52]. Still, there is a great interest in the industry to find some simple and cheap methods that could allow estimating the fouling propensity of biological fluids such as MBR supernatants. Many analytical tools, including three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy and high performance liquid chromatography-size exclusion chromatography (HPLC-SEC) equipped with different detectors (UV, refractive index, fluorescence and organic carbon), have been used to characterize SMP in supernatant. Table 1 summarizes the advantages and disadvantages of different techniques for SMP characterization.



Table 1. SMP characterization methods and their features

Method	Principle	Advantages	Disadvantages	Reference
EEM fluorescence	Fluorescence spectroscopy	Sensitive and selective detection of proteins and humic substances	No molecular weight distribution analysis; Not for polysaccharide detection.	[53-55]
HPLC-SEC-UV	HPLC-SEC with UV detection	Molecular weight distribution analysis of proteins and humic substances	Not selective; Not for polysaccharide detection; Not sensitive for compounds in biopolymer range.	[30, 43, 53-54, 56]
HPLC-SEC-RI	HPLC-SEC with refractive index detection	Molecular weight distribution analysis of organic compounds including polysaccharides	Not selective; Not sensitive for compounds in biopolymer range.	[53, 57-58]
HPLC-SEC-fluorescence	HPLC-SEC with fluorescence detection	Sensitive and selective molecular weight distribution analysis of proteins and humic substances	Not for polysaccharide detection.	[43, 54, 56]
LC-OCD	HPLC-SEC with organic carbon detection	Molecular weight distribution analysis of organic compounds including proteins, polysaccharides and humic substances	Not selective	[53-54]

By integrating the advantages of EEM fluorescence spectroscopy with HPLC-SEC analysis, HPLC-SEC-fluorescence could achieve accurate detection of protein-like substances in different molecular weight ranges, especially for the compounds in biopolymer range (MW

bigger than 10 kDa), which could help to improve understanding the effects of protein-like substances and their molecular weight distribution on membrane fouling. Shon et al. [56] used HPLC-SEC-fluorescence to characterize the composition of effluent organic matter (EfOM) present in biologically treated sewage effluent (BTSE) and attributed the high fouling propensity of hydrophobic (HP) fractions to the presence of big peak of high MW protein-like substances. Thanh et al. [59] used HPLC-SEC-fluorescence to study the effects of aeration shear stresses on the characteristics of sludge supernatant of an aerobic granular sludge bioreactor and attributed higher fouling propensity of the sludge under higher aeration stress to the production of large MW soluble microbial products (30–50 kDa). Teychene et al. [43] employed HPLC-SEC-fluorescence to investigate the interaction of macromolecular compounds in MBR supernatant with added fine particles and attributed the reduced fouling propensity of supernatants after addition of fine particles to the reduction of protein-like substances in higher than 100 kDa range. However, no study has been carried out to evaluate HPLC-SEC-fluorescence as a tool for estimating fouling propensity of MBR supernatants and fouling of MBR during the different fouling conditions.

The objective of this study was to evaluate the possibility of HPLC-SEC-fluorescence as a tool for rapid estimation of the modification of fouling in MBRs, when some variations in fouling conditions happen. For example, the operating conditions of MBRs such as organic loading rates may vary a lot in real industrial applications. It was then decided to obtain information on HPLC-SEC fluorescence and on fouling propensity using the same biological fluids. During different operational periods, various fouling situations in a lab-scale MBR

were observed. Then, HPLC-SEC-fluorescence analysis was performed on the supernatants and permeates obtained from the MBR under these different fouling situations. Supernatants of MBR in these different fouling situations were also sampled for filterability tests performed with a dead-end filtration cell and HPLC-SEC-fluorescence analysis of both the MBR supernatants and the permeates obtained during the filtration tests was performed. Then, the relation between HPLC-SEC-fluorescence analysis and fouling rate or fouling parameters was investigated for both the sludge in the MBR and the supernatants in the filterability tests. The organization of this study is shown in Fig. 1.

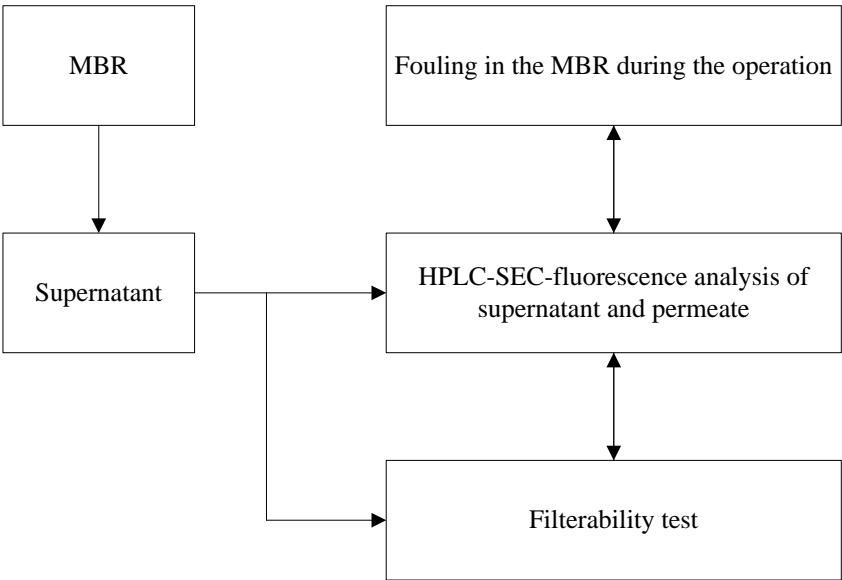


Fig. 1. Schematic diagram of study plan

## 2. Materials and methods

### 2.1 Membrane bioreactor set-up

A lab-scale submerged MBR set-up consisting of an anoxic reactor (5.4 L) and an aerobic reactor (12.6 L) was used in this study (see Fig. 2). A flat sheet microfiltration membrane ( $L_{p0} = 1110 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at  $20 \text{ }^\circ\text{C}$ , Kubota, Japan) was submerged inside the aerobic reactor. It has an effective filtration area of  $0.1 \text{ m}^2$ , with an average pore size of  $0.2 \text{ }\mu\text{m}$ . This MBR was operated at the high sludge retention time (SRT) of 40 days, with a sludge concentration in the aerobic reactor between 4-6 g/L. The instantaneous permeate flux was fixed at  $12.5 \text{ L/h/m}^2$  with filtration/relaxation cycles of 8 min/2 min, leading to a net permeate flux of  $10 \text{ L/h/m}^2$ . Two aeration systems were used in the aerobic reactor. An aeration system with big air bubble was installed below the membrane to reduce fouling and it was operated continuously at flow rate of 1.5 L/min, i.e.  $\text{SAD}_m$  of  $0.9 \text{ m}^3 \text{ air/h/m}^2$  and  $\text{SAD}_p$  of  $90 \text{ m}^3 \text{ air/m}^3$  permeate. Besides, another aeration system with fine air bubbles was used to maintain the dissolved oxygen concentration in the sludge at about 2-3 mg/L and automatically controlled by an oxygen sensor. The sludge recirculation ratio was fixed at 4. The MBR was fed with a municipal wastewater taken from a wastewater treatment plant close to Toulouse, France, after a primary physical treatment.

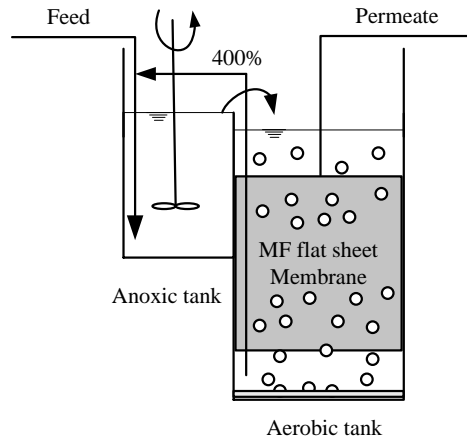


Fig. 2. MBR set-up used in this study

Table 2 shows the MBR performances in this study. During the MBR operation, when the permeability of membrane reached to about  $200 \text{ L/h/m}^2/\text{bar}$ , membrane was taken out from the aerobic tank, cleaned by water and then wiped with a soft sponge. Particularly, starting from set 2, after physical cleaning the membrane was soaked in 0.5 % Sodium hypochlorite ( $\text{NaClO}$ ) solution for 2 hours to check the effects of a chemical cleaning. The MBR reached high removal of COD, more than 95% during the 4 sets. Whereas, a limited removal of TN, of about 40%, was observed during set 1 and set 2 when the MBR was operated at an organic loading rate of about  $0.1 \text{ kg COD/kg MLSS/d}$ , due to the insufficiency of the organic source for heterotrophic denitrification [60].

Table 2. Sludge sampling procedures and MBR performances in this study

Set	Experiment duration	Organic loading rates (kg COD/kg MLSS/d)	MLSS (g/L)	Average COD removal	Average TN removal
1	14 days	0.1	4-6	96.7 %	41.4 %
2	14 days	0.1	4-6		
3	10 days	0.2	4-6	97.4 %	81.6 %
4	6 days	0.4	4-6	98.1 %	83.2 %

## 2.2 Analytical methods

The concentration in mixed liquor suspended solids (MLSS) was measured according to the standard method AFNOR NFT 90-105. Chemical oxygen demand (COD) and total nitrogen (TN) were measured by spectrometry with reagent kits. Dissolved organic carbon (DOC) was measured by a Total Organic Carbon analyser (Shimadzu, FR). Prior to analysis of DOC, each sample was prefiltered on a 0.45  $\mu\text{m}$  membrane. Supernatants were prepared by centrifugation of the sludge samples at 4000 g during 10 minutes at room temperature.

