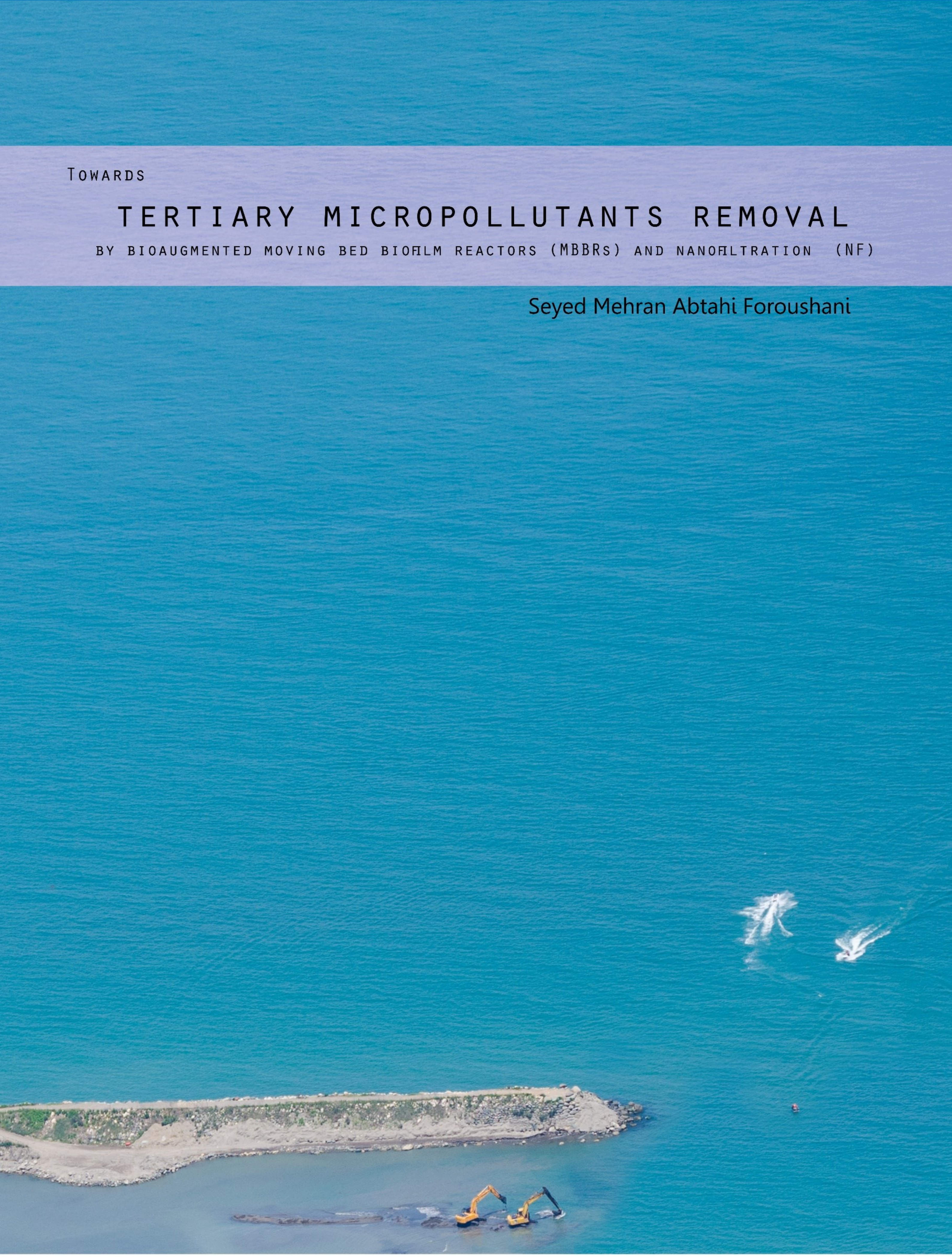

Towards

TERTIARY MICROPOLLUTANTS REMOVAL

by bioaugmented moving bed biofilm reactors (MBBRs) and nanofiltration (NF)

Seyed Mehran Abtahi Foroushani

MESA+ INSTITUTE FOR NANOTECHNOLOGY
UNIVERSITY OF TWENTE.
KU LEUVEN
LABORATOIRE DE GÉNIE CHIMIQUE TOULOUSE • UMR 5503
UNIVERSITÉ TOULOUSE III PAUL SABATIER
Université de Toulouse

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TOWARDS TERTIARY MICROPOLLUTANTS REMOVAL BY BIOAUGMENTED MOVING BED BIOFILM REACTORS (MBBRS) AND NANOFILTRATION (NF)

DISSERTATION

to obtain
the degree of doctor at the University of Twente,
on the authority of the rector magnificus,
Prof. dr. T.T.M. Palstra
on account of the decision of the graduation committee,
to be publicly defended
on Monday 18th of June 2018 at 08:45.

by
Seyed Mehran Abtahi Foroushani
born on 20th March, 1982,
in Khomini Shahr, Iran.

For the University of Twente, this dissertation has been approved by:
Prof. dr. ir. H.D.W. Roesink (Supervisor)
Prof. dr. ir. W.M. de Vos (Co-supervisor)

The tale of an idea from conception to birth

TOWARDS TERTIARY MICROPOLLUTANTS REMOVAL BY BIOAUGMENTED MOVING BED BIOFILM REACTORS (MBBRS) AND NANOFILTRATION (NF)

DISSERTATION

Prepared under the framework of EUDIME program to obtain multiple doctorate degrees issued by

the University of Toulouse (Laboratory of Chemical Engineering),

the University of Twente (Faculty of Science and Technology), and

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to be publicly defended on Monday 18th of June, 2018 at 08:45.

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EUDIME Doctorate Board

Prof. dr. C. Albasi (Supervisor)	University of Toulouse
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“Human beings are members of a whole,

In creation of one essence and soul,

If one members is afflicted with pain,

Other members uneasy will remain.

If you’ve no sympathy for human pain,

The name of human you cannot retain.”

Saadi Shirazi

(famous Persian poet, 1208-1291)

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List of Abbreviations and Symbols

List of Abbreviations

AOB: ammonia oxidizing bacteria

AOP: advanced oxidation process

ASM1: Activated Sludge Model 1

ASFBBR: aerated submerged fixed-bed bioreactor

Allo-BA: allochthonous bioaugmentation

Auto-BA: autochthonous bioaugmentation

BAC: biological activated carbon

BAF: biological aerated filter

bMBBR: bioaugmented-moving bed biofilm reactor

BS: biofilm solids

CAS: conventional activated sludge

CEC: contaminants of emerging concern

cMBBR: control-moving bed biofilm reactor

DO: dissolved oxygen

DOC: dissolved organic carbon

EDG: electron donating groups

EMR: enzymatic membrane reactor

EPS: extracellular polymeric substance

EWG: electron withdrawing groups

FBBR: fluidized bed biofilm reactor

FO: forward osmosis

F/M: food to microorganism ratio

FISH: Fluorescent in situ hybridization

GAC: granular activated carbon

Gen-BA: gene bioaugmentation

HMDS: hexamethyldisilazane

HRT: hydraulic retention time

HSSF wetland: horizontal subsurface flow wetland

IFAS: integrated fixed-film activated sludge

IR: inoculation rate

LbL: layer by layer

LCA: life cycle assessment

LMEs: lignin modifying enzymes

LQ: limit of quantification

MATH: microbial adhesion to hydrocarbon

MBBR: moving bed biofilm reactors

MBR: membrane bioreactor

MF: microfiltration

MLSS: mixed liquor suspended solids

MLVSS: mixed liquor volatile suspended solids

MPA: minimum projection area

MPs: micropollutants

MOB: methane oxidizing bacteria

MWCO: molecular weight cut-off

NF: nanofiltration

NOB: nitrite oxidizing bacteria

NOM: natural organic matter

OLR: organic loading rate

OBP: oxidation by-products

OTP: ozonation transformation products

qPCR: quantitative polymerase chain reaction assay

PAA: poly(acrylic acid)

PAH: poly(allylamine hydrochloride)

PAC: powdered activated carbon

PAH: polycyclic aromatic hydrocarbon

PEM: polyelectrolyte multilayer

PSA: protective surface area

PSD: particle size distribution

RBC: rotating biological contactor

RO: reverse osmosis

SAT: salt aggregation test

SBBGR: sequencing batch biofilter granular reactor

SEM: scanning electron microscopy

SF: sand filtration

SF wetland: surface flow wetland

SMP: soluble microbial products

SRT: solids (sludge) retention time

TMP : Trans membrane pressure

TP: transformation product

UF: ultrafiltration

UV: ultraviolet

VSSF wetland: vertical subsurface flow wetland

WFD: water framework directive

WRF: white-rot fungi

WWTP: wastewater treatment plant

List of Symbols

F_{biod}: mass flow of the biotransformed compound

F_{inf}: mass flow of MPs in the influent

F_{eff}: mass flow of MPs in the effluent

$F_{stripped}$: mass flow of air-stripped MPs

F_{sor}: mass flow of MPs sorbed onto the suspended and/or attached biomass

H: Henry’s law constant

k_{biol}: pseudo-first order degradation constant

k_{sor}: sorption kinetic constant

k_d: solid-water partitioning coefficient

k_{de}: detachment rate constant

k_H: henry's law constants

K_{oc}: Carbon–Water Partitioning Coefficient

log_D: logarithm of the octanol-water distribution coefficient

q: the air supply per unit of wastewater

Q: the feed flow rate

r_{biol}: MPs transformation rate

r_d: detachment rate of the biofilm

r_{sor}: MPs sorption rate

V: volume of the reactor

X_S: sum of the volatile suspended solids and the volatile biofilm solids

PREFACE

The tale of an idea from conception to birth

Preface

1. *Framework of the thesis*

This PhD thesis was performed under the framework of the EUDIME program (doctoral contract No. 2014-122), funded by the European Commission - Education, Audiovisual and Culture Executive Agency (EACEA). The R&D sections at VeoliaWater Technology (Toulouse, France) and Biovitis (Saint-Étienne-de-Chomeil, France) were also financial supporters of the research.

2. *The tale of an idea from conception to birth*

The potential risk of emerging micropollutants (MPs), constantly discharged from municipal wastewater treatment plants, is now under active evaluation among researchers. An integrated layout of a multi-component tertiary system, comprised of moving bed biofilm reactors (MBBRs) and a nanofiltration (NF) membrane, was our initial layout to cope with MPs. As shown in Fig. 1, secondary-treated wastewater is split into two streams. The main stream is used for feeding the MBBRs, while NF membrane is fed by a partial fraction of the stream.

In such a configuration, concentrate stream produced by NF membrane is utilized for acclimation of bacterial strains to the target MPs in a so-called "adaptation process". Although existing high-efficient NF membranes are seen very proficient in MPs removal, high salinity of their concentrate can be very harmful to the bacterial strain because the increased osmotic pressure damages bacterial cell walls (plasmolysis of the organisms). In other words, high salt concentration of the retentate deteriorates the process of adaptation. Hence, the main challenge of this part was to prepare a unique NF membrane with a high level of MPs removal along with a low level of salts rejection under realistic condition. Meanwhile, such a low-saline concentrate can be easily bio-treated in activated sludge-based reactors. To achieve a low-saline concentrate containing high concentrations of MPs, we decided to study a polyelectrolyte multilayer (PEM)-based NF membrane in terms of salts and MPs removal.

The bacterial strain selected for the bioaugmentation of MBBRs was "*Pseudomonas fluorescens*" (provided by Biovitis) that has a proven capability in both aspects of the biofilm formation, and in metabolizing the industrial pollutants. After re-activation and adaptation of the biomass to target MPs, adapted strains are directly imported into two out of three identical-sized MBBRs. The remained MBBR would work as a control reactor for evaluating the influence of bioaugmentation on the reactors' performance. Microbial biofilm is developed on the saddle-shaped surface of newly-born Z-MBBR carriers, produced by AnoxKaldnes company.

This thesis aimed at elucidating the potential of bioaugmented MBBRs and PEM-based NF membranes, for the removal of MPs from conventionally-treated municipal wastewater. Three scientific groups at three universities of Toulouse, Twente and KU Leuven were in-depth involved to understand the key parameters behind the removal of MPs in order to optimize tertiary treatment technologies. The outline of the work is explained in Chapter (I).

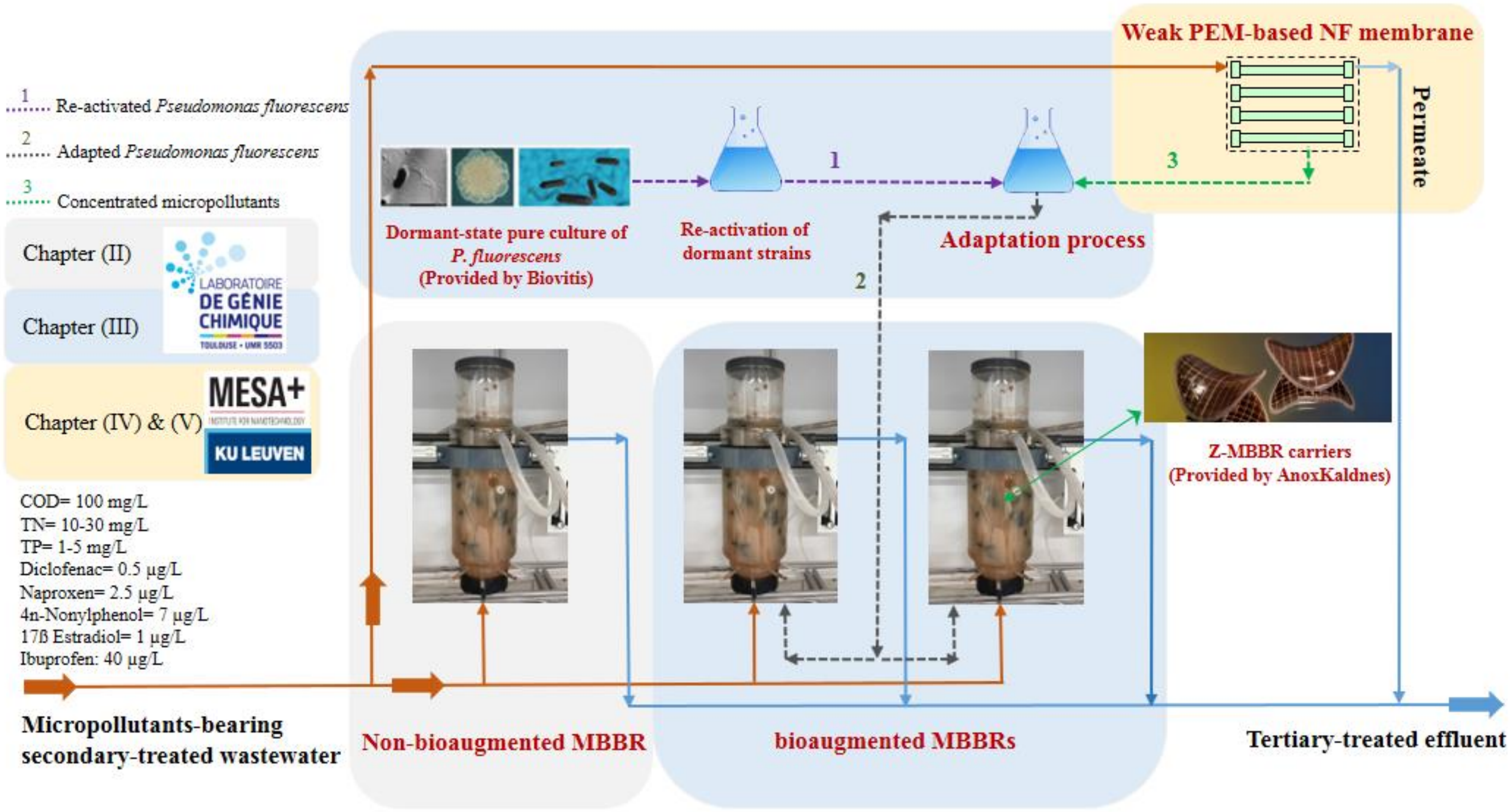


Fig. 1. The concept of an integrated layout, comprised of a coupled MBBR-NF system, for the elimination of target MPs from secondary-treated municipal wastewater

CHAPTER (I)

Bibliographic focus on tertiary treatment technologies & Outline for tertiary removal of target micropollutants

Table of Contents

Preface

This Chapter is devoted to a holistic literature review dealing with micropollutants (MPs) removal processes, with a special emphasis on tertiary treatment technologies. The strategies used for tertiary elimination of MPs are then discussed. In the first part, the fate of target MPs in wastewater treatment is briefly discussed. An overview on tertiary treatment technologies for MPs removal is then given in the second part. In this part, short fundamental discusions along with a focus on the efficiency of tertiary bioreactors are given. The third part deals with the performance of biofilm reactors for tertiary MPs removal. This part is started with a summarized description about the biofilm formation, and continued with configurations of the biofilm reactors. Also, the third part encompasses "the bioaugmentation" from the definition to its application in the biofilm reactors for MPs removal. In the fourth part, we report on the strategies used in this thesis for tertiary MPs removal, including bioaugmented moving bed biofilm reactors (MBBRs) and nanofiltration (NF). This part ends up with several objectives and scientific questions, that will be connected to the next chapters of the thesis.

1. The occurrence and fate of target micropollutants (MPs) in wastewater treatment

1.1. General classification of MPs

MPs are usually defined as "chemical compounds present at extremely low concentrations i.e. from ng.L^{-1} to µg.L^{-1} in the aquatic environment, and which, despite their low concentrations, can generate adverse effects for living organisms" [1]. Sources of MPs in the environment are diverse and many of those originate from mass-produced materials and commodities [2]. Table 1 summarizes the sources of the major categories of MPs in the aquatic environment [2–4]. Controlling the main resources of pollution, as well as developing new wastewater treatment options, are the primary solutions in order to prevent further damage to the environment [5,6].

1.2. European legislation on the *issue of MPs*

The huge impact of natural and anthropogenic organic substances that are constantly released into the environment, has persuaded the scientists and decision-makers to develop several environmental standards worldwide. Moreover, water quality is one of the priority issues of the environmental policy agenda due to the increasing demand for the safe and clean water [5]. European environmental regulations have been legislated to establish a framework for the water protection policy. The European water framework directive (WFD) is probably the most significant mark in the European Union (EU) legislation on water, intending to intensify the monitoring of pollutants in ecosystems and enhance the control of contaminants release [7]. The first list of the EU's environmental quality standards was published in 2008 under the Directive 2008/105/EC [8]. Five years later, the Directive 2013/39/EU was launched to update the previous documents [9]. This directive suggested the monitoring of 49 priority substances and 4 metals, and also proposed the first European Watch List which was then published in the Decision 2015/495/EU of 20 March 2015 [10]. This list comprises 17 organic compounds, named “contaminants of emerging concern (CECs)”, unregulated pollutants for which Union-wide monitoring data need to be gathered for the purpose of supporting future prioritization exercises [5,11]. In addition to these compounds, there are some organic compounds that are not still listed in the European environmental regulations. According to the review paper of Sousa et al. [5], 28 organic MPs not listed in the European legislation, were found at concentrations above 500 ng.L^{-1}, therefore more research about occurrence and fate is also needed for many of these emerging compounds.

1.3. Justification of the choice of MPs

Several parameters were involved in the selection of MPs, including: i) the most commonly detected compounds at the outlet of conventional wastewater treatment plants (WWTPs) as depicted in many papers [2–5,7,12–60], ii) recent European legislations, and iii) analytical costs as well as considerations/limitations for measuring the concentration of MPs. Diversity of MPs in the aspects of physico-chemical properties and biodegradability (from the easy-biodegradable to recalcitrant MPs) was also taken into account.

In the present work, the removal of five MPs (listed in Table 2 with physico-chemical characteristics shown in Table 3) from synthetic secondary-treated municipal wastewater was deeply studied. As working with 17ß-Estradiol was forbidden in the Universities of Twente and KU Leuven, we decided to study the rejection of Ibuprofen instead.

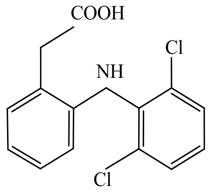
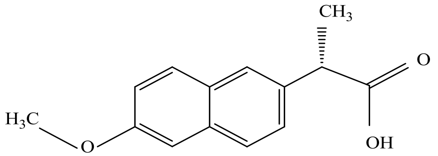
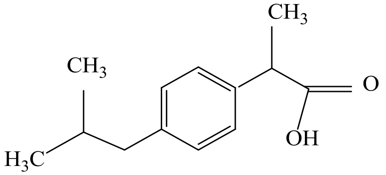
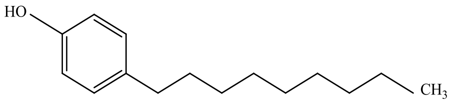
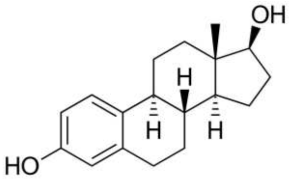
Table 1. The general classification and main sources of MPs in the aquatic environment [2–4]

Main categories	Sub-clauses	Examples	Main sources
Pharmaceuticals	Analgesic and anti-inflammatory	Diclofenac, Naproxen, Ibuprofen, Acetaminophen, Ketoprofen, Mefenamic acid, Salicylic acid	Municipal wastewater, hospital wastewater, run-off from aquaculture, run-off from concentrated animal feeding operation, industrial wastewater (mostly from drugs manufacturing discharges)
	Lipid regulator	Bezafibrat, Clofibric acid, Gemfibroz	
	Antibiotics	Erythromycin, Sulfamethoxazole, Trimethoprim	
	ß-blockers	Atenolol, Metoprolol	
	Nervous stimulants	Caffeine	
	Anticonvulsants	Carbamazepine	
Personal care products	Musk fragrance	Galaxolide, Tonalide	Municipal wastewater (mostly from bathing, shaving, spraying, swimming and etc.), industrial wastewater (mostly from the sanitary manufacturing discharges)
	Disinfectant	Triclosan	
	Insect repellant	DEET	
	UV filter	Benzophenone-3	
Steroid hormones	Estrogens	Estrone, Estradiol, 17α-Ethynylestradiol, Estriol	Municipal wastewater (from excretion), run-off from aquaculture, run-off from concentrated animal feeding operation
Surfactants	Non-ionic surfactants	Nonylphenol, Octylphenol	Municipal wastewater (from bathing, laundry, dishwashing and etc.), Industrial wastewater (from industrial cleaning discharges
Industrial chemicals	Plasticizers	Bisphenol A, DBP (di-butyl phthalate), DEHP (di(2 ethylhexyl) phthalate), DMP (di-methyl phthalate)	Municipal wastewater (by leaching out of the material)
	Fire retardant	TCEP (tris(2-chloroethyl) phosphate), TCPP (tris(1-chloro-2-propyl) phosphate)	
Pesticides	Herbicide	Atrazine, Diuron	Municipal wastewater (from improper cleaning, run-off from gardens, lawns and roadways and etc.) Agricultural runoff
	Insectcide	Diazinon	
	Fungicide	Clotrimazole, Tebuconazole	

Table 2: Our target MPs in this study

Target MPs	Category	European legislation	MPs concentration at the outlet of conventional WWTPs (µg. L^{-1}) (min-average-max)	Tertiary treatment process studied
Diclofenac	analgesic and anti-inflammatory pharmaceuticals	Decision 2015/495/EU [10]	0.035 - 0.477 - 1.72 [13] 0.040 - 0.679 - 2.448 [4] 0.21 - 0.34 - 0.62 [14] 0.013 – 0.024 – 0.049 [15] 0.044 – 0.173 – 0.329 [16] 0.006 – 0.179 – 0.496 [17] 0.131 – 0.263 – 0.424 [17] 0.006 – 0.220 – 0.431 [18] 0.15 – 0.41 – 1.1 [19] average: 0.485 [20]	MBBR & NF
Naproxen		not listed in the European legislations [5]	0.017 – 0.934 – 2.62 [4] 0.09 – 0.13 – 0.28 [14] 0.037 – 0.111 – 0.166 [15] 0 – 0.0165 – 0.0918 [16] 0. 54 – 2.74 – 5.09 [21] 0.22 – 1.64 – 3.52 [21] 0.83 – 2.18 – 3.64 [21] 0.29 – 1.67 – 4.28 [21] 0.234 – 0.370 – 0.703 [17] 0.002 – 0.170 – 0.269 [17] 0.359 – 0.923 – 2.208 [18]	
Ibuprofen			0.03 - 3.48 - 12.6 [4] 0.015 - 0.04 - 0.079 [15] 0 - 0.0489 - 0.111 [16] 0 - 4.13 - 26.5 [21] 0 - 26.69 - 40.2 [21] 0 - 50.16 – 55 [21] 0 - 7.62 - 48.2 [21] 0.131 - 0.263 - 0.424 [17] 0.065 - 0.143 - 0.491 [17] 0 - 0.135 - 0.653 [18] Average: 0.0805 [22] Average: 0.952 [61] Average: 42.885 [20] Maximum: 55 [2]	NF
4n-Nonylphenol	endocrine disrupting compound/surfactant	Directive 2008/105/EC [8] and 2013/39/EU [9]	0.5 – 0.5 – 7.8 [23] 2.515 – 6.138 – 14.444 [24] 1.084 – 1.885 – 3.031 [24] Maximum: 7.8 [2] Average: 0.786 [25] Average: 7.19 [26] Average: 2 [27] Average: 1.42 [28]	MBBR & NF
17ß-Estradiol	steroid hormone	Decision 2015/495/EU [10]	<0.001 – 0.019 – 0.007 [23] 0.0005 – 0.0015 – 0.0029 [29] 0.0003 – 0.0009 – 0.0021 [29] 0.0007 – 0.0024 – 0.0035 [29] Average: 0.0025 [20] Average: 0.0036 [30] Average: 0.001 [31] 0 [32] 0 [15]	MBBR

Table 3. General physico-chemical characteristics of target MPs [2,62–69]

Compound	CAS number	Formula	Molecular Weight (g/mol)	Molar volume (cm^3/mol)	Molecular dimension Length × Width ×Height (nm)	Minimum Projection Area (Å^2)	log K_{OW}	log D (pH:7)	pKa	Henry's law constant (atm.m^3.mol^{-1}) [68,69]	Molecular structure
Diclofenac	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.15	182	0.829× 0.354 × 0.767	43.3	4.548	1.77	4.18	4.73E-12	
Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.26	192.2	1.37 × 0.78 × 0.75	34.8	3.18	0.34	4.3	3.39E-10	
Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.28	200.3	1.39 × 0.73 × 0.55	35.4	3.97	0.77	5.2	1.5E-007	
4n-Nonylphenol	104-40-5	$C_{15}H_{24}O$	220.35	279.8	1.558 × 0.395 × 1.559	NA	6.142	6.14	10.15	4.7E-3	
17ß-Estradiol	50-28-2	$C_{18}H_{24}O_2$	272.38	232.6	1.39 × 0.85 × 0.65	NA	4.13	4.15	10.27	3.64E-11	

NA: not available in literature

1.4. The fate of target MPs in WWTPs

Over the last few decades, conventional WWTPs have been designing based on primary treatment to separate screenings, grits, suspended solids and greases, and secondary biological treatment to remove suspended solids and organic matters. Moreover, biological nutrient removal (BNR) processes have been also developed to decrease the amount of Nitrogen and Phosphorous compounds of the effluent [70]. At present, effluent streams of WWTPs can be considered as one of the most important sources of MPs in the environment because they, especially recalcitrant compounds e.g. Carbamazepine and Diclofenac, are not efficiently removed during the physical and biological wastewater treatment processes [61]. In Fig. 1, we do see the insufficiency of the conventional WWTPs for polishing of MPs-bearing municipal wastewater. It is, therefore, necessary to apply tertiary treatment technologies to remove remaining MPs from WWTP effluents, thereby the subsequent hazardous effects of MPs on humans and the environment will be lowered [36].

The elimination of MPs during the conventional activated sludge (CAS) processes is governed by the abiotic and biotic reactions. Photodegradation, air stripping (volatilization) and sorption onto the biosolids (both suspended and attached biomass) constitute the abiotic MPs removal, whilst metabolism and co-metabolism are recognized as the biodegradation mechanisms involved in the biotic MPs removal [71]. For instance, Fig. 2 illustrates how Galaxolide (a polycyclic musk compound) is removed during the activated sludge process by different pathways. To date, the importance of the biotic MPs removal has been attracted much higher attentions than the role of abiotic section [72], probably due to this fact that MPs biodegradation is a sustainable process and potentially can form end products consisting of inorganic compounds, i.e. mineralization [73]. Additionally, MPs biodegradation is often the dominant removal process for the majority of compounds, as compared with abiotic removal drivers [74]. According to the review paper published by Verlicchi et al. [39], sorption onto the secondary activated sludge is reported up to maximum 5% for most of the analgesic and anti-inflammatory pharmaceuticals, beta-blockers, and steroid hormones which is too much lower than the role of biodegradation in MPs removal (even up to 100%). On the contrary, the removal percentage of some antibiotics like Ciprofloxacin and Norfloxacin is reported in the range of 70-90% due to the sorption, while below than 10% of these compounds were abated by the biodegradation mechanisms [75]. Some studies have pointed out the significance of MPs sorption onto the biosolids, as this factor is found to have an impact on the MPs bioavailability [73] and causes the occasional negative mass balance of MPs, where MPs desorption from the suspended or attached biomass occurs during the treatment process [76]. When the waste sludge is going to be used as a fertilizer on an agricultural land, this factor should be also taken into account, knowing that sludge digestion is likely not able to remove the most of persistent MPs [77].