High performance liquid chromatography—size exclusion chromatography (HPLC—SEC) analysis was performed by an Akta Purifer (GE Healthcare, USA), equipped with a column Protein Kw804 (Shodex, Japan), a silica-based column with an exclusion limit of 1000 kDa, and a fluorescence detector (Varian, USA). The eluent solution for the mobile phase was prepared with 25 mM  $\text{Na}_2\text{SO}_4$  and a phosphate buffer (2.4 mM  $\text{NaH}_2\text{PO}_4$  and 1.6 mM

Na<sub>2</sub>HPO<sub>4</sub> at pH 6.8). The flow rate of eluent passing through the column was 1.0 mL/min. Excitation/emission wavelengths were set at 280/350 nm, which were confirmed as the required values by EEM fluorescence results [43, 54], to detect protein-like substances. The column was calibrated using standard proteins: thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa) from GE Healthcare. Prior to analysis of HPLC-SEC-fluorescence, each sample was prefiltered on a 0.45 µm membrane.

### **2.3 Filterability tests**

Ultrafiltration membranes (from Orelis, FR), made of polyethersulfone (PES), with a nominal pore size of 0.01 µm ( $L_{p0} = 50-80 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at 20 °C) and ultrafiltration membranes (Microdyn-Nadir GmbH, Germany), made of polyethersulfone (PES), with a molecular weight cutoff (MWCO) 150 kDa ( $L_{p0} = 400-600 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  at 20 °C) were used for the filterability tests of supernatant, because the 0.2 µm PS membranes could retain very little components in the MBR supernatant. In addition, in MBRs that employ microfiltration membranes, the deposits formed on the membrane surface and inside the pores constitute an additional barrier and makes the apparent pore size of the fouled microfiltration membrane be in the ultrafiltration (UF) range [61]. A new membrane sample was used for each filtration test. All membranes were soaked in ultra pure water over night to maintain pores wetted. Then they were rinsed by filtration of ultra pure water at 1 bar during 15 min prior to filtration test. All filtration tests were carried out in an Amicon cell (Millipore), with an effective

membrane filtration surface of about 13.4 cm<sup>2</sup>. For the 0.01 μm PES membranes, the filtration tests were performed at three constant transmembrane pressures (TMP): 0.5 bar, 0.8 bar, and 1.0 bar. For 150 kDa PES membranes, all filtration tests were carried out only at constant TMP of 0.5 bar. For each filtration test, supernatant or sludge was filtered through selected membranes until 70 ml permeate was produced (corresponding to a filtered permeate volume of about 50 L m<sup>-2</sup>); temperature was measured and flux was normalized to the flux at 20 °C. Membrane fouling resistance was determined according to Eq. (1).

$$J = \frac{TMP}{\mu(R_m + R_f)} \quad (1)$$

In this equation, J is the permeate flux (m<sup>3</sup>/s/m<sup>2</sup>) at 20 °C, with R<sub>m</sub> the membrane resistance (m<sup>-1</sup>), R<sub>f</sub> the fouling resistance (m<sup>-1</sup>), TMP the transmembrane pressure (Pa) and μ is the dynamic viscosity at 20 °C (Pa s).

Then in the dead-end filtration cell, αC can be determined by cake deposit model as expressed in Eq. (2).

$$R_f = \alpha C \frac{V}{A} \quad (2)$$

In this equation, α is the specific cake resistance (m kg<sup>-1</sup>), C is the deposited mass per filtered permeate volume (kg m<sup>-3</sup>), V is the cumulated permeate volume (m<sup>3</sup>) and A is the effective membrane area for filtration (m<sup>2</sup>). For supernatant filtration, C could be calculated by the retained DOC (kg m<sup>-3</sup>).



### 3. Results

#### 3.1. Fouling in MBR versus HPLC-SEC results

Fouling in MBR was characterized by the decline of membrane permeability during MBR operation. Fig. 3 presents the time-variation of permeability during operation of the MBR with the numbers in the figure to indicate the sampling of supernatants (sample 1, 2, 3 and 4). The MBR was operated under varied organic loading rates. Between every two successive sets, membrane cleaning was performed to recover the permeability. As shown in Fig.3, when the organic loading rate was increased from 0.1 kg COD/kg MLSS/d (set 1 and set 2) to 0.2 kg COD/kg MLSS/d (set 3) and to 0.4 kg COD/kg MLSS/d (set 4), increased average fouling rate, expressed as the decrease rate of permeability, in MBR was observed, i.e. from about 40 L/h/m<sup>2</sup>/bar/d (set 1 and set 2) to about 90 L/h/m<sup>2</sup>/bar/d (set 3), and to about 140 L/h/m<sup>2</sup>/bar/d (set 4).

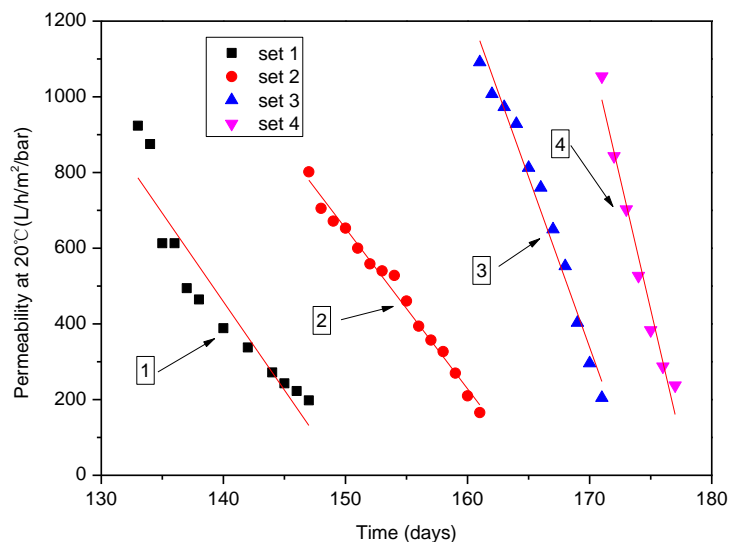


Fig. 3. Permeability of MBR operated under varied organic loading rates

To investigate the MW range of biopolymer that is responsible for MBR fouling, HPLC-SEC-fluorescence analysis of a supernatant and permeate obtained from the MBR was performed, as shown in Fig. 4. The molecular weight of protein-like substances in MBR supernatants showed bimodal distribution. Indeed, two peaks were observed in the HPLC-SEC results which represent two groups of protein-like molecules, i.e. molecular size of 100-1000 kDa and 10-100 kDa, respectively. Since Her et al. [54] and Huber et al. [62] identified components of SMP with molecular weight higher than 10 kDa as the biopolymer fraction, the two detected peaks are both in the range of biopolymers. As shown in Fig. 4, the protein-like substances in supernatant with molecular size of 100-1000 kDa could be almost completely retained by the MBR membrane, whereas almost all of the protein-like substances with molecular size of 10-100 kDa passed through MBR membrane. This indicates that the 100-1000 kDa protein-like SMPs may play an important role in MBR fouling through the biofilm formation.

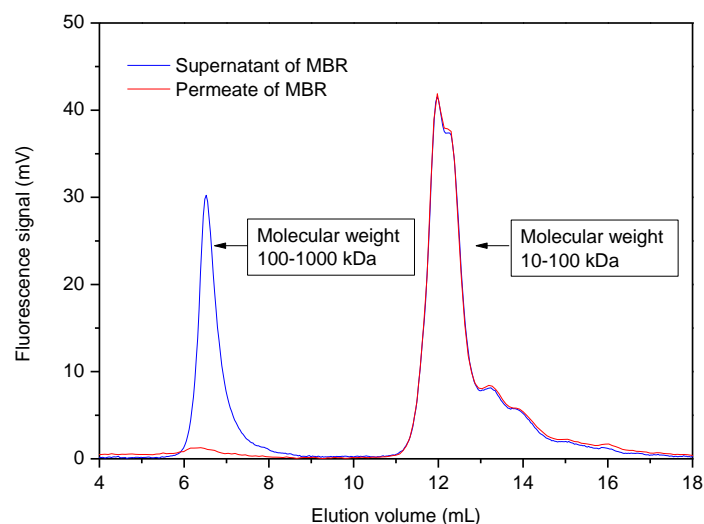


Fig. 4. HPLC-SEC analysis of supernatant and permeate of MBR

To investigate the relation between 100-1000 kDa protein-like SMPs and MBR fouling, HPLC-SEC-fluorescence analysis of supernatants sampled from MBR under different organic loading rates was performed. As shown in Fig. 5, a significant increase of protein-like substances with molecular size of 100-1000 kDa was observed in MBR supernatant, from sample 1 and sample 2 to sample 3 when the organic loading rate of MBR was increased, from 0.1 kg COD/kg MLSS/d to 0.2 kg COD/kg MLSS/d, and also from sample 3 to sample 4 when 0.2 kg COD/kg MLSS/d to 0.4 kg COD/kg MLSS/d. This may be explained by a growth of the microbial activity in activated sludge when the organic loading rate was increased. Zhou et al. [63] reported that the MBR sludge in the rapid growth phases with high microbial activities produced significantly higher BAP than the MBR sludge in stationary growth phase and more large MW compounds (higher than 100 kDa) were observed in MBR supernatant in the rapid growth phase. Ni et al. [64] reported that BAP could be further classified into the growth-associated BAP (GBAP), which are produced in the microbial growth phase, and the endogeny-associated BAP (EBAP), which are generated in the endogenous phase. Therefore, the growth of microbial activity might increase the growth-associated BAPs in the supernatant which is considered as having a molecular size between 100-1000 kDa. Taking into account the almost complete retention of 100-1000 kDa protein-like SMPs in supernatant by MBR membrane (see Fig. 4), the significant increase in protein-like 100-1000 kDa SMPs may lead to the higher fouling rate of MBR membrane, as indicated in Fig. 3. Thus, HPLC-SEC-fluorescence could characterize the variation of 100-1000 kDa protein-like SMPs when the change in operating conditions of MBR occurs and help to

estimate the fouling in MBR that is caused by variation of 100-1000 kDa protein-like SMPs in MBR supernatant. In Fig. 5, a peak of protein-like compounds with elution volume between 14 mL to 16 mL was also observed in sample 4. The molecular size of compounds in this elution volume is less than 10 kDa and their effect on fouling of MBR is of minor importance.