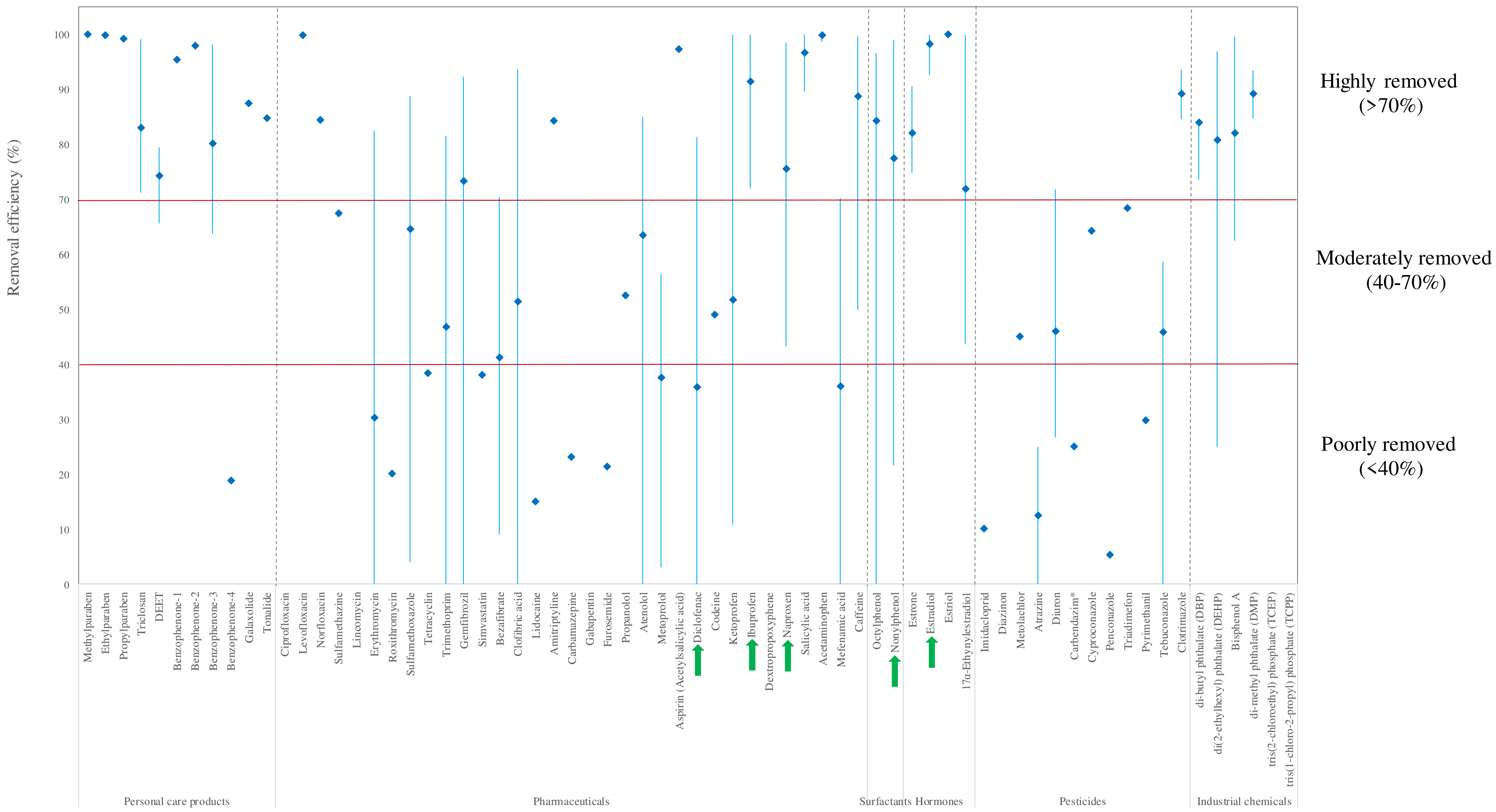


Fig 1. The range of MPs Removal by conventional WWTPs found in the literature reviews [2–4,15,16,22,35,78–80] , and MPs classification according to their elimination [2]

(the arrows show our target MPs in this study)

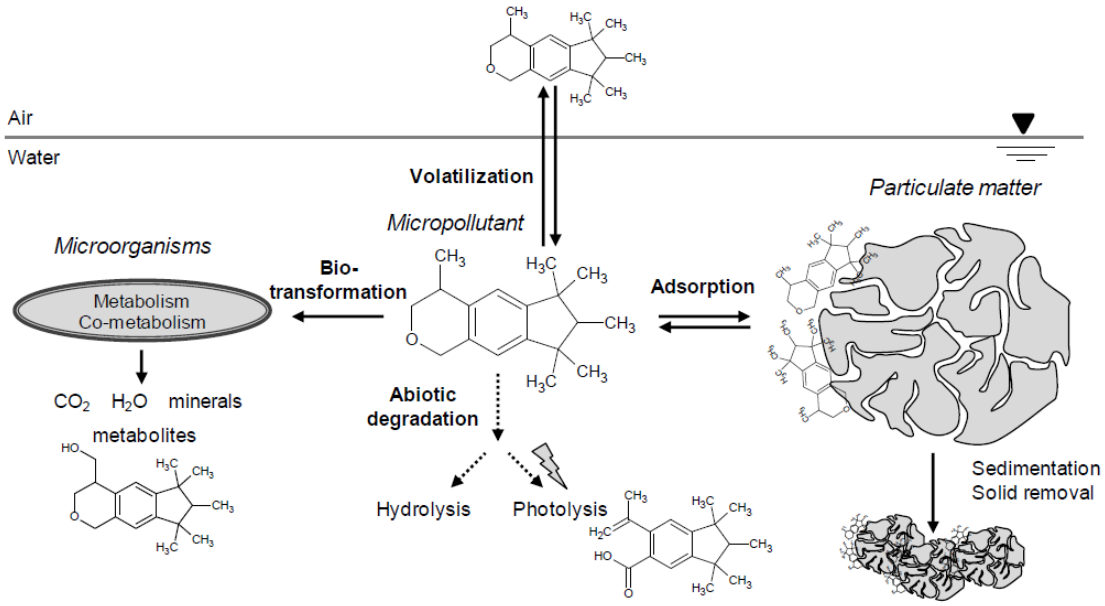


Fig. 2. The main removal mechanisms of MPs (here: Galaxolide) in CAS processes (adapted from [1])

1.4.1. The contribution of photodegradation in MPs removal

Photodegradation consists of direct and indirect natural photolysis. Direct photolysis (direct absorption of light photons by the MPs) is found not affective in wastewater treatment plants because sunlight range is between 290 and 800 nm, while wavelengths for light absorption of many MPs are usually below 280 nm [35,43]. In the case of indirect photolysis, two different strategies are expressed in literature: (I) suspended solids and dissolved organic matters reduce the photodegradation efficiency by the light screening [81], and (II) when wastewater compounds (organic matters and carbonates) absorb sunlight form very reactive intermediates such as carbonate radical ($CO°_3^{-}$) and hydroxyl radical (°OH) which can somehow transform some types of photo-sensitive MPs [82]. In general, in conventional WWTPs, photolysis of MPs by natural sunlight is very restricted because of the low surface-to-volume ratio available for sunlight irradiation (only the surface of the clarifiers or the biological tanks) and the high turbidity of the wastewater, that deeply confines the penetration of light into the water. Hence, photodegradation of MPs is not expected to be an important degradation mechanism in conventional WWTPs. In the case of constructed wetlands and sewage lagoons where a high surface-to-volume ratio is available for sunlight irradiation, the contribution of Photolysis would be more remarkable in the overall MPs removal [47]. For instance, Matamoros et al. [83] who studied the effect of solar radiation on MPs removal in the wetlands, compared two similar surface-flow constructed wetlands systems fed with the same influent, one of which was completely covered, and found that Diclofenac, Ketoprofen and Triclosan were removed at similar rates as the advanced oxidation processes (AOPs) or NF and reverse osmosis (RO) membranes investigated by Kimura et al. [84] and Rosal et al. [18] in uncovered wetlands.

1.4.2. The contribution of volatilization in MPs removal

Volatilization of MPs in conventional WWTPs is performed via surface volatilization and mostly air stripping [44]. Surface volatilization at the surface of the biological reactor is often not taken into account, although it is not negligible [85]. The fraction of compound volatilized in the aeration tank mainly depends on the flow of air getting in contact with wastewater and Henry's law constants (k_H) of MPs [41]. Taking into account the typical air flow rates used in CAS systems (5 – 15 m^3 air. m^{-3} wastewater according to Joss et al. [54]) and also the low Henry's law constants (k_H) of the most of MPs, losses due to the stripping are nearly negligible for the vast majority of MPs [41]. Operation conditions of the process (type of aeration, temperature and atmospheric pressure) are also involved in the volatilization of MPs [44].

1.4.3. The contribution of sorption in MPs removal

In general, two types of sorption profoundly occur in activated sludge systems: I) adsorption i.e. electrostatic interactions of the oppositely charged groups (positively charged groups of MPs with the negatively charged surfaces of the microorganisms and sludge), and II) absorption i.e. hydrophobic interactions between the aliphatic and aromatic groups of a compound and the lipophilic cell membrane of microorganisms [1,2,61,65,79]. In addition, other mechanisms like cationic exchanges, cationic bridges, surface complexation and hydrogen bridges may also have an impact on the MPs sorption [44]. As a whole, sorption onto the sludge or particulate matter can be a dominant removal mechanism for hydrophobic or positively charged MPs, in particular when they are slightly biodegradable [50,54]. A comprehensive study by Stevens-Garmon et al. [74] on the sorptive behavior of MPs onto the primary and secondary activated sludge indicates that positively-charged compounds such as Amitriptyline and Clozapine have the highest sorption potential as compared to the neutral and negatively-charged ones. Moreover, sorption onto the biofilm in a nitrifying MBBR was recognized significant only for positively charged MPs such as Atenolol and Erythromycin in the batch experiments of Torresi et al. [86]. Theoretically, sorption is a physicochemical process and consequently, it is greatly influenced by i) the colloidal fraction of organic matter that increases solubility of some substances [87], and ii) available surface for the interaction. Nevertheless, within activated sludge, typical variation of pH is low, between 6 and 8, and induces limited modification of sorption [44].

So far, most of the researchers have described the phenomenon of sorption by means of the solid-water partitioning coefficient (K_d) i.e. the ratio of the equilibrium concentration of the chemical on the solids to the corresponding equilibrium aqueous concentration [74,77]. Some K_d values reported from different studies on the CAS reactors showed a great variability, particularly for pharmaceutical compounds; e.g. for Diclofenac, Ternes et al. [77] found a value of 2 $L.kg_{ss}^{-1}$, whereas Urase and Kikuta [88] found a range of 16–701 $L.kg_{ss}^{-1}$. According to the various K_d values reported in the literature (Fig. 3), it is required to differentiate K_d values according to i) the type of solid matrix (e.g., activated sludge, particular content of raw/treated wastewater, etc.) that dramatically influences the sorption phenomenon., and ii) the type of activated sludge system [89]. K_d values can be also related to the ratio of MPs concentration/available biomass. To date, some researchers have tried to establish a kind of classification scheme in order to describe the phenomenon of MPs sorption in activated sludge systems [1,50,74]. In brief, Stevens-Garmon et al. [74] noticed that compounds with $K_d < 30$ $L.kg_{ss}^{-1}$ are compounds with a poor sorption potential on inactivated sludge [74]. Meanwhile, Joss et al. [50] by preparation of a mass balance of a municipal WWTP proved that MPs sorption onto the secondary sludge is not relevant for compounds showing K_d value below 300 $L.kg_{ss}^{-1}$. Nevertheless, the best classification is apparently prepared by Margot et al. [90] whose main conclusion is summarized in Table 4.

Table 4. The classification scheme proposed by Margot et al. [90] on the issue of MPs sorption in CAS reactors

K_d ($L.kg_{ss}^{-1}$)	The rate of MPs removal by the sorption	Examples
$K_d < 400$	Negligible removal (< 10%)	Diclofenac, Carbamazepine [50]
$400 < K_d < 4000$	Low to moderate removal (10-50%)	Azithromycin, Oxazepam [91,92]
$4000 < K_d < 40000$	Moderate to high removal (50-90%)	Ciprofloxacin, Norfloxacin, Fluoxetine [91,92]
$K_d > 40000$	High removal (> 90%)	Heptachlor, Hexachlorobenzene [92,93]

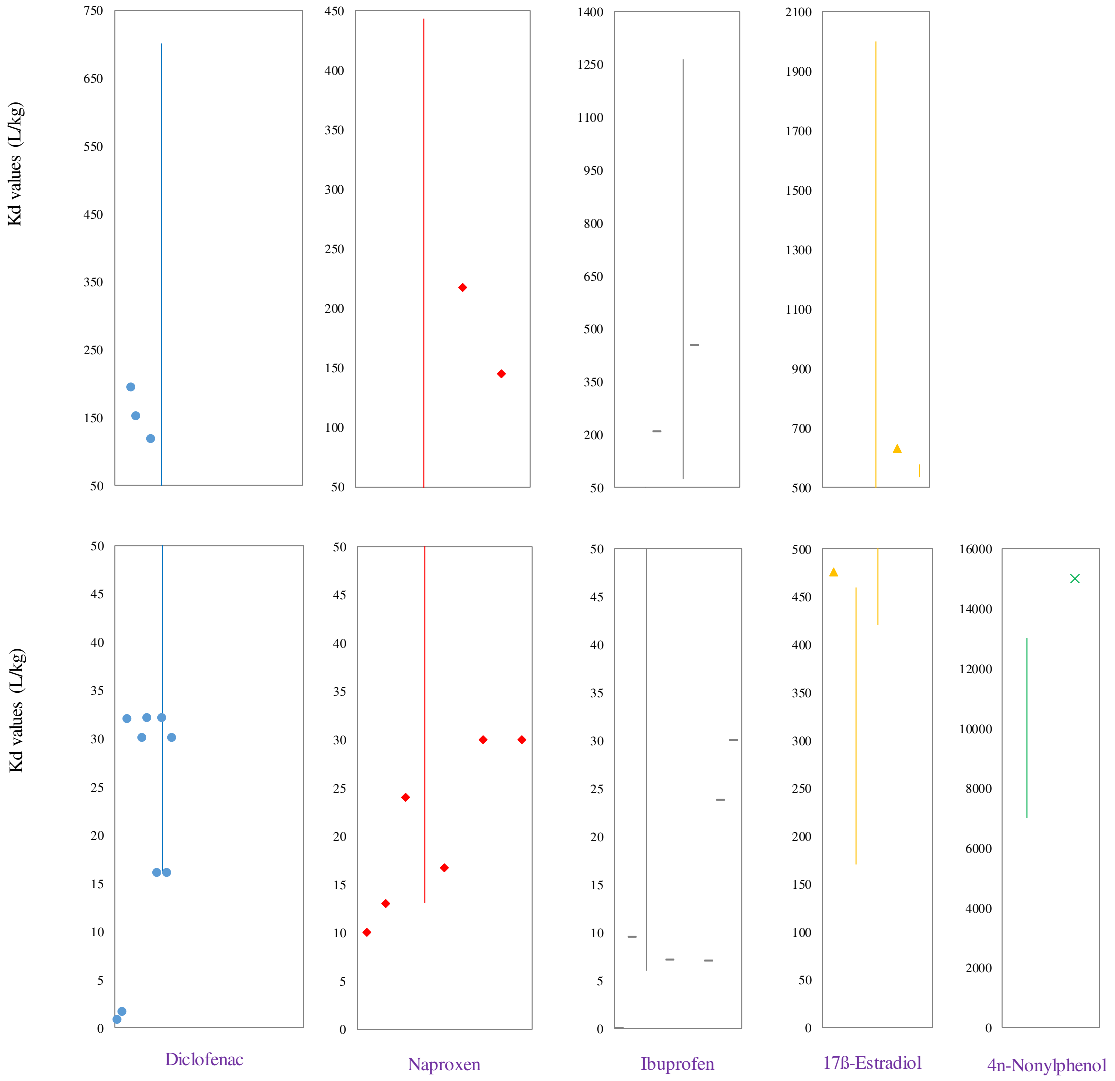


Fig. 3. Minimum to maximum (vertical bars) and average (scattered points) values of K_d (L.kg$_{ss}^{-1}$), related to the target MPs reported for CAS reactors (adapted from literature review of Pomiès et al. [44], Lue et al. [2], Horsing et al. [91], Stevens-garmon et al. [74], Joss et al. [50], and Barret et al. [89])

1.4.4. The contribution of biodegradation in MPs removal

Generally, microorganisms have been observed to employ two main catalytic processes when participating in biologically-mediated reactions with MPs. Firstly, microorganisms can interact with MPs in metabolic reactions; these are growth-linked processes that often result in mineralization of the MP. Secondly, microorganisms can interact with MPs in co-metabolic reactions; these are reactions that do not sustain growth of the responsible microorganisms and often lead to the formation of transformation product that may possibly be used as growth substrates for other microorganisms. To be relevant for MPs removal, the microorganisms participating in co-metabolic reactions must have enzymes with a vast substrate specificity and competition for the enzyme between the MPs and growth substrates should not lead to a disadvantage for the survival of the organisms [94]. A schematic of the metabolic and co-metabolic strategies is provided in Fig 4.

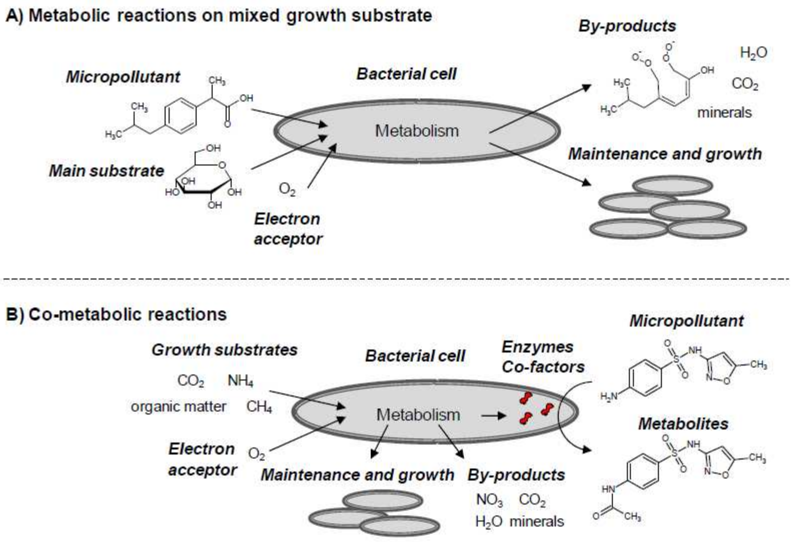


Fig. 4. Metabolic and co-metabolic pathways of MPs biodegradation in CAS reactors (a: Ibuprofen, b: Sulfamethoxazole) (adapted from [90])

In the co-metabolic mechanism, higher concentration of the substrate is seen to accelerate the biodegradation rate of MPs [95]. As stated above, during this mechanism, MPs are not used as a growth substrate but are biologically transformed, by side reactions catalyzed by unspecific enzymes or cofactors produced during the microbial conversion of the growth substrate [96]. Casas et al. [95] evaluated the ability of a staged MBBR (three identical reactors in series) on the removal of different pharmaceuticals (including X-ray contrast media, b-blockers, analgesics and antibiotics) from hospital wastewater. As a whole, the highest removal rate constants were found in the first reactor while the lowest were found in the third one. The authors noticed that the biodegradation of these pharmaceuticals occurred in parallel with the removal of COD and nitrogen that suggest a co-metabolic mechanism. Besides, in the research of Tang et al. [97] on a polishing MBBR, the removal rate constant of some pharmaceuticals such as Metoprolol and Iopromide was dramatically enhanced by adding humic acid salt (30 mg.L^{-1} dissolved organic carbon (DOC)), indicating the role of substrate availability in co-metabolic degradation of these MPs.

In contrast to the co-metabolism, higher concentration of the substrate decelerates the biodegradation rate of some MPs in the scenario of competitive inhibition i.e., competition between the main growth substrate (carbon and nutrients) and the pollutant to the nonspecific enzyme active site [1,98]. For instance, Joss et al. [51] showed the substrate present in the raw wastewater competitively inhibits the degradation of Estrone and 17ß-Estradiol in CAS systems. These compounds were then mainly removed in activated sludge compartments with a low substrate loading.

During the metabolic pathways, MPs are metabolized to varying degrees, and their excreted metabolites and unaltered parent compounds can be under the further modifications [39]. These intermediate metabolites might be more persistent and toxic than their parent compounds, thus it is important to understand the biotransformation pathways of MPs and to identify the transformation products accumulated [99]. Quintana et al. [100] reported that most of these intermediate metabolites are then further degraded, even to complete mineralization in a membrane bioreactor (MBR) treating municipal wastewater. A recent research by Ooi et al. [101] showed that tertiary nitrifying MBBRs do not completely mineralize Clindamycin and its main transformation product (clindamycin sulfoxide) is persistent. However, little is still known on the fate of MPs' intermediate metabolites in the bioreactors, thereby unlocking this not yet well-defined aspect of MPs degradation remains a challenge to researchers.

To describe the issue of MPs biodegradation in activated sludge-based reactors, we are able to refer to a simple classification scheme suggested by Joss et al. [54] who characterized the biological degradation of MPs using pseudo-first order degradation constant (k_{biol}). They obtained k_{biol} values of 35 MPs from a nutrient-removing activated sludge system (shown in Fig. 5), and then revealed that MPs with $k_{biol} <$ 0.1 L. gVSS^{-1}. d^{-1} are not removed to a significant extent (<20%), while compounds with $k_{biol} >$10 L. g VSS^{-1}.d^{-1} are transformed by > 90%, and in-between a moderate removal is expected [54]. In Fig. 6, we give k_{biol} values for target MPs found in the literature for secondary biological wastewater treatment. According to the above-mentioned classification, Fig. 6 and Fig. 1, we can roughly conclude that the high rate of biodegradation seen for Ibuprofen and 17ß-Estradiol, the moderate rate for Naproxen and 4n-Nonylpenol, and also the low rate for Diclofenac are nearly justifiable in the secondary biological wastewater treatment.

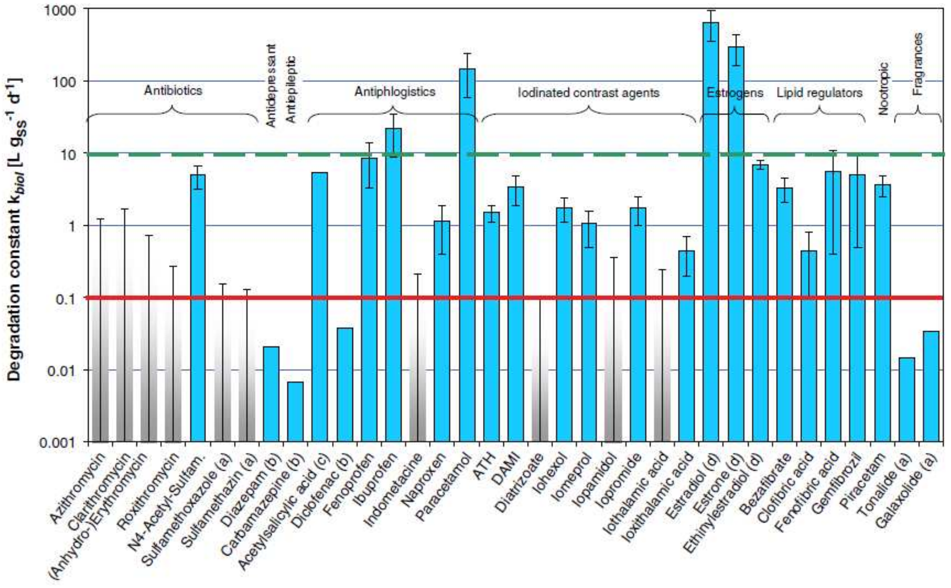


Fig. 5. k_{biol} values of several MPs obtained in nutrient-removing municipal WWTPs by Joss et al. [54]

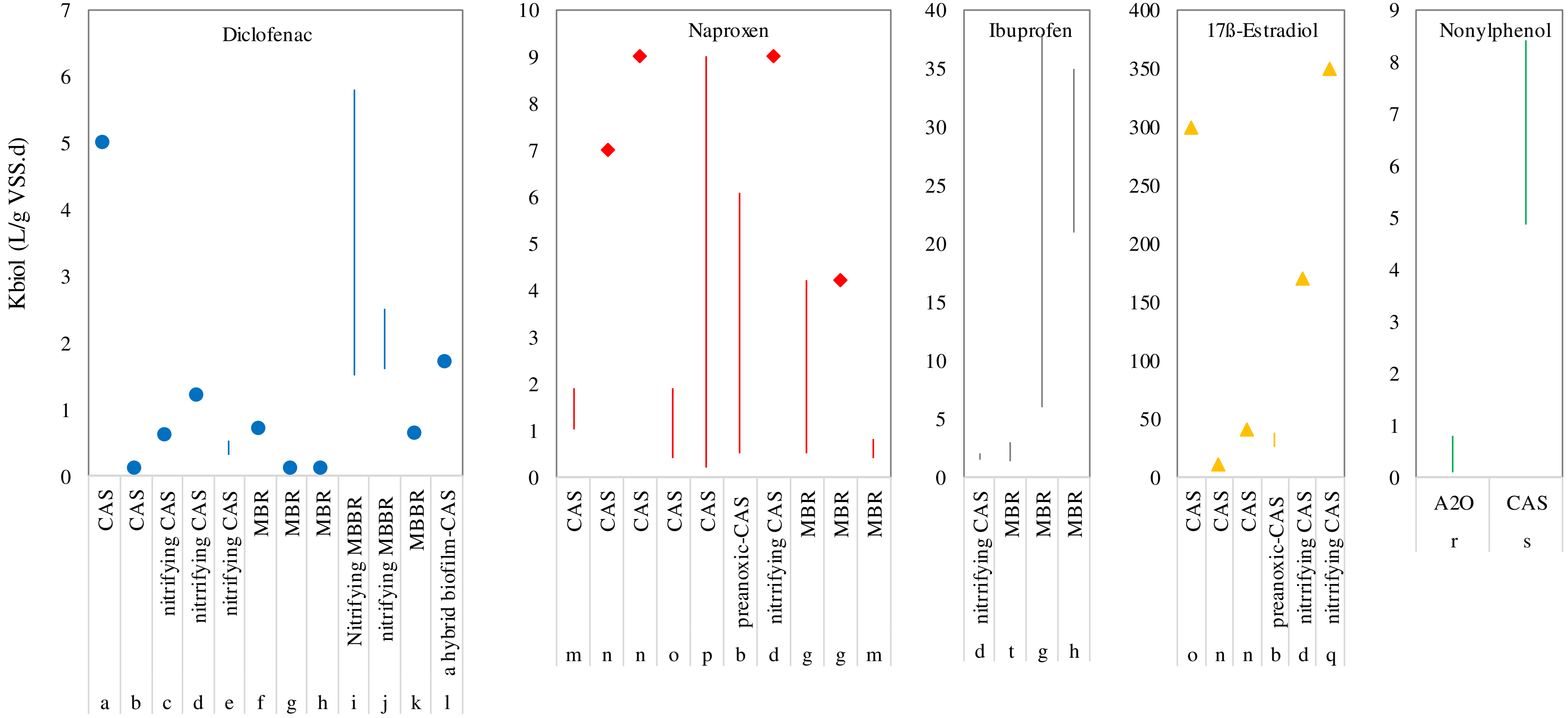
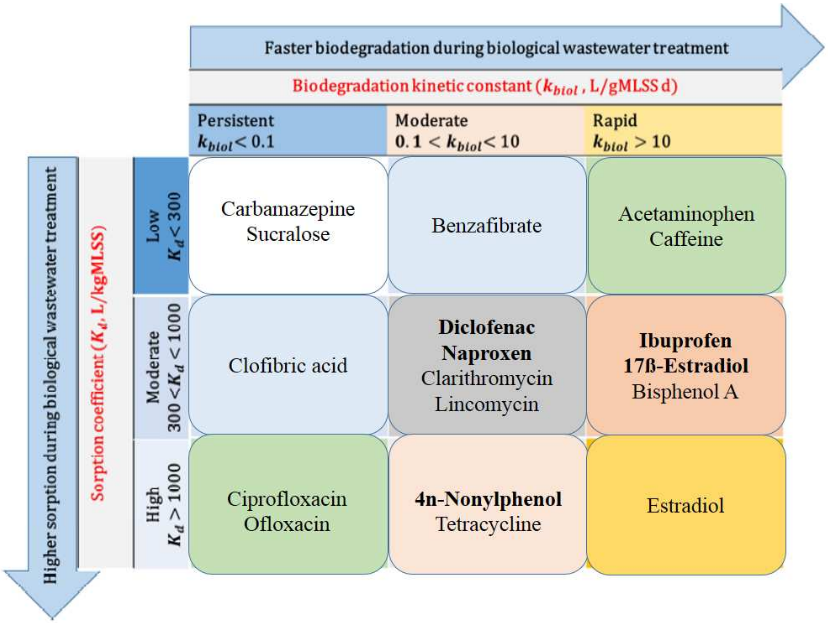


Fig. 6. k_{biol} values of target MPs found the literature for the secondary biological wastewater treatment

Abbreviations: CAS: conventional activated sludge, MBR: membrane bioreactor, MBBR: moving bed biofilm reactor, A^{2}O: anaerobic anoxic aerobic activated sludge

References: [a][102], [b][57], [c][103], [d][104], [e][40], [f][105], [g][60], [h][54], [i][106], [j][107], [k][95], [l][52], [m][88], [n][55], [o][108], [p][39], [q][51], [r][109], [s][110], [t][111] Clear separation between metabolism and co-metabolism is hardly feasible in complex systems such as activated sludge as both reactions probably occur simultaneously due to the diversity of microorganisms present. Indeed, co-metabolic and metabolic reaction steps might be closely interrelated and substitutable, since they are part of a metabolic network [96]. The discrimination between metabolic and co-metabolic processes becomes more difficult when some MPs are degraded via the both mechanisms. For instance, Çeçen et al. [112] found that chlorinated aliphatic compounds such as Trichloroethylene are degradable via the both metabolic and co-metabolic pathways, depending on the species composition of the microbial community and on the reaction conditions [112]. Table 5 lists the k_{biol} values obtained from the literature review of Yifeng Xu et al. [99] to compare metabolic pathways in MPs biodegradation. Although the inoculum/activated sludge and the experimental conditions were various among these findings, it could be roughly concluded that the co-metabolic biodegradation rate constants were significantly higher than the metabolic biodegradation rate constants for majority of the MPs studied [99].

Although both biodegradation and sorption are evidently two dominant mechanisms for MPs removal in WWTPs (Fig. 7), MPs removal efficiencies vary depending on the operating conditions applied in the WWTP, such as hydraulic retention time (HRT), sludge retention time (SRT), food to microorganism ratio (F/M) and temperature; even though the influence of these parameters is not always clearly understood [44]. Despite the fact that MPs' k_{biol} values are not strongly affected by the SRT [49], a longer SRT may promote the diversity of bacterial communities, as well as the presence of slower growing species, thus increasing the biodegradation potential of the biomass [104]. On the other hand, low F/M ratio emerged by the high amount of biomass and the relative shortage of biodegradable organic matter may force microorganisms to metabolize some MPs with the competitive inhibition mechanism [58]. In the case of HRT, Joss et al. [50] observed a better removal efficiency for MPs when they applied longer HRTs that bring longer contact time between wastewater and sludge [50].



	Persistent $k_{biol} < 0.1$	Moderate $0.1 < k_{biol} < 10$	Rapid $k_{biol} > 10$
Low $K_d < 300$	Carbamazepine Sucralose	Benzafibrate	Acetaminophen Caffeine
Moderate $300 < K_d < 1000$	Clofibric acid	**Diclofenac** **Naproxen** Clarithromycin Lincomycin	**Ibuprofen** **17ß-Estradiol** Bisphenol A
High $K_d > 1000$	Ciprofloxacin Ofloxacin	**4n-Nonylphenol** Tetracycline	Estradiol

Fig. 7. The contribution of biodegradation and sorption in MPs removal, according to the classification introduced by Tran et al. [45] (bold-written compounds are placed in the graph according to our literature review already given in Fig. 3 and Fig. 6)

Table 5. The metabolic and co-metabolic k_{biol} constants of several MPs, prepared according to the literature review of Yifeng Xu et al. [99]

MPs	k_{biol} (L. gVSS^{-1}. d^{-1})		Description of the process	Reference
	Metabolism	Co-metabolism		
Diclofenac	0.064	0.41-0.69	Batch degradation experiments were conducted with enriched nitrifying cultures under various initial conditions such as in the presence of different growth substrates and the inhibitors	[113]
Carbamazepine	0.028	0.09-0.19		
Ketoprofen	0.10	0.91-2.12		
Gemfibrozil	0.099	1.35-2.45		
Fenoprofen	0.083	1.57-2.23		
Indomethacin	0.022	1.52-2.16		
Clofibric acid	0.009	0.04-0.09		
Propyphenazone	0.014	0.11-0.23		
Acetaminophen	0.81	1.3	Nitrifier enrichment culture inoculated in a MBR with 100 µg. L^{-1} Acetaminophen in the influent.	[114]
Ibuprofen	1.22	-	Ibuprofen was used as a sole carbon and energy source by one isolated environmental bacteria from a WWTP	[115]
	0.53	-	laboratory scale activated sludge reactor with initial Ibuprofen concentration of 100 µg.L^{-1}	[88]
	-	2.43-3.01	Batch degradation experiments were conducted with enriched nitrifying cultures under various initial conditions such as in the presence of different growth substrates and the inhibitors	[113]
	-	36	Biomass from nitrification/denitrification tanks of a sewage treatment plant as an inoculum. Synthetic feeding in order to develop autotrophic nitrifying biomass with Ibuprofen concentration (80 – 320 µg.L^{-1}) introduced	[116]
Naproxen	0.084	-	Batch degradation experiments were conducted with enriched nitrifying cultures under various initial conditions such as in the presence of different growth substrates and the inhibitors	[113]
	-	19	Biomass from nitrification/denitrification tanks of a sewage treatment plant as an inoculum. Synthetic feeding in order to develop autotrophic nitrifying biomass with Naproxen concentration (80 – 320 µg.L^{-1}) introduced.	[116]

2. Tertiary treatment technologies for MPs removal

According to the descriptions above, conventional treatment methods do not lead to sufficient removal of MPs, and the upgrading of WWTPs by the implementation of additional advanced or tertiary treatment technologies, prior to discharge into the environment, has arisen as practice for the total mineralization of MPs, or by converting them into less harmful compounds [36]. To date, identification of technically and economically feasible advanced wastewater treatment options for the elimination of MPs from secondary-treated effluent is ongoing. In view of this, scientists have been trying various types of tertiary treatment technologies such as AOPs [117,118], adsorption processes [36] and membrane filtrations [65] throughout the last decade. In addition to these costly methods in the aspects of investment and operation [119], lower attentions have been paid to biological treatment of secondary-treated effluents due to not-satisfactory growth of microbial strains at very low substrate concentrations i.e. low carbon sources and nutrients [120]. Here, we briefly report on the most-frequently used treatment technologies for removal of MPs from secondary-treated municipal wastewater.