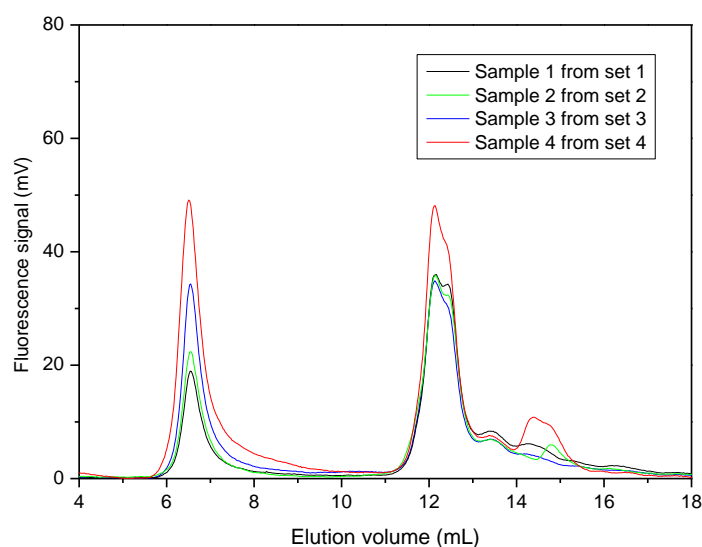


Fig. 5. HPLC-SEC analysis of supernatants of MBR under different organic loading rates

As the peak height is a simple and effective indicator to stand for the fluorescence intensity and to quantify the constituent content by the HPLC-SEC-fluorescence analysis [54] and almost complete retention of protein-like compounds with molecular size of 100-1000 kDa in supernatant is obtained by MBR membrane as indicated in Fig. 4, the fluorescence peak height of 100-1000 kDa protein-like compounds could be considered as a fouling indicator to estimate fouling in MBRs.

### **3.2 Fouling propensity of supernatants versus HPLC-SEC results**

To further validate the relation between fouling propensity of supernatants and fluorescence peak height of the protein-like compounds with molecular size of 100-1000 kDa detected by HPLC-SEC-fluorescence, filterability tests of the same supernatant samples obtained from MBR under different fouling conditions were performed together with HPLC-SEC-fluorescence analysis.

Fig. 6 shows one example of HPLC-SEC-fluorescence results for a MBR supernatant (used as feed for supernatant filtration) and its corresponding permeate obtained on a 0.01  $\mu\text{m}$  PES membrane during supernatant filtration test. The result showed the 0.01  $\mu\text{m}$  PES membrane retained almost all protein-like compounds that are in 100-1000 kDa range and let pass almost all the protein-like compounds in 10-100 kDa range. This could be explained by the fact that a 0.01  $\mu\text{m}$  pore size is often considered as corresponding to the 100 kDa molecular weight cut-off (MWCO) for ultrafiltration membranes. This result also emphasizes the importance of protein-like compounds in 100-1000 kDa range relative to the fouling propensity of supernatants.

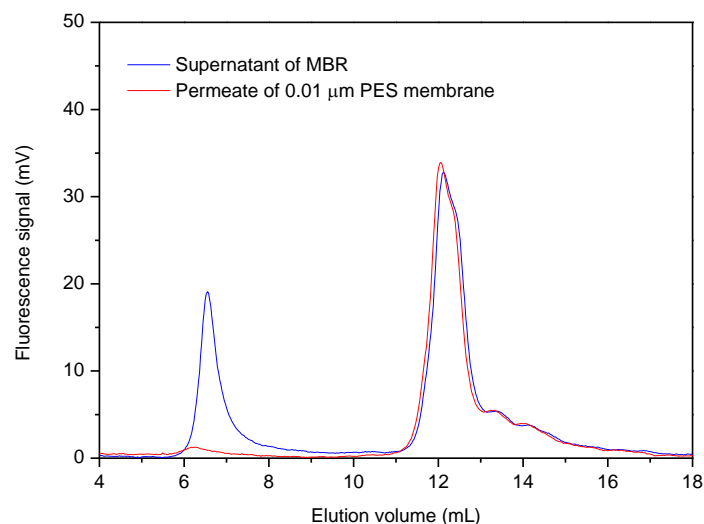


Fig. 6. HPLC-SEC analysis of MBR supernatant of and permeate of 0.01  $\mu\text{m}$  PES membrane

To investigate the fouling propensity of supernatant samples, the filterability tests of supernatant samples (sample 1, 2, 3 and 4) obtained from MBR under different fouling situations using 0.01 $\mu\text{m}$  PES membranes were performed at 1.0 bar, as shown in Fig. 7. Considering the content of 100-1000 kDa protein-like SMPs in these supernatant samples (see Fig. 5), the results from Fig. 7 indicates that supernatant with higher peak height of 100-1000 kDa protein-like substances had higher fouling rate, defined as the fouling resistance produced for a fixed volume of permeate filtered during the filtration tests. Notably, the fouling rate of supernatant samples from set 3 and set 4 were significantly higher than that from set 1 and set 2.

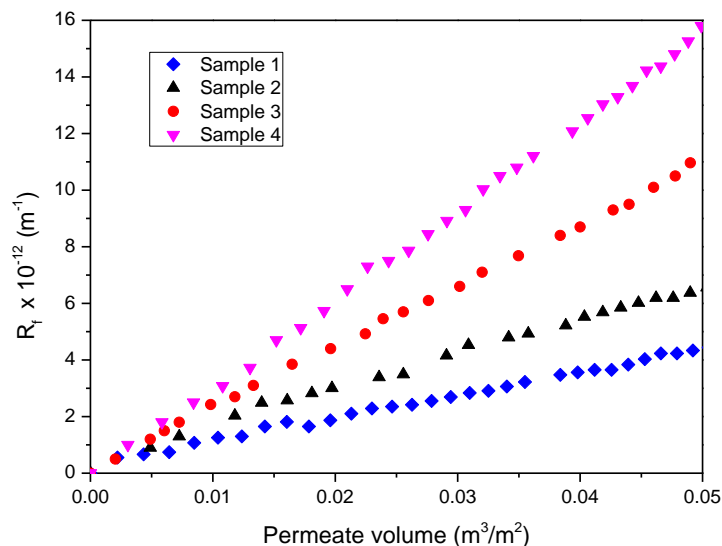


Fig. 7 Filterability tests of selected MBR supernatants at 1.0 bar

To relate the peak height of 100-1000 kDa protein-like compounds in MBR supernatants with the fouling propensity of corresponding supernatants (sample 1, 2, 3 and 4), the peak height and calculated  $\alpha C$  in filterability tests using 0.01 $\mu$ m PES membranes at three different TMP (0.5 bar, 0.8 bar, 1.0 bar) are plotted in Fig. 8. The result showed good linear relation between the peak height and  $\alpha C$  at all three TMP for all selected samples, which further confirmed the feasibility of using HPLC-SEC-fluorescence as a tool to estimate fouling propensity of MBR supernatants.

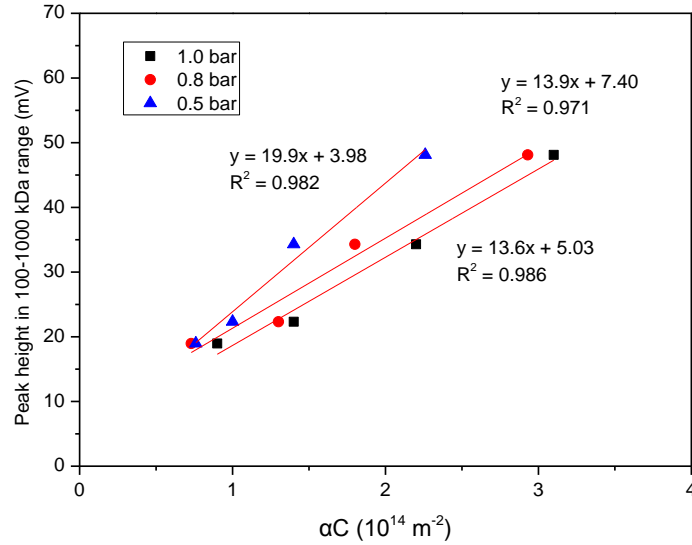


Fig. 8. Peak heights versus  $\alpha C$  values for selected MBR supernatants (0.01 $\mu\text{m}$  PES membranes)

Fig. 9 plots peak height of 100-1000 kDa protein-like substances in MBR supernatants versus the calculated  $\alpha C$  in filterability tests of corresponding MBR supernatants, using 150 kDa PES membranes at TMP of 0.5 bar. The results also showed good linear relation between the peak height and  $\alpha C$  value for the 150 kDa PES membranes.

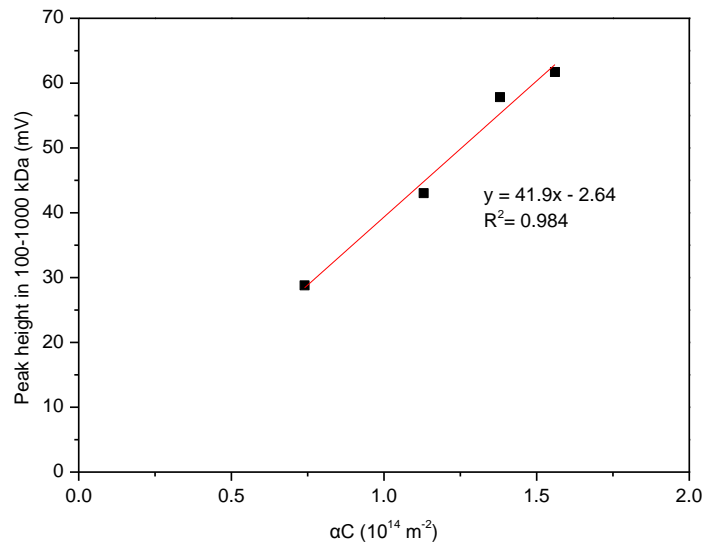


Fig. 9. Peak heights versus  $\alpha C$  values for MBR supernatants (150 kDa PES membranes)



## 4. Conclusions

Since membrane fouling remains the major limitation for MBR. Understanding fouling is still a key issue for a more sustainable operation of MBRs. There is a need for simple and cheap methods that could allow estimating the fouling in MBRs when some modification in fouling situations occurs. This study related the HPLC-SEC-fluorescence analysis to membrane fouling rate in MBR as well as fouling propensity (as indicated by the calculated  $\alpha_C$  value) of MBR supernatants. The results showed good linear relation between fluorescence peak height of protein-like substances in 100-1000 kDa range and fouling propensity of MBR supernatants sampled. Also, HPLC-SEC-fluorescence was found to be a simple and useful tool to relate the fouling in MBRs operated under varied organic loading rates with the peak height of 100-1000 kDa protein-like SMPs in supernatant. Thus, the peak height of protein-like compounds in 100-1000 kDa range was identified as a fouling indicator. More studies still need to be performed to validate the use of HPLC-SEC-fluorescence as a tool for estimating fouling of MBR in different fouling situations caused by modified operating conditions such as SRT and MLSS concentration. Also, the possibility of using HPLC-SEC-fluorescence analysis to optimize the MBR fouling conditions should be further investigated.

## References

- [1]. T. Melin, B. Jefferson, D. Bixio, C. Thoeye, W. De Wilde, J. De Koning, J. Van der Graaf, T. Wintgens, 2006. Membrane bioreactor technology for wastewater treatment and reuse. *Desalination* 187, 271-282.
- [2]. B. Lesjean, E. Huisjes, 2008. Survey of the European MBR market: trends and perspectives. *Desalination* 231, 71-81.

- [3]. C. Visvanathan, R.B. Aim, K. Parameshwaran, 2000. Membrane separation bioreactors for wastewater treatment. *Critical Reviews in Environmental Science and Technology* 30, 1-48.
- [4]. P. Le-Clech, A. Fane, G. Leslie, A. Childress, 2005. MBR focus: the operators' perspective. *Filtration & separation* 42, 20-23.
- [5]. S. Judd, 2008. The status of membrane bioreactor technology. *Trends in biotechnology* 26, 109-116.
- [6]. P. Le-Clech, V. Chen, T.A. Fane, 2006. Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science* 284, 17-53.
- [7]. A. Drews, 2010. Membrane fouling in membrane bioreactors—characterisation, contradictions, cause and cures. *Journal of Membrane Science* 363, 1-28.
- [8]. S. Ognier, C. Wisniewski, A. Grasmick, 2002. Influence of macromolecule adsorption during filtration of a membrane bioreactor mixed liquor suspension. *Journal of Membrane Science* 209, 27-37.
- [9]. C. Wisniewski, A. Grasmick, 1998. Floc size distribution in a membrane bioreactor and consequences for membrane fouling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 138, 403-411.
- [10]. E.H. Bouhabila, R. Ben Aim, H. Buisson, 2001. Fouling characterisation in membrane bioreactors. *Separation and Purification Technology* 22, 123-132.
- [11]. C.S. Lapidou, B.E. Rittmann, 2002. A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. *Water Research* 36, 2711-2720.
- [12]. S. Rosenberger, C. Laabs, B. Lesjean, R. Gnirss, G. Amy, M. Jekel, J.C. Schrotter, 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. *Water Research* 40, 710-720.
- [13]. J.R. Pan, Y.-C. Su, C. Huang, H.-C. Lee, 2010. Effect of sludge characteristics on membrane fouling in membrane bioreactors. *Journal of Membrane Science* 349, 287-294.

- [14]. Z. Wang, Z. Wu, S. Tang, 2009. Characterization of dissolved organic matter in a submerged membrane bioreactor by using three-dimensional excitation and emission matrix fluorescence spectroscopy. *Water Research* 43, 1533-1540.
- [15]. D.J. Barker, D.C. Stuckey, 1999. A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Research* 33, 3063-3082.
- [16]. S. Liang, C. Liu, L. Song, 2007. Soluble microbial products in membrane bioreactor operation: behaviors, characteristics, and fouling potential. *Water Research* 41, 95-101.
- [17]. T. Jiang, S. Myngheer, D.J. De Pauw, H. Spanjers, I. Nopens, M.D. Kennedy, G. Amy, P.A. Vanrolleghem, 2008. Modelling the production and degradation of soluble microbial products (SMP) in membrane bioreactors (MBR). *Water Research* 42, 4955-4964.
- [18]. R.S. Trussell, R.P. Merlo, S.W. Hermanowicz, D. Jenkins, 2006. The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. *Water Research* 40, 2675-2683.
- [19]. K. Kimura, N. Yamato, H. Yamamura, Y. Watanabe, 2005. Membrane fouling in pilot-scale membrane bioreactors (MBRs) treating municipal wastewater. *Environmental science & technology* 39, 6293-6299.
- [20]. A. Masse, M. Spérandio, C. Cabassud, 2006. Comparison of sludge characteristics and performance of a submerged membrane bioreactor and an activated sludge process at high solids retention time. *Water Research* 40, 2405-2415.
- [21]. D. Al-Halbouni, J. Traber, S. Lyko, T. Wintgens, T. Melin, D. Tacke, A. Janot, W. Dott, J. Hollender, 2008. Correlation of EPS content in activated sludge at different sludge retention times with membrane fouling phenomena. *Water Research* 42, 1475-1488.
- [22]. B.G. Choi, J. Cho, K.G. Song, S.K. Maeng, 2013. Correlation between effluent organic matter characteristics and membrane fouling in a membrane bioreactor using advanced organic matter characterization tools. *Desalination* 309, 74-83.

- [23]. M. Stricot, A. Filali, N. Lesage, M. Spárandio, C. Cabassud, 2010. Side-stream membrane bioreactors: Influence of stress generated by hydrodynamics on floc structure, supernatant quality and fouling propensity. *Water Research* 44, 2113-2124.
- [24]. A. Menniti, S. Kang, M. Elimelech, E. Morgenroth, 2009. Influence of shear on the production of extracellular polymeric substances in membrane bioreactors. *Water Research* 43, 4305-4315.
- [25]. A. Avella, L.F. Delgado, T. Görner, C. Albasi, M. Galmiche, P. De Donato, 2010. Effect of cytostatic drug presence on extracellular polymeric substances formation in municipal wastewater treated by membrane bioreactor. *Bioresource technology* 101, 518-526.
- [26]. L. Zhou, Z. Zhang, W. Jiang, W. Guo, H.-H. Ngo, X. Meng, J. Fan, J. Zhao, S. Xia, 2013. Effects of low-concentration Cr(VI) on the performance and the membrane fouling of a submerged membrane bioreactor in the treatment of municipal wastewater. *Biofouling* 30, 105-114.
- [27]. E. Reid, X. Liu, S. Judd, 2006. Effect of high salinity on activated sludge characteristics and membrane permeability in an immersed membrane bioreactor. *Journal of Membrane Science* 283, 164-171.
- [28]. S. Tang, Z. Wang, Z. Wu, Q. Zhou, 2010. Role of dissolved organic matters (DOM) in membrane fouling of membrane bioreactors for municipal wastewater treatment. *Journal of hazardous materials* 178, 377-384.
- [29]. M. Hernandez Rojas, R. Van Kaam, S. Schetrite, C. Albasi, 2005. Role and variations of supernatant compounds in submerged membrane bioreactor fouling. *Desalination* 179, 95-107.
- [30]. B. Teychene, C. Guigui, C. Cabassud, G. Amy, 2008. Toward a better identification of foulant species in MBR processes. *Desalination* 231, 27-34.
- [31]. E. Filloux, J. Labanowski, J. Croue, 2012. Understanding the fouling of UF/MF hollow fibres of biologically treated wastewaters using advanced EfOM characterization and statistical tools. *Bioresource Technology* 118, 460-468.

- [32]. Y.-x. Shen, K. Xiao, P. Liang, J.-y. Sun, S.-j. Sai, X. Huang, 2012. Characterization of soluble microbial products in 10 large-scale membrane bioreactors for municipal wastewater treatment in China. *Journal of Membrane Science* 415, 336-345.
- [33]. N. Yamato, K. Kimura, T. Miyoshi, Y. Watanabe, 2006. Difference in membrane fouling in membrane bioreactors (MBRs) caused by membrane polymer materials. *Journal of Membrane Science* 280, 911-919.
- [34]. U. Metzger, P. Le-Clech, R.M. Stuetz, F.H. Frimmel, V. Chen, 2007. Characterisation of polymeric fouling in membrane bioreactors and the effect of different filtration modes. *Journal of Membrane Science* 301, 180-189.
- [35]. Y. Shen, W. Zhao, K. Xiao, X. Huang, 2010. A systematic insight into fouling propensity of soluble microbial products in membrane bioreactors based on hydrophobic interaction and size exclusion. *Journal of Membrane Science* 346, 187-193.
- [36]. S. Arabi, G. Nakhla, 2010. Impact of molecular weight distribution of soluble microbial products on fouling in membrane bioreactors. *Separation and Purification Technology* 73, 391-396.
- [37]. T. Jiang, M.D. Kennedy, V.D. Schepper, S.-N. Nam, I. Nopens, P.A. Vanrolleghem, G. Amy, 2010. Characterization of soluble microbial products and their fouling impacts in membrane bioreactors. *Environmental science & technology* 44, 6642-6648.
- [38]. S. Rosenberger, H. Evenblij, S. Te Poele, T. Wintgens, C. Laabs, 2005. The importance of liquid phase analyses to understand fouling in membrane assisted activated sludge processes—six case studies of different European research groups. *Journal of Membrane Science* 263, 113-126.
- [39]. G. Ferrero, I. Rodríguez-Roda, J. Comas, 2012. Automatic control systems for submerged membrane bioreactors: A state-of-the-art review. *Water Research* 46, 3421-3433.
- [40]. M. Jacob, Réutilisation des eaux usées épurées par association de procédés biologiques et membranaires, PhD thesis, INSA de Toulouse, 2011.