2.1. Advanced oxidation processes for tertiary MPs removal

AOPs are quite efficient novel methods for advanced treatment of wastewater. These processes involve the use and generation of powerful transitory species, principally the hydroxyl radical (HO°) that is a powerful oxidizing agent leading to oxidation and mineralization of organic matter, while this species is characterized by lack of selectivity of attack [121]. The versatility of the AOPs is enhanced by the fact that there are different ways of producing HO° radicals, facilitating compliance with the specific treatment requirements [80]. Regarding the methodology to generate HO° radicals, AOPs can be divided into chemical, electro-chemical, sono-chemical and photo-chemical processes. Conventional AOPs can be also classified as homogeneous and heterogeneous processes, depending on whether they occur in a single phase or they make use of a heterogeneous catalyst like metal supported catalysts, carbon materials or semiconductors such as TiO_2, ZnO, and WO_3 [78]. In addition to the MPs removal, AOPs have also been used as pre-treatment of industrial wastewater to improve biodegradability before the subsequent biological process [122]. The properties of most common AOPs (mainly at bench or pilot-scales) that have been so far evaluated for MPs removal are given in Table 6. Also, Table 7 show the capability of AOPs for tertiary MPs removal. It is worth noting the fact that most studies do not include information on the by-products formed during the application of AOPs. Therefore, AOPs should be carefully monitored and ecotoxicological investigations should be accompanied to investigate the formation of potentially toxic transformation products [123]. The integration of different AOPs in a sequence of complementary processes is also a common approach to achieve a better removable compound. For instance, Perfluorooctane sulfonic acid (an industrial compound) was studied in two reclamation plants located in Australia, differing in the effluent load and in the process applied, UV/H_2O_2 and membrane processes leading to removals below detection limit [124], while alkaline ozonation was unsuccessfully tested for the removal of Perfluorooctane sulfonic acid [125]. This type of integration can also produce a biodegradable effluent that can be further treated by a cheaper and conventional biological process, reducing the residence time and reagent consumption in comparison with AOPs alone [126]. In such cases, a biological pre-treatment (removing biodegradable compounds) followed by an AOP (converting the non-biodegradable portion into biodegradable compounds with less chemical consumption) and a biological polishing step may prove to be more useful [127]. However, it is important to completely eliminate the oxidizing agents before any biological treatment, since they can inhibit the growth of microorganisms [78]. A monitoring of 550 substances by Bourgin et al. [128] who treated secondary-treated effluent of municipal WWTPs by ozonation, confirmed that applying ozone dose of 0.55 g O_3/g DOC (dissolved organic carbon) was very efficient to abate a broad range of MPs by >79% on average. After ozonation, an additional biological post-treatment was applied to eliminate possible negative ecotoxicological effects generated during ozonation caused by biodegradable ozonation transformation products (OTPs) and oxidation by-products (OBPs).

Table 6. A summary of the AOPs properties for tertiary MPs removal

Type of AOP	Advantages	Disadvantages/limitations	References
Ozonation	Remarkable capability for removing most of the pharmaceuticals and industrial chemicals	As O_3 is a highly selective oxidant, ozonation often cannot ensure the effective removal of ozone-refractory compounds such as Ibuprofen.	[129]
	It has been successfully applied in many full-scale applications in reasonable ozone dosages.	Ozonation produces carcinogenic bromate from bromide that exists in secondary-treated effluents.	[129,130]
Fenton oxidation	This kind of system is attractive because it uses low-cost reagents, iron is abundant and a non toxic element and hydrogen peroxide is easy to handle and environmentally safe.	In this process, the low pH value often required in order to avoid iron precipitation that takes place at higher pH values. This process is not convenient for high volumes of wastewater in full-scale applications.	[78,131]
Heterogeneous photocatalysis with TiO_2	The principle of this methodology involves the activation of a semiconductor (typically TiO_2 due to its high stability, good performance and low cost) by artificial or sunlight.	The need of post-separation and recovery of the catalyst particles from the reaction mixture in aqueous slurry systems can be problematic.	[131]
		The relatively narrow light-response range of TiO_2 is one of the challenges in this process. This process is not convenient for high volumes of wastewater in full-scale applications.	
photolysis under ultraviolet (UV) irradiation	Photo-sensitive compounds can be easily degraded with this method.	UV irradiation is a high-efficient process just for effluents containing photo-sensitive compounds. This process is not convenient for high volumes of wastewater in full-scale applications.	[131]
		The addition of H_2O_2 to UV is more efficient in removing MPs than UV alone, but UV/H_2O_2 is a viable solution for the transformation of organic MPs with low O_3 and $\circ OH$ reactivity.	
Ultrasound irradiation (Sonolysis)	It is a relatively new process and therefore, has unsurprisingly received less attention than other AOPs. But it seems that this process is economically more cost-effective.	There are very few studies and consequently rare experience about Sonolysis of the effluent MPs.	[80]

Table 7. The efficiency of AOPs for target MPs removal (%) from secondary-treated municipal wastewater found in the literature

Type of AOP	The main properties	Initial concentration of MPs	Diclofenac	Naproxen	4n-Nonylphenol	17ß-Estradiol	Ibuprofen	Reference
Ozonation	Ozone dose: 2.8 ± 30%	2.6-5.8 µg/L	80					[132]
	Ozone dose: 0.55 g O_3/g DOC	5 µg/L	96					[128]
	g O_3/g DOC = 0.25−1.5., the contact time: 20 min	2 µg/L	100	100			75	[130]
	No detail is given about the ozonation.	4n-Nonylphenol: 0.66 µg/L Naproxen: 0.06 µg/L Diclofenac: 0.63 µg/L	98.4	100	78.8	100	100	[59]
	Ozone dose: 5-40 mg/L., the contact time: 20 min	4.68 ± 0.89 ng/L				99.99		[133]
	a 5-L glass jacketed reactor operating in semi-batch mode., gas flow of 0.36 Nm^3/h containing 9.7 g/Nm^3 ozone	Diclofenac: 232 ng/L Ibuprofen: 2.7 µg/L Naproxen: 2.4 µg/L	61.5	60.9			95	[18]
Ozonation - activated carbon filtration	Ozone dose: 0.25 to 0.50 mg O_3/mg DOC	10 µg/L	94	100				[134]
electro- peroxone process	current: 80 mA, inlet O_3 gas phase concentration: 6 mg/L, sparging gas flow rate: 0.25 L/min	1 µg/L	90				90	[129]
Photo-fenton	5 mg/L of Fe^{2+} and 50 mg/ L of H_2O_2., contact time: 50 min., The total illuminated area: 9 m^2., the irradiated volume: 108 L	Diclofenac: 1.3 µg/L Naproxen: 1.4 µg/L	97	97.3				[135]
solar photocatalysis & TiO_2	a suntest solar simulator equipped with a 765–250 W/m^2 Xe lamp., 20 mg/L of TiO_2., Contact time: 100 min.	Diclofenac: 4.5 µg/L Naproxen: 4.5 µg/L Ibuprofen: 0.75 µg/L	78	35			100	[136]
Electron beam irradiation	an electron beam accelerator (500 kV; 25 mA; 1.2 m scan width), Maximum penetration of 500-keV electrons in water: 1.4 mm.	3.95 µg/L			87			[137]
UV	No detail is given about the UV.	6 µg/L			66.7			[27]

2.2. Adsorption processes for tertiary MPs removal

Among tertiary treatment technologies, today, adsorption of MPs onto the powdered activated carbon (PAC) or granular activated carbon (GAC), followed by a final polishing step (using sand filtration (SF) or UF membranes), have shown a great potential in terms of MPs removal, large-scale feasibility, and costs [36,61,138]. Full-scale trials of this process have not only demonstrated good removal of a broad range of MPs, but also contributed to reducing the effluent toxicity [132,139]. Activated carbon processes involve physical adsorption onto the activated carbon resulting in the removal of nearly all adsorbed contaminants retained by the filtration and the spent carbon must then be regenerated or disposed of [36]. The efficiency of an integrated GAC – filtration system to remove MPs has been studied in some WWTPs, showing a mitigated efficiency depending on the compound and the frequency of GAC regeneration/replacement [134,140,141]. PAC adsorption, with a dosage of 10–20 mg.L^{-1}, has been proposed as a more efficient alternative compared to GAC treatment in some researches [142,143]. Despite an acceptable performance of these systems for elimination of a broad range of MPs [12,139], there are some problematic issues observed in terms of spent carbon, sorption efficiency and operational costs. In the case of GAC, a regeneration process of the spent carbon is required, while spent PAC must be incinerated or dumped after filtration process [61]. Moreover, as MPs adsorption onto the activated carbon is strongly under the control of hydrophobic and electrostatic interactions, hydrophilic and/or negative charged MPs are not well removed by this process [139]. Economically, a research conducted by Moser. R [144] in Switzerland estimated the cost of several methods to upgrade municipal WWTPs for MPs removal, sand filtration and ozonation were in the same range, 5.9 to 32.2 and 4.8 to 36.7 CHF.EP^{-1}.a^{-1} respectively (depending on the plant size) whereas activated carbon adsorption cost was higher, between 21.5 and 95 CHF.EP^{-1}.a^{-1} (Swiss Franc. Population-year) [145].

Adsorption processes are not only confined to the MPs adsorption onto the PAC and GAC media. For instance, some researchers have used the biological activated carbon (BAC) filtration as a tertiary treatment system for MPs removal [145,146]. A BAC filter consists of a fixed bed of GAC supporting the growth of bacteria attached on the GAC surface [145]. This technology has been already used for many years for drinking water treatment, usually after ozonation, and has proven to be able to significantly remove natural organic matter, ozonation by-products, and precursors of the disinfection by-products [147]. The impact of BAC, sand filtration (SF) and biological aerated filter (BAF) for removal of the selected organic MPs such as Diclofenac, Naproxen and 4n-Nonylphenol from secondary-treated effluent was studied by Pramanik et al. [146]. Ultimately, BAC led to greater removal of DOC (43%) than BAF (30%) which in turn was greater than SF (24%). All systems could effectively remove most of the selected organic MPs, and there was a greater removal of these MPs by BAC (76–98%) than BAF (70–92%) or SF (68–90%).

The use of different types of clays as MPs adsorbents has also attracted the attentions of some researchers in order to abate MPs from the wastewater effluents [148–150]. Advantages of the clays come from their characteristics such as a large specific surface area, cation exchange capacity, low costs, low toxicity and also environmental friendliness [151]. The MPs adsorption to the clays is influenced by various water quality parameters such as organic matter and particle concentrations in wastewaters. It is expected that MPs removal mechanisms via hydrophobicity adsorption and charge interactions are predominant with the use of clay [148]. Although the performance of the Clay-based adsorption processes is still seen inconvenient in MPs removal (e.g. Diclofenac and Naproxen were removed up to 53% and 22%, respectively, by their adsorption onto an integrated clay-starch system [148]), but working on it seems worthy due to its low investment and operational costs.

In Table 8, we brought some examples of the capability of adsorption processes for target MPs removal from secondary-treated wastewater. A glance through this data and also Table 7 shows the efficiency of adsorption processes is not yet as high as AOPs. Further optimization, however, is still needed to achieve an adsorption-based system to remove MPs containing different physico-chemical properties.

Table 8. The efficiency of adsorption processes for target MPs removal (%) from secondary-treated municipal wastewater found in the literature

Adsorption process	The main properties	Initial concentration of MPs	Diclofenac	Naproxen	4n-Nonylphenol	17ß-Estradiol	Ibuprofen	Reference
PAC	PAC dose: 2.5-5 mg/L	2.6-5.8 µg/L	80					[132]
	The addition of PAC (1 g/L) into the sequential membrane bioreactor was applied.	10 µg/L		42-64			71-97	[143]
	PAC dose: 10 mg/L	40 µg/L	96	98				[142]
PAC/SF	PAC dose: 10-12 g/m^3 of the effluent., HRT: 2-3 h in the contact time., filtration rate: 4-15 m/h	Not given	92	95			100	[139]
PAC/NF	PAC concentration: 10-100 mg/L, 1.5 mm capillary Nanofiltration NF50 M10 from Norit X-Flow with TMP: 1.5 - 4 bar	10 ng/L - 10 µg/L	51.4					[152]
PAC/UF	PAC concentration: 20 mg/L, PES-UF membrane: permeability: 80-200 L/(m^2.h.bar) and water flux: 23 L/(m^2.h)	1.3 - 9.1 µg/L	85					[153]
GAC	A borosilicate glass column filled with 7.5 g of GAC was used as a post-treatment unit for the MBR permeate. The column had an internal diameter of 1 cm and an active length of 22 cm	5 µg/L	75	71			10	[140]
	a full-scale GAC (Volume: 1900 m^3)., The GAC used had the following properties: 0.50 g/mL apparent density, 1.0 mm effective size, 920 mg/g iodine number	Estradiol: 2 ± 1 ng/L Diclofenac: 10 ng/L	98			100		[141]
BAC filtration	Media: GAC; media height: 80 cm; diameter: 22.5 cm; Empty bed contact time: 18 min	3 µg/L	91					[145]
	The surface area, total pore volume and micropore volume of the activated carbon are 800 m^2/g, 0.865 cm^3/g and 0.354 cm^3/g, respectively.	Diclofenac: 1700 ng/L Naproxen: 1500 ng/L 4n-Nonylphenol: 1400 ng/L	76.5	80	92.9			[146]
Activated carbon	Dose: of 20-160 mg/L, the response time: 30 h	4.68 ± 0.89 ng/L				83.33		[133]
Clay-starch	Clay dosage: 0-60 mg/L of Smectite, Starch dosage: 20 mg/L of Nalco Starch EX10704	Diclofenac: 30.6 ng/L Naproxen: 12.8 ng/L	53.00	22				[148]

2.3. Membrane filtration *for tertiary MPs removal*

In wastewater reclamation, microfiltration (MF) and UF membranes are often used for tertiary treatment of WWTPs to obtain a high-quality effluent for some applications such as groundwater recharge or reuse for irrigation and industry especially for areas suffering from the water shortage. These membranes ensure an efficient removal of suspended solids and disinfection [41]. However, they cannot generally retain MPs because the molecular weight of the most of the MPs range between 200 and 800 Da while typical molecular weight cut-off (MWCO) of MF and UF membranes are well above several thousand Daltons. Size exclusion of MPs in MF and UF membranes, therefore, cannot occur. However, the initial adsorption of MPs to membrane surface may occur which cannot be interpreted as removal rate since the concentration of solute in permeate will gradually increase after a short time [65]. Snyder et al. [154] concluded that the vast majority of pharmaceuticals (Diclofenac, Carbamazepine, Ibuprofen, etc.) spiked to a secondary effluent were not rejected when passing through an UF system, although estrogens (Estradiol, Estrone and Ethinylestradiol) were well removed (91–99%) which was attributed to their relatively high sorption properties, even though other compounds as for example Galaxolide did not follow this pattern [154]. Jermann et al. [155] investigated the fate of Ibuprofen and 17ß-Estradiol during an UF process and the effects of fouling by natural organic matter (NOM). Without NOM, UF with hydrophilic membrane showed insignificant removal for Ibuprofen and low removal for 17ß-Estradiol (~8%), while hydrophobic membrane retained much larger amount of 17ß-Estradiol (~80%) and Ibuprofen (~25%). The higher retention of 17ß-Estradiol was attributed to the higher Carbon–Water Partitioning Coefficient (K_{oc}) value of the compound [155]. The integration of MF or UF membranes with NF or RO membranes is, therefore, essential for enhanced elimination of MPs. As an example, Garcia et al. [156] combined MF with RO to remove MPs for effluent reuse. MF alone was found to be able to reduce the concentrations of some compounds, such as bis-(2-ethylhexyl) phthalate (DEHP) by more than 50%. With the combination of MF with RO, the removal efficiency was dramatically improved, ranging from 65% to 90% for most MPs [156].

If membrane filtration is required as a post-treatment technique for an efficient removal of MPs, pressure-driven membranes i.e. NF and RO membranes constitute an interesting alternative [41] that have attracted a great interest because of high removal rates of low molecular weight MPs, excellent quality of treated effluent, modularity and the ability to integrate with other systems. A lower energy consumption and higher permeate fluxes for NF membranes in comparison to RO membranes have encouraged the use of NF membranes for several commercial purposes, such as wastewater reclamation, water softening, and desalination [157,158]. Also for MPs removal, NF membranes are seen as a more cost effective alternative to RO membranes [65,67]. Yangali-Quintanilla et al. [159] compared the various MPs removal by NF and RO membranes. The elimination efficiency of NF membranes was very close to that achieved by RO membranes. The average retention efficiency by the tight NF was

82% for neutral MPs and 97% for ionic compounds, while RO was able to achieve 85% removal of neutral contaminants and 99% removal of ionic contaminants [159].

Table 9 summarizes the efficiency of membrane technologies for the removal of target MPs from secondary-treated municipal wastewater. Nevertheless, prediction of compounds removal is quite difficult since it depends on physico-chemical properties of the compound, membrane properties, membrane-solute interactions and also influent matrix [42,160]. Regarding the usage of NF membrane in the present study, the mechanisms of solute transport in NF membranes including electrostatic interaction, hydrophobic interaction and size exclusion are briefly discussed in following sections. Although many researchers have focused on these mechanisms, still further studies are required to understand the mechanism which is affected by solute properties, membrane parameters, feed water composition and operating parameters [65]. The key membrane properties affecting rejection identified include MWCO, pore size, surface charge, hydrophobicity, and surface roughness. In addition, water characteristics such as pH, ionic strength, hardness, and organic matter also have an influence on solute rejection [46].

Table 9. The efficiency of membrane filtrations for target MPs removal (%) from secondary-treated municipal wastewater found in the literature

Type of membrane	The main properties	Initial concentration of MPs	Diclofenac	Naproxen	4n-Nonylphenol	17ß-Estradiol	Ibuprofen	Reference
UF	Polyethersulfone flat-sheet, 100 kDa; TMP = 0.5 ± 0.01 bar	100 ng/L				80	25	[155]
	a dead-end UF unit at an average flow-rate of 2.5 m^3/h	2.9 µg/L		12.4				[161]
FO	The supplier: Hydration Technology Innovations (HTI, Albany, OR)	10 µg/L		100				[162]
NF	Polyethersulfone NF, TMP = 0.3-0.7 bar, Permeability: 1.4-7.3 L/m^2.h.bar	0.5 - 1 µg/L	60	60				[163]
NF 200	Operating flux: 13 L/m^2.h, 483 kPa	7-18 µg/L			70	70		[159]
NF 90	Operating flux: 13 L/m^2.h, 345 kPa				80	90		
NF (TFC-SR2)	Operating flux: 500 ± 20 L/h, TMP: 5 bar, at 25 ± 2 ºC	Diclofenac: 0.3 µg/L Naproxen: 0.3 µg/L Ibuprofen: 1 µg/L	60	62			55	[160]
NF270			95	95			90	
NF (SelRO)			100	100			95	
NF (NE 40)	MWCO: 1000 Da, Cross flow velocity: 6 µm/s	Ibuprofen: 110 ng/L Naproxen: 82 ng/L Diclofenac: 138 ng/L	86.1	44.3			39.1	[164]
NF (NE 70)	MWCO: 350 Da, Cross flow velocity: 8 µm/s	70 ng/L					27.3	
NF (NE 90)	MWCO: 210 Da, Cross flow velocity: 10.9 µm/s	50 ng/L					96.9	
NF 90	flow rate of 500-700 L/h., TMP: 5 bar	15 µg/L					99-100	[165]
NF 90	Pure water permeability: 2.49 L/m^2 d kPa, applied feed pressure: 414 kPa	0.3 µg/L	100	98			100	[166]
NF 200	Pure water permeability: 1.20 L/m^2 d kPa, applied feed pressure: 345 kPa		100	95			95	
Polyelectrolyte multilayers-based NF	NF membranes made by layer by layer (LbL) assembly of weak polyelectrolytes (TMP: 1.5 bar, Cross-flow velocity: 4.5 m/s)	Diclofenac: 0.5 µg/L Naproxen: 2.5 µg/L Nonylphenol: 7 µg/L Ibuprofen: 40 µg/L	77	55.6	70		48	[167]
RO	Filmtec TW30; TMP = 9.5–10.2 bar	7-18 µg/L	95					[159]
	a low pressure gradient: (ΔP = 11 bar)., and constant feed flowrate: 2.4 m3/h	Naproxen: 2.9 µg/L		98.2				[161]
	No detail is given about the RO membranes.	Nonylphenol: 0.66 µg/L Naproxen: 0.06 µg/L Diclofenac: 0.63µg/L	98.4	83.3	66.7	100		[59]

2.3.1. The role of size exclusion

Size exclusion (steric hindrance) is defined as a sieving mechanism in which solutes with size larger than the MWCO of the membrane are efficiently retained, whereas smaller solutes may pass through the membrane [168]. In the aspect of MPs retention by NF membranes, the rejection of uncharged and hydrophilic compounds is seen to be influenced by steric hindrance/size exclusion [169]. Radjenovic et al. [170] studied the rejection of several pharmaceuticals by NF90 membranes in a water treatment plant. They revealed that because the molecular weight of Acetaminophen (an uncharged compound at neutral pH) was lower than MWCO of the employed NF membrane, its rejection was obtained low (~ 45%). On the other hand, Diclofenac (a negative compound at neutral pH) with its higher molecular weight had the highest rejection (~100%). However, low rejection of Gemfibrozil (~ 50-70%) despite its high molecular weight and the presence of charge repulsion effect was surprising [170]. Kimura et al. [171] demonstrated through rejection experiments with pharmaceuticals that the rejection of uncharged compounds was influenced by their molecular size. However, their next study revealed that steric hindrance may not be the only factor to quantify the rejection of organic MPs [172].

Often, molecular weight is used to reflect molecular size. However, it does not truly reflect the size [67]. Consequently, spatial dimensions of MPs such as molecular length [67], molecular width [173,174] and recently minimum projection area (MPA) [63,175] are also under the attention for studying the rejection behavior of the NF membranes. MPA, calculated from van der Waals radius, is defined as the smallest two-dimensional projection area of a three-dimensional molecule. By projecting the molecule on an arbitrary plane, two-dimensional projection area can be calculated and the process is repeated until the minimum of the projection area is obtained [63].

Quintanilla et al. [67] concluded that rejection of hydrophilic neutral solutes such as Acetaminophen, Phenacetine and Metronidazole can be linearly correlated to their molar volume and molecular length, but no correlation was observed between their rejections and equivalent width [67]. Conversely, Agenson et al. [176] observed a better correlation between the rejection of above-mentioned MPs and their relevant molecular width [176]. Similarly, Kiso et al. [177,178] performed rejection experiments using hydrophobic compounds including aromatic pesticides, non-phenolic pesticides, and alkyl phthalates with NF membranes and concluded that compound rejection was correlated significantly with molecular width in addition to compound hydrophobicity [177,178]. Fujioka et al. [63,175] and demonstrated that the MPA is a better surrogate parameter to assess the rejection of hydrophobic neutral (e.g. Bisphenol A) and positively-charged MPs (e.g. Atenolol) by both ceramic and polymeric NF membranes in comparison to the molecular weight. In contrast, the rejection of negatively charged MPs (e.g. Naproxen and Ibuprofen) was independent of their MPA [63,175]. These findings prove that MPs retention by NF membranes is not solely governed by the molecular geometry, and other rejection mechanisms should be also taken into account.

2.3.2. The role of electrostatic interaction

To date, the rejection of uncharged MPs by NF membranes is considered to be predominantly caused by size exclusion, while charged molecules are also rejected by the electrostatic interactions with the charged membranes [42]. The most of the thin film composite NF membranes have a negatively-charged surface at neutral pH due to deprotonated acidic functional groups which are added during the manufacturing process to increase the selectivity and water permeability [179]. A couple of studies on the issue of electrostatic interactions between membrane surface and charged organic solutes have reported high rejection values for negatively-charged MPs, which could be explained by electrostatic repulsion between a negatively charged membrane surface and negatively charged solutes. In the case of positively-charged MPs attractive forces between the solutes and the negatively charged membrane surface cause an increase in the concentration of solute at the membrane surface, and therefore lead to lower observed rejections [170–172,180–182]. Verliefde et al. [181] examined the removal of several pharmaceuticals by means of negatively-charged Trisep TS-80 and Desal HL NF membranes operated at low recovery of 10%. They concluded that size exclusion was the main mechanism for rejection of neutral compounds, but higher and lower rejection of negatively and positively-charged compounds was attributed to electrostatic repulsions and attractions, respectively [181].

In NF membranes, the rejection of charged MPs is, however, dependent on the feed water parameters such as pH [179], divalent cations [179,183], and also NOMs of the feed water [184,185]. Both membrane surface charge and MP charge vary according to the pH of feed water by the dissociation of the functional groups as a function of the solutes disassociation constant (*pKa*) [179]. The presence of divalent cations appears to act as a “shield” and thus reduces the effective membrane surface charge [179,183]. In the case of organic matters, some studies have reported an increased, others a decreased negative membrane surface charge due to the deposition of NOMs [184,185]. Comerton et al. [186] studied the effect of NOM and divalent cations (Ca^{+2} & Mg^{+2}) on the rejection of pharmaceuticals from an MBR effluent by NF membranes. They observed that divalent cations did not have important effects on the rejection of Acetaminophen and Carbamazepine (uncharged MPs), but significantly decreased the rejection of Gemfibrozil (a charged MP). On the other hand, NOM increased the rejection of these pharmaceuticals [186]. Majewska-Nowak et al. [187] indicated that pesticides such as Atrazine could adsorb to organic matter present in the feed water, increasing rejection as a result of increased size and the electrostatic interaction between the organic and the membrane.

From bibliographic review, it seems that less knowledge is still available on the rejection behavior of negatively-charged NF membranes for positively-charged MPs. It is interesting to see whether electrostatic interactions will also play a role in their high removal or whether rejection will be mainly determined by steric effects [181].

For uncharged MPs, intrinsic physicochemical properties of the molecules can substantially affect their retention in NF membranes [168,188]. For example, high retention of Carbamazepine (an uncharged molecule at neutral pH) by a tight NF membrane was attributed to its high polarity (represented by the dipole moment) in the research of Nghiem et al. [188]. The authors concluded that polarity can influence the separation of molecules that are cylindrical in shape because they can be directed to approach the membrane pores head-on due to attractive interaction between the molecule polar centers and fixed charged groups on the membrane surface. They also indicate that this phenomenon is probably inherent for high dipole moment organic MPs, and the governing retention mechanism remains steric in nature [188].

2.3.3. The role of hydrophobic *interaction*

Polymeric NF membranes are usually hydrophobic in nature. Hydrophobic MPs can thus adsorb onto these membranes. The higher hydrophobicity of a compound results in the higher adsorption onto the membrane surface, especially when compounds are electrostatically neutral [42]. No strong correlation has been observed between the hydrophobicity of negatively charged MPs and their rejection due to charge repulsion that prevented solutes from approaching the membrane surface [66]. Many hydrophobic organic MPs are also able to form hydrogen bonds with membrane surface which probably conduct to the adsorptive mechanism [189]. For instance, Han et al. [190] showed that Estrone can form hydrogen bonds with polyamide resulting in initial retention due to adsorption [190].

When wastewater is, however, used as feed solution, the existing interactions between the molecules and membranes may be influenced by the effluent organic matters and then the separation mechanism of MPs could not be simply attributed to the sieving effect and surface charge. In this case, hydrophobic interactions that take place between the fouled membrane surface and such solutes gain predominance [160]. Regarding the hydrophilic or hydrophobic character of MPs, the octanol-water partition coefficient (K_{OW}) can be used as an indicator of hydrophobicity. This parameter is usually considered as a pH independent factor and only reflects hydrophobic interactions. But unlike other properties of target compounds, hydrophobicity is strongly linked to electrostatic interactions, surface complexation or hydrogen bonding, which can significantly change with variation of pH, especially the *pKa* [160]. As a result, a pH-corrected value of log K_{OW}, known as log D, has been employed in this study to predict the MPs' hydrophobicity and it can be defined as the ratio between the ionized and unionized form of the solute at a specific pH value (here the pH is adjusted at 7) [62]. Compounds with log D>2.6 are referred as hydrophobic, and hydrophilic when log D ≤ 2.6 [162]. In the present work, Diclofenac, Naproxen and Ibuprofen are hydrophilic ($\log_{D}$: 1.77, 0.34 and 1.44, respectively [65]), while 4n-Nonylphenol and 17ß-Estradiol ($\log_{D}$: 6.14 and 4.15, respectively [62]) are hydrophobic compounds.

In the research of Dang et al. [62] on a loose NF membrane (NF270), most of the hydrophobic MPs significantly adsorbed onto the membranes after 24 hours of filtration, while the hydrophilic compounds exhibited much lower and more variable adsorption levels. They adsorbed much less compared to hydrophobic species and many compounds did not adsorb onto the membrane at all pH conditions employed [62]. In contrast, Braeken et al. [191] who studied the fate of MPs in a tight NF membrane (Desal-HL) reported that hydrophilic compounds are solvated in water phase and consequently their effective diameter might be larger. Therefore, on a size exclusion basis, hydrophilic compound could be rejected more effectively than hydrophobic ones [191]. In a good agreement with the outcomes of Dang et al. [62] and Braeken et al. [191] previously discussed, Comerton et al. [186,192] concluded that the effect of hydrophobicity was more apparent for NF membranes with larger pores than NF with smaller ones because larger pores allowed compounds to access adsorption sites in the membrane surface and pores [186,192].

The removal of several steroid hormones including 17ß-Estradiol and Estrone by two different NF membranes (NF90 and NF270) was studied by Nghiem et al. [193]. At the first stages of filtration, adsorption (or partitioning) of hormones to the membrane polymer was the dominant removal mechanism. The final retention stabilized when the adsorption of hormones into the membrane polymer has reached equilibrium because of the limited adsorptive capacity of the membrane. The overall hormone retention was eventually lower than that expected based solely on the size exclusion mechanism. That behavior is attributed to partitioning and the subsequent diffusion of hormone molecules in the membrane polymeric phase, which ultimately resulted in a lower retention [193,194]. Consequently, a precise evaluation of a NF membrane in terms of the rejection of a hydrophobic MP is not possible until saturation of the membrane with the compound is accomplished. In other words, a relatively large volume of MPs-bearing feed water must be filtered to reach saturation conditions to avoid an overestimation of rejection [194].

2.4. Biological treatment for tertiary MPs removal

Many currently used tertiary treatment processes still exhibit unsatisfactory levels of MPs removal, and may produce some by-products that can be even more harmful than the parent molecules. As a result, advanced MPs-oriented wastewater treatments have lately become an area of active research focus [48]. In Table 10, the results of our literature review about the capability of biological-based systems for tertiary MPs removal are given. These methods are often hybrid systems e.g. MBRs (activated sludge/membrane filtration), wetlands (biological/sorption/filtration), and bio-filters (biological/sorption/filtration). In areas where there is sufficient land, wetlands have been often used for tertiary MPs removal, mainly due to their simplicity of operation and cheapness. On the other hand, the lower attention of researchers paid to the activated sludge-based reactors perhaps comes from the low amount of carbon and nutrients of the secondary-treated wastewater providing an unfavorable condition for an appropriate growth of microorganisms. Such a reason might explain why there are only few papers in the literature, dealing with tertiary activated sludge-based reactors. A review on the biological-based methods is given in following sections, with an emphasis on their advantage/disadvantages for MP removal.

2.4.1. Wetlands

Over the last decades, constructed wetlands have been applied to wastewater treatment, due to their advantages including simplicity, eco-friendliness, and low operation and maintenance costs. These systems containing water, substrate, plants, and native microorganisms are able to efficiently remove total suspended solids, organic matter, nutrients and metals [195]. Constructed wetlands are classified according to the hydrology (water surface flow and subsurface flow); plants growth form (free-floating, floating leaved, and submerged); and flow path (horizontal and vertical)., and three main types of i) surface flow (SF), horizontal subsurface flow (HSSF) and vertical subsurface flow (VSSF) constructed wetlands are often used for wastewater treatment [196].