- [41]. J. Lee, W.-Y. Ahn, C.-H. Lee, 2001. Comparison of the filtration characteristics between attached and suspended growth microorganisms in submerged membrane bioreactor. *Water Research* 35, 2435-2445.
- [42]. N. Lesage, M. Sperandio, C. Cabassud, 2008. Study of a hybrid process: Adsorption on activated carbon/membrane bioreactor for the treatment of an industrial wastewater. *Chemical Engineering and Processing: Process Intensification* 47, 303-307.
- [43]. B. Teychene, C. Guigui, C. Cabassud, 2011. Engineering of an MBR supernatant fouling layer by fine particles addition: A possible way to control cake compressibility. *Water Research* 45, 2060-2072.
- [44]. J.H. Roorda, J.H. Van der Graaf, 2005. SUR test used for optimisation of membrane filtration plants treating wastewater effluents. *Desalination* 179, 131-150.
- [45]. H. Evenblij, S. Geilvoet, J. Van der Graaf, H. Van der Roest, 2005. Filtration characterisation for assessing MBR performance: three cases compared. *Desalination* 178, 115-124.
- [46]. T. de la Torre, M. Mottschall, B. Lesjean, A. Drews, A. Iheanaetu, M. Kraume, 2010. Filterability assessment in membrane bioreactors using an in-situ filtration test cell. *Water Science & Technology* 61.
- [47]. C. Huyskens, E. Brauns, E. Van Hoof, H. De Wever, 2008. A new method for the evaluation of the reversible and irreversible fouling propensity of MBR mixed liquor. *Journal of Membrane Science* 323, 185-192.
- [48]. J.-X. Li, R. Sanderson, G. Chai, 2006. A focused ultrasonic sensor for in situ detection of protein fouling on tubular ultrafiltration membranes. *Sensors and Actuators B: Chemical* 114, 182-191.
- [49]. J. Mendret, C. Guigui, P. Schmitz, C. Cabassud, 2009. In situ dynamic characterisation of fouling under different pressure conditions during dead-end filtration: compressibility properties of particle cakes. *Journal of Membrane Science* 333, 20-29.

- [50]. P. Loulergue, C. André D. Laux, J.-Y. Ferrandis, C. Guigui, C. Cabassud, 2011. In-situ characterization of fouling layers: which tool for which measurement? *Desalination and Water Treatment* 34, 156-162.
- [51]. P. Loulergue, M. Weckert, B. Reboul, C. Cabassud, W. Uhl, C. Guigui, 2014. Mechanisms of action of particles used for fouling mitigation in membrane bioreactors. *Water Res.* 66, 40-52.
- [52]. P. Loulergue, Caractérisation In-Situ de dépôts formés en filtration membranaire de suspensions particulaires et de biofluides: interaction entre structure locale et performances du procédé INSA de Toulouse, 2012.
- [53]. F. Meng, B. Liao, S. Liang, F. Yang, H. Zhang, L. Song, 2010. Morphological visualization, componential characterization and microbiological identification of membrane fouling in membrane bioreactors (MBRs). *Journal of Membrane Science* 361, 1-14.
- [54]. N. Her, G. Amy, D. McKnight, J. Sohn, Y. Yoon, 2003. Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection. *Water Research* 37, 4295-4303.
- [55]. R.H. Peiris, C. Hallé H. Budman, C. Moresoli, S. Peldszus, P.M. Huck, R.L. Legge, 2010. Identifying fouling events in a membrane-based drinking water treatment process using principal component analysis of fluorescence excitation-emission matrices. *Water Research* 44, 185-194.
- [56]. H. Shon, S. Vigneswaran, I.S. Kim, J. Cho, H. Ngo, 2006. Fouling of ultrafiltration membrane by effluent organic matter: A detailed characterization using different organic fractions in wastewater. *Journal of Membrane Science* 278, 232-238.
- [57]. H.Y. Ng, T.W. Tan, S.L. Ong, 2006. Membrane fouling of submerged membrane bioreactors: impact of mean cell residence time and the contributing factors. *Environmental science & technology* 40, 2706-2713.
- [58]. P. Wang, Z. Wang, Z. Wu, S. Mai, 2011. Fouling behaviours of two membranes in a submerged membrane bioreactor for municipal wastewater treatment. *Journal of Membrane Science* 382, 60-69.

- [59]. B.X. Thanh, M. Spérandio, C. Guigui, R.B. Aim, J. Wan, C. Visvanathan, 2010. Coupling sequencing batch airlift reactor (SBAR) and membrane filtration: Influence of nitrate removal on sludge characteristics, effluent quality and filterability. *Desalination* 250, 850-854.
- [60]. M. Kumar, P.-Y. Lee, T. Fukusihma, L.-M. Whang, J.-G. Lin, 2012. Effect of supplementary carbon addition in the treatment of low C/N high-technology industrial wastewater by MBR. *Bioresource technology* 113, 148-153.
- [61]. S. Judd, *The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment*, Elsevier. 2010
- [62]. S.A. Huber, A. Balz, M. Abert, W. Pronk, 2011. Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography – organic carbon detection – organic nitrogen detection (LC-OCD-OND). *Water Research* 45, 879-885.
- [63]. Z. Zhou, F. Meng, S. Liang, B.-J. Ni, X. Jia, S. Li, Y. Song, G. Huang, 2012. Role of microorganism growth phase in the accumulation and characteristics of biomacromolecules (BMM) in a membrane bioreactor. *RSC Advances* 2, 453-460.
- [64]. B.-J. Ni, R.J. Zeng, F. Fang, W.-M. Xie, G.-P. Sheng, H.-Q. Yu, 2010. Fractionating soluble microbial products in the activated sludge process. *Water Research* 44, 2292-2302.





# Résumé long en français



## 1. Introduction

De nos jours, le stress hydrique est un grand défi auquel l'humanité dans de nombreux endroits à travers le monde doit faire face. Pour résoudre ce problème, les eaux usées municipales ont été considérées comme une source d'eau alternative pour diverses applications après un traitement approprié. Parmi tous les types de procédés de traitement des eaux usées, le bioréacteur à membrane (BAM) occupe une place de choix dans un contexte de réutilisation de ces eaux usées traitées en raison de sa haute qualité de l'effluent. Cependant, le colmatage de la membrane inhérent à ses propriétés de rétention reste encore la principale limitation pour le développement du BAM. Le colmatage des membranes se traduit par le déclin de la perméabilité en cours de fonctionnement du BAM. Le colmatage de la membrane est un phénomène complexe. On peut y retrouver un dépôt, formé sur la surface de la membrane, un blocage des pores en raison des plus petites particules, colloïdes et solutés des composés adsorbés sur la surface ou encore le développement d'une couche de gel. Le surnageant de la boue activée, qui contient des colloïdes et des solutés, s'est révélé jouer un rôle plus important dans le colmatage de la membrane du BAM que les floccs biologiques dans de nombreuses études. Ainsi, les produits microbiens solubles (PMS), les principaux constituants organiques du surnageant de boues, ont été reconnus comme les principaux responsables du colmatage des membranes du BAM. Plusieurs facteurs peuvent influencer sur la production de PMS : les conditions de fonctionnement du BAM telles que la charge organique traitée, l'âge de boues, les conditions hydrodynamique comme l'aération ou l'agitation mécanique, ou encore la présence de stress environnementaux (pour les bactéries) dans les

eaux usées comme les métaux lourds, la pression osmotique (sels), et les micropolluants comme les produits pharmaceutiques. Dans la dernière décennie, une grande attention a été accordée aux micropolluants pharmaceutiques présents dans les eaux usées domestiques en raison de leurs effets négatifs potentiels sur la santé humaine ou les organismes aquatiques. Cependant, il y a un manque de connaissances sur les effets des micropolluants pharmaceutiques sur le colmatage du BAM.

Ainsi, l'objectif principal de cette thèse était d'examiner les effets sur la performance de filtration de la présence de micropolluants pharmaceutiques qui sont fréquemment trouvés dans les eaux usées domestiques. La carbamazépine (CBZ), un médicament anti-épileptique, a été choisi comme représentant des micropolluants pharmaceutiques dans cette étude. Il a été choisi en raison de sa présence dans les eaux usées domestiques et sa persistance après certains traitements. En outre, la CBZ a été reconnue pour avoir des effets d'inhibition sur l'activité microbienne des boues. Dans cette étude, les effets de la CBZ sur le colmatage du BAM ont été étudiés de deux manières: un pic de pollution (la CBZ a été ajoutée dans un réacteur discontinu qui utilise de la boue à partir d'un BAM) pour étudier les effets des pics de CBZ à court terme sur le pouvoir colmatant et une pollution continue (la CBZ a été ajoutée en continu dans un BAM) pour examiner les effets de CBZ à long terme sur le colmatage du BAM. L'étude se veut faire un lien entre le colmatage de la membrane et le changement des caractéristiques de la boue et du surnageant après l'ajout CBZ. Enfin, la possibilité d'utiliser HPLC-SEC-fluorescence comme un outil pour une estimation rapide de la filtrabilité du colmatage des boues a été proposée

## 2. Effets de la CBZ sur le colmatage

### 2.1 Effets de la CBZ sur le pouvoir colmatant des boues

Le pouvoir colmatant des boues activées est défini comme la résistance de colmatage produit, qui est mesurée par le test de filtrabilité qui utilise une cellule de filtration, où un volume fixe de perméat a été filtré. Ainsi, l'effet à court terme de la CBZ sur le pouvoir colmatant des boues activées a été examiné dans les tests de filtrabilité à l'aide de boues prélevées dans des réacteurs discontinus (réacteur-avec CBZ et de contrôle-réacteur). Comme le montre la Fig. 1, pour la boue provenant du réacteur avec CBZ, le pouvoir colmatant était significativement plus élevé, par rapport à celle de la boue provenant du réacteur-contrôle.

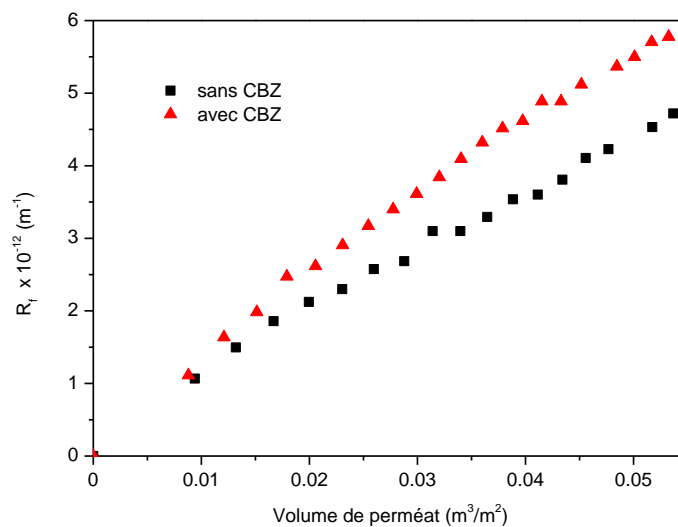


Fig. 1. L'effet à court terme de la CBZ sur le pouvoir colmatant des boues activées

(CBZ = 100 µg L<sup>-1</sup>, membrane PS 0.2 µm, 1.0 bar)

En ce qui concerne les effets à long terme, des essais de filtrabilité des boues provenant du BAM avant et après addition de la CBZ ont été réalisés. Comme montre la Fig. 2, l'addition continue de la CBZ dans le BAM augmenté le pouvoir colmatant des boues.

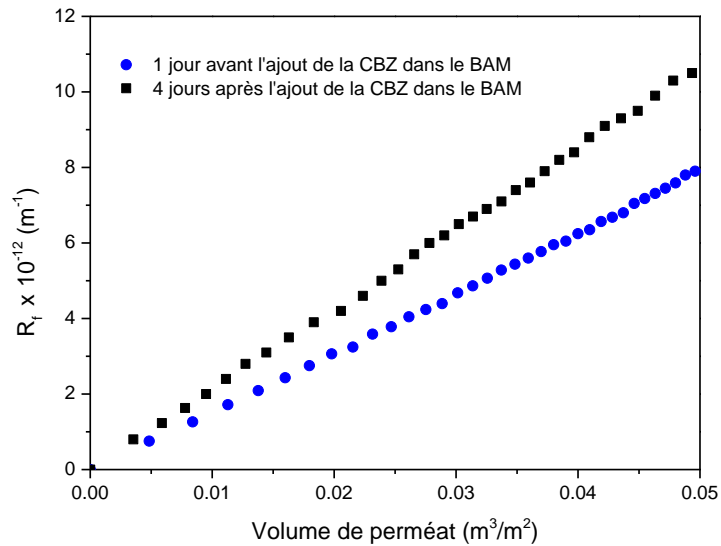


Fig. 2. L'effet à long terme de la CBZ sur le pouvoir colmatant des boues activées

(CBZ = 90 µg L<sup>-1</sup>, membrane PES 150 kDa, 0.5 bar)

## 2.2 Effets de la CBZ sur le colmatage du BAM

L'effet à long terme de la CBZ sur le colmatage du BAM pilote était caractérisé par la pression transmembranaire (PTM). La Fig. 3 présente les variations dans le temps de la PTM pendant le fonctionnement du BAM. Quatre périodes et comportements différents peuvent être distingués:

- Avant l'ajout de la CBZ dans le BAM, des conditions de filtrabilité stable ont été atteintes, et la PTM augmente très légèrement.

- 1 jour après l'addition de la CBZ dans le BAM, une augmentation significative de la PTM a été observée : Il apparaît clairement comme une réponse du processus de bioéparation à l'effet d'un stress sur la biomasse.

- Entre 1 jour et 8 jours après l'addition de la CBZ, la PTM a augmenté progressivement avec une vitesse d'augmentation plus élevée par rapport à celle avant l'addition de la CBZ.

- 8 jours après l'addition de la CBZ, un saut de PTM se produit, et la PTM augmente continuellement à une vitesse significativement plus élevée.

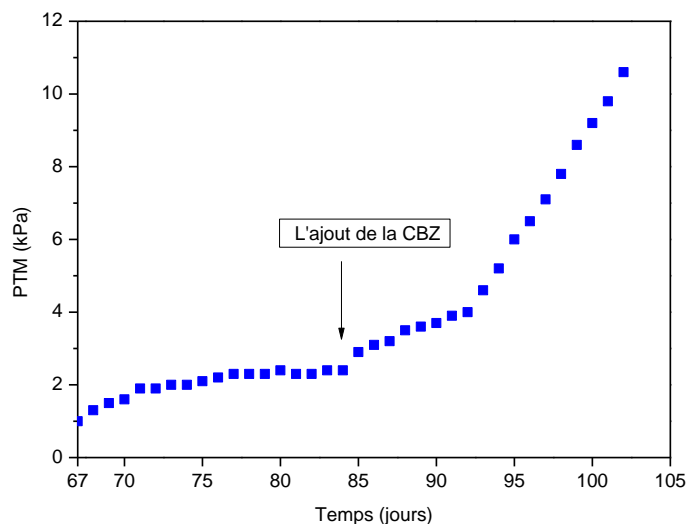


Fig. 3. Effet de la CBZ sur la PTM du BAM

En conclusion, le contact de boues activées avec la CBZ augmente la capacité de colmatage des boues activées et le colmatage du BAM. Trois hypothèses peuvent être proposées pour expliquer cette augmentation du pouvoir colmatant: i) un changement de l'activité microbienne liée à une contrainte sur les micro-organismes; ii) un changement des propriétés physico-chimiques de la biomasse, comme la distribution de la taille moyenne des floes; iii)



un changement de la qualité de surnageant. Ces trois hypothèses seront discutées dans les paragraphes suivants qui visent à évaluer les effets de la CBZ sur les propriétés de surnageant et de la biomasse.

### **3. Effets de la CBZ sur les caractéristiques de la biomasse**

L'effet de la CBZ sur l'activité microbienne de la boue dépend du temps de contact. Pendant une courte période de contact avec  $100 \mu\text{g L}^{-1}$  de CBZ dans le réacteur respirométrique, aucune inhibition de l'activité microbienne des boues n'a été observée, ce qui indique que les bactéries n'ont pas modifié leur mode de dégradation ou encore que la croissance bactérienne compense le faible effet d'inhibition induit par la CBZ. En revanche, l'addition continue de CBZ ( $90 \mu\text{g L}^{-1}$  CBZ) dans le BAM a causé environ 20% d'inhibition de l'activité microbienne des boues après plusieurs jours. Cette inhibition peut être causée par un changement de comportement de la croissance bactérienne ou par l'évolution de la population de la communauté microbienne. 4 jours après l'ajout de la CBZ, pas plus de l'inhibition (c'est toujours 20%) n'a été observé, ce qui indique que l'adaptation ou acclimatation des boues actives a été atteinte. Cependant, après l'addition de CBZ dans le BAM aucun changement significatif des performances d'élimination de la DCO et de l'azote n'a été observé, par rapport à celle avant l'addition de CBZ, ce qui aussi indique que l'inhibition n'est pas significative.

Concernant l'effet de la CBZ sur la distribution de taille des floes, aucun effet significatif n'a été observé lors d'un pic d'injection ainsi que pendant l'injection continu. Cela peut indiquer

qu'aucune décomposition bactérienne importante n'a eu lieu en réponse au stress induit par la présence de la CBZ que ce soit sous forme d'un pic ponctuel ou d'une pollution continu, ce qui est en accord avec les résultats de l'analyse de l'activité microbienne obtenue.

#### 4. Effets de la CBZ sur les caractéristiques de surnageant

Les effets à court terme de la CBZ sur la composition surnageant ont été examinés sur des surnageants prélevés dans les réacteurs batch. La Fig. 4 donne les concentrations de protéines et de polysaccharides dans les surnageants utilisant les boues du BAM. Une légère augmentation de la concentration en protéines (environ 20%) a été observée dans le surnageant provenant du réacteur-avec CBZ, par rapport à celle du réacteur témoin. En revanche, aucune différence significative de la concentration en polysaccharide a été trouvée dans le surnageant des deux réacteurs.

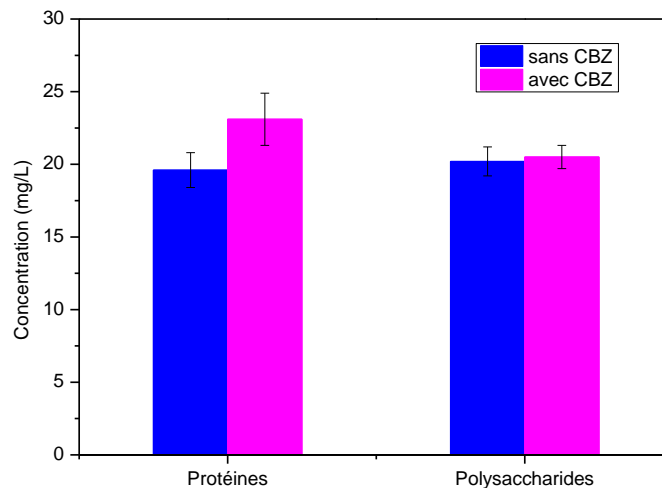


Fig. 4. Effet à court terme de la CBZ sur la composition de PMS dans le surnageant

Les effets à long terme de la CBZ sur la composition du surnageant ont été examinés également. La Fig. 5 montre qu'une augmentation significative de la concentration en protéines dans le surnageant a été observée 1 jour après l'addition de CBZ, puis la concentration en protéines reste quasiment constante pendant plusieurs jours avant de diminuer à partir du 7<sup>ème</sup> jour environ après l'addition de CBZ dans le BAM. Par ailleurs, aucune augmentation significative de la rétention membranaire en protéine dans le BAM a été observée, ce qui indique que l'excès de protéine induit par la CBZ est essentiellement constitué de molécules relativement petites qui peuvent passer à travers la membrane et le dépôt présent sur celle-ci. En revanche, aucune modification significative n'a été observée pour la concentration de polysaccharide dans le surnageant après addition de CBZ en BAM.