Constructed wetlands have also shown a good capability for the elimination of a broad range of MPs from the secondary-treated wastewater by means of physical, chemical and biological processes, such as volatilization, sorption and sedimentation, photodegradation, plant uptake and microbial degradation. In addition, wetland's design and operating parameters such as configuration, water depth, plant species, operating mode (batch or continuous) and HRT can also affect the removal of pollutants [7,43]. According to the Zhang et al. [43], SF wetlands show better performance for compounds susceptible to photodegradation because water can be directly exposed to sunlight, while SSF systems have a higher potential to eliminate biodegradable compounds, because their substrate promotes higher adsorption and interactions between wastewater, soil, plants and microorganisms [43]. Compared to the HSSF, VSSF wetlands have an enhanced microbial degradation as result of a higher oxygenation originated by the wastewater drainage in the different soil layers [7]. Regarding Table 10, free water SF systems seem more efficient for tertiary MPs removal, because of the larger storage capacity as compared to SSF constructed wetlands [43].

Table 10. The efficiency of biological treatments for target MPs removal (%) from secondary-treated municipal wastewater found in the literature

Treatment type	Brief description of the treatment	Initial MPs concentration	Diclofenac	Naproxen	4n-Nonylphenol	17ß-Estradiol	Ibuprofen	Reference
Wetland	a pilot-scale subsurface flow wetland, flow rate: 11.4 m^3/d, HRT: 4 d	32.80- 55.54 ng/L				27		[197]
	a pilot-scale floating aquatic wetland, flow rate: 11.4 m^3/d, HRT: 4 d					13		
	A full-scale free water surface constructed wetland, Gravel depth: 0.3-0.4 m, loading rate: 100 m^3/d	Diclofenac: 35 g/d Naproxen: 10 g/d Ibuprofen: 5 g/d	73-96	52-92			95-96	[198]
	A full-scale free water surface constructed wetland, hydraulic loading rate: 1800 m^3/d	Diclofenac: 100-400 ng/L Naproxen: 100 ng/L	38-87	~80				[199]
	A full-scale free water surface constructed wetland, Water depth: 0.4-1.5 m, loading rate: 250 m^3/d	Diclofenac: 1.25 µg/L Naproxen: 0.34 µg/L Ibuprofen: 0.04 µg/L	85	72			96	[200]
	A full-scale free water surface constructed wetland, loading rate: 1620-48000 m^3/d	Diclofenac: 0.004-0.51 µg/L Naproxen: 0.064-0.29 µg/L Ibuprofen: 1.2-0.66 µg/L	30-36	34-75			38-88	[201]
	A full-scale and batch-mode vertical subsurface flow constructed wetlands, HRT: 3 h, Gravel depth: 1 m.	3-9 µg/L	84.00	84			89	[202]
	A full-scale and batch-mode Horizontal subsurface flow constructed wetland, HRT: 7 d, Water depth: 0.3 m.	2 µg/L					82-96	[203]
	A full-scale hybrid polishing pond and free water surface constructed wetland, depth of gravel: 0.2-0.5 m, loading rate: 3700 m^3/d	Diclofenac: 0.5-1.2 µg/L Ibuprofen: 40-60 µg/L Naproxen: 0.5 µg/L	86-98	72-96			79-97	[204]
	A full-scale hybrid wetland-ground water flow-through system, Water depth: 2-3 m	180 ng/L				67		[205]

Continue of Table 10. The efficiency of biological treatments for target MPs removal (%) from secondary-treated municipal wastewater found in the literature

Treatment type	Brief description of the treatment	Initial MPs concentration	Diclofenac	Naproxen	4n-Nonylphenol	17ß-Estradiol	Ibuprofen	Reference
Bio-filter	a soil bio-filter column with 14.15 m of height (including 12 m of saturated zone (medium sand), and 2.15 m unsaturated zone (fine sand)) and 80 mm of inner diameter	Diclofenac: 2 µg/L Ibuprofen: 3.4 µg/L	33				96	[206]
	Media (quartz sand: 0.210–0.297 mm particle size)., HRT: 5 h., hydraulic loading rate: 0.012 m^3.m^{-2}. h^{-1}	0.24 ± 0.047 µg/L	41.00					[207]
	a pilot-scale bio-filter filled by sand (height: 3m, internal diameter: 22.5 cm), Empty bed contact time: 30-120 min	2 µg/L	20.00					[145]
Algal bioreactor	a pilot-scale algal bioreactor, algal strain: *Scenedesmus dimorphus*, HRT: 24 h	0.2-17 ng/L				70		[208]
	a lab-scale bioreactor in batch mode, algae genera: *Anabaena cylindrica*, *Chlorococcus*, *Spirulina platensis*, *Chlorella*, *Scenedesmus quadricauda*, and *Anaebena var*	1 µg/L				54		[209]
	a pilot-scale microalgae-based reactor., a surface loading rate of 7-29 g of COD m^{-2}. d^{-1}, HRT: 4-8 d	9 µg/L	40-60	60-90			90	[210]
MBR	The hollow fibre polyvinylidene fluoride membrane modules (nominal pore size: 0.04 µm, total membrane area: 182.9 m2), MLSS: 11.5 g/L	4n-Nonylphenol: 4.2 ng/L 17B-Estradiol: 144.3 ng/L			50	86.7		[211]
	a pilot-scale tertiary MBR system equipped by hollow-fiber UF membrane (pore size: 0.03-0.1 µm), HRT: 10 h, SRT: 20-25 d.	80 µg/L	7 µg.g VSS^{-1}h^{-1}	71µg.g VSS^{-1}h^{-1}			248 µg.g VSS^{-1}h^{-1}	[212]
	No detail is given about the MBR.	4n-Nonylphenol: 0.66 µg/L Naproxen: 0.06 µg/L Diclofenac: 0.63 µg/L	35	50	60			[59]
MBBR	lab-scale polishing MBBRs with intermittent feeding, filling ratio: 50% (AnoxKaldnes K5 carriers), HRT: 4 h	3-20 µg/L	100.00				100	[213]
	lab-scale Polishing MBBRs with adding humic acid (30 mgC/L) to the effluent, filling ratio: 23% (AnoxKaldnes K5 carriers)	1.2-14.6 µg/L					100	[97]

2.4.2. *Bio-filters*

Bio-filters are often defined as columns filled by a media or even combination of medias such as sand, soil, GAC, wood chips, straw or peat in order to support the growth of microorganisms as well as provide sorptive sites for the enhanced pollutants removal [214,215]. So far, this cost-effective process has been mostly used for decentralized storm water and wastewater treatment [215]. Excellent removal of particulate matters, nutrients and heavy metals from storm waters is reported in bio-filters in several studies [216,217]. Such systems have been also used for wastewater treatment [218,219]. In the research of Heistad et al. [218] on a septic tank followed by a bio-filter, 97% of BOD_7, 30% of total nitrogen, 99.4% of total phosphorous, and 70.8% of total suspended solids were removed from the outlet of septic tank. Meanwhile, no *Escherichia coli* or somatic coliphages was detected in the effluent of bio-filter during three years of operation [218]. Hoang et al. [219] investigated the performance of a bio-filter filled by GAC for removing organic matter from wastewater. The results show that performance of GAC bio-filters decreased with shallower filter bed depths. Furthermore, the GAC bio-filter performed better at lower influent concentration and lower filtration rates. The daily backwash adopted to avoid the physical clogging of the bio-filter did not have any significant effect on the organic removal efficiency of the filter [219].

So far, few studies have been published on the potential of such systems for tertiary MPs removal. For instance, Ternes et al. [206] demonstrated that under certain conditions bio-filters (soil filtration) can be utilized for the high elimination of several pharmaceuticals such as Ibuprofen (~ 96%) and Bezafibrat (~ 97%). A moderate removal for Clarithromycin (~ 54%) and Clofibric acid (~ 52%), a low removal for Diclofenac (~ 33%) and no elimination for some compounds like Carbamazepine and Diatrizoate from a real wastewater already treated in a municipal WWTP were also observed [206]. The elimination of several recalcitrant MPs from secondary-treated wastewater was studied by Escolà Casas and Bester [207]. By operating the set-up at a hydraulic loading rate of 0.012 m^3. m^{-2}. h^{-1}, the reactor removed 41%, 94%, 58%, 57% and 85% of Diclofenac, Propranolol, Iopromide, Iohexol and Iomeprol respectively. For these compounds, the removal efficiency was dependent on HRT. Only 59% and 21% of the incoming Tebuconazole and Propiconazole respectively were removed but their removal did not depend on HRT [207].

In the modified type of the above-mentioned system, plants or reeds are also implanted on the top of the biofilter. This so-called "biologically activated soil filters" are very similar in principle to SSF constructed wetlands, in which, wastewater is infiltrated through beds of sand and gravel with reeds growing on top. Chemical constituents are retained within the filter matrix by forces of sorption. Within the soil filter system, the compounds are also exposed to chemical transformation or biodegradation by soil microorganisms and plants [214,220]. Several works have focused on the performance of such systems for MPs removal from wastewater [214,215,220]. Janzen et al. [220] investigated the performance of a pilot-scale biofilter made of peat, sand and gravel. The upper layer was planted with a reed named *phragmites australis* to prevent clogging and was spiked with activated sludge to enhance microbial biomass and biodegradation potential. MPs-bearing synthetic wastewater was then fed into the set-up, operated at a low hydraulic load (61 L. m^{-2}. d^{-1}) and HRT of 48 h. The elimination of all tested industrial MPs such as Butylated hydroxytoluene, Dibenzyl and Benzophenone were obtained more than 97%. The analysis of the peat layer was subsequently performed to find out whether sorption or biodegradation processes are predominant. For the compound Butylated hydroxytoluene, only a minor fraction of the input was found in the peat layer, thus sorption to the peat layer was not predominant for this compound., and probably chemical transformation or biodegradation occurred. The rest of compounds were found with a high concentration in the peat layer, revealing that sorption was the predominant removal mechanism [220]. In another research by Bester et al. [215], a similar bio-filter was evaluated for removal of biocides like Diuron and Terbutryn. By applying the hydraulic loading rate of 61 L. m^{-2}. d^{-1}, the removal rate of these compounds were achieved by 82% to 100% [215]. A moderate to high removal (64%-99%) for Xenobiotics such as Benzothiazoles and Triclosan was also observed by Bester et al. [214] who worked with the same bio-filtration set-up [214]. Unfortunately, according to our literature review, no work has been yet carried out on the issue of tertiary MPs removal by the plant-based biofilters.

Perhaps, the most innovative type of bio-filters is sequencing batch biofilter granular reactor (SBBGR) that is characterized by high biomass concentration (up to 40 g.L^{-1}), high sludge retention times (up to 6 months) and low sludge production [221,222]. Balest et al. [222] compared two systems of a pilot-scale SBBGR and a full scale WWTP (CAS treatment process) for the elimination of several endocrine disrupter compounds and steroid hormones from wastewater. The results showed SBBGR achieved higher removal efficiency than the CAS process for all tested MPs. The removal efficiencies for Bisphenol A, Estrone, 17ß-Estradiol and 4-tert- Octylphenol were 91.8%, 62.2%, 68% and 77.9% for the SBBGR system and 71.3%, 56.4% 36.3% and 64.6% for the CAS process, respectively. The authors attributed the excellent performance of the SBBGR to the high sludge age (~160 d) achieved in the reactor [222]. In literature, there is no publication yet in the case of tertiary MPs removal by SBBGR systems.

Regardless of the fact that there are no enough publications in the aspect of tertiary MPs removal by the biofilters, it appears that such systems are not still considered as efficient systems, in particular when removal of persistent MPs in desired. Although this approach is too space intensive, but is a more cost-effective treatment option in decentralized and small applications than AOPs, MBRs and membrane filtrations. Meanwhile, it should be noted that when disassembling such bio-filter the peat layer may be contaminated and not so easy to dispose of [214].

2.4.3. Algal bioreactors

In a coupled WWTP- algal bioreactor system, secondary-treated wastewater provides a growth medium rich in macro and micro nutrients for algal growth [223]. The algal ponds/bioreactors are shallow raceway reactors in which microalgae and bacteria grow in symbiosis. In such systems, organic matter is degraded by heterotrophic bacteria, which consume oxygen provided by microalgal photosynthesis; therefore, no aeration is needed [224]. As one of the innovative applications, harvested algal biomass can be then used for the production of biofuels and biogas [223,225]. Except this benefit, considerable potential of the algal section is also reported in few studies for the purpose of MPs polishing, mostly by two mechanisms of biodegradation and photolysis [210,226]. Also, Tam et al. [227] revealed that bio-sorption (the physico-chemical adsorption that occurs at the cell surface) was an important removal mechanism of a biocide named Tributyltin in both dead and living algal cells. Interestingly, dead cells were generally more efficient in removing Tributyltin during a three-day exposure [227], probably due to the reactor's high SRT where more dead but sorption-friendly cells are found as compared to the low SRT.

More than fifty years ago, the contribution of microalgae in bioremediation of phenolic compounds has been proposed by Oswald et al. [228], but over the last two decades capabilities of some algae species for biodegradation of phenolic compounds gained interest [229]. Despite the acute toxicity of phenols for some algae species, both cyanobacteria and eukaryotic microalgae such as *Chlorella* sp., *Scenedesmus* sp are capable of biotransforming phenolic compounds [230].

In the lab-scale trials, toxicological studies reviewed by Faramarzi et al. [231] indicate that some green microalgae can mediate transformation of steroid hormones [231]. Other studies have shown that *Chlorella sorokiniana* can greatly reduce Salicylic acid added to a synthetic medium up to ~ 93% [232], and several other MPs such as Diclofenac and Ibuprofen from urine and anaerobically digested black water by around 60-100% [226].

In the pilot or full-scale trials, the success of algal bioreactors is also proved by some researches. For instance, de Godos et al. [233] showed that levels of veterinary antibiotics such as Tetracycline can be reduced by 69% in a high-rate algal pond coupled with a WWTP [233]. Moreover, in the research of Matamoros et al. [210] involving growing algae on a municipal wastewater in a pilot-scale high-rate algal pond during cold and warm seasons, the ability of algae to remove emerging organic contaminants was demonstrated. In their study, MPs removal efficiencies were enhanced during the warm season, while the HRT effect on MPs removal was only noticeable in the cold season. The authors then reported the average removal percentages of 40-60%, 60-90%, and more than 90% for Diclofenac, Naproxen and Ibuprofen, respectively [210].

As a whole, although Algal–bacterial systems seem efficient for the tertiary elimination of MPs but the high land requirement of open systems, the high construction costs of enclosed photo-bioreactors, and the difficulty of harvesting the biomass include their main disadvantages. Nevertheless, when parameters of the safety and energy savings are considered, the additional costs brought from by land use, reactor construction and biomass harvesting will be justified [234].

2.4.4. Membrane bioreactors (MBRs)

Among the various wastewater treatment processes, the MBR, composed of a membrane and activated sludge process, is one of the most effective wastewater treatment processes, due to its high quality effluent and small foot print [235]. Today's experience indicates that this process is strongly able to treat wastewater e.g. Xing et al. [236] reported that a pilot-scale MBR (using a ceramic tubular UF membrane) can remove approximately 97% of COD, 96% of ammonia nitrogen, and 100% of total suspended solids from an urban wastewater [236]. Indeed, the superiority of the MBR system over CAS in terms of basic effluent quality has been widely reported [140,237]. By the aspect of MPs polishing, MBR systems implemented as an end-of-pipe polishing step in the effluent of existing WWTPs appears to be a logical means to prevent MPs dispersion in the environment via insufficiently treated wastewater [140]. The high solids retention time (SRT) and high accumulation of active biomass found in MBR systems make it possible to create an adapted microbial community with high ability to remove MPs [46,238]. The high SRT obtained in MBRs allows MPs to be removed mainly by means of adsorption followed by biodegradation mechanisms [239].

Although better removal of moderate to high biodegradable MPs is seen in MBR reactors [238], significant variation in MBR removal performance has been also noted, particularly for biologically persistent hydrophilic compounds [50,240]. In the research of Reif et al. [241] who investigated the removal of several pharmaceuticals in a MBR operated in SRT of 44-72 d, Ibuprofen and Naproxen were removed by 98% and 84%, respectively, while a very low removal (< 9%) was observed for recalcitrant Carbamazepine and Diclofenac. They also concluded that the biodegradation has been the dominant mechanism in removal of all pharmaceuticals [241]. The outcomes of the study of Joss et al. [54] demonstrated that there is no significant advantage of the MBR compared to the CAS process operating at similar operating conditions on the elimination of recalcitrant MPs [54]. Recalcitrant behavior of Carbamazepine, Diazepam, Diclofenac, and Trimethoprim in MBRs was also reported by Serrano et al. [143]. It is likely that operating conditions could play a salient role in these cases, where the characteristics of MBRs (i.e. high biomass concentration, high SRTs, etc.) promote the development of slowly-growing bacteria for sustained biodegradation of refractory compounds when a sufficient acclimation time is applied [48]. The advantage of operating the MBR at very high SRT values to promote the biodegradation of recalcitrant compounds is usually offset by the increased operating expenses associated with the higher oxygen requirements of biomass [242]. The positive correlation between MPs removal and SRT in MBRs is shown in some studies [243,244]. As an example, Bernhard et al. [243] observed that by increasing the SRT from 20 d to 62 d, the removal of Diclofenac enhanced from 8% to 59% [243]. Similarly, the MBR with longer SRT of 65 d had a better performance than the

MBR with a shorter SRT of 15 d for the removal of Diclofenac (82% against 50%) in the study of Kimura et al. [244]. Generally speaking, MBRs show erratic results for removal of MPs, stating that different parameters are involved such as applied SRT, HRT, pH, temperature, MBR configuration, type of membrane used, type of wastewater, molecular structure of MPs, etc [245].

From bibliographic review, it appears that there is a lack of comprehensive study about the performance of tertiary (polishing) MBRs for the purpose of MPs removal. Arriaga et al. [212] compared three pilot-scale reactors of CAS, MBR and tertiary MBR in terms of MPs removal. Interestingly, the MPs biodegradation rates of all tested MPs was higher in polishing MBR compared to the rest of systems. In the polishing MBR, the biodegradation rates of Diclofenac, Naproxen and Ibuprofen was obtained up to 7.3, 71.1 and 247.9 µg.g $VSS^{-1}h^{-1}$, respectively, whereas, for the MBR the related values of 0.1, 0.4 and 2.7 µg.g $VSS^{-1}h^{-1}$, and for the CAS the corresponding values of 0, 0.8 and 2.6 µg.g $VSS^{-1}h^{-1}$ were observed. They also revealed that artificial addition of exogenous MPs during the start-up phase accelerates the adaptation of the biomass, leading to have a better performing tertiary MBR in terms of MPs removal [212]. In another work published by Wu et al. [211] who worked on a pilot-scale A^2O-MBR system treating municipal wastewater, MBR could remove 4n-Nonylphenol and 17ß-Estradiol up to around 50% and 86.7%, respectively. In this regard, calculated K_d values of the MPs showed that sludge adsorption significantly contributed to the removal of MPs as though K_d values of 4n-Nonylphenol and 17ß-Estradiol were reported by 58670 and 69080 L. kg_{SS}^{-1}, respectively [211]. It is important to note that MPs with K_d values > 40000 L. kg_{SS}^{-1} are compounds that will be highly removed by the sorption mechanism, according to the classification scheme proposed by Margot et al. [90] already shown in Table 4.

To say a huge obstacle preventing the wide implantation of MBR plants, we can refer to its economic aspect. MBR systems still remain more expensive compared to some types of the tertiary treatment methods, particularly for most of the small and decentralized schemes. The average specific energy requirements concerning MBR operation which have been reported are usually in the range of 0.6-2.3 $kWh.m^{-3}$ of treated effluent, depending on the size and operating conditions of the plant. Another major barrier is related to the staff expertise, as this process needs a skillful workforce, especially with respect to the process control, and operation/maintenance of membrane modules [242].

2.4.5. Biofilm reactors

In wastewater treatment, it is well documented that biofilm reactors outperform suspended biomass-based reactors [246,247]. As such, high biomass concentration and the presence of slow-growing bacterial strains found in biofilm reactors can increase the removal of a broad range of MPs [56]. Regarding the subject of this thesis (Chapters (II) and (III): Tertiary MBBRs), MPs removal in biofilm reactors is widely studied in the following part.

3. Tertiary MPs removal in biofilm reactors

3.1. Biofilm formation and development

Biofilms are complex biostructures that appear on all surfaces that are regularly in contact with water. They are structurally complex, dynamic systems with attributes of primordial multicellular organisms and multifaceted ecosystems [248]. To date, many researchers have found that the process of biofilm formation could be frequently affected by the environmental and operational conditions, such as carbon & nutrients availability, fluid velocity, MLSS, temperature, pH, and surface roughness [249]. According to the description given by Gottenbos et al. [250] and Andersson et al. [251], biofilm formation and development in aquatic environments, as simply illustrated in Fig. 8, include several steps. At first, a conditioning film comprising inorganic solutes and organic molecules is formed on the abiotic or biotic surfaces. This occurs prior to the arrival of the first microorganisms (Fig 8a). Then, microorganisms are transported to the surface and initial microbial adhesion occurs. At this step, several forces are involved including hydrophobic interactions and covalent, hydrogen and ionic bonding. The initially adhered cells rarely come in direct contact with the surface because of repulsive electrostatic forces, instead the secreted polymers link the cells to the surface substratum (Fig 8b). In the following step, attachment of adhering microorganisms is strengthened through the production of extracellular polymeric substance (EPS). This state will be irreversible in the absence of physical or chemical forces [250,251]. In general, EPS is a complex, high-molecular-weight mixture of polymers that are present in pure cultures, activated sludge, granular sludge and biofilms. The term EPS refers not only to extracellular polymeric substances but also to extracellular polysaccharides, exopolysaccharides and exopolymers. EPS is believed to play an important role in the physico-chemical properties of microbial aggregates via the promotion of the initial cell attachment to solid surfaces and the formation and maintenance of microcolonies and mature biofilm structures, in addition to protecting the biofilm from toxic substances (Fig 8c) [247,252,253] . The growth and development of attached microorganisms due to the cell division and recruitment of planktonic bacteria, combined with continued secretion of exopolymers, results in maturation of the biofilm. Here, the attached microorganisms consume the nutrients of the conditioning film and the aqueous bulk to grow and produce more EPS resulting in the formation of microcolonies. Ultimately, the microcolonies expand to form a layer covering the surface. The maturation of biofilm is slow and depending on the process takes several days or even months to reach structural maturity [254]. The final porous structure of the mature biofilm leads to a better substrate penetration into the deeper areas of the biofilm especially in a low substrate availability [255,256]. J. Guo et al. [257] concluded that porous biofilms are convenient for immobilizing of numerous microorganisms and perform well against the biofilm wash-out along with the effluent (Fig 8d). A mature biofilm is a vibrant structure, that continuously adapts itself to the surroundings. This means that microorganisms, under unfavorable conditions, may leave the biofilm community (i.e. detachment) in the search for a new and favorable habitat to settle down in. High fluid shear or other detachment forces are also involved at this stage (Fig 8e). Meanwhile, as the number of biofilm organisms increases, growth rates will decrease due to nutrient and oxygen limitations and accumulation of organic acids, eventually leading to a stationary biofilm thickness, where adhesion and growth counterbalance detachment [251]. M. Plattes et al [258] who developed a zero-dimensional biofilm model for dynamic simulation of MBBRs using Activated Sludge Model 1 (ASM1), proposed that detachment rate of the biofilm is equal to the biofilm growth rate in a steady state condition [258].

Regarding the behavioral complexity of the biofilm, biofilm models are becoming a salient research and engineering tool for researchers & designers who are interested in biofilm reactors [248]. Despite the outcomes of two research groups of i) Wanner et al. [259] who compared the existing biofilm models to give a consensus description and ii) Boltz et al. [260] who developed one dimensional biofilm models as an engineering tool, there is still a lack of comprehensive study about the biofilm models that are necessary for the development and implementation of biofilm reactors in real scales as well as future's research.

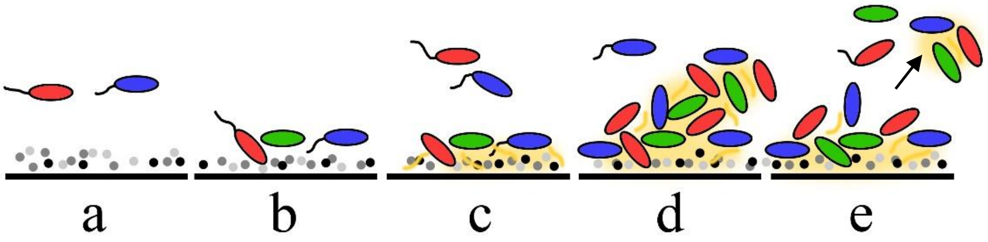


Fig. 8. Biofilm formation and development in aquatic environments, a: conditioning film, b: initial adhesion of microorganisms, c: attachment of microorganism, e: biofilm maturation, f: biofilm detachment [250,251].

3.2. Configurations of biofilm reactors

To date, it is well documented that biofilm reactors have surpassed suspended biomass-based reactors in regard to biomass productivity and wastewater treatment efficiency [246,247]. Operational flexibility, low space requirements, reduced HRT, resilience to changes in the environment, increased biomass residence time, high active biomass concentration, enhanced ability to degrade recalcitrant compounds as well as a slower microbial growth rate resulting in lower sludge production are some of the benefits with biofilm treatment processes [251]. In general, biofilm reactor configurations applied in wastewater treatment are divided into two main categories: i) fixed-bed reactors such as trickling filter, biological aerated filter, integrated fixed-film activated sludge (IFAS), and aerated submerged fixed-bed bioreactor (ASFBBR)., and ii) moving-bed reactors such as MBBR, rotating biological contactor (RBC) and fluidized bed biofilm reactor (FBBR) [261].

3.3. MPs removal in biofilm reactors

Regarding MPs removal, it seems likely that biofilm is a more promising technology for MP biodegradation than suspended biomass since it is now clearer that older and more mature biomass performs better. The only possibility to achieve the old age of biomass is to use biofilms [262]. For instance, Kim et al. [263] compared two pilot-scale systems of IFAS and CAS for the purpose of MPs removal from wastewater as well as effluent's overall estrogenic activity. Both systems performed similarly in terms of COD removal, but the IFAS system provided a better nitrification. Compared to the CAS system, five compounds (Bisphenol A, Triclosan, Carbamazepine, 4n-Nonylphenol and Octylphenol) demonstrated improved removal in the IFAS system. By means of a method called "yeast estrogen screen (YES) assay", the authors also demonstrated that the effluent estrogenic activity of the IFAS was 70% lower than CAS, proving a high Estrogen removal by IFAS. No explanation was given by the authors for this finding, but the higher SRT and the presence of attached biomass, in addition to the suspended biomass, are today recognized as the main reasons for the higher capability of IFAS as compared to the CAS.

A glance through the articles and reports published in the last decade exhibits an ever-increasing attention of researchers to the potential of biofilm reactors for an enhanced MPs removal from wastewater. The biofilm related publications are mostly experimental, and only a handful of studies are fundamental [86,106,107,248,258,264]. Hence, little is still known about the biomass capacity to remove MPs in biofilm reactors and whether this capacity differs from that of activated sludge process [265]. Here, we attempted to give an outlook of the recent findings that may help to have a better judgment about the efficiency of biofilm reactors for MPs removal from wastewater.

Beginning steps of the studies related to the MPs removal went hand in hand with some variable and erratic results. The influence of process parameters was not often evaluated in most of biofilm's studies, where only the elimination rate of MPs has been the main goal. For instance, Göbel et al. [266] found that a FBBR using polystyrene balls was rather inefficient in removing a broad range of MPs, with removal rates ranging from negative for Erythromycin to about 80% removal for N4-Acetylsulphamethoxazole [266]. Unfavorable performance of FBBR filled with a porous sintered glass carrier was also shown by González et al. [267] who observed no Diclofenac and Bentazone biodegradation within 25 days of operation at applied HRT of 11 h [267]. Accinelli et al. [268] evaluated the feasibility of using MBBRs filled by the carriers manufactured from cutting the screw neck of water bottles for the removal of several MPs from wastewater. Without the carriers, elimination rates for Bisphenol A, Oseltamivir and Atrazine were relatively low, i.e. 18% ,7% and 3.5%, respectively. While, addition of incubated carriers enhanced the removals by 34%, 49% and 66%, respectively [268]. The influence of process parameters such as HRT and SRT has been recently studied in several studies. In Table 11, in which the efficiency of biofilm reactors for MPs removal is summarized, applied process parameters are also given. Nevertheless, analysis of existing findings shows there is still a kind of data scattering in this filed. Hence, further researches seem necessary in order to understand the explicit role of process parameters on the issue of MPs removal by biofilm reactors.

According to Clara et al. [269], the effluent concentration of some organic MPs is dependent on the selected/operated SRT and independent of influent concentrations [269]. Indeed, in biofilm reactors, higher biomass concentration and the presence of slower growing species, both resulting from higher SRTs, have led to higher removal efficiencies of a broad range of MPs. Meanwhile, high SRTs are achievable in low HRTs in biofilm reactors [56]. Joss et al. [51] compared two systems of CAS and a submerged biofilm reactor (Biostyr™, VeoliaWater Technology) to evaluated the removal of Estrone, Estradiol, and Ethinylestradiol from an urban wastewater. The results show around 90% removal of all compounds in the CAS system, while only slightly lower removal of the compounds was observed in the biofilm reactor (77% for Estrone, and 90% for Estradiol and Ethinylestradiol). This finding becomes more interesting when we know that the biofilm reactor was operated at a low HRT of 35 min, against a much longer HRT of 12 h applied in the CAS system [51]. The shorter HRT in the biofilm reactor can be compensated by a higher biomass concentration and/or a higher MPs removal capacity per unit of biomass, probably associated to the development of slowly-growing bacteria in the biofilm [56]. Development of slowly-growing bacteria seen in biofilm reactors are found effectual in MPs removal [52,55,56,58,106,265,270]. For instance, in a series of batch experiments conducted by Falås et al. [265] to evaluate the capability of biofilms for MPs removal, the presence of biofilm-coated carriers (AnoxKaldnes K1) could enhance the overall biodegradation of some compounds. Clofibric acid, Mefenamic acid and Diclofenac were not removed in the bare reactors only containing suspended biomass, whereas the carrier reactors containing both suspended and attached biomass showed too much higher removals of at least 60% after 24 h for the above compounds [265]. In their subsequent experiments, Falås et al. [52] evaluated the efficiency of a hybrid biofilm-CAS process for MPs removal and concluded that the attached biomass can remarkably contribute to the removal of some MPs such as Diclofenac. In this process, two different communities of bacteria were observed such as a slow growing community in the attached biomass, and ammonia and nitrite oxidizing bacteria in the suspended biomass [52]. Similarly, the advantage of the biofilm reactors regarding the presence of slow-growing bacteria was shown by Escolà Casas and Bester [207]. The biodegradation of some recalcitrant MPs was studied in a biofilm reactor (slow sand filtration) treating a real secondary-treated wastewater. By applying the hydraulic loading rate of 0.012 $m^3.m^{-2}.\ h^{-1}$, the reactor removed 41, 94, 58, 57 and 85% of Diclofenac, Propranolol, Iopromide, Iohexol and Iomeprol respectively [207]. Again, to investigate the potential of a hybrid system for the removal of pharmaceuticals from a hospital wastewater, Escolà Casas et al. [271] investigated a pilot plant consisting of a series of one activated sludge reactor, two Hybas™ (VeoliaWater Technology) reactors and a polishing MBBR during 10 months of continuous operation. Removal of organic matter and nitrification mainly occurred in the first reactor. When the removal rate constants were normalized to biomass amount, the last reactor (MBBR) appeared to have the most effective biomass in respect to removing pharmaceuticals. They concluded that the polishing MBBR combines a fast growing biomass with low sludge age in free activated sludge flocs, and a slow-growing biomass with high sludge age on MBBR carriers [271]. Despite the benefit of enhanced MPs removal, the time required for development of slowly-growing bacteria is long because these strains are often autotrophic, grow slowly and have limited abilities to produce EPS [272] which is known as the main factor of biofilm formation [273].