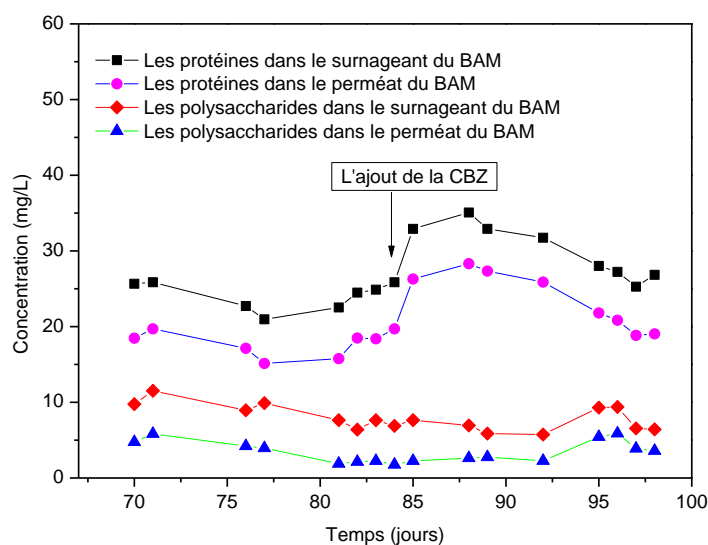


Fig. 5. Effet à long terme de la CBZ sur PMS dans le surnageant et perméat du BAM

En conclusion, quel que soit le type de contamination de CBZ (pic ou continu) dans le BAM, la présence de CBZ induit une augmentation de la concentration en protéines mais pas de changement significatif sur la concentration en polysaccharides. L'augmentation de la

résistance de colmatage des boues activées des réacteurs batch et du BAM avec l'addition de la CBZ peut être alors attribuée à la modification de la teneur en protéines dans le surnageant, car aucun effet significatif n'a été observé dans la distribution de la taille des flocons et l'activité microbienne en cas de court-terme ainsi que les conditions de contact continu à long terme.

Pour approfondir le rôle des protéines dans le colmatage, la distribution de taille moléculaire des protéines est caractérisée par SEC-HPLC-fluorescence avec des longueurs d'onde d'excitation/émission fixées à 280/350 nm. Dans le cas d'un pic de CBZ, l'analyse HPLC-SEC des surnageants préparés à partir des réacteurs discontinus est donnée Fig. 6. Le poids moléculaire des substances protéiques dans les surnageants du BAM présente une distribution bimodale. En effet, deux pics sont présents : un premier correspondant à un poids moléculaire de 100-1000 kDa et un second à 10 à 100 kDa. Un pic nettement plus élevé des protéines de 100-1000 kDa dans le surnageant du réacteur-avec CBZ peut être observé par rapport à celui du surnageant du réacteur sans CBZ. En revanche, aucune différence significative de la hauteur des pics détectés des protéines avec une taille moléculaire de 10-100 kDa entre les deux surnageants n'a été observée. Sachant que les protéines de taille moléculaire de 100-1000 kDa sont entièrement retenues par des membranes au cours des essais de filtrabilité des boues (voir publication 2), on peut conclure que l'augmentation de la résistance du colmatage des boues induit par le pic de CBZ (réacteur batch) (voir Fig. 1) est induite par l'augmentation de protéines de taille moléculaire de 100-1000 kDa dans le surnageant qui suit l'injection de CBZ.

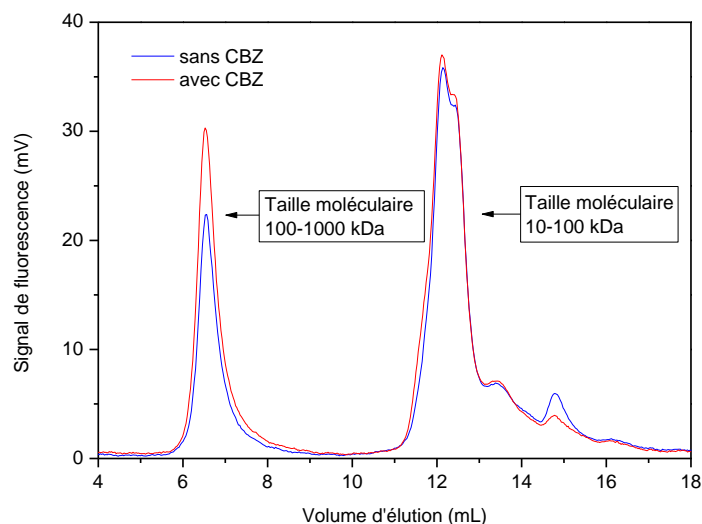


Fig. 6. Effet à court terme de la CBZ sur des protéines dans le surnageant

En ce qui concerne l'effet à long terme de la CBZ, les surnageants du BAM avant et après l'ajout de la CBZ dans le BAM ont été analysés par HPLC-SEC, comme représenté sur la Fig.

7. On peut constater que la présence de  $90 \mu\text{g L}^{-1}$  CBZ dans le BAM pendant une longue durée induit deux effets mesurables sur des protéines :

- Une diminution de la concentration de protéines de taille moléculaire de 100-1000 kDa.

Plus précisément, un jour après l'addition de la CBZ dans le BAM, une diminution importante (environ 16%) de protéines de 100-1000 kDa a été observée dans le surnageant du BAM.

Ensuite, seulement une légère baisse de 100-1000 kDa protéines a été observée entre 1 jour et 7 jours après l'addition de la CBZ.

- Une augmentation plus importante de protéines de taille moléculaire de 10-100 kDa.

Plus précisément, un jour après l'addition de la CBZ dans le MBR, une augmentation très significative de protéines de 10-100 kDa, environ 23%, a été observée dans le surnageant du

BAM. Seule une légère augmentation de 10-100 kDa protéines a été observée dans le surnageant du BAM entre 1 jour et 7 jours après l'addition de la CBZ dans le BAM.

Par ailleurs, étant donné que la rétention des protéines de taille moléculaire de 100-1000 kDa est quasi-complète, que celle des protéines de taille moléculaire de 10-100 kDa est partielle lors des essais de filtrabilité de boues, et que une diminution de protéines avec une taille moléculaire de 100-1000 kDa et une augmentation plus important de protéines avec une taille moléculaire de 10-100 kDa est observée après l'ajout de CBZ, on peut conclure que l'augmentation de la résistance de colmatage est issue d'une modification par les protéines de 10-100 kDa de la structure du dépôt formé lors de la filtration de la boue.

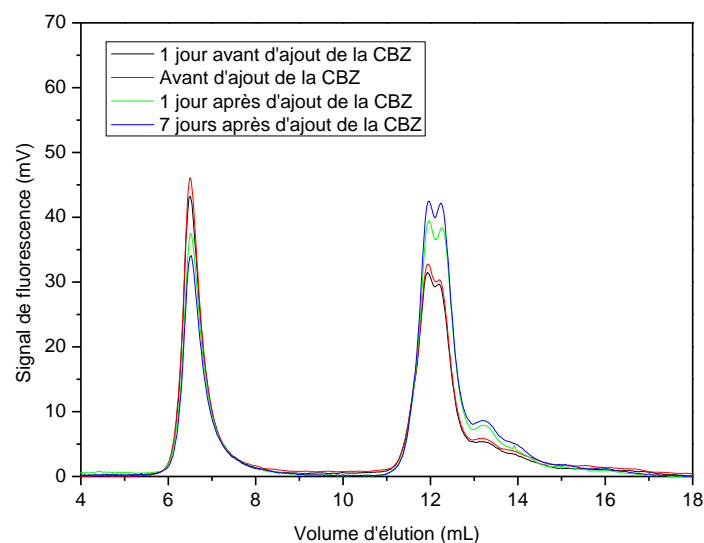


Fig. 7. Effet à long terme de la CBZ sur des protéines dans le surnageant

En conclusion, l'effet de la CBZ sur la distribution de poids moléculaire des protéines dans le surnageant dépend du temps de contact. Lors d'un pic ponctuel de pollution, la CBZ induit une augmentation des protéines de 100-1000 kDa. Par contre, lors d'un contact en continu, la

CBZ induit une augmentation des protéines de 10-100 kDa. Ces deux types de protéines (taille moléculaire de 10-100 kDa et de 100-1000 kDa) du surnageant pourraient contribuer à l'augmentation de la capacité du colmatage des boues. Les protéines de 100-1000 kDa pourraient être retenues par le dépôt sur la surface de la membrane et donc former d'une couche de gel, alors que les protéines de 10-100 kDa pourraient être bloquées dans le dépôt et donc modifier sa structure et en particulier sa porosité.

## **5. Effets de CBZ sur les caractéristiques de dépôt du BAM**

Les caractéristiques de dépôt du BAM pourraient être examinées par la rétention des PMS dans le surnageant. Comme représenté sur la Fig. 8, l'analyse HPLC-SEC a montré qu'avant d'ajouter la CBZ, la membrane du BAM retient presque toutes les protéines de 100-1000 kDa. En revanche, presque toutes les protéines de 10-100 kDa ont traversé la membrane du BAM. Cependant, 11 jours après l'ajout de la CBZ dans le BAM, les protéines de 10-100 kDa dans le surnageant ont été partiellement retenues par la membrane du BAM, comme le montre la Fig. 9. On peut supposer que l'augmentation importante des protéines de 10-100 kDa dans le surnageant du BAM après l'ajout de la CBZ induit une accumulation de ces petits biopolymères à l'intérieur du dépôt, qui réduit donc sa porosité et induit finalement une rétention plus importante de ces composés dans le BAM.