Redox conditions within the biofilm are also able to influence on the removal of MPs. For instance, Zwiener and Frimmel [274] compared short-term biodegradation of Diclofenac and Clofibric acid in oxic (aerobic) and anoxic biofilm reactors at a HRT of 48 h. In the aerobic biofilm reactor, Clofibric acid and Diclofenac were not eliminated and reached a level of approximately 95% of their initial concentration. Conversely, the anoxic biofilm reactor achieved to higher removal of Diclofenac (~ 38%) and Clofibric acid (~ 30%) [274]. In an opposite trend, the reduction of the Nonylphenol ethoxylate was higher in the aerobic biofilm reactors (50-70%) compared to the anoxic biofilm reactors (30-50%) reported by Goel et al. [275]. Under the both aerobic and anoxic conditions, removal of recalcitrant compounds such as Carbamazepine, Sulfamethoxazole and Diclofenac was low (~ 25%) in the research of Suárez et al. [104]. These results demonstrate that anoxic redox conditions are not necessarily less favorable environments for MPs removal [2], and even can improve the elimination of some MPs [274,276]. Recent studies show biofilm reactors can lead to different redox conditions at different biofilm thicknesses [52,86,106]. The co-existence of oxic and anoxic conditions in the overall biofilm volume can facilitate nutrient removal, and enhance the elimination of a broad range of MPs [56].

The biological removal of 17α-ethinylestradiol in an ASFBBR was evaluated under the ammonium starvation by Forrez et al. [277]. Removal efficiencies higher than 96% were obtained at a HRT of 4.3 d and a volumetric loading rate of 11 µg. L^{-1}. d^{-1}. Increasing the volumetric loading rate up to 40 and 143 µg. L^{-1}. d^{-1} led to slightly lower removal efficiencies i.e. 81 and 74%, respectively. Interestingly, the elimination of 17α-ethinylestradiol was not affected by the absence of ammonium in the feed, suggesting that ammonia oxidizing bacteria (AOB) were able to maintain their population density and their activity, even after several months of ammonium starvation [277].

3.4. MPs removal in tertiary MBBRs

From the invention of MBBR by Hallvard Ødegaard and co-workers at the Norwegian University of Science and Technology (NTNU) [278], the acceptable performance of these reactors have been proved for carbon oxidation, nitrification, denitrification, and deammonification [248,279,280]. Compared to other biofilm reactors, regarding our review summarized in Table 11, it seems that more scientists have focused on the use of MBBRs for MPs removal from wastewater. In Table 11, the efficiency of biofilm reactors for the both secondary and tertiary treatment is given. From this table, it is apparent that working on tertiary MBBRs is very young, and its wide implantation in full-scale applications still needs more research.

In general, MPs removal in MBBRs depends on the process parameters such as SRT, HRT and F/M. Despite the fact that (I) MPs' k_{biol} values are not strongly affected by the SRT [49], and (II) the correlation between the SRT and elimination of target MPs is still not clear [40,50,57], some authors [95,106,265] have noted that possible high SRTs in MBBR reactors enable them to remove MPs more efficiently than other tertiary biological methods for the biotic MPs removal. Longer SRTs allow bacterial population to become more diversified and more capable of degrading MPs either by direct metabolism or by co-metabolic degradation via enzymatic reactions [49]. On the other hand, low F/M ratio emerged by the high suspended and attached biomass and the relative shortage of biodegradable organic matter may force microorganisms to metabolize some MPs with the competitive inhibition mechanism [58].

Working on tertiary MBBRs is still stood on the beginning steps. Tang et al. [97] investigated the effect of humic acid, as a model for complex organic substrate, on the biodegradation of 22 pharmaceuticals by a tertiary MBBR. From the results of the batch incubations of MBBR carriers, the biodegradation rate constants of ten of those compounds (e.g. Metoprolol and Iopromide) were increasing with increased humic acid concentrations. At the highest humic acid concentration (30 mgC. L^{-1}), the biodegradation rate constants were four times higher than the biodegradation rate constants without added humic acid. They concluded that the presence of complex substrate stimulates degradation of some MPs via a co-metabolism mechanism. Also, biodegradation improvement of some compounds such as Carbamazepine and Ibuprofen was not observed by adding humic acid [97]. In their next study [213], the authors ran a tertiary MBBR in the continuous mode with a novel strategy. To overcome that effluent contains insufficient organic matter to sustain enough biomass, the reactor was intermittently fed by raw wastewater. By this method, the removal of the majority of pharmaceuticals such as Diclofenac, Metoprolol and Atenolol was dramatically enhanced. As an example, the effluent concentration of Diclofenac was detected up to below than limit of quantification (LOQ) that somehow means a complete removal. Degradation of Diclofenac occurred with a half-life of only 2.1h and was much faster than any hitherto described wastewater bioreactor treatment [213]. In our point of view, this strategy (intermittent feeding by raw wastewater) leads in the beneficial renewal of the both attached and suspended biomass, but can disturb the acclimation process. In other words, adaptation of the biomass to MPs can be negatively influenced by the periodic entrance of raw wastewater to the reactor, but renewing the biomass can be beneficial for the microbial population dynamics. Torresi et al. [281] have lately noticed high potential of tertiary nitrifying MBBRs in MPs removal. They used ammonium-rich secondary-treated wastewater for feeding the reactor, and concluded that the thickest nitrifying biofilm (500 µm), attached on Z-MBBR carriers, has the highest specific biotransformation rate constants for a broad range of organic MPs due to the high biodiversity found in thick biofilms [281].

In addition to MPs biodegradation (i.e. biotic removal), the potential of MBBRs for MPs sorption onto the both suspended and attached biosolids should also be taken into account. Considering a series of batch experiments performed by Falas et al. [265], sorption of MPs onto biosolids is a fast process and can reach equilibrium within just 30 min for acidic pharmaceuticals such as Diclofenac and Naproxen. In the study of Y. Luo et al. [282] on a sponge-based MBBR, some MPs like 4n-Nonylphenol and 17ß-Estradiol were eliminated up to 80% during the first two hours in the batch experiments with acclimatized sponge, indicating that sorption has a remarkable role in abiotic removal of these compounds. Sorption onto the biofilm in a nitrifying MBBR was recognized significant for positively charged MPs in the batch experiments of Torresi et al. [86]. Some studies about particle size distribution (PSD) of the suspended solids [283–285] revealed that MBBR reactors contain smaller solids than CAS systems and MBRs. In two parallel-operated MBRs one without carriers and one with carriers (both had the equal MLSS ≈ 5 $g.L^{-1}$), an average diameter of suspended solids without carriers was around 95 µm, whereas with carriers (Filling ratio:5%) an average diameter of them decreased to 68.3 µm after 72 hours of operation [284]. The reason of this occurrence is that circulating carriers are continuously shattering the suspended biomass and thereby higher accumulation of MPs in MBBRs' suspended biomass is expected than the CAS systems and MBRs. It is noteworthy that PSD of MBBR reactors is a function of operational conditions, e.g. lowering HRT in MBBR reactors causes a shift in the average particle size of *suspended* solids towards smaller particles [283,285] that can affect the sorption capacity of MPs. Further studies are, however, required to substantiate this phenomenon, and desorption of MPs from the biosolids should be also taken into account.

3.5. MPs removal in Hybrid biofilm reactors

Hybrid biofilm reactors which are a combination of two or more treatment processes with biofilm reactors have been recently studied that may appear to be more effective than the sole biofilm reactors to remove MPs. Logically, the removal of some recalcitrant compounds can be improved with the combination of two processes due to synergistic effects [56]. As an example, Escola Casas et al. [271] whose study was about pharmaceuticals biodegradation in a hybrid biofilm- CAS system (Hybas^{TM}, VeoliaWater Technology) treating an hospital wastewater, recommended to add an ozonation process before the Hybas system in order to facilitates the subsequent removal of recalcitrant MPs by biodegradation [271]. Also, hybrid biofilm systems are seen advantageous in other aspects. For instance, Lue et al. [286] concluded that adding a MBBR prior to a MBR can not only enhance MPs elimination but also mitigate membrane fouling. They compared a hybrid MBBR–MBR system and a conventional MBR in terms of MPs removal efficiency and membrane fouling propensity. The results show the hybrid MBBR–MBR system could effectively remove most of the selected MPs. By contrast, the conventional MBR system showed lower removals of Ketoprofen, Carbamazepine, Primidone, Bisphenol A and Estriol by 16.2%, 30.1%, 31.9%, 34.5%, and 39.9%, respectively. During operation, the MBBR–MBR system exhibited significantly slower fouling development as compared to the conventional MBR system, which could be ascribed to the wide disparity in the soluble microbial products (SMP) levels between MBBR–MBR (4.02–6.32 mg. L^{-1}) and conventional MBR (21.78 and 33.04 mg. L^{-1}) [286]. Algal or fungal biofilm reactors are another type of hybrid biofilm reactors. From bibliographic review, the role of algae or fungi in biofilms in relation to MPs elimination is basically not researched [246,262]. A short review on the capability of several hybrid biofilm reactors for MPs removal from wastewater is given in Table 12. By this sight, as only a handful of researches are so far studied, it is clear that further technical and economical investigations are yet needed to advance in the design of hybrid biofilm reactors. To date, there is no report about the application of hybrid biofilm reactors for tertiary MPs removal although these systems beseem a promising technology for an enhanced elimination of MPs.

Table 11. A short review on the capability of biofilm reactors for MPs removal

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the biofilm reactor	Type of wastewater	Specification of the reactor	Process parameters	MPs removal	Reference
a submerged biofilm reactor (Biostyr™, Veolia Technology)	A real municipal wastewater, MPs concentration: 7 ng/L	Reactor's volume: 190 m^3 Continuous mode of operation	HRT: 35 min DO: 2-3 mg/L Temperature: 15-16 °C	Estrone: 90% 17ß-Estradiol: 95% Ethinylestradiol: 69%	[51]
IFAS (Bioportz®, Entex Technologies Inc.)	A real municipal wastewater passed from the primary clarifier, MPs concentration: 26-910 ng/L	Reactor's volume: 1370 L Filling ratio: 50% with Bioportz media (HDPE, with a biologically active surface area of 576 $m^2.m^{-3}$) MLSS: 2692 mg. L^{-1} Attached biomass: 16.9 g. m^{-2} Continuous mode of operation	SRT: 8 d HRT: 6.4 h	Bisphenol A: 90% Triclosan: 84% 4n-Nonylphenol: 65% Estrone: ~70% 17ß-Estradiol: ~ 90%	[263]
ASFBBR	A synthetic municipal wastewater, spiked with 50-100 µg/L of MP.	Reactor's volume: 1.4 L (filled with 130 g of media AnoxKaldnes K1) Continuous mode of operation	HRT of 4.3 d DO: 6.9 ± 0.8 mg. L^{-1} pH: 7.8 ± 0.2 Temperature: 26 ± 2°C volumetric loading rate: 11 µg. L^{-1}. d^{-1} Upflow velocity: 1 $m.h^{-1}$	17α-ethinylestradiol: 96%	[277]
FBBR	A real municipal wastewater passed from the primary clarifier, MPs concentration: 90-1600 ng/L	Reactor's volume: 1500 m^3 consisting of 8 Biostyr up-flow cells filled with 3.6 mm Styrofoam beads as biofilm support. Continuous mode of operation	HRT: 1 h Average temperature: 19 °C	Sulfapyridine: -29-20% Sulfamethoxazole: -21-5% N^4-acetylsulfamethoxazole: 9-21% Trimethoprim: -13-31% Azithromycin: 10-33% Erythromycin: -8-4% Clarithromycin: 11-14% Roxithromycin: 3-9%	[266]
	A real municipal wastewater passed from the primary clarifier, MPs concentration: 10 µg/L	Reactor's volume: 10 L filled with particles of native, porous sintered glass as media. Continuous mode of operation	HRT: 11 h	Diclofenac: 0% Bentazone: 0% Pesticides MCPP: ~ 50% Pesticides MCPA: ~ 50%	[267]

Continue of Table 11. A short review on the capability of biofilm reactors for MPs removal

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the biofilm reactor	Type of wastewater	Specification of the reactor	Process parameters	MPs removal	Reference
Biofilm sand filter	A real secondary-treated municipal wastewater, MPs concentration: 0.1 – 20.8 µg/L	Reactor's volume: 142 mL Filling ratio: 100% with Quartz sand (0.210–0.297 mm particle size)	pH: 8 Temperature: 20°C HRT: 4 h-39 h	Diclofenac: 0-82% Propranolol: 45-98% Propiconazole: 0-21% Iohexol: 25-91% Iomeprol: 17-93% Iopromide: 0-91%	[207]
MBBR	A real municipal wastewater, spiked with 10 µg/L of each MP.	Reactor's volume: 2 L filled with the carriers manufactured from cutting the screw neck of water bottles. Batch mode of operation	Temperature: 25°C	Bisphenol A: 34% Oseltamivir: 49% Atrazine: 66%	[268]
	A real municipal wastewater, spiked with 100 µg/L of each MP.	Reactor's volume: 5 L Filling ratio: 23% with AnoxKaldnes K1 Batch mode of operation	HRT: 24 h DO: 5-9 mg. L^{-1} pH: 5.5-8 Ambient temperature: 18°C	Ibuprofen: 100% Naproxen: 60 %	[265]
	A synthetic municipal wastewater, spiked with 5 µg/L of each MP.	Reactor's volume: 40 L Filling ratio: 30% with sponge cubes Continuous mode of operation	HRT: 24 h pH: 7 DO: 5.5-6.5 mg. L^{-1} Feed flowrate: 27.8 mL.min^{-1} COD loading rate: 0.40 kg.m^{-3}d^{-1} Operation time: 100 d	Carbamazepine: 25.9% Diclofenac: 45.7% Gemfibrozil: 62.4% Ibuprofen: 93.7% Ketoprofen: 58.2% 4n-Nonylphenol: 95.7% 17ß-Estradiol: 96.2% Pentachlorophenol: 78.9% Bisphenol A: 77.8% Acetaminophen: 71.4%	[282]
	A synthetic municipal wastewater, spiked with 10 µg/L of each MP.	Reactor's volume: 25 L Media:Pumice stones (1–2 mm particles) Continuous mode of operation	HRT: 10 h DO: 3.8 ± 0.3 mg. L^{-1} pH: 7.5 Total attached biomass: 2.5 g.L^{-1}	Clofibric acid: 100% Diclofenac: 40% Ibuprofen: 100%	[274]

Continue of Table 11. A short review on the capability of biofilm reactors for MPs removal

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the biofilm reactor	Type of wastewater	Specification of the reactor	Process parameters	MPs removal	Reference
MBBR	A synthetic municipal wastewater, spiked with 1 µg/L of each MP.	Reactor's volume: 4 L Filling ratio: 30 % with AnoxKaldnes K1 Continuous mode of operation	HRT: 48 h DO: 8.4 mg. L^{-1} pH: 6.3-7.8 Temperature: 19 °C Attached biomass concentration: 0.49 g. L^{-1}	Clofibric acid: 28% Ibuprofen: 94% Naproxen: 70% Ketoprofen: 73% Carbamazepine: 1% Diclofenac: 74%	[287]
		Reactor's volume: 4 L Filling ratio: 5 % with Mutag BioChip™ (specific surface area of 3000 $m^2.m^{-3}$) Continuous mode of operation	HRT: 48 h DO: 8.4 mg. L^{-1} pH: 6.3-7.8 Temperature: 19 °C Attached biomass concentration: 0.21 g. L^{-1}	Clofibric acid: 5% Ibuprofen: 94% Naproxen: 80% Ketoprofen: 63% Carbamazepine: 0% Diclofenac: 85%	
	A real municipal wastewater, spiked with 30 µg/L of each MP.	Two MBBRs in series Reactor's volume for each MBBR: 4.5 L Filling ratio: 30 % with AnoxKaldnes K3 Continuous mode of operation	HRT: 26.4 h (for MBBR 1) HRT: 10.8 h (for MBBR 2) DO: 4 mg. L^{-1} (for both MBBRs) Operation time: 5 months Attached biomass: 1079 mg. L^{-1} (for MBBR 1) Attached biomass: 726 mg. L^{-1} (for MBBR 2)	Benzotriazole: 78% 5-methy-1H lbenzotriazole: 55% 5- chlorobenzotriazole: 40% 4-methyl-1H-benzotriazole: 70% Xylytriazole: 42% 2-hydroxybenzothiazole: 96%	[288]
	A hospital wastewater, MPs concentration: 14 µg/L	Reactor's volume: 9 L (3 × 3 L in series) Filling ratio: 50% with AnoxKaldnes K5 Continuous mode of operation	HRT: 6 h pH: 7.5-8.5 Temperature: 15-18°C	Ibuprofen: 100% Iohexol: 60% Iomeprol: 55% Atenolol: 40% Sulfamethizole: 25% Sulfamethoxazole: -20% Venlafaxine: 12% Propranolol: 8% Carbamazepine: 10% Clindamycin: 98%	[95]

Continue of Table 11. A short review on the capability of biofilm reactors for MPs removal

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the biofilm reactor	Type of wastewater	Specification of the reactor	Process parameters	MPs removal	Reference
MBBR	An oilfield wastewater, MPs concentration: 78-216 μg. L^{-1}	Reactor's volume: 5 L Filling ratio: 50% (suspended ceramic granules as media) Specific surface area: 3.8-4.1 m^2. g^{-1} Continuous mode of operation	HRT: 10-36 h SRT: 10 d DO: 3 mg. L^{-1} COD loading rate: 1.2-4.2 kg. m^{-3}. d^{-1}	Naphthalene: 79% Phenanthrene: 80% Fluoranthrene: 84% Chrysene: 57%	[289]
	a detergent wastewater, 528-561 μg. L^{-1}	Reactor's volume: 20 L filled with ceramic particles (mean particle sizes: 3-5 mm)	HRT: 5-20 h Temperature: 20-25 °C Air flowrate: 40 L. h^{-1}	Linear Alkylbenzene Sulfonate (a surfactant): at HRT of 5 h: 98.5% at HRT of 29 h: 99%	[290]
	A real secondary-treated municipal wastewater, MPs concentration: 3-20μg/L	Two MBBRs in series Reactor's volume for each MBBR: 3 L Filling ratio: 50 % with AnoxKaldnes K5 Continuous mode of operation	HRT: 4 h pH: 7.4-8 DO: 7.2-8.3 mg. L^{-1} Air flowrate: 300 L. h^{-1}	Diclofenac ~ 100% Ibuprofen ~ 100% Trimethoprim ~ 30% Atenolol ~ 55% Propranolol ~ 25% Sulfamethazine ~ 30%	[213]

Table 12. A short review on the capability of hybrid biofilm reactors for MPs removal from wastewater, regarding the process parameters

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of biofilm reactor	Type of wastewater	Specification of the reactor	Process parameters	MPs removal in the biofilm part of the hybrid system			Reference
				Removal	Biodegradation	Sorption	
a hybrid MBBR-MBR system	A medium-strength synthetic wastewater, each MP concentration: 5 µg/L	Reactor's volume: 4 L Filling ratio: 20% with sponge cubes Continuous mode of operation Membrane: polyvinylidene fluoride MF TMP: 35 kPa	pH: 7 HRT: 24 h (for MBBR) HRT: 6 h (for MBR) Feed flowrate: of 28 mL/min Operation: 90 days Biomass attached: 0.41 g/g sponge	Carbamazepine: 30%	15%	15%	[286]
				Diclofenac: 45%	25%	20%	
				Ibuprofen: 88%	85%	3%	
				17ß-Estradiol: 96%	95%	1%	
				Nonylphenol: 97%	95%	2%	
				Bisphenol A: 89%	85%	4%	
				Salicylic acid: 96%	94%	2%	
				Metronidazole: 37%	30%	7%	
				Ketoprofen: 80%	60%	20%	
				Naproxen: 82%	77%	5%	
				Gemfibrozil: 70%	60%	10%	
a hybrid UASB-biofilm MBR system	A synthetic wastewater, MPs concentration: 1-40 µg/L	Reactor's volume: 120 L Filling ratio:50% with AnoxKaldnes K3 Continuous mode of operation Membrane: hollow-fiber UF (pore size of 0.04 mm, total surface of 0.9 m^2)	SRT: 60 d (for biofilm MBR) HRT: 12 h (for UASB) HRT: 5 h (for biofilm MBR) pH: 7.5 Ambient temperature: 20 - 22°C Biofilm in UASB: 30 g. L^{-1} Biomass in biofilm MBR: 5-7 g.L^{-1} Operation: 180 days	Diclofenac: 0%	0%	0%	[291]
				Naproxen: 0%	0%	0%	
				Ibuprofen: 67%	66%	1%	
				Sulfamethoxazole: 38%	38%	0%	
				Roxithromycin: 7%	7%	0%	
				Ethynilestradiol: 3%	3%	0%	
				17ß-Estradiol: 92%	92%	0%	
				Galaxolide: 0%	0%	0%	
				Tonalide: 8%	0%	8%	
				Celestolide: 7%	0%	7%	
				Diazepam: 25%	25%	0%	
				Carbamazepine: 8%	8%	0%	
				Estrone: 84%	84%	0%	

Continue of Table 12. A short review on the capability of hybrid biofilm reactors for MPs removal from wastewater, regarding the process parameters

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of biofilm reactor	Type of wastewater	Specification of the reactor	Process parameters	MPs removal	Reference
a hybrid biofilm-activated sludge process	A real municipal wastewater, spiked with 1 µg/L of each MP.	Reactor's volume: 30 L Filling ratio: 35% with AnoxKaldnes K1 Continuous mode of operation	SRT: 3-4 d HRT: 12 h DO: 3.5 ± 0.5 mg. L^{-1} Ambient temperature: 16°C	Diclofenac: 20% Carbamazepine: 0% Mefenamic acid 58% Atenolol: 25% Trimethoprim: 2% Clarithromycin: 0%	[52]
a hybrid MBBR-MF system	A synthetic municipal wastewater, spiked with 10 µg/L of the MP.	A lab-scale coupled anaerobic MBBR, two-aerobic MBBRs, and MF membrane was used. The volume of anaerobic MBBR: 12 L The volume of each aerobic MBBR: 16 L Filling ratio for each MBBR: 30% (Media: High-density polyethylene) Continuous mode of operation	HRT: 8 h (in anaerobic MBBR) HRT: 8 h (at each aerobic MBBR) DO: 4.49 mg. L^{-1} (at aerobic MBBR 1) DO: 4.24 mg. L^{-1} (at aerobic MBBR 1) MLSS ~ 2.7 g. L^{-1} (in anaerobic MBBR) MLSS ~ 3.3 g. L^{-1} (in aerobic MBBR 1) MLSS ~ 3.5 g. L^{-1} (in aerobic MBBR 2) Feed flowrate: 0.1 L. min^{-1} Temperature: 18 ± 3°C	Polychlorinated Biphenyls: At anaerobic MBBR: 73% At aerobic MBBRs: 83%	[292]
a hybrid GAC-Sequencing batch biofilm reactor (SBBR)	A wastewater from paper industry, concentration of MP: 12-52 µg.L^{-1}	Reactor's volume: 2.2 m^3 Volume occupied by the GAC: 0.14 m^3 Volume occupied by the plastic balls (as media with diameter of 3 cm): 0.02 m^3	Filling time: 0.5 h Reaction time (HRT): 21.5 h Settling time: 1 h Draw time: 1 h pH: 6.4-7.8 Air flowrate: 2.1-3.4m^3.min^{-1} Attached biomass ~ 1600 mg. L^{-1} MLSS ~ 1000 mg. L^{-1}	2,4-dichlorophenol: 100%	[293]

3.6. MPs removal in bioaugmented biofilm reactors

3.6.1. Definition and concept of bioaugmentation

Bioaugmentation is generally the implantation of indigenous or allochthonous wild type or genetically modified organisms to bioreactors or polluted hazardous waste sites in order to accelerate the removal of undesired compounds [294,295]. This process is generally identified as a straightforward and high-efficient bioremediation technology, which could improve the traditional bio-treatment processes and reduce the energy consumption [296].

In a simple language, first, a suitable inoculum needs to be selected and produced. This step can be itself problematic depending on the pollutant of interest and the availability of known degraders [297]. In wastewater treatment, once the inoculum is furnished, it must be adapted and then delivered to the bioreactor, which it requires some feats of expertise and engineering. With the inoculum in place, the microbes in it need to thrive and degrade the pollutant. In the past, bioaugmentation was not well regarded due to a lack of controlled studies and scientific reasoning behind the inocula. With the ever-growing understanding of microbial systems, and by means of investigation procedures such as metagenomics and metatranscriptomics, we might be now able to make better feasible hypotheses about what would make a good bioaugmenting inoculum and how to control its behavior in the bioreactor. These scenarios have to be yet tested under field situations for many persistent pollutants [298].

3.6.2. Criteria & metabolic pathways of candidate microorganisms

The initial screening/selection step of the microorganism should be based on the metabolic potential of the microorganism and also on essential features that enable the cells to be functionally active and persistent under the desired environmental conditions [299]. The ability of the microorganism for biodegradation of the target compound should be also considered. The problems associated with strain selection for bioaugmentation are reviewed by Thompson et al. [297]. In a right selection, the introduced inoculum would have to contend with the autochthonous microbes for resources and to avoid predation [298]. Augmented microorganisms may be added to cooperate with autochthones or to replace them, so survival of the cells is the bottleneck to success [300]. As reported by Yu and Mohn [301], candidate microorganisms should meet at least three main criteria: firstly, to be catabolically able to degrade the pollutant, even in the presence of other potentially inhibitory pollutants; secondly, they must persist and be competitive after their introduction into the bioreactor; and thirdly, they should be compatible with the indigenous microbial communities [301]. In addition, they should not be closely related to human pathogens e.g. *Pseudomonas aeruginosa* [296].

According to the metabolic pathways described by Benner et al. [94], bioaugmented microorganisms employ two main catalytic processes of metabolic and co-metabolic strategies when participating in biologically-mediated reactions with MPs (Fig.9).

Bioaugmented microorganisms, involved in metabolic biodegradation of MPs, are often enriched and isolated from environments that are repeatedly exposed to specific MPs such as WWTPs [302], and agricultural soils [303]. In the metabolic strategy, microorganisms interact with target MPs in growth-linked processes that result in complete mineralization of the MP. A variety of individual bacterial strains have been isolated that can use specific MPs as growth substrates and thereby mineralize these compounds to biomass, CO_2, water, and other benign chemicals. Bioaugmentation with individual strains selected to mineralize target MPs would require pre-culturing of the strain to attain an optimal cell density followed by inoculation into the bioreactor [94].

Co-metabolic strategy are reactions that do not sustain growth of the responsible microorganisms and often lead to the formation of transformation products (oxidized metabolites). These metabolites can be subsequently used as primary substrates for heterotrophic bacteria. In other words, this strategy would be to consider organisms that transform MPs into compounds that can be utilized as growth substrates by other members of the microbial community [94]. For example, Khunjar et al. [304] studied the biological fate of 17a-Ethinylestradiol in a bioreactor containing an AOB culture, two enriched heterotrophic cultures, and nitrifying activated sludge cultures. Interestingly, AOB oxidized 17a-Ethinylestradiol to transformation products that were subsequently mineralized by heterotrophs. They finally concluded that AOBs and heterotrophs may cooperatively enhance the reliability of treatment systems where efficient removal of 17a-Ethinylestradiol is desired [304]. It is well documented that the AOB and methane oxidizing bacteria (MOB) catalyze co-metabolic reactions leading to biotransformation of MPs [113,305]. To be relevant for MPs removal, the microorganisms participating in co-metabolic reactions should have enzymes with broad substrate specificity. Also the competition for the enzyme between MP and growth substrates should not lead to a disadvantage for the survival of the bioaugmented microorganisms [94].

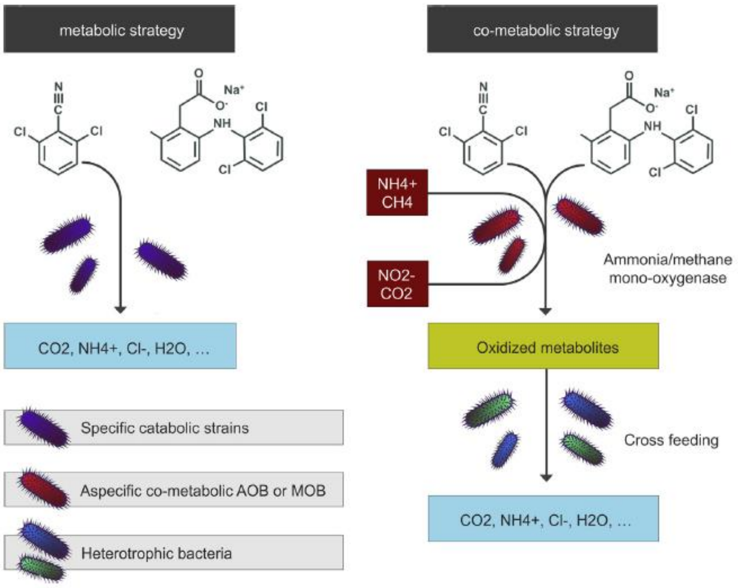


Fig. 9. Metabolic and co-metabolic reactions involved in bioaugmentation (adapted from Benner et al.[94])

3.6.3. Bioaugmentation failure

From the bioaugmentation studies, we observe that the number of exogenous microorganisms decreases shortly after addition to the bioreactor. In this regard, according to the Gentry et al. [306], the reasons that hamper microbial growth may include biotic and abiotic stresses. Fluctuations or extremes in temperature, water contents, pH, and nutrient availability, along with potentially toxic pollutant levels in the bioreactor include the abiotic stresses. In the aspect of biotic stresses, the added microorganisms almost always face with a competition from indigenous microorganisms for limited nutrients, accompanied with antagonistic interactions including antibiotic production by competing organisms, and predation by protozoa and bacteriophages [306]. Biotic factors are often more consequential [307]. Nevertheless, over the last few years, bioaugmentation has remained debatable as a scientific and technological endeavor.

Although bioaugmentation seems simple in principle, many attempts with bioaugmentation have failed due to poor survival or low activity of the bioaugmentation strains [308]. For instance, a nitrifying SBR, studied by Bouchez et al. [309], was inoculated twice with the aerobic denitrifying bacterium *Microvirgula aerodenitrificans* and fed with acetate. No improvement was obtained on nitrogen removal. Fluorescent in situ hybridization (FISH) with rRNA-targeted probes revealed that the added bacteria almost disappeared from the reactor within 2 days. These results were attributed to the predator-prey interactions (between protozoa and *Microvirgula aerodenitrificans*) happened in the SBR [309]. In another study, Goldstein et al. [310] found that *Pseudomonas* species having potential to degrade 2,4-dichlorophenol and p-nitrophenol in cultures failed to do the same in the target lake water. Problems concerning the adaptation of the inoculated microorganisms and competition between introduced and indigenous biomass are ascribed to the performance failure [310]. Hence, seeding alone is generally not enough and should be accompanied by suitable physical and environmental alterations [299].

3.6.4. General classification of bioaugmentation

According to the classification suggested by El Fantroussi et al. [307], the most common pathways for adding exogenously grown strains, either singly or in the form of consortia, into a bioreactor include: i) the addition of a pre-adapted microorganism, ii) the addition of pre-adapted consortia, iii) the introduction of genetically engineered bacteria, iv) and the addition of biodegradation-relevant genes that are packaged in a vector in order to be transferred by conjugation into microorganisms already present in the biotope under remediation [307].

As Semrany et al. [300] proposed, the process of bioaugmentation can be also classified based on the origin of candidate microbes i.e. i) autochthonous bioaugmentation (Auto-BA), ii) allochthonous bioaugmentation (Allo-BA), and gene bioaugmentation (Gen-BA). Isolation of the candidate microorganism(s) from the contaminated soil, water or wastewater, followed by preparation in an enriched culture, and then re-injection of the adapted microorganism(s) in the original environment is defined as Auto-BA. There is increasing evidences from the literature that the best way in which to overcome the ecological barriers is to look for microorganisms from the same ecological niche as the polluted area (i.e. augmentation with indigenous microorganisms) [307]. In the approach of Allo-BA or “bioenrichment”, the candidate microorganism(s) are isolated from another medium. In successful Allo-BA studies, the introduced strain vanished with time but after shifting its degradation capacities to some autochthonous strains. This is explained by the presence of “mobile DNA elements” carrying the genes involved in biodegradation process. These “plasmids” can be transferred between two bacteria via conjugation. In the advance method of Gen-BA that is somehow faces with some legal and social restrictions, “Genetically Engineered Microorganisms (GEM)” are used for bioaugmentation. This type of microorganism will carry plasmids in order to enhance the capacity of pollutants biodegradation [300].

For both classifications, we will bring several examples of the studies that have used these pathways in the following sections.