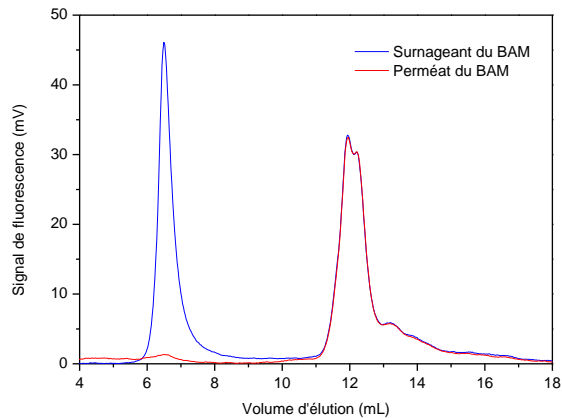


Fig. 8. Analyse de surnageant et perméat du BAM par HPLC-SEC avant d'ajouter CBZ dans le BAM

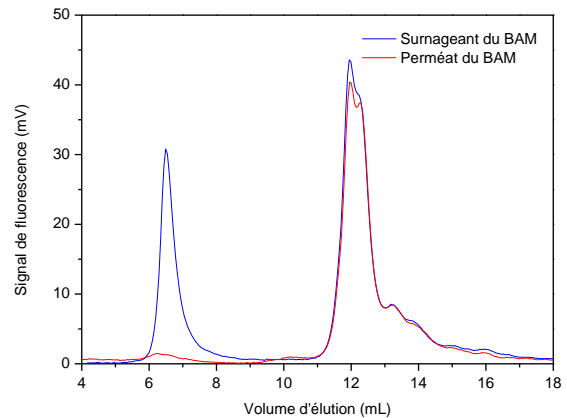


Fig. 9. Analyse de surnageant et perméat du BAM par HPLC-SEC 11 jours après l'ajout le la CBZ dans le BAM

Ainsi, la stabilisation de la PTM observée avant l'addition de la CBZ dans le BAM peut être attribuée à la rétention de PMS de 100-1000 kDa et donc à la formation d'une couche de gel sur la surface de la membrane, puisqu'aucune rétention de protéines de 10-100 kDa dans le surnageant a été trouvée avant l'addition de la CBZ dans le BAM. Le saut TMP qui est apparu un jour après l'addition de la CBZ peut être causé par l'augmentation significative de protéines de 10-100 kDa dans le surnageant. L'augmentation soudaine et importante de protéines de 10-100 kDa peut provoquer un blocage interne du dépôt présent sur la membrane du BAM. La vitesse d'augmentation de la PTM plus élevée entre 1 jour et 8 jours après l'addition de la CBZ pourrait être expliquée par l'accumulation continue des PMS de 10-100 kDa dans le dépôt. Le saut de PTM qui est apparu après l'augmentation progressive de la PTM peut être due à ce que l'accumulation de protéines de 10-100 kDa provoque une réduction de la porosité jusqu'à une valeur critique du dépôt induisant des résistances locales importantes. Le résultat présenté sur la Fig. 9 pourrait étayer cette hypothèse concernant la rétention partielle des protéines de 10-100 kDa observée au cours de cette période. Par ailleurs, les résultats ont



montré que le dépôt formé par la boue du BAM est compressible et sa compressibilité peut aussi contribuer à la PTM saut continu.

## **6. HPLC-SEC-fluorescence pour l'estimation du colmatage du BAM**

Les résultats obtenus pendant le fonctionnement du BAM ont montré que la HPLC-SEC-fluorescence peut être utilisée pour caractériser la variation de la teneur en protéines de 100-1000 kDa induite par des variations des conditions opératoires du BAM (charge massique, temps de séjour des boues..). Etant donné que ces protéines jouent un rôle sur le pouvoir colmatant des surnageants et boues, cet outil HPLC-SEC fluorescence peut être utilisé pour une aide à l'évaluation du colmatage. Ainsi la hauteur du pic de fluorescence de protéines de 100-1000 kDa pouvait être considérée comme indicateur de colmatage dans les BAM.

Ainsi la hauteur du pic de protéines de 100-1000 kDa présentes dans les différents surnageants et les valeurs de  $\alpha C$  de surnageant obtenus dans les tests de filtrabilité à l'aide d'une membrane PSE de 0,01  $\mu\text{m}$  sont tracés Figure 10 pour trois PTM différentes (0,5 bar, 0,8 bar, 1,0 bar). On peut observer une relation linéaire entre les hauteurs du pic de protéines de 100-1000 kDa dans le surnageant et les valeurs de  $\alpha C$  à tous les trois TMP ce qui confirme la possibilité d'utiliser HPLC-SEC-fluorescence comme un outil pour estimer le pouvoir colmatant des surnageants du BAM.

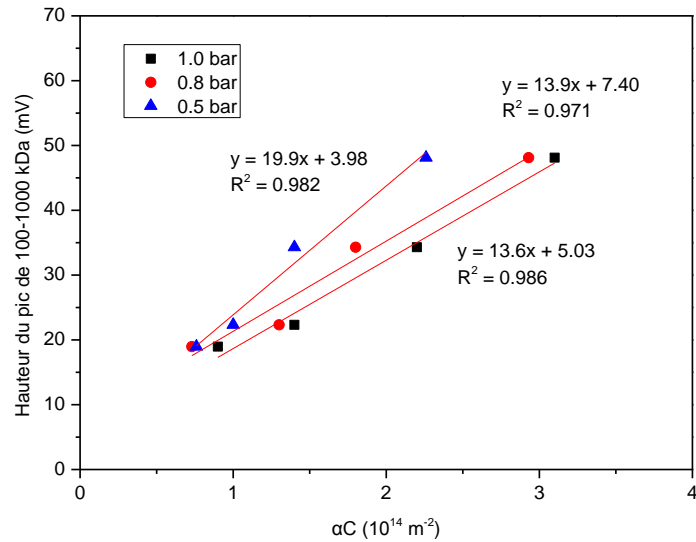


Fig. 10. Hauteur du pic par rapport aux valeurs  $\alpha C$  de surnageant

## 7. Conclusions et perspectives

La présence de micropolluants dans les eaux usées domestiques pharmaceutiques pose la question de l'impact de la présence de micropolluants pharmaceutiques sur le colmatage des BAM. Cette étude a examiné d'une part les effets de la CBZ sur la capacité colmatante des boues dans les expériences en batch et d'autre part sur le colmatage du BAM dans un fonctionnement continu à l'échelle du BAM.

Les résultats ont montré que les effets de la CBZ sur la boue activée du BAM dépendent du temps de contact. Le contact à court terme de la CBZ avec des boues conduit à augmenter la proportion de protéines de 100-1000 kDa dans le surnageant en raison de la décomposition de bactéries. Au contraire, le contact à long terme (injection en continu de la CBZ) avec des boues dans le BAM induit la production de protéines de 10-100 kDa en raison des réactions de défense des bactéries face le stress de la CBZ. En raison des différents effets de CBZ sur les caractéristiques des boues et des surnageants du BAM, les mécanismes de colmatage impliqués dans ces deux situations sont très différents. A la fois des protéines de 100-1000 kDa et de 10-100 kDa sont susceptibles de contribuer au colmatage des membranes.

Les protéines de 100-1000 kDa qui sont complètement retenues par les membranes MF/UF ou les membranes utilisées dans les BAM lorsque peuvent conduire à la formation d'un dépôt colloïdale qui contribuera à l'augmentation de le pouvoir colmatant des boues et l'augmentation progressif de colmatage du BAM. Les protéines de 10-100 kDa qui sont partiellement retenues par les membranes MF/UF ou les membranes du BAM lors de la filtration de boues peuvent induire un colmatage interne des dépôts présents sur la membrane et qui pourrait conduire à l'augmentation du pouvoir colmatant jusqu'à un saut de PTM dans le BAM.

Cette étude propose une méthodologie et des outils pour comprendre les interactions complexes entre les micropolluants pharmaceutiques, les boues activée et le colmatage de la membrane dans les différentes conditions. Le rôle important des protéines dans ces interactions a été démontré. L'utilisation de HPLC-SEC-fluorescence comme un outil utile pour étudier les phénomènes impliqués dans ces interactions a également été démontré. Cependant, de nombreuses questions sont restées sans réponse. D'autres études doivent être effectuées pour distinguer les deux effets de stress impliqués: la décomposition des cellules et les réponses défensives par les bactéries viables. En outre, des études sur les effets des micropolluants pharmaceutiques sur le changement des structures des communautés microbiennes et les effets correspondants sur le potentiel du colmatage devraient être étudiés.

Pour améliorer la capacité du BAM sur l'élimination des micropolluants pharmaceutiques, d'autres études doivent être menées pour identifier les espèces bactériennes qui favorisent l'élimination de la CBZ ainsi que d'autres produits pharmaceutiques dans le BAM et déterminer les conditions de fonctionnement optimales qui contribuent à enrichir ces espèces bactériennes. En outre, le transfert de masse et de la biodégradation de produits pharmaceutiques en phase de boue et également dans les dépôts de la surface de la membrane

doit être examiné afin de mieux comprendre les mécanismes d'élimination des micropolluants pharmaceutiques dans des BAM. Autres outils tels que LC-OCD, CLSM, ainsi que les analyses du dépôt in situ comme LSGI doivent être utilisés pour analyser plus en détails la structure et la composition du dépôt pour valider les hypothèses faites dans cette étude.

En outre, des études ultérieures avec les objectifs d'accroître l'élimination des micropolluants pharmaceutiques et en même temps de réduire le colmatage du BAM en présence des stress pharmaceutiques doivent être effectués. Par exemple, le charbon actif en poudre (CAP) pourrait être ajouté dans le BAM pour augmenter l'élimination de la CBZ par adsorption et donc de réduire les effets du stress induits. En outre, la filière intégrée BAM-OI-ozonation pourrait être examinée.

Enfin, d'autres études concernant l'évaluation des risques des micropolluants pharmaceutiques sur la santé humaine et les organismes aquatiques devraient être menées afin d'évaluer la nécessité de traitement avancé dans les systèmes de traitement des eaux usées et de réutilisation.