3.6.5. Common applications of bioaugmentation in wastewater treatment

From bibliographic review, scientists have widely used the process of bioaugmentation in order to fulfill one or more purposes including i) to enhance reactor performance and accelerate the onset of pollutants biodegradation in wastewater [311] sewage sludge [312], and soil [313], ii) to compensate for pH shock loadings as well as organic or hydraulic overloading [314], iii) to protect the existing microbial community against adverse effects [315], iv) to accelerate the start-up phase of the bioreactors [316], and v) to increase the biogas production from anaerobic processes [317]. On the issue of wastewater treatment, several studies related to the addition of pre-adapted consortia to the activated sludge-based systems for improving the performance of bioreactors are summarized in Table 13. However, due to the intricacy of the practical operational conditions, full-scale application of the activated sludge systems bioaugmented by specialized microorganisms has been rarely reported [318]. In the next sections, we will focus on the field of MPs removal form wastewater using bioaugmented bioreactors.

Table 13: Bioaugmentation of activated sludge-based systems with the addition of pre-adapted consortia to improve the performance of bioreactor

Type of the reactor	Type of wastewater	Primary seed	Augmented seed	Main results	Reference
a 165-L pilot-scale SBR	Municipal wastewater	Inoculation with activated sludge	Three heterotrophic nitrification–aerobic denitrification bacteria named *Agrobacterium tumefaciens LAD9, Comonas testosteroni GAD3* and *Achromobacter xylosoxidans GAD4* were used.	The bioaugmentation system exhibited stable and excellent carbon and nutrients removal, the averaged effluent concentrations of COD, NH_4^+ -N, TN and TP were 20.6, 0.69, 14.1 and 0.40 mg/L, respectively. In addition, the introduced bacteria greatly improved the structure of original microbial community and facilitated their aerobic nutrients removal capacities.	[319]
a 10-L pilot-scale modified sequencing batch biofilm reactor			Specialized mixed bacteria belonged to *Pseudomonas sp. KW1*., *Pseudomonas aeruginosa* and *Bacillus sp. YW4*.	Bioaugmentation dramatically enhanced the removal efficiency of COD, TP, and TN up to 84%, 68% and 59% respectively, compared to non-augmented reactor that had lower values.	[320]
a 112 L pilot-scale biofilm airlift reactor			Bioaugmentation with nitrifying activated sludge taken from a pilot plant operated with full nitritation (85 ± 7% AOB, <1 ± 1% NOB and 15 ± 5% heterotrophs).	The length of the start-up period was significantly reduced while the stability of operation was increased, in comparison with non-bioaugmented reactor. Moreover, the specialized nitrifying biomass added to the Bioaumented-reactor remained in the biofilm throughout the start-up period.	[321]
Three full-scale WWTPs with MLE, SBR and oxidation ditch processes		Inoculation with dewatered waste activated sludge	Specialized mixed bacteria belonged to *Proterobacteria, Bacterioieds, Nitrospirales*, *Cyanobacteria*, *Bacillus sp. F2*, and *Bacillus sp. F6* was used.	Rapid start-up and the following stable performance of WWTPs at low temperatures were observed. The bioaugmented specialized bacteria were predominant in the biological systems.	[322]
2-L lab-scale MBRs		Inoculation with activated sludge	Bioaugmentation with nitrifying activated sludge taken from a side-stream MBR fed with a synthetic high nitrogen-loaded influent (no name of a special strain is given in the study).	The bioaugmentation process caused an increase of nitrifying bacteria of the genera *Nitrosomonas* and *Nitrobacter* (up to more than 30%) in the inoculated MBR reactor. The overall structure of the microbial community changed in the main stream MBR as a result of bioaugmentation. The effect of bioaugmentation in the shift of the microbial community was also verified through statistical analysis.	[323]
a full-scale pure oxygen activated sludge municipal WWTP		Natural microbial community	Bioaugmentation with nitrifying activated sludge (no name of a special strain is given in the study)	An ammonia-nitrogen removal rate of 0.21 mg-N/g MLVSS-h was observed, while the rate increased to 0.54 mg-N/g MLVSS-h with an introduction of 6% bioaugmented nitrifiers, indicating that the integrated side-stream nitrifiers bioaugmentation process was beneficial in improving nitrification efficiency.	[324]

Continue of Table 13: Bioaugmentation of activated sludge-based systems with the addition of pre-adapted consortia to improve the performance of bioreactor

Type of the reactor	Type of wastewater & pollution level	Primary seed	Augmented seed	Main results	Reference
a 4-L lab-scale and a 110-L pilot scale conventional activated sludge	Dairy wastewater, COD = 1500 mg/L	No information about the primary seed is given.	A filamentous fungal consortium including *Aspergillus niger, Mucor hiemalis and Galactomyces geotrichum*	The positive impact of fungal addition on COD removal was confirmed when fungi was beforehand accelerated by pre-cultivation on the same medium, since COD removal increased from 55% in absence of fungi to 75% after their addition. Moreover, there was a clear impact of fungal addition on the 'hard' or non-biodegradable COD owing to the significant reduction of the increase of the COD on BOD_5 ratio between the inlet and the outlet of the biological tank.	[325]
a 100-L Pilot-scale conventional activated sludge	tannery wastewater	Inoculation with activated sludge	Commercial microbial consortium of *BM-S-1* containing *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Planctomycetes* and *Deinococcus-Thermus*	The removal efficiencies of COD, TN and TP were 91.4%, 77.9%, and 89.4%, respectively.	[326]
a 5-L lab-scale biofilm-activated sludge (filled with porous polyurethane foam as carriers)	petrochemical wastewater at low temperatures (13-15℃)	Inoculation with activated sludge taken from the petrochemical wastewater treatment plant	Mixed bacteria belonged to *Pseudomonas, Bacillus, Acinetobacter, Flavobacterium and Micrococcus.*	The COD and NH_4^+-N removal was obtained up to 75.80% and 70.13% respectively. The application of polyurethane foam as carrier in the bioaugmentation practice is promising for the retention of sufficient biomass and prevention mechanisms to the immobilization cells.	[257]
The full-scale conventional activated sludge	petrochemical wastewater	Natural microbial community	Mixed bacteria belonged to *Pseudomonas, Bacillus, Acinetobacter, Flavobacterium and Micrococcus*	Bioaugmentation was successful for the rapid upgrade of the activated sludge process to the contact oxidation process.	[327]
A 5.3 m^3 biofilm oxidation ditch reactor	Nitrogen-rich stream water, TN: 45 mg/L and NH_4^+–N: 30 mg/L.	Inoculation with activated sludge taken from a WWTP with a hybrid biofilm-activated sludge process	Bioaugmentation with the enrichment cultures of nitrifying bacteria (enriched ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in the water and onto the surface of the AquaMats carriers).	Enhancement of the removal efficiency of TN and NH_4^+–N from 25.9% to 50.3%, and from 34.5% to 60.1%, respectively was observed. Moreover, Augmentation of nitrifying bacteria could significantly increase the quantity of AOB and NOB both in water and on biofilm.	[328]

3.6.6. Capability of bacterial and fungal bioaugmentation for MPs removal

In general, fungi and bacteria can both degrade and transform organic contaminants. One might therefore ask which characteristics or environmental circumstances make fungi particularly suitable for application in environmental biotechnology. Obviously, fungal degradation should be considered for pollutant classes that are inefficiently degraded by bacteria. Wastewater treatment involving bacteria is, however, considered to be more stable, as bacteria generally tolerate a broader range of habitats and grow faster than fungi [329]. Principal methods used by fungi to degrade organic chemicals are complicated, and are well reviewed by Harms et al. [329] (Section S1 and Fig.1S in supplementary data).

In Table 14, several examples of the application of single-strain bioaugmentation for the purpose of pollutants removal from wastewater are given. For instance, Roh and Chu [330] investigated the performance of lab-scale SBRs that were firstly inoculated with a nitrifying activated sludge and then bioaugmented with a *Sphingomonas* strain KC8 (a 17ß-Estradiol degrading bacterium). The SBRs were operated under three SRTs of 5, 10, and 20 d. Higher 17ß-Estradiol removals (>99%) were observed for the SBRs operated in SRTs of 10 and 20 d. Neither estrogens nor estrogenic activity was detected in the treated water, except some samples from the SBR operated at SRT of 5 d. The results suggested that bioaugmented bioreactors operating at long SRTs (10 and 20 d) were effective in removing 17ß-Estradiol to the non-estrogenic treatment endpoint [330].

Using a microbial consortium rather than a pure culture for the bioremediation is more advantageous, since they can share biochemical steps in order to completely mineralize recalcitrant and/or toxic substrates. Also, they can better overcome the barriers present in the new ecological and physicochemical environments [331]. A couple of researches correspond to the addition of pre-adapted consortia to the activated sludge-based systems for pollutants removal from wastewater are presented in Table 15.

As remarked in Table 14 and Table 15, apparently, fungal bioaugmentation has been so far used more than bacterial bioaugmentation for MPs removal. The main reason is probably found in the ability of fungal strains in the production of strong enzymes that are able to degrade a vast majority of MPs. In general, MPs removal by a fungal-bioaugmented bioreactor depends on various factors such as fungal species, culture medium and also the chemical structure of MPs present [332].

Among various fungal species, white-rot fungi (WRF) (either whole-cell WRF or their lignin modifying enzymes (LMEs)) has attracted more attention of the scientists for MPs removal [333]. WRF secrete three main classes of LME including lignin peroxidases (LiPs), manganese-dependent peroxidases (MnPs) and laccase [334]. In this regard, MPs with strong electron donating groups (EDG) such as hydroxyl (–OH) and amine (–NH_2) have been found to be extensively/effectively removed (e.g. Nonylphenol and 17ß-Estradiol). Conversely, compounds containing strong electron withdrawing groups (EWG) like halogen, nitro, azepine and triazine are difficult to be removed (e.g. Carbamazepine). It should be noted that some MPs containing both EDGs and EWGs have been reported to be readily degraded (e.g. Diclofenac and Naproxen), while some other MPs have exhibited rather poor removal (e.g. Atrazine). For MPs containing both EDGs and EWGs, the overall influence of these functional groups and particularly their opposing effects on the MPs biodegradability is complicated and needs to be studied further [333]. To show the potency of fungal species for MPs removal from wastewater, Tables 1S in supplementary data highlights the comparative removal data of some frequently reported MPs by different fungal species (whole-cell), obtained in batch experiments. This table has been prepared with a focus on the effect of functional groups of MPs on their removal. Section 2S in supplementary data also gives several examples on the application of fungus species and enzymes for MPs removal from secondary-treated wastewater.

A mixture of fungal and bacterial strains developed in non-sterile conditions of fungal-bioaugmented bioreactors is seen efficient in MPs removal. To bring an example, based on a series of batch tests performed by Hai et al. [335], an enhanced removal of three widely used recalcitrant pesticides from their liquid mixture was demonstrated by implementing a non-acclimated mixed culture of bacteria and fungi. During an incubation period of 14 days, the mixed fungus–bacterial culture achieved 47%, 98%, and 62% removal of Aldicarb, Atrazine and Alachlor from the liquid phase, respectively [335]. In the case of continuous-mode of operation, in the study of Nguyen et al. [336] who added WRF *Trametes Versicolor* in a non-sterile lab-scale MBR for purifying a malt-based synthetic wastewater, a mixed culture of fungi and bacteria gradually developed in the reactor. They finally concluded that white-rot fungal enzyme (laccase), coupled with a redox mediator (1-hydroxy benzotriazol) could degrade 51% Diclofenac, 70% Triclosan, 99% Naproxen and 80% Atrazine [336].

Table 14: Several examples of the single-strain bioaugmentation of activated sludge-based systems for removal of industrial pollutants and MPs from wastewater

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the reactor	Type of wastewater & pollution level	Primary seed	Augmented seed	Main results	Reference
A 5.5-L lab-scale MBR	A malt-based synthetic wastewater (pH=4.5), Concentration of Diclofenac, Triclosan, Naproxen and Atrazine were 5 $\mu g.L^{-1}$.	Incoculation with sludge from another lab-scale fungus-augmented MBR *(Trametes Versicolor).*	Bioaugmentaion with the pure white rot fungus *Trametes Versicolor (ATCC 7731).* Moreover, in the last 30 days, reactor was conducted with continuous dosing of 5 μM of HBT (redox mediator).	Because the original MBR, used for seeding the reactor, was operated under non-sterile conditions, a mixed culture of fungi and bacteria gradually developed in the reactor. The results show that white-rot fungal enzyme (laccase), coupled with a redox mediator (1-hydroxy benzotriazol, HBT), could degrade target MPs that are resistant to bacterial degradation (Diclofenac: 51%, Triclosan: 70%, Naproxen: 99% and Atrazine: 80%).	[336]
A 5.5-L lab-scale MBR	A malt-based synthetic wastewater (pH=4.5), Bisphenol A: 1585±270 $\mu g.L^{-1}$ Diclofenac: 1526±366 $\mu g.L^{-1}$	Bioaugmentaion with the pure white rot fungus *Trametes Versicolor (ATCC 7731)*		Stable removal of Bisphenol A (80-90%) and Diclofenac (55%) was observed by applying an HRT of 2 d. Generally, removal of these MPs was highly affected by HRT.	[337]
A 11.8-L lab-scale MBR	Real textile wastewater, Acid Orange II, 100 $mg.L^{-1}$	white-rot fungus *Coriolus versicolor (NBRC 9791*		This fungal MBR achieved 93% removal during long-term non-sterile operation at a HRT of 1d. This study also demonstrated the occurrence of enzyme washout from MBR and its HRT-specific detrimental influence on removal performance.	[338]
A 3-L lab-scale RBC	Synthetic wastewater, a mixture of azo dyes including Direct Red-80 (DR-80) and Mordant Blue-9 (MB-9), 25-200 $mg.L^{-1}$	white-rot fungus *Phanerochaete chrysosporium*		The system could completely decolourize the wastewater at HRT of 48 h. The effect of increase in the disc rotation speed from 2 to 6 rpm in the study revealed no large differences in the decolourization efficiencies of the wastewaters	[339]
A 2-L lab-scale bioreactor filled with porous polyether foam	Effluent of a municipal WWTP, COD: 28 $mg.L^{-1}$ Carbamazepine: 1 $mg.L^{-1}$	Bioaugmentaion with white rot fungus *Phanerochaete chrysosporium (BKM F-1767)*		It was found that the sufficient supply with nutrients is crucial for an effective elimination of Carbamazepine. Given the conditions, a high elimination of Carbamazepine (60–80%) was achieved. The effective elimination was stable in a continuous operation for a long term (around 100 days).	[340]
A 1.5-L lab-scale fluidized bioreactor	Synthetic wastewater, Carbamazepine: 200 $\mu g.L^{-1}$	Bioaugmentaion with the pure white rot fungus *Trametes Versicolor* (ATCC 42530)		With a HRT of 3 d, 54% of the inflow concentration was reduced at the steady state (25 d) with a CBZ degradation rate of 11.9 mg CBZ g^{-1} dry weight d^{-1}.	[341]

Continue of Table 14: Several examples of the single-strain bioaugmentation of activated sludge-based systems for removal of industrial pollutants and MPs from wastewater

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the reactor	Type of Pollutant & pollution level	Primary seed	Augmented seed	Main results	Reference
Three identical and parallel 2-L lab-scale SBRs	Synthetic wastewater, 17ß- Estradiol: 1 $mg.L^{-1}$	Inoculation with nitrifying activated sludge	*Sphingomonas* strain KC8 (a 17ß-Estradiol degrading bacterium)	The SBRs were operated under three SRTs of 5, 10, and 20 d. Higher 17ß-Estradiol removals (>99%) were observed for the SBRs operated in SRTs of 10 and 20 d. Neither estrogens nor estrogenic activity was detected in the treated water, except some samples from the SBR operated at SRT of 5 d. The results suggested that bioaugmented bioreactors operating at long SRTs (10 and 20 d) were effective in removing 17ß- Estradiol to the non-estrogenic treatment endpoint.	[330]
a 2.2-L lab-scale SBR	Synthetic wastewater, Pyridine: 1000-4000 $mg.L^{-1}$	No information about primary seed is given.	The aerobic granules containing *Rhizobium sp. NJUST18*	The aerobic granules could degrade pyridine at extremely high volumetric degradation rate (between 1164.5 $mg.L^{-1}.h^{-1}$ and 1867.4 $mg.L^{-1}.h^{-1}$), demonstrating excellent pyridine degradation performance.	[342]
a 22-L anoxic and oxic activated sludge system	Quinoline (N-heterocyclic aromatic compound): 500 $mg.L^{-1}$	Inoculation with activated sludge	*Bacillus sp. Q2* (isolated from petroleum-contaminated soil)	100% removal in 22 h versus <5% in 45 h for non-augmented sludge	[343]
A 10-L lab-scale MBR	Synthetic dye wastewater, Bromoamine acid: 150-300 $mg.L^{-1}$		*Sphingomonas xenophaga QYY*	The augmented MBR showed the color and COD removal of 90% and 50%, respectively. The augmented MBR possessed relatively stable treatment abilities, in which the introduced strain *QYY* could be persistent and co-exist well with the indigenous populations.	[344]
a 2-L lab-scale activated sludge	Tobacco wastewater, Nicotine: 1000 $mg.L^{-1}$		*Acinetobacter sp. TW* as a nicotine-degrading strain	COD and Nicotine removal reached up to 90% and 98%, respectively. Moreover, compared with the non-bioaugmented system, the amounts of protein carbonyls and 8-OHdG were significant lower in the bioaugmented systems, which suggested that strain *TW* played an important role in eliminating the nicotine toxicity from the bioreactors.	[345]
A 11.8-L lab-scale MBR	Real textile wastewater, Acid Orange II, 100 $mg.L^{-1}$	white-rot fungus *Coriolus versicolor (NBRC 9791)*		This fungal MBR achieved 93% removal during long-term non-sterile operation at a HRT of 1d.	[338]

Continue of Table 14: Several examples of the single-strain bioaugmentation of activated sludge-based systems for removal of industrial pollutants and MPs from wastewater

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the reactor	Type of Pollutant & pollution level	Primary seed	Augmented seed	Main results	Reference
a 1.5 L lab-scale SBRs	Synthetic wastewater, 2,4 Dichlorophenoxyacetic acid, 30-90 mg/L	Aerobic granular sludge seed	Plasmid pJP4 mediated bioaugmentation by *Pseudomonas putida SM1443* as a carrier	2,4 Dichlorophenoxyacetic acid was removed up to 97%	[346]
a 2-L lab-scale SBR	Tobacco wastewater, Nicotine: 250 mg.L^{-1}	Inoculation with activated sludge	*Pseudomonas sp. HF-1* as a nicotine-degrading strain	Compared to the non-bioaugmented system, the bioaugmented system exhibited considerably stronger pollution disposal abilities, with 100% nicotine degradation and more than 84% COD removal. Moreover, bioaugmentation of strain *HF-1* resulted in the maintenance of high treatment activity by minimizing the Nicotine toxicity for other microbes in the bioaumented system.	[347]
a 3.5-L lab-scale SBR	Synthetic wastewater, O-Nitrobenzaldehyde: 100 mg.L^{-1}		*Pseudomonas putida ONBA-17*	In addition to the shorter required time for start-up, 100% degradation of o-nitrobenzaldehyde was obtained as compared with 23.5% of the non-inoculated control.	[348]
a 2.5-L lab-scale SBRs	Synthetic wastewater, 2,4,6-Trichlorophenol: 250–760 µM	Granular sludge previously acclimated to 2,4 -dichlorophenol	*Desulfitobacterium sp.*	Bioaugmentation did not significantly improve the anaerobic biodegradation of 2,4,6-trichlorophenol.	[349]
A 2-L lab-scale SBR	Synthetic wastewater, Phenol in alkaline Medium: 550 mg.L^{-1}	Inoculation with activated sludge (The optimal proportion of activated sludge and strain *JY-2* was controlled as 20:1 (dry weight))	*Pseudomonas JY-2* (isolated from Activated sludge)	90% of phenol was degraded within 1.5 days in bioaugmented system, while only 65% of phenol was degraded in the non bioaugmented one	[350]
a full-scale municipal aerated lagoon	Pulp and paper wastewater, Dehydroabietic acid (DhA): 20 mg.L^{-1}	Natural microbial community	*Zoogloea resiniphila DhA-35*, a DhA-degrading bacterium	This bacterium was persistent after introduction into the lagoon microbial community, and its cellular rRNA:rDNA ratio increased during the period of DhA removal. The introduction of strain *DhA-35* changed the microbial community structure, but did not adversely affect the TOC removal by the community.	[351]

Table 15: Bioaugmentation of activated sludge-based systems with the addition of pre-adapted consortia for removal of industrial pollutants and MPs from wastewater

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the reactor	Type of wastewater & pollution level	Primary seed	Augmented seed	Main results	Reference
a 2-L lab-scale conventional activated sludge	Synthetic wastewater, COD: 250 mg.L^{-1}, Tetrahydrofuran (a polar reagent): 20 mM	Inoculation with activated sludge	A bacterial consortium including *Rhodococcus sp. YYL, Bacillus aquimaris MLY2, Bacillus cereus MLY1*	After bioaugmentation of the reactor, strain *YYL* quickly became dominant in the system and was incorporated into the activated sludge. The concentration of MLSS increased from 2.1 g/L to 7.3 g/L in 20 d, and the efficiency of Tetrahydrofuran removal from the system was remarkably improved (95%).	[352]
A 6-L lab-scale MBR	Synthetic wastewater, COD: 240 mg.L^{-1} Atrazine: 15–20 mg.L^{-1}		Atrazine-degrading GEM (genetically engineered microorganism) of *Pseudomonas sp. ADP* and *Escherichia coli DH5α*	The removal efficiency of Atrazine was above 90%. High initial influent atrazine loading, high operation temperature and large initial density of genetically engineered microorganism were favorable to shorten the start-up period up to 2 days.	[353]
a 8-L lab-scale conventional activated sludge	The synthetic Oil-Containing wastewater, Mixture of lipids: 250 – 1000 mg.L^{-1}.	Inoculation with activated sludge	A commercial consortium of *Bacillus, Pseudomonas, Rhizobium, Acinetobacbacter, Comamonas* and *Lactobacillus.*	The mixture of lipids removal efficiency in the reactor with microbial supplement was higher than in the reference reactor. In addition, the bioreactor with microbial supplement is characterized by higher microbial community diversity than non-bioaugmented bioreactor and there was a significant difference between the beta and gamma-proteobacteria content in the reactor with microbial supplement.	[354]
a 20-L pilot-scale MBR	Synthetic wastewater, Acetaminophen: 100 µg.L^{-1}	Inoculation with nitrifying activated sludge	*Delftia tsuruhatensis* *Pseudomonas aeruginosa*	>99.9% abatements were observed and isolation of *D. tsuruhatensis* able to use Acetaminophen as sole carbon source	[114]

3.6.7. *Bioaugmentation of biofilm reactors for MPs removal*

In bioremediation, the use of carriers provides a physical support for biomass, accompanied with a better access to nutrients and moisture, which extends the survival rate of the microorganisms. Under field conditions, extended survival of the microbes is essential for efficient degradation of the pollutants, especially the recalcitrant ones, because they are not often degraded during the early stage of the bioremediation process [355]. Therefore, the strategies of microbial cell encapsulation [356] and immobilization [357] can lead to a better survival rate by shielding cells under stressed environmental conditions, usually enabling a faster and more efficient biodegradation as compared to suspended biomass [299]. In Section 3S in supplementary data, several examples about the capability of immobilization technique for pollutants removal from wastewater are given.

In wastewater treatment, the immobilization of microorganisms has been proposed as a novel strategy for preventing wash-out of the degraders [358]. In both configurations of biofilm reactors i.e. fixed-bed and moving-bed bioreactors already introduced in Section 2.4.5.2, biofilm can be considered as a convenient place for immobilizing of pre-selected MPs-degrading bacterial and fungal strains [262]. To date, many attempts for bioaugmentation of biofilm reactors have failed [262]. For instance, in the study of Feakin et al. [359], two bacterial strains of *Rhodococcus rhodochrous* and *Acinetobacter junii* capable of biodegrading Atrazine and Simazine (1-10 µg. L^{-1}) were inoculated into a fixed-bed reactor pre-filled with silanized glass wool and GAC. The reactors (one as a control and the other one as a bioaugmented reactor), continuously operated at an empty bed contact time of 20 min, did not show a satisfying biodegradation rate i.e. the removal rate ranged from 19.5 to 32% of each herbicide for both inoculated and non-inoculated reactors [359].

In spite of the point that bioaugmentation of biofilm reactors needs some feats of bio-technological expertise [298], Table 16 demonstrates its outstanding capability for purification of industrial wastewaters and also the removal of MPs from wastewater.

In the case of the treatment of industrial wastewaters, as an example, Anastasi et al. [360] inoculated a fungal strain named *Bjerkandera adusta* in a packed-bed bioreactor (filled with the colonized sponges) and achieved an effective decolorization of real textile wastewater [360]. To give another example, Yang et al. [361] inoculated a fungal consortium into a continuous biofilm reactor filled with polyethylene fiber wads. The optimal nutrient feed to this bioreactor was 0.5 g. L^{-1} glucose and 0.1 g. L^{-1} $(NH_4)_2SO_4$ when 30 mg. L^{-1} reactive black 5 was used as an initial dye concentration. Dye mineralization rates of 50–75% and color removal efficiencies of 70–80% were obtained at HRT of 12h. Additionally, the microbial community on the biofilm was monitored in the whole running process. The results indicated that fungal strains are dominant populations in the biofilm with the ratio of fungi to bacteria 6.8:1 to 51.8:1 under all the tested influent conditions [361].

As seen in Table 16, studies associated to the MPs removal using bioaugmented biofilm reactors are still limited. As an example, Jelic et al. [341] studied the aerobic biodegradation of Carbamazepine in a fluidized bed bioreactor bioaugmented by WRF *Trametes versicolor*. Around 96% of Carbamazepine was removed after 2 days in the batch mode of operation. In the continuous mode, at HRT of 3 d, 54% of the influent Carbamazepine was reduced at the steady state condition with a Carbamazepine degradation rate of 11.9 µg Carbamazepine g^{-1} dry weight d^{-1}. No metabolites resulted from the Carbamazepine biodegradation were detected in both batch and continuous mode of operation. Also, no assessment was presented by the authors to see whether *Trametes versicolor* has been dominant in microbial population of the bioreactor [341].

In a novel strategy recently used for bioaugmentation of biofilm reactors, immobilizing specific-pollutant degrading strains into the biofilm is mediated by biofilm-forming bacteria. A handful of studies have shown that this strategy might be an efficient approach for colonization of the degraders into the biofilm. For instance, bioaugmentation of sequencing batch biofilm reactors with bacterial strains of *Comamonas testosteroni* and *Bacillus cereus* and their impact on reactor bacterial communities was investigated by Cheng et al. [362]. The reactors, filled by sphere-like porous PVC carriers, were firstly inoculated with activated sludge and continuously fed by a synthetic wastewater containing 100-500 mg. L^{-1} 3,5-dinitrobenzoic acid. After the start-up stage, the reactors were inoculated by *Bacillus cereus* G5 as a biofilm-forming bacteria and *Comamonas testosteroni* A3 as a 3,5 dinitrobenzoic acid (DNB)-degrading bacteria, and continuously operated at a HRT of 24 h. In the bioaugmented reactor, the removal efficiency of 3,5-dinitrobenzoic acid was achieved up to 83% after 28 days of operation, while this value was reported by 75.9% after 33 days of operation in non-bioaugmented reactor. Although, the difference between removal efficiencies is low, but the bioaugmented reactor exhibited obvious resistance to shock loading with 3,5-dinitrobenzoic acid. The microbial diversity in the reactors was also explored. *C. testosteroni* A3 was predominant in the bioaugmented reactor, indicating the effect of *B. cereus* G5 in promoting immobilization of *C. testosteroni* A3 cells in the biofilm. They finally concluded that those microbial strains, e.g. *B. cereus* G5, which can stimulate the self-immobilization of the degrading bacteria offer an innovative method for immobilization of degraders in bioaugmented biofilm reactors [362]. The same strategy was also used by Chunyan Li et al. [363], whereby a unique biofilm consisting of three bacterial strains with high biofilm-forming capability (*Bacillus subtilis* E2, E3, and N4) and an acetonitrile-degrading bacteria (*Rhodococcus rhodochrous* BX2) was established for acetonitrile-containing wastewater treatment in MBBR reactors. Activated sludge was first used for inoculation of reactors and then the above strains were added to the reactors. Continuous operation of reactors lasted for 30 days at HRT of 24h. The bioaugmented MBBR exhibited strong resistance to Acetonitrile loading shock and completely depleted the initial concentration of Acetonitrile (800 mg. L^{-1}). The immobilization of *R. rhodochrous* BX2 cells in the biofilm was also confirmed by PCR–DGGE method. Similar to Cheng et al. [362], they revealed that biofilm-forming bacteria can promote the immobilization of contaminant-degrading bacteria in the biofilms and can subsequently improve the degradation of contaminants in wastewater [363]. Even to be more cost-effective and less laborious that this strategy, Dvorak et al. [364] used only one strain for bioaugmentation of full-scale MBBRs treating an industrial wastewater containing Aniline and Cyanide. They used *Rhodococcus erythropolis* CCM that has a proven ability to catabolize a wide range of compounds and metabolize harmful environmental pollutants. Furthermore, this strain has a good biofilm-forming ability and have a high resistance to extreme conditions (e.g. salinity 2–3% and temperatures of 10–38 °C). Over a long operation time of 5 years, the removal rates of Aniline and Cyanide were obtained up to 75-99% and more than 88% respectively [364]. From our literature review, no report has been so far published in terms of MPs removal by this strategy.

Table 16: Bioaugmentation of biofilm reactors for removal of industrial pollutants and MPs from wastewater

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the reactor	Type of wastewater & pollution level	Primary seed	Augmented seed	Main results	Reference
A 2 L lab-scale plate bioreactor filled with a porous polyether foam	Effluent of a municipal WWTP, COD: 28 mg. L^{-1} Carbamazepine: 1 mg. L^{-1}	Bioaugmentaion with white rot fungus *Phanerochaete chrysosporium (BKM F-1767)*		It was found that the sufficient supply with nutrients is crucial for an effective elimination of Carbamazepine. A high elimination of Carbamazepine (60–80%) was achieved. The effective elimination was stable in a continuous operation for a long term (around 100 days).	[340]
A 1.5 L lab-scale fluidized bioreactor	Synthetic wastewater, Carbamazepine: 200 µg. L^{-1}	Bioaugmentaion with the pure white rot fungus *Trametes Versicolor* (ATCC 42530)		With a HRT of 3 d, 54% of the inflow concentration was reduced at the steady state condition (SRT: 25 d) with a Carbamazepine degradation rate of 11.9 µg Carbamazepine g^{-1} dry weight d^{-1}.	[341]
A lab-scale 3 L RBC	Synthetic wastewater, a mixture of azo dyes including Direct Red-80 and Mordant Blue-9, 25-200 mg. L^{-1}	white-rot fungus *Phanerochaete chrysosporium*		The system could completely decolorize the wastewater at 48 h HRT. The effect of increase in the disc rotation speed from 2 to 6 rpm in the study revealed no large differences in the decolourization efficiencies of the wastewaters.	[339]
A 10 L lab-scale MBBR	Synthetic wastewater, COD: 400 mg. L^{-1} Acetonitrile = 800 mg. L^{-1}	Inoculation with activated sludge	Three bacterial strains of *Bacillus subtilis E2, E3,* and *N4* with high biofilm-forming capability., and *Rhodococcus rhodochrous BX2* as an acetonitrile-degrading bacterium	This biofilm exhibited strong resistance to Acetonitrile loading shock and displayed a typical spatial and structural heterogeneity and completely depleted the initial concentration of acetonitrile within 24 h. Furthermore, that biofilm-forming bacteria can promote the immobilization of contaminant-degrading bacteria in the biofilms and can subsequently improve the degradation of contaminants in wastewater.	[363]
A 520 m^3 full-scale MBBR (two reactors in series)	Industrial wastewater, COD: 40-10340 mg. L^{-1} Aniline:78-4970 mg. L^{-1}, Cyanides:0.8-850 mg. L^{-1} (During a five-year operation)		*Rhodococcus erythropolis CCM2595* chosen for its good biofilm-forming ability and good degradation efficiency of Cyanides and Aniline.	Cyanide removal efficiency: 75% to 99%, Aniline removal efficiency: more than 85%, and COD removal efficiency fluctuated considerably throughout MBBR operation, ranging from 31% to 87%.	[364]

Continue of Table 16: Bioaugmentation of biofilm reactors for removal of industrial pollutants and MPs from wastewater

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the reactor	Type of wastewater & pollution level	Primary seed	Augmented seed	Main results	Reference
a 5 L lab-scale biofilm-activated sludge (filled with porous polyurethane foam as carriers)	petrochemical wastewater at low temperatures (13-15°C) (type of pollutant is not given in the study)	Inoculation with activated sludge taken from the petrochemical wastewater treatment plant	Mixed bacteria belonged to *Pseudomonas, Bacillus, Acinetobacter, Flavobacterium and Micrococcus.*	The COD and NH_4^+-N removal was obtained up to 75.80% and 70.13% respectively. The application of polyurethane foam as carrier in the bioaugmentation practice is promising for the retention of sufficient biomass and prevention mechanisms to the immobilization cells.	[257]
A 5.3 m^3 biofilm oxidation ditch reactor	Nitrogen-rich water, TN: 45 mg. L^{-1} NH_4^+–N: 30 mg. L^{-1}	Inoculation with activated sludge taken from a WWTP with a hybrid biofilm-activated sludge process	Augmentation with the enrichment cultures of nitrifying bacteria (AOB and NOB) onto the surface of the AquaMats carriers).	Enhancement of the removal efficiency of TN and NH_4^+–N from 25.9% to 50.3%, and from 34.5% to 60.1%, respectively was observed. Moreover, Augmentation of nitrifying bacteria could significantly increase the quantity of AOB and NOB both in water and on biofilm.	[328]
A 5 L lab-scale sequencing batch biofilm reactor	Synthetic wastewater, 3,5 dinitrobenzoic acid: 100-500 mg. L^{-1}	Inoculation with activated sludge	*Bacillus cereus G5* as biofilm-forming bacteria and *Comamonas testosteroni A3* as 3,5 dinitrobenzoic acid -degrading strain	*Comamonas* was predominant in the reactor, indicating the effect of *G5* in promoting immobilization of *A3* cells in biofilms. Those microbial resources, e.g. *G5*, which can stimulate the self-immobilization of the degrading bacteria offer a novel strategy for immobilization of degraders in bioaugmentation systems and show broader application prospects. In other words, immobilizing specific-pollutant degrading strains into biofilms mediated by biofilm forming bacteria might be an efficient approach for colonization of the degraders in bioaugmentation treatment systems. In this study, removal efficiency of 3,5 dinitrobenzoic acid obtained up to 83%.	[362]
a 5 L lab-scale SBR filled with modified zeolite	Coke wastewater, Pyridine: 41.0 mg. L^{-1}, Quinoline: 45 mg. L^{-1}.	Inoculation with activated sludge taken from coking wastewater treatment plant.	A bacterial consortium including two pyridine-degrading bacteria (*Paracoccus sp. BW001* and *Shinella zoogloeoides BC026*) and a quinoline-degrading bacterium (*Pseudomonas sp. BW004*)	During a 120-day operation, the bioaugmented reactor removed over 99 % Pyridine and 99 % Quinoline,	[365]

Continue of Table 16: Bioaugmentation of biofilm reactors for removal of industrial pollutants and MPs from wastewater

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the reactor	Type of wastewater & pollution level	Primary seed	Augmented seed	Main results	Reference
a 4 L lab-scale biological aerated filters filled with zeolite	Coke wastewater, COD: 1700 mg. L^{-1}, NH_3-N: 86 mg. L^{-1}. Phenol: 200 mg. L^{-1}, Naphthalene: 59 mg. L^{-1} Carbazole: 12.5 mg. L^{-1}.	Inoculation with activated sludge taken from coking wastewater treatment plant.	Bioaugmentation with free and magnetically immobilized cells of *Arthrobacter sp. W1* as a Phenol-degrading bacterium	The introduced strain *W1* remained dominant in the bioaugmented reactor, indicating both strain *W1* and the indigenous degrading bacteria played the most significant role in the treatment. The removal efficiency of the phenolic compounds were between 70-80%.	[366]
A 3 L lab-scale aerobic sequencing batch biofilm reactor	Coke wastewater, Quinoline: 100 mg. L^{-1}.		*Brevundimonas sp. K4* as a Quinoline -degrading strain	The results showed that bioaugmentation by both free and immobilized *K4* strains enhanced Quinoline removal efficiency, and especially, the latter could reach its stable removal after a shorter accommodation period, with 94.8% of mean quinolone removal efficiency.	[367]
a 1280 m^3 full-scale Bio-SAC process (a novel fluidized bed reactor)	Coke wastewater, Ferric cyanide:14 mg. L^{-1}.	No information about the primary seed is given.	a cyanide-degrading yeast *(Cryptococcus humicolus)* and unidentified cyanide- degrading microorganisms	Continuous operation showed poor removal efficiency than expected owing to poor settling performance of microbial flocs, slow biodegradation rate of ferric cyanide and lack of organic carbon sources within the wastewater.	[368]
Two lab-scale 1.7 and 4 L agitated and biofilm SBRs	Synthetic wastewater, 2,4, dichlorophenoxyacetic acid: 45-500 mg. L^{-1}	Aerobic granular sludge seed or the strains *E. coli DH5*$_a$, *Alcaligenes sp.*, mixed culture of aerobic granular sludge, respectively.	Plasmid pJP4 mediated bioaugmentation by *Pseudomonas putida SM1443* as a carrier or transconjugant *Alcaligenes sp.* (with plasmid pJP4)	In biofilm approach, 2,4-D was completely degraded as sole carbon source by bioaugmented biofilm versus 86% degradation in acclimated controls. In agitated reactors, bioaugmented reactor showed enhanced degradation kinetics on the first days, but lost this superiority with time compared to control. Finally, bioaugmentation increased 2,4-D average removal rate significantly with an enhancement of 12–14 and 98% respectively with the three mentioned primary seeds.	[369]
a 2 L lab-scale MABR (membrane-aerated biofilm reactor)	Synthetic dye wastewater, Acid Orange 7: 50–200 mg. L^{-1}	Inoculation with activated sludge	*Shewanella sp. XB* (quinone reducer)	Decolorization reached 98% in 6 h with colorless effluent against only 57.8% with yellow effluent by conventional method	[370]

Continue of Table 16: Bioaugmentation of biofilm reactors for removal of industrial pollutants and MPs from wastewater

(Grey rows: secondary-treatment of raw sewage., Blue rows: secondary-treatment of industrial or hospital wastewater., Green rows: tertiary treatment of sewage)

Type of the reactor	Type of wastewater & pollution level	Primary seed	Augmented seed	Main results	Reference
a 38 L pilot-scale sequencing batch biofilm reactors	synthetic wastewater, Benzyl alcohol: 162 mg. L^{-1}	Inoculation with activated sludge taken from a biofilm reactor treating municipal wastewater	TOL plasmid mediated bioaugmentation by *Pseudomonas putida KT2442*	Benzyl alcohol degradation rate was enhanced after inoculation from 0.98 prior to inoculation to 1.9 mg Benzyl alcohol /min on the seventeenth day of operation.	[295]
a 10 L lab-scale RBC	Synthetic wastewater, 2-Fluorophenol: 50 mg. L^{-1}	Inoculation with activated sludge	2-Fluorophenol-degrading bacterial strain named strain *FP1*	Complete biodegradation was observed throughout the study.	[371]
a 0.5 L lab-scale anaerobic biofilter	Dairy wastewater Pollutants (not mentioned in the study)	Inoculation with activated sludge	Commercial inocula: *HydroPacks, Bilikuk & Laktazym*	Bioaugmentation with commercial inocula did not improve the performance of the biofilter.	[372]
A 1.4 L lab-scale anaerobic sequencing batch biofilm reactor	Sulphate bearing chemical wastewater COD: 6000 mg. L^{-1}, Sulphates: 1600 mg. L^{-1}	Inoculation with anaerobic seed acquired from a lab-scale UASB treating chemical wastewater.	The reactor was augmented with enriched Sulphate Reducing Bacteria (SRB) consortia entrapped in the alginate matrix (the name of bacteria is not given).	After augmentation, COD removal efficiency enhanced from 35% to 78% and sulphate reduction from 27% to 80%. Concomitant increase in the biogas yield and reduction in VFA concentration in the system were also observed.	[315]
a 1-L lab-sclale anaerobic biofilm-based column reactors	Strong municipal synthetic wastewater, COD: 1000 mg. L^{-1}	Inoculation with anaerobic sludge of another acidogenic reactor	*Ethanoligenens harbinense B49*	Specific hydrogen production rate was obtained up to around 1.36 $L.g^{-1}.VSS^{-1}.d^{-1}$ versus 1.10 $L.g^{-1}.VSS^{-1}.d^{-1}$ for non-bioaugmented reactor.	[322]

4. Outline of the strategies used for tertiary removal of target MPs

If, on one hand, development of tertiary treatment technologies is becoming an inevitable part of today's research, on the other hand, they may produce negative environmental impacts in terms of the energy and chemical consumption. From bibliographic review aforesaid, many tertiary treatment technologies are also yet faced with some problematic issues, such as the lack of selectivity (e.g. AOPs [121]), high energy consumption (e.g. RO [373]), biofouling (e.g. MBRs [242]), high land requirements (e.g. wetlands [196]), physical clogging (e.g. biofilters [219]), regeneration process of the spent carbon in GAC filters [36], difficulty of harvesting the biomass in algal bioreactors [234]), etc. Apart from that, these processes still exhibit unreliable or unsatisfactory levels of MPs removal [48].

How green are environmental technologies? This is an important question for scientists in order to prioritize their efforts to develop tertiary treatment technologies. Indeed, research on such technologies must be switched from non environment-friendly methods to the ways, in which, environmental considerations are taken into account. To broaden the green horizon of tertiary treatment technologies, two different approaches were examined in this thesis, including i) MBBRs, and ii) polyelectrolyte multilayer (PEM)-based NF membranes.

Of tertiary treatment technologies, MBBR is recently seen as a proficient approach in MPs removal [97,213]. This dual-biomass reactor achieves a high SRT in a low HRT, eliminates microbial wash out by the biofilm, and encourages the growth of slow-growing microbes that have a proven capability in MPs removal [374]. Many above-mentioned problematic issues seen for other technologies do not exist for such a system. Regardless of an inevitable aeration that needs energy, no adverse environmental impact is expected in MBBRs. Hence, MBBRs seem to be a promising alternative compared to other technologies for the elimination of MPs.

As discussed in Section 2.3 and shown in Table 9, existing RO and high-efficient NF membranes (such as NF90) are completely proficient in the tertiary removal of target MPs. As compared to the RO, NF requires lower energy and has higher permeate fluxes for several commercial purposes, such as wastewater reclamation [157,158]. Also for MPs removal, NF membranes are seen as a more cost effective alternative to RO membranes [65,67]. One of the major drawbacks of such membranes is the production of an unwanted stream named "concentrate" containing all the retained compounds [375]. Simple and non eco-friendly methods such as land application and discharge to a surface water, deep wells, and evaporation ponds have been so far used in many plants worldwide [376]. Since the direct discharge of an untreated concentrate poses a significant risk to the environment, over the last decade, several labor-intensive and costly methods like AOPs, adsorption and ion exchange have been well developed to reduce its harmful effects on the environment [376–378]. In addition, biological treatment of the concentrate has been recently taken into account by some researchers as cost-effective and environment-friendly alternatives [379,380]. The main obstacle for the biological treatment of MP-bearing concentrates is their high salinities, that can cause high osmotic stress for the involved microorganisms or the inhibition of the reaction pathways in the organic degradation process [381,382]. Indeed, the efficiency of MPs biodegradation drastically declines due to the high salt content of the concentrate steam [383–385]. As a remedial study, we aimed at preparing an innovative type of PEM-based NF membranes to combine two abilities of “low salts rejection” and “high MPs retention”. Low rejection of salts leads to the production of a low-saline concentrate, something that will facilitate its biological treatment.

4.1. Tertiary MBBRs

To date, it is been demonstrated that high removal of MPs in a tertiary MBBR necessarily entails the intermittent feeding the reactor by raw wastewater to provide enough carbon and nutrients [213]. A positive correlation has been also shown between the biofilm thickness and the removal of a broad range of MPs in tertiary nitrifying MBBRs. To form a thick nitrifying biofilm, secondary-treated wastewater must be enriched by ammonium in order to stimulate the growth of slowly-growing bacterial species of AOBs and NOBs [106]. In **Chapter II**, we investigated the performance of tertiary MBBRs that were not intermittently fed by either raw wastewater or ammonium-rich secondary-treated wastewater. Instead, we focused on the formation of a thin and viable biofilm that was well adapted to the target MPs. Abiotic and biotic removals of MPs were comprehensively studied in this chapter.

4.2. Tertiary bioaugmented MBBRs

Like any technique, there are positive and negative aspects to the use of bioaugmentation for MPs removal. The main advantage provided by bioaugmentation is that it can remove pollutants that might otherwise be very costly and time-consuming to remediate. For full-scale applications, this point converts to a benefit when the inocula is produced in a short time and in a cost-effective approach [277]. On the other hand, the process of bioaugmentation is always accompanied with several challenges. For instance, the presence of several contaminants can sometimes decelerate their biodegradation. Therefore, pollutants that inhibit the degradation of other compounds should be removed first, even if they have lower toxicity than the others [353]. Another challenge is the survival of inocula during the wastewater treatment [334].

Although the attempts to use “bioaugmentation of biofilm reactors” did not hitherto show reliable results to improve MP biodegradation [262], this area of research remains fascinating and potentially promising, for example, to understand the proper and viable implantation of bioaugmented strains into the biofilm’s microbial community, and to assess its subsequent effects on MPs removal. A glance through the literature indicates that low attention has been so far directed towards the application of bacterial/fungal bioaugmentation for tertiary MPs removal. Taking this into account, the continued development of knowledge discussed briefly above, proves that bacterial/fungal bioaugmentation can be estimated as promising technologies if, of course, some feats of biotechnological science are employed. Nevertheless, the issue of tertiary MPs removal in bioaugmented bioreactors is still young, and needs to be studied in detail. In **Chapter III**, we aimed at determining whether bacterial bioaugmentation of tertiary MBBRs could successfully enhance MPs removal from conventionally-treated municipal wastewater. Along with assessing the biotic and abiotic aspects of MPs removal, implantation of newly-introduced microbial strains into the biofilm and liquid phase was also monitored by DNA extraction and quantitative polymerase chain reaction assay (qPCR).

Laboratory experiments of tertiary MBBRs (**Chapters II & III**) were carried out at the "Laboratory of Chemical Engineering (LGC)" located in "Institut National Polytechnique (INP)" of Toulouse (France).

4.3. PEM-based NF

In **Chapter IV**, we aimed at preparing and studying a NF membrane that combines a low salt rejection with a high MPs rejection for the treatment of secondary-treated municipal wastewater. This strategy would lead to make membrane processes with a low-saline concentrate stream which is more convenient for the biological treatment in activated sludge systems. This membrane was prepared using layer by layer (LbL) deposition of two weak and oppositely-charged polyelectrolytes on the surface of a hollow fiber dense UF membrane. The impact of ionic strength of the coating solutions was then evaluated on the properties of the formed PEMs (e.g. hydration ratio) followed by the performance of the PEM-based membranes in terms of ions and MPs retention. All laboratory experiments and filtration tests of **Chapter IV** were performed at the group "Membrane Science and Technology (MST)" of the "Faculty of Science and Technology" in the University of Twente (the Netherlands).

In **Chapter V**, we evaluated the effect of PEMs' post-treatment on the properties and performance of weak PEM-based NF membranes. PEMs were coated on the surface of flat-sheet polyacrylonitrile (PAN) UF membranes. They were then post-treated by the thermal and/or salt annealing, and were carefully characterized before and after annealing by ions and MPs rejection over a long filtration time. All filtration tests and laboratory experiments of **Chapter V** were performed at the group "Membrane Technology Group (COK)" of the "Department of Molecular and Microbial Systems" in the KU Leuven (Belgium).

Chapter VI gives the main outcomes of the present study, along with some recommendations and ideas for future of the work.

Note that experimental works for MBBRs and PEM-based NF membranes were carried out independently for each given concept, and our final aim was not the comparison of such processes.

Supplementary data of Chapter (I)

Micropollutants removal from wastewater: Focus on tertiary treatment technologies

Section S1: Principal methods used by fungi to degrade organic chemicals

In brief, as described by Harms et al. [329] (Fig. 1S), initial pollutant attack may occur extracellularly or intracellularly. Metabolites generated during extracellular pollutant oxidation may be subject to intracellular catabolism. Metabolites arising from intracellular initial attack may be excreted and can then either undergo further extracellular enzymatic reactions or form bound residues through abiotic oxidative coupling. Ultimately, it may result in mineralization or metabolite excretion that can further form bound residues [329].

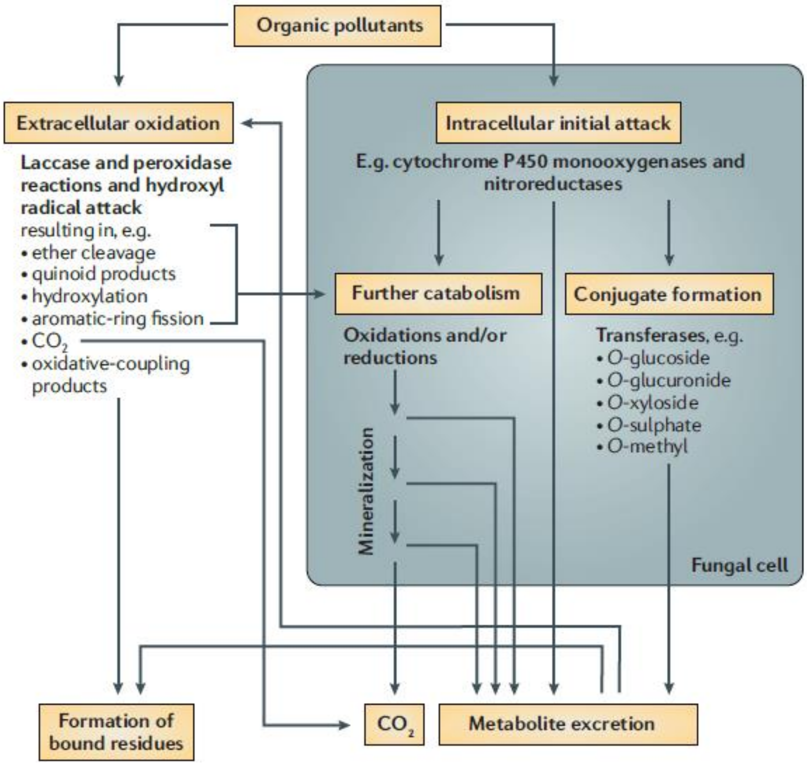


Fig. 1S. Principal methods used by fungal species to degrade organic chemicals (adapted from Harms et al. [329])

Table 1S. Potential of different fungal species for MPs removal from wastewater

	Removal (%)	Initial Concentration (mg.L^{-1})	Incubation time (d)	Removal (%)	Initial Concentration (mg.L^{-1})	Incubation time (d)	Removal (%)	Initial Concentration (mg.L^{-1})	Incubation time (d)	Removal (%)	Initial Concentration (mg.L^{-1})	Incubation time (d)	Removal (%)	Initial Concentration (mg.L^{-1})	Incubation time (d)	Removal (%)	Initial Concentration (mg.L^{-1})	Incubation time (d)	Removal (%)	Initial Concentration (mg.L^{-1})	Incubation time (d)
	Trametes versicolor (Laccase, LiP, MnP)			*Bjerkandera adusta* (Laccase, LiP, MnP)			*Irpex lacteus* (Laccase, MnP)			*Pleurotus ostreatus* (Laccase, MnP)			*Pycnoporus cinnabarinus* (Laccase, MnP)			*Dichotomitus squalens* (Laccase, MnP)			*Phanerochaete chrysosporium* (LiP, MnP)		
	MPs with strong EDG (mainly high removal)																				
4-Nonylphenol[1]	0	2.5	0.58	81	2.5	0.58	100	2.5	0.58	100	2.5	0.58	100	2.5	0.58	100	2.5	0.58	100	2.5	0.58
Nonylphenol[1]	100	2.5	0.58	100	2.5	0.58	100	2.5	0.58	100	2.5	0.58	100	2.5	0.58	100	2.5	0.58	100	2.5	0.58
Bisphenol A[1]	100	2.5	0.58	20	2.5	0.58	100	2.5	0.58	100	2.5	0.58	100	2.5	14	100	2.5	0.58	3	2.5	0.58
17 a-Ethynylestradiol[2]	100	10	14	100	10	14	100	10	14	100	10	14	100	10	14	100	10	14	38	10	14
	MPs with strong EWG (mainly low removal)																				
Carbamazepine[3,4,5,6,7]	76-80	0.01–0.05	1 - 2	100	1	14	2	10	7	100	0.035	7	-	-	-	-	-	-	0	10	7
	MPs with EDG and EWG (mainly highly removal)																				
Diclofenac[3,4,5, 8, 9, 10]	100	0.01-10	0.02-2	100	1	7	-	-	-	-	-	-	-	-	-	-	-	-	100	1	14
Naproxen[3,5, 10, 11]	100	0.01-10	0.25-2	100	1	14	-	-	-	-	-	-	-	-	-	-	-	-	100	1	14
Ibuprofen[3,6,8,10]	100	0.01-10	2 - 7	100	1	14	100	10	7	-	-	-	-	-	-	-	-	-	70-88	10	7
Triclosan[1]	15	2.5	0.58	10	2.5	0.58	96	2.5	0.58	92	2.5	0.58	-	-	-	-	-	-	-	-	-
	MPs with EDG and EWG (mainly low removal)																				
Diuron[12,13]	99	10	42	31-47	10	42	-	-	-	12	10	42	-	-	-	21	10	42	-	-	-
Atrazine[13]	-	-	-	-	-	-	-	-	-	58	10	42	-	-	-	25	10	42	86	10	42
Terbuthylazine[13]	63	10	42	-	-	-	-	-	-	31	10	42	-	-	-	52	10	42	-	-	-
Clofibric acid[3,6]	75-97	0.01-10	2-7	-	-	-	21	10	7	-	-	-	-	-	-	-	-	-	0-24	10	7

References:[1][386], [2][387], [3][388], [4][389], [5][332], [6][390], [7][391], [8][392], [9][393], [10][394], [11][395], [12][396], [13][397].

Section S2: Several examples about the application of fungal species and enzymes for MPs removal from secondary-treated wastewater

A novel plate bioreactor filled with a sheet of open-cell porous polyether foam was designed and operated by Zhang and Geißen [340] to eliminate Carbamazepine from secondary-treated effluent of a municipal WWTP in Berlin, Germany. The WRF of *Phanerochaete chrysosporium* was grown on polyether foam under non-sterile conditions. Then, in the continuous mode of operation, the biodegradation rate and removal of Carbamazepine was obtained by 9 $mg.m^{-2}.d^{-1}$ and 60-80%, respectively. The effective elimination was stable in the continuous operation for a long term (around 100 days). It was also found that the sufficient supply with nutrients is crucial for an effective elimination of Carbamazepine [340].

While the extensively studied WRF such as *Trametes versicolor* are attractive candidates with their high production rates of LMEs such as laccase, very little is still known about the potential of bacterial laccases for bioremediation applications [398]. Laccases from bacterial strains of *Streptomyces psammoticus* and *Streptomyces ipomoea* showed high activity at slightly alkaline pH values (i.e. 7–8) found in wastewater, as well as tolerance to high NaCl concentrations (i.e. > 1 M) [399,400].

Margot et al. [398] compared fungal and bacterial laccase for MPs removal from secondary-treated wastewater. Four strains of the bacterial genus *Streptomyces* (*S. cyaneus*, *S. ipomoea*, *S. griseus* and *S. psammoticus*) and the WRF of *Trametes versicolor* were compared to understand their ability to produce active extracellular laccase in municipal secondary-treated wastewater with different carbon sources. Among the *Streptomyces* strains evaluated, only *S. cyaneus* produced extracellular laccase with sufficient activity to envisage its potential use in WWTPs. Laccase activity produced by *T. versicolor* was more than 20 times greater. The laccase preparation of *S. cyaneus* (L_{Sc}) and laccase from *T. versicolor* (L_{Tv}) were further compared in terms of their activity and MPs oxidation efficiency. L_{Sc} and L_{Tv} showed highest activities under acidic conditions (i.e. pH: 3 - 5), but L_{Tv} was active over wider pH and temperature ranges than L_{Sc}, especially at neutral pH and temperature between 10°C and 25°C (typical conditions found in WWTPs). Furthermore, both L_{Sc} and L_{Tv} oxidized three MPs of Diclofenac, Bisphenol A, and Mefenamic acid, with faster degradation kinetics observed for L_{Tv}. As a consequence, *T. versicolor* appeared to be the better candidate to remove MPs from secondary-treated wastewater [398].

Section S3: Several examples about the potential of immobilization technique for pollutants removal from wastewater

In the study of Liu et al. [401], *Acinetobacter sp.* XA05 and *Sphingomonas sp.* FG03 strains with high biodegradation activity of phenol were isolated from the activated sludge and phenol-contaminated soils, respectively. Then, the biodegradation of phenol by free and immobilized cells of both strains were compared. Strains XA05 and FG03 were mixed at the ratio of 1:1, and polyvinyl alcohol (PVA) was used as a gel matrix to immobilize mixed cells of two strains by repeated freezing and thawing. Both free suspended and immobilized cells showed high phenol degradation efficiencies, i.e. higher than 95% within 35h with an initial concentration of 800 mg. L^{-1} phenol, and the immobilized cells showed better performance and stability than that of the suspended cells. The authors reported that the toxicity of phenol at high concentrations could inhibit the growth of free cells, and the carrier material of the immobilized cell could act as a protective shelter against the toxicity of phenol [401]. Activity enhancement of immobilized cells has been also reported by Chung et al. [402] who revealed that immobilization of living cells could alter their physiological features in metabolism such as enhanced enzyme induction [402].

In addition to the microbes' immobilization, a great attention has been also paying to the enzymes' immobilization (especially for laccase). The enzymes, if available at large quantity and stable in sufficient time, however, need to be retained in a bioreactor by means of membranes or immobilization, which makes the process complex to be developed and operated [340]. Regardless of the membranes, in order to avoid the cost related to the large amount of free enzyme required in full-scale applications (due to losses during the treatment) [398], two strategies have been proposed: i) enzyme's immobilization on solid supports in order to reuse them for several times with one of the following methods including entrapment, encapsulation, adsorption, covalent binding, and self-immobilization [403], and ii) production of the enzyme during the wastewater treatment by means of laccase-producing microorganisms and cheap substrates [404]. The first option faces with the expensive immobilization techniques, while the latter option needs growing and maintaining the laccase-producing organisms during the wastewater treatment, a process that seems complicated and is still little studied [398]. Both options, in full-scale applications where sterilization is not feasible, are usually confronted with the contamination of other microorganisms in the wastewater matrix. This contamination might hinder the removal of pollutants. Therefore, development of bioaugmented processes working under non-sterile conditions seems necessary for the purpose of an efficient MPs removal [340].

CHAPTER (II)

Abiotic and biotic removal of micropollutants in tertiary moving bed biofilm reactors (MBBRs)

This chapter has been recently accepted as:

S. Mehran Abtahi, Maike Petermann, Agathe Juppeau Flambard, Sandra Beaufort, Fanny Terrisse, Thierry Trotouin, Claire Joannis Cassan, Claire Albasi; “Micropollutants removal in tertiary moving bed biofilm reactors (MBBRs): Contribution of the biofilm solids and suspended biomass.” *Science of the Total Environment., 2018*

Table of Contents

Abstract

The performance of tertiary moving bed biofilm reactors (MBBRs) was evaluated in terms of micropollutants (MPs) removal from secondary-treated municipal wastewater. After stepwise establishment of a mature biofilm, monitored by scanning electron and confocal microscopies, abiotic and biotic removals of MPs were deeply studied. Since no MPs reduction was observed by the both photodegradation and volatilization, abiotic removal of MPs was ascribed to the sorption onto the biosolids. Target MPs i.e. Naproxen, Diclofenac, 17ß-Estradiol and 4n-Nonylphenol, arranged in the ascending order of hydrophobicity, abiotically declined up to 2.8%, 4%, 9.5% and 15%, respectively. MPs absorption onto the suspended biomass was found around two times more than the biofilm, in line with MPs' higher sorption kinetic constants (k_{sor}) found for the suspended biomass. When comparing abiotic and biotic aspects, we found that biotic removal outperformed its counterpart for all compounds as Diclofenac, Naproxen, 17ß-Estradiol and 4n-Nonylphenol were biodegraded by 72.8, 80.6, 84.7 and 84.4%, respectively. The effect of the changes in organic loading rates (OLRs) was investigated on the pseudo-first order degradation constants (k_{biol}), revealing the dominant biodegradation mechanism of co-metabolism for the removal of Diclofenac, Naproxen, and 4n-Nonylphenol., while 17ß-Estradiol obeyed the biodegradation mechanism of competitive inhibition. Biotic removals and k_{biol} values of all MPs were also seen higher in the biofilm as compared to the suspended biomass. To draw a conclusion, a quite high removal of recalcitrant MPs is achievable in tertiary MBBRs, making them a promising technology that supports both pathways of co-metabolism and competitive inhibition, next to the abiotic attenuation of MPs.

1. Introduction

Nowadays, the high-risk occurrence of micropollutants (MPs), as priority hazardous substances in the aquatic environment, has created a global demand for developing innovative and cost-effective technologies to upgrade current wastewater treatment plants (WWTPs). Since most of the WWTPs are not designed to efficiently eliminate the majority of MPs [1], secondary-treated effluents have been world-widely recognized as the main source of these hazardous compounds in the water bodies [2]. To overcome this anxiety, scientists have been trying various types of tertiary treatment technologies such as advanced oxidation processes (AOPs) [3,4], adsorption processes [5] and membrane filtrations [6] throughout the last decade. In addition to these costly methods in the aspects of investment and operation [7], lower attentions have been paid to biological treatment of secondary-treated effluents due to not-satisfactory growth of microbial strains at very low substrate concentrations i.e. low carbon sources and nutrients [8]. In spite of this fact, recently, moving bed biofilm reactors (MBBRs) are under the sharp-eyed investigation to see their capability in tertiary treatment of wastewater [9,10]. Indeed, the acceptable performance of these versatile reactors have been already proved for carbon oxidation, nitrification, denitrification, and deammonification [11–13]. In addition, Torresi et al. [14] have lately noticed high potential of tertiary nitrifying MBBRs in MPs removal. They concluded that the thickest nitrifying biofilm (500 μm), attached on Z-MBBR carriers, has the highest specific biotransformation rate constants for a broad range of organic MPs due to the high biodiversity found in thick biofilms. Despite this benefit, the time required for development of nitrifying biofilm is long because both types of "ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB)" are autotrophic, grow slowly and have limited abilities to produce extracellular polymeric substance (EPS) [15] which is known as the main factor of biofilm formation [16]. Furthermore, thick nitrifying biofilms which goes hand-in-hand with high-efficient MPs removal may also be connected to confine substrate diffusion in the biofilm [17] and higher levels of inorganic precipitates in the biofilm (i.e. scaling) causing the blockage of biofilm surface by the precipitates and enhancement of the carriers' weight for maintaining in suspension [18]. In view of these points, in this study, we aimed to develop and examine heterotrophic biofilm in MBBRs for the purpose of MPs removal from secondary-treated effluent. At low substrate availability, however, generation of a thin biofilm is expected which is logically encountered with lower problematic issues such as scaling and limitations in substrate diffusion into the biofilm. Meanwhile, conversely to autotrophic bacteria, heterotrophic bacteria can have a doubling time of a few hours making the biofilm establishment faster [18–20].

The fate of MPs during the activated sludge processes is controlled by the abiotic and biotic reactions. Photodegradation, air stripping and mostly sorption onto biosolids constitute the abiotic removal of MPs [21], whilst metabolism and co-metabolism are recognized as the biodegradation mechanisms involved in the biotic MPs removal [22]. To date, the importance of the biotic MPs removal has been attracted much higher attentions than the role of its counterpart [23], probably due to this fact that MPs biodegradation is a sustainable process and potentially can form end products consisting of inorganic compounds, i.e. mineralization [24]. Additionally, MPs biodegradation is often the dominant removal process for the majority of compounds, as compared with abiotic removal drivers [25]. According to the review paper published by Verlicchi et al. [26], sorption onto the secondary activated sludge is reported up to maximum 5% for most of the analgesic and anti-inflammatory pharmaceuticals, beta-blockers, and steroid hormones which is too much lower than the role of biodegradation in MPs removal (even up to 100%). On the contrary, the removal percentage of some antibiotics like Ciprofloxacin and Norfloxacin is reported in the range of 70-90% due to the sorption, while below than 10% of these compounds were abated by the biodegradation mechanisms [27]. Some studies have pointed out the significance of MPs sorption onto the biosolids, as this factor is found to have an impact on the MPs bioavailability [24] and causes the occasional negative mass balance of MPs, where MPs desorption from the suspended or attached biomass occurs during the treatment process [28]. When the waste sludge is going to be used as a fertilizer on an agricultural land, this factor should be also taken into account, knowing that sludge digestion is likely not able to remove the most of persistent MPs [29].

In MBBR reactors, today's knowledge on the mechanisms of MPs removal is still insufficient in terms of abiotic and biotic aspects [30–32]. Apart from that, individual contributions of the biofilm and suspended biomass have been rarely studied in MPs removal. The main objective of this study was to evaluate the removal of four MPs including two analgesic and anti-inflammatory pharmaceutical compounds (Diclofenac and Naproxen), a steroid hormone (17ß-Estradiol) and an endocrine disrupting compound (4n-Nonylphenol) by means of tertiary pilot-scale MBBRs, and thereby assess the distinct role of the biofilm and suspended biomass in abiotic and biotic elimination of MPs. To describe an outline for this research, we firstly tried to develop an efficient biofilm in the reactors that ever worked on the continues mode. At the same time, the steady-state situation of the reactors fed by the MPs-bearing secondary-treated municipal wastewater was achieved. Subsequently, distributional removal of MPs was comprehensively studied.

2. Materials and methods

2.1. Chemical compounds

All chemicals used in this study including all salts ($CaCl_2.2H_2O$, NaCl, K_2HPO_4, $MgSO_4.7H_2O$, $NaHCO_3$, $KMnO_4$, NaOAc, NaN_3), allylthiourea, peptone, meat extract, sucrose, acetone, methanol, hexamethyldisilazane (HMDS), glutaraldehyde, and also all MPs were analytical grade and obtained from Sigma-Aldrich.

2.2. Synthetic wastewater

Mother stock solution of the chemicals for simulating the secondary-treated municipal wastewater were weekly prepared according to the “OECD Guideline for the Testing of Chemicals, Part 303B-Biofilms”(Alcantara et al., 2015; OECD, 2001). This solution, fed continuously into the MBBR reactors, was diluted with the tap water in order to achieve desirable amount of COD, nutrients and MPs. The pH of stock solution was tried to keep at 7 ± 0.5 by using 300 mg. L^{-1} of $CaCO_3$ (in the form of $NaHCO_3$ to provide alkalinity) and NaOH (10 mg.L^{-1}) [14]. By the way, mother stock solutions of MPs were separately prepared in high-pure methanol with concentration of 1 g.L^{-1}, stored in 15-mL amber glass bottles and kept in freezer (-18°C). An appropriate amount of each MP was added to the mother stock solutions of the wastewater to reach to the target concentration of MPs. Here, the final concentrations of Diclofenac, Naproxen, 17ß-Estradiol and 4n-Nonylphenol were considered 0.5, 2.5, 1 and 7 µg. L^{-1}, respectively, based on available data in literature about concentration of target MPs in effluents of conventional municipal WWTPs, presented in Table 1S in supplementary data along with their physico-chemical characteristics.

2.3. Biofilm carriers

Saddle-shaped Z-carriers, produced by AnoxKaldnes company (Lund, Sweden), with a 30 mm diameter, 2190 mm^2/carrier protective surface area (PSA), 400 µm grid height and compartment size of 2.3 mm × 2.3 mm were used in this study. Compared to other types of available carriers in the market, I) biofilm expands on the outside of the Z-carriers instead of inside voids, and the exposed biofilm is covered on the entire surface of the carrier [35], and II) these carriers are less prone to the scaling phenomenon, as the formed biofilm is shown to be filled by lower amounts of inorganic precipitates [18].

2.4. *MBBR configuration and operation*

2.4.1. MBBR set-up

Two identical pilot-scale glass MBBR reactors, each with an effective volume of 3.1 L, were operated in parallel under the ambient temperature. Coarse-bubble air distribution was provided from the bottom of each reactor to maintain dissolved oxygen (DO) concentration between 4 to 5 mg. L^{-1} (Honeywell DO probe), and also provide a proper circulation of the whole carriers inside the reactors. During the continuous running, concentrated wastewater was fed into the reactors by means of an adjustable peristaltic pump (Minipuls 3, GILSON) and a rotameter-based system was used for entering the tap water into the reactors. Applying different ratios between the flowrates of the concentrated wastewater and tap water allowed us to operate MBBRs with favorable values of hydraulic retention times (HRTs) and influent's COD. A glance through the literature indicates that MBBRs have been so far operated in a wide range of HRTs [11–13] and a definitive value has not stablished yet, in particular, for tertiary MBBRs which are in the beginning steps of the attention. However, in the continuous running of the set-up, HRTs and influent's COD values were stepwisely changed from 20 to 4 h and 500 to 100 mg. L^{-1}, respectively.

2.4.2. Start-up procedure & biofilm formation

The start-up strategy is explained in Table 1a. In brief, in order to increase the surface roughness for better biofilm attachment, bare Z-carriers were initially washed with 1 mg.L^{-1} $KMnO_4$ for 24 h [14]. Indeed, bacterial attachment to the solid surfaces is promoted by the enhancement of the surface roughness because irregular surfaces I) are able to protect the biofilm from the detachment in the high shearing forces, and II) provide more available surface for the bacterial attachment [36]. MBBRs were firstly filled by the pre-washed carriers at a total filling ratio of approximately 40%, and secondly inoculated by the activated sludge (4743.1 ± 9.2 mg. L^{-1}), taken from a municipal WWTP (Toulouse, France) with a conventional activated sludge (CAS) system, up to the half of the reactors' effective volume. Afterwards, reactors were fed with a synthetic wastewater with COD of 500 mg. L^{-1}, and run in batch mode (around 24 h) for acclimation of the biomass to the wastewater. Continuous feeding of the reactors was then applied at HRT of 20 h until a steady-state condition was achieved in terms of COD removal (> 80%). For the biofilm formation, a special strategy was used in this study. As indicated in Table 1 and Fig. 1S in supplementary data, MBBRs were continuously operated for 22 weeks at a nearly constant organic loading rate (OLR) of 1.9 g COD.d^{-1} across four HRTs of 20, 14.8, 9.8, and 4 h. At each step, both HRT and influent COD were declined when COD removal obtained more than 80%. At the final step (HRT: 4h and influent COD: 100 mg.L^{1}), MBBRs' operation were continued until the achievement of a stable biofilm growth rate for approximately one month. Furthermore, 50 mg.L^{-1} $CaCl_2.2H_2O$ was added to the influent of the reactors during the first month of the continuous running because cations, such as magnesium and calcium, actively contribute to the biofilm cohesion and act as lipopolysaccharide cross-linkers [37,38]. On the other hand, target MPs were added to the reactors from the beginning of the 12th week, for the purpose of biomass adaptation to the MPs. It should be noted that according to the findings of Falås et al. [39], long-term exposure to MPs at typical municipal wastewater concentrations is generally not a necessary trigger for the MPs degradation in CAS processes.

2.4.3. Methodology for the assessment of MBBR performance

2.4.3.1. Overall removal of MPs- Contribution of the biofilm and suspended biomass

After biofilm formation, reactors' feeding with a MPs-bearing secondary-treated wastewater was continued in order to assess the overall removal of MPs at different OLRs. As shown in Table 1b, the reactors worked continuously for 5 days at HRT: 4 h, 7.5 days at HRT: 6 h, 10 days at HRT: 8 h and 12.5 days at HRT: 10 h to have the same ratio between the operation time and HRT. Influent and effluent samples were collected in the last two days of each HRT for COD and MPs analysis. In addition, we also investigated the individual role of the biofilm and suspended biomass in the overall removal of MPs at these applied HRTs (Table 1c). For this purpose, colonized carriers from one reactor were transmitted into another identical clean MBBR (filling ratio: 40%), pre-filled with an autoclaved MPs-bearing secondary-treated wastewater. The continuous feeding of the reactor was subsequently started with MPs-bearing secondary-treated wastewater, and parameters of COD and MPs were measured in two days in a row. From the difference between the overall removal and MPs removal by the biofilm, we obtained the MPs removal by the suspended biomass.

2.4.3.2. Abiotic and biotic removal of MPs

Overall removal of MPs consists of abiotic and biotic aspects of MPs removal. In this research, the biotic removal was obtained from the difference occurred between the overall and abiotic removal. Table 1d and Table 2 briefly summarizes our strategy for the assessment of abiotic removal.

Taking this into account that sorption onto the suspended and attached biomass, air stripping and photodegradation are involved in abiotic removal of MPs [21], four pre-autoclaved and sealed 1000-mL Erlenmeyer flasks, as described in Table 2, were incubated in batch mode for 2 hours in 120 rpm. Falas et al. [40] found that sorption of MPs onto the biosolids is a fast process in an activated sludge system and can reach equilibrium within just 30 min for acidic pharmaceuticals such as Diclofenac and Naproxen. In the study of Y. Luo et al. [41] on in a sponge-based moving bed bioreactor, some MPs like 4n-Nonylphenol and 17ß-Estradiol were eliminated up to 80% during the first two hours in the batch experiments with colonized sponge, that proves sorption has a remarkable role in abiotic removal of these compounds. Moreover, on the basis of a research conducted by Anderson et al. [23] on the sorption capacity of suspended biomass for steroid estrogens, equilibrium is almost reached after only 30 min and concentrations in the water phase did not change after 2 h. The time used for this batch experiment was therefore set at 2 h to ensure that equilibrium was reached in this test and homogenous samples were collected at regular intervals for MPs analysis. In order to avoid MPs biodegradation throughout the batch experiment, we used 500 mg.L^{-1} sodium azide (NaN_3) to suppress aerobic microbial activity, and 5 mg.L^{-1} allylthiourea to inhibit nitrification [14,42].

First, each flask was filled with the pre-autoclaved synthetic secondary-treated wastewater (500 mL) with COD = 100 mg. L^{-1} containing MPs (Diclofenac, Naproxen, 4n-Nonylphenol and 17ß-Estradiol: 0.5, 2.5, 7 and 1 µg. L^{-1}, respectively). Since, we have already filled/operated MBBRs with filling ratio of 40%, we put 82 colonized carriers in the fourth flask to have the same filling ratio with MBBR reactor. Regarding final amount of the attached biomass (~ 7.9 mg/carrier), concentration of the attached biomass in the fourth flask was around 1300 mg. L^{-1}. To have the same amount of the biosolids in the second & fourth flasks, concentration of the suspended biomass in the second flask was selected equal with 1300 mg. L^{-1}. Additionally, for the purpose of assessing the possible sorption of MPs onto the non-colonized carriers, third flask was filled with the same filling ratio of the bare carriers, pre-treated by 1 mg. L^{-1} $KMnO_4$ for 24 h. Furthermore, first flask did not contain any type of suspended or attached biomass to investigate the role of photodegradation and air stripping in abiotic removal of MPs during duration of the experiment. Finally, we could calculate I) the sorption of MPs onto the suspended biomass from the difference observed between flasks 1 & 2, and II) the sorption of MPs onto the biofilm from the difference seen between flasks 1 & 4. Also, subtracting the results of flasks 1 from flask 3 could give us the sorption onto the non-colonized carriers.

2.4.3.3. Modeling of biofilm formation

To go deeper into the biofilm behavior, we used Eq. (1) introduced by M. Plattes et al [43] who developed a zero-dimensional biofilm model for dynamic simulation of MBBRs using Activated Sludge Model 1 (ASM1). They proposed that detachment rate of the biofilm is equal to the biofilm growth rate in a steady state condition.

$$r_d = k_{de}.(BS)^2 \tag{1}$$

Where, BS is concentration of the biofilm solids (g BS.m^{-3}), r_d is detachment rate of the biofilm (g BS. m^{-3}. d^{-1}), and k_{de} is detachment rate constant (m^3. g BS^{-1}. d^{-1}).

2.4.3.4. Pseudo-first order degradation kinetics

Biological transformation of MPs in activated sludge-based systems, can be described by pseudo-first order kinetics as expressed as Eq. (2) [44,45].

$$k_{biol} = \frac{F_{inf} - (F_{eff} + F_{stripped} + F_{sor})}{X_S.S.V} \tag{2}$$

Where, F_{inf}, F_{eff}, $F_{stripped}$ and F_{sor} indicate the mass flows of MPs in the influent, effluent, air-stripped compound, and sorbed onto the suspended and/or attached biomass, respectively (µg. d^{-1}). Meanwhile,

k_{biol} is pseudo-first order degradation constant (L. g VSS^{-1}. d^{-1}), V is the volume of the reactor (L), and S is soluble compound concentration in the reactor (µg. L^{-1}). In the present work, in addition to the total k_{biol} (calculated for the both biofilm and suspended biomass), k_{biol} was separately calculated for the biofilm and suspended biomass. For the total k_{biol}, X_S is sum of the volatile suspended solids and the biofilm solids (g. L^{-1}). Furthermore, X_S is the biofilm solids for the biofilm's k_{biol} (g BS. L^{-1}), while is the volatile suspended solids for the k_{biol} related to the suspended biomass (g VSS. L^{-1}).

Parameter of $F_{stripped}$ can be calculated according to the Eq. (3).

$$F_{stripped} = Q.H.q.S \tag{3}$$

Where, Q is the feed flow rate (L. d^{-1}), H is Henry's law constant (dimensionless), and q is the air supply per unit of wastewater (L_{air}. L^{-1} influent).

As we calculated k_{biol} at steady-state condition, F_{sor} was not considered in Eq. (2) (because F_{sor} is constant with time, $F_{sor} = 0$ at steady-state condition).

2.4.3.5. Sorption kinetics

In order to determine MPs' sorption kinetic constants, Eq. (4) was used as proposed by [46].

$$r_{sor} = k_{sor}.X_{TSS}.S \tag{4}$$

Where, r_{sor} is MPs sorption (µg. L^{-1}. d^{-1}) and k_{sor} is sorption kinetic constant (L. g TSS^{-1}. d^{-1}). We evaluated both k_{sor} values for the suspended and attached biomass.

Table 1. Detailed steps of the reactors operation as well as biotic and abiotic removal of MPs*

Main stages		Operation time	Feeding regime	Feeding type	Influent COD (mg.L^{-1})	HRT (h)	OLR (g COD. d^{-1})	Explanations
a	Start-up and biofilm formation	24 h	-	-	-	-	-	Washing of carriers with KMnO$_4$
		24 h	Acclimation in Batch mode	synthetic wastewater without MPs	500	-	-	Filling of the reactors with pre-washed carriers, and Inoculation with activated sludge
		6 weeks**	Continuous mode	synthetic wastewater without MPs	500	20	~1.90	Stepwise reduction of HRT, after achieving COD removal > 80%.
		2 weeks			375	14.8		
		7 weeks		MPs-bearing synthetic wastewater	250	9.8		
		7 weeks			100	4		
b	Overall removal of MPs	5 days	Continuous mode	MPs-bearing synthetic wastewater	100	4	~1.93	MBBR operation for measuring overall removal of MPs
		7.5 days				6	~ 1.23-1.3	
		10 days				8	~ 0.94-0.99	
		12.5 days				10	~ 0.77	
c	Overall removal of MPs by the biofilm and suspended biomass	2 days	Continuous mode	MPs-bearing autoclaved-synthetic wastewater	100	4	~1.93	MBBR operation for evaluating the individual contribution of the biofilm and suspended biomass in MPs removal
		3 days				6	~ 1.23-1.3	
		4 days				8	~ 0.94-0.99	
		5 days				10	~ 0.77	
d	Abiotic removal of MPs	2 h	Batch mode in erlenmeyer flasks	MPs-bearing synthetic wastewater	100	-	-	Abiotic MPs removal by the biofilm and suspended biomass, described in Table 2.

*The biotic removal of MPs, reported in the text, is obtained from the difference between the overall and abiotic removal values.

**$CaCl_2.2H_2O$ was added to the feed to speed up the process of biofilm formation [37,38].

Table 2. Experimental design for evaluating the abiotic removal of MPs (batch incubation of pre-autoclaved and sealed flasks at 120 rpm for 2 h)

Flask	contents	The aim
1	Pre-autoclaved wastewater + MPs + NaN_3 + allylthiourea	The role of photodegradation & air stripping
2	Pre-autoclaved wastewater + MPs + suspended biomass + NaN_3 + allylthiourea	The role of photodegradation, air stripping & sorption onto suspended biomass
3	Pre-autoclaved wastewater + MPs + non-colonized carriers + NaN_3 + allylthiourea	The role of photodegradation, air stripping & sorption onto non-colonized carriers, pre-washed with $KMnO_4$ (1 mg. L^{-1} for 24 h)
4	Pre-autoclaved wastewater + MPs + colonized carriers + NaN_3 + allylthiourea	The role of photodegradation, air stripping & sorption onto the biofilm

2.5. Viability of the biofilm and suspended biomass

During the continuous running of MBBRs, the bacterial viability of the suspended biomass and biofilm was distinguished using the "LIVE/DEAD® BacLight™ L7012 Bacterial Viability Kits" (Molecular Probes, Invitrogen Detection Technologies). In order to assess the viability of the suspended biomass, according to the protocol of manufacturer, 3 µL of pre-combined stains (1.5 µL of each stains including SYTO®9 and propidium iodide) was added to 1 mL of the mixed liquor in an amber glass bottle. After mixing, this solution was incubated at room temperature for 15 minutes. Subsequently, 5 µL of the stained bacterial suspension was trapped between a slide and an 18 mm square coverslip and observed by epifluorescence microscope (LSM 800, ZEISS) equipped with UV light (HXP 200C) [47]. On the other hand, for viability assessment of the biofilm, 3 µL of each stain was added to 1 mL of demineralized water. Then 200 µL of staining solution was gently added onto the biofilm sample immediately after picking up the target carrier from MBBRs. Afterwards, the staining dish was covered by the aluminum paper and incubated for 30 minutes at room temperature. The sample was gently rinsed by demineralized water for removing all excess stain and observed using the confocal microscope (Leica SP2-AOBS) [48].

2.6. Biofilm morphology

Throughout the study, the biofilm morphology and its coverage on the surface of carriers were monitored by the Scanning Electron Microscopy (SEM). After gentle cutting of each biofilm-coated carrier into the small pieces, each piece was initially fixed with 2 mL of 4% glutaraldehyde, 1 mL of phosphate buffer (pH: 7.4) and 1 mL of demineralized water for 20 minutes, and then washed 2 times in 1 mL of phosphate buffer, 2 mL of 0.4 M sucrose and 1 mL of demineralized water for 15 minutes. In the step of dehydration, sample was immersed in 2-mL acetone-water solution (50%:50%) for 5 minutes, 2-mL acetone-water solution (70%:30%) for 5 minutes, and 2-mL acetone-HMDS solution (50%:50%) for 5 minutes. Finally, the sample was dried overnight under the evaporation of 2 mL HMDS solution. For the following step of metallization, dried sample was coated with 10-nm gold for 60 seconds via a compact sputter coater (The Scancoat Six, EDWARDS) according to the protocol of manufacture. It was then observed by means of a mini-SEM microscope (TM 3000 tabletop, HITACHI) with different magnifications to assess the biofilm structure.

2.7. Quantification of biomass - MLSS and MLVSS

To measure the biofilm solids mass, four carriers from each reactor were situated on an aluminum-wrapped cup, dried overnight at 105 °C in a drying oven (Memmert Oven), and weighed. Dried carriers were then washed in 3 M NaOH solution to detach the whole biofilm, and cleaned with demineralized water to rinse excess NaOH solution. Samples were dried again at 105 °C overnight and weighed. Finally, the biofilm solids were calculated as the weight difference before and after washing of carriers [49]. The biomass per area was calculated knowing that each carrier (Z-carriers with maximum biofilm thickness of 400 µm) has a PSA of 2194 mm^2 [35]. Moreover, mixed liquor suspended solids (MLSS) were measured by filtering through a paper filter (VWR, 516-0348, France) with 0.70 µm pore size succeeded by drying overnight at 105 °C and weight determination. By the way, overnight heating under the temperature of 550 °C in a furnace (Salvis Lab Thermocenter, TC40) was applied in order to measure mixed liquor volatile suspended solids (MLVSS) [49].

2.8. Dissolved COD and nutrients measurements

Samples were firstly filtered through 0.70 µm glass fiber filters (VWR, 516-0348, France). Then, the analysis process were done using HACH LANGE kits of LCI 500 or LCK 514 for COD, LCK 341 for total Nitrogen, LCK 304 for NH_3-N, and LCK 341 for $P\text{-}PO_4^3$, along with DR3900 Benchtop VIS Spectrophotometer equipped with HT200S oven (HACH LANGE, Germany). These parameters were measured in duplicate and the average values are reported.

2.9. MPs analysis

For MPs analysis, samples (each with a volume of 250 mL) were firstly filtered using 0.70 µm glass fiber filters (VWR, 516-0348, France), secondly collected in 500-mL amber glass bottles and finally kept in freezer (-18°C). They were then shipped to the LaDrôme laboratory (France) in a freeze box for analysis within 24 h under the analyzing license of COFRAC ESSAIS. A multi detection procedure including Gas Chromatography (coupled with ECD/NPD mass spectrometry) and Liquid Chromatography (along with DAD, fluorescence, tandem mass spectrometry) was applied for all MPs with Limit of Quantification (LQ) of 0.01 µg/L for Diclofenac, Naproxen and 17ß-Estradiol, and 0.04 µg/L for 4n-Nonylphenol. Removal values R were calculated according to the Eq. (5), where C_i and C_e are MP concentration in the influent and effluent of the reactors, respectively. Each measurement was performed in duplicate and the average of values with standard deviation are reported.

$$R = \left(1 - \frac{C_e}{C_i}\right) \times 100 \qquad (5)$$

3. Results and discussion

3.1. Biofilm formation

To date, many researchers have found that the process of biofilm formation could be frequently affected by the environmental and operational conditions, such as carbon & nutrients availability, fluid velocity, MLSS, temperature, pH, and surface roughness [36]. In this research, since we were facing with the challenge of low COD and nutrients availability, the OLR was almost kept constant at different HRTs in order to provide enough food for the biomass generation and maintenance. Fig. 1 indicates that once the COD removal increased more than 80%, the HRT was reduced to the next step. This procedure was repeated to the final HRT of 4 h, where a stable COD removal and also the food to microorganisms ratio (F/M) (Fig. 1S in supplementary data) were observed for five weeks in a row.

In addition to this fact that suspended biomass contribute considerably to the overall performance of the MBBR [18], M. Plattes et al. [43] reported that attachment rate of the biomass is a function of the square of the suspended solids ($MLSS^2$) and an attachment rate constant (k_a). Hence, both parameters of MLSS and MLVSS/MLSS ratio were monitored during the biofilm formation. As plotted in Fig. 2, at the final HRT of 4h, the MLSS concentration was remained around 1340 mg. L^{-1} by the conventional recirculation of the gravitational-sedimentated activated sludge into the MBBRs. We also always tried to keep MLVSS/MLSS ratio above 0.7, for instance, about 300 mL of a fresh activated sludge, got from a municipal WWTP, was added into each MBBR in 13th week. Moreover, result of the viability test on the suspended biomass (Fig. 2S in supplementary data) shows that live cells dramatically overcome dead cells at the end of the process of biofilm formation i.e. an HRT of 4 h (pictures are related to 20th week).

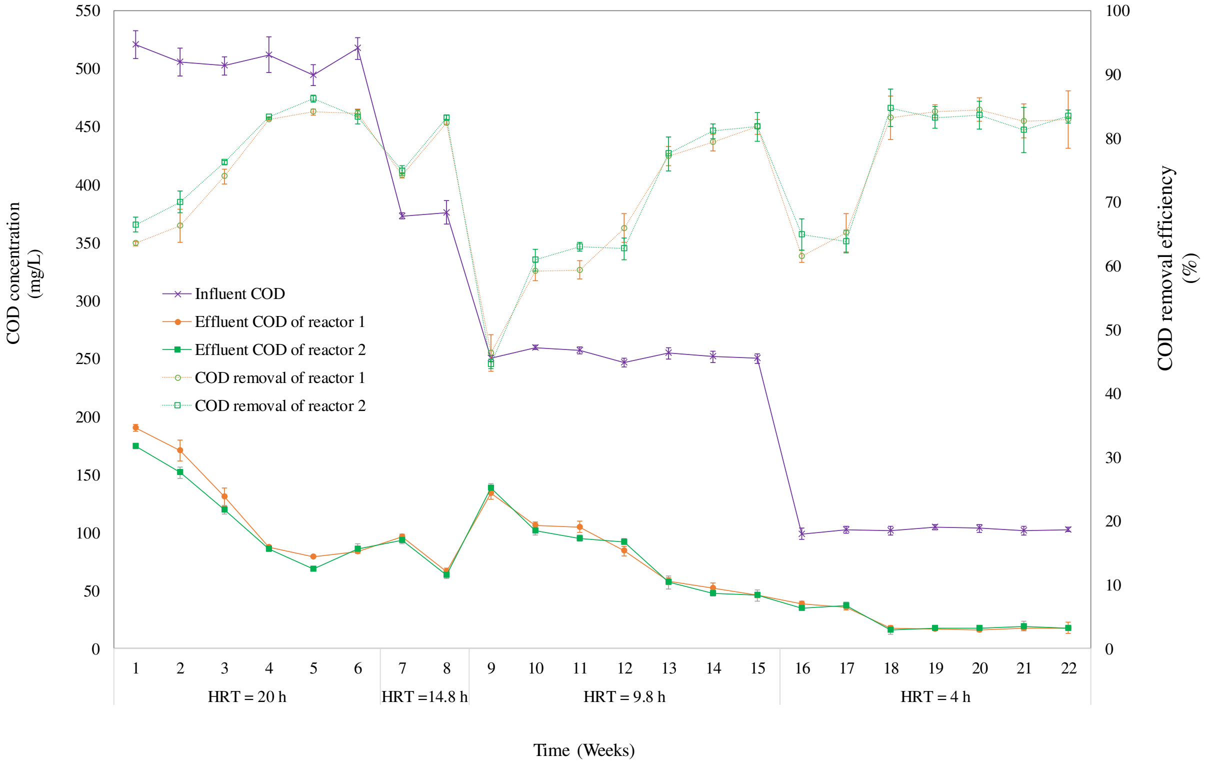


Fig. 1. Overall COD conversion in MBBR reactors during the process of biofilm formation

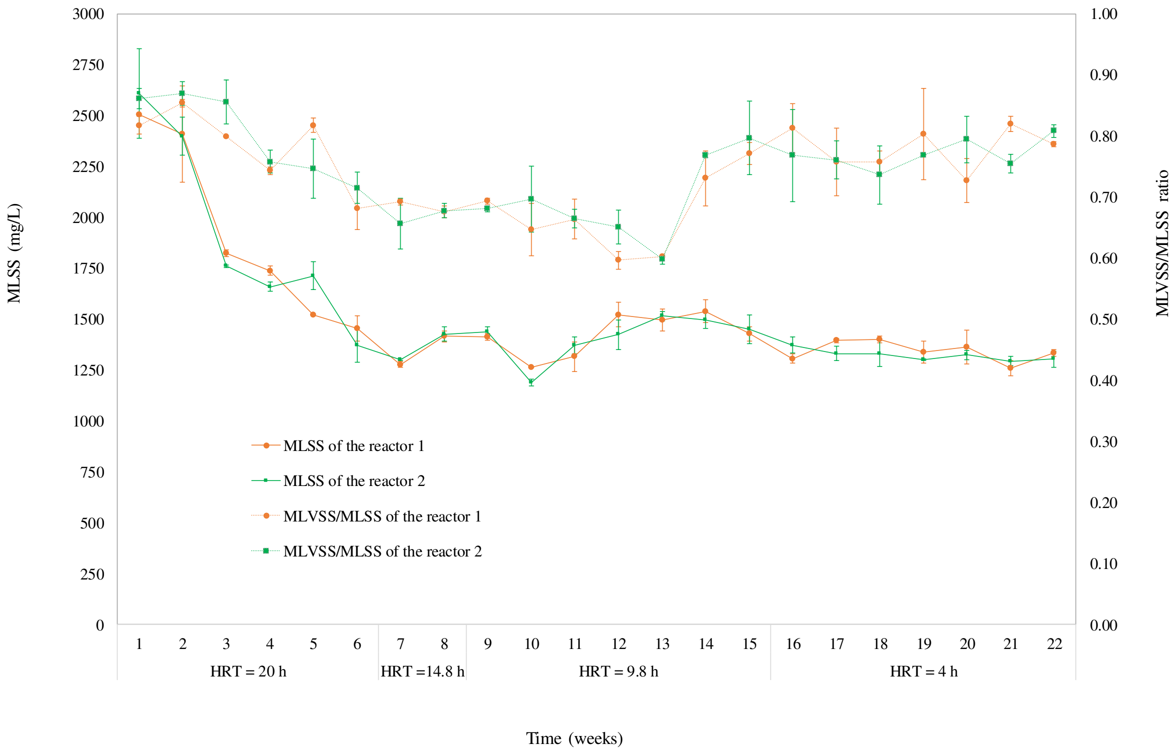


Fig. 2. Monitoring of the MLSS and MLVSS/MLSS ratio during the process of biofilm formation

In Fig. 3a, we clearly indicate how the biofilm has gradually developed on the surface of carriers up to approximately 7.9 mg/carrier, corresponding to about 1275 mg. L^{-1} biofilm solids inside each MBBR (calculated based on 500 carriers placed in a 3.1-L reactor). In spite of still ongoing studies about the meaning of steady-state condition in biofilm reactors [50], assuming that MBBRs are at steady-state condition at the end of HRT: 4 h (COD removal ≈ 84% for five weeks in a row), the detachment rate of biomass can be considered equal to the biofilm growth rate [18]. Here, this hypothesis was used to evaluate the overall and individual biofilm growth rate at each HRT under the steady-state condition. As it can be seen in Fig. 3b, the biofilm growth rate has not fluctuated or changed a little for the last five weeks of the process of biofilm formation. On the other hand, according to Fig. 3c, lower biofilm growth rates were observed in the first applied HRTs compared to the last applied HRT, indicating that initial steps of the biofilm formation are slow and time-consuming. These initial steps are firstly characterized by the loose adhesion of planktonic cells to the surface, secondly the production of EPS, and then the cellular aggregation and the subsequent growth [37]. The highest proportion of the overall biofilm growth rate belongs to the lowest applied HRT i.e. HRT of 4h (~ 67%). Secondary-treated wastewater inherently provide a low mass transfer driving force between the substrate and attached biomass. The use of shorter HRTs in tertiary MBBRs, however, probably promotes substrate diffusion into the biofilm and therefore seems to be more convenient than long HRTs. To better understand the biofilm behavior, Fig. 4 was also plotted using Eq. (1) developed by M. Plattes et al [43]. Again, we do see a stable k_{de} for the last five weeks of this process (~ 0.0048 m^3. g BS^{-1}. d^{-1}). This value has not been previously reported for tertiary MBBRs in literature, but it is higher than reported values for nitrifying secondary MBBRs (0.001 m^3.g BS^{-1}.d^{-1}) [43]. In this study, invariable biofilm growth rate and k_{de} in the last weeks probably show a type of balance in the attachment and detachment of the biomass solids from the colonized carriers. After observing this stable situation, next to the steadfast and high COD removal efficiency, we assessed the detailed performance of MBBRs at different HRTs (4, 6, 8 and 10 h) for MPs removal that is discussed in section 3.2.

Fig. 5 shows different magnifications of SEM images acquired at various HRTs to demonstrate the quantized changes in the biofilm morphology. Under the evolutionary point of view, it is evident that biofilm coverage has increased step by step across the surface of each compartment (magnification of 50x). A filamentous structure with considerable empty spaces was observed in high HRTs by paying a close attention to bigger magnifications in the first steps of the biofilm formation. Then, reduction of HRT appears to reduce the filamentous and openness structure of the biofilm, likely due to the production of EPS that gradually fills the empty spaces [15,36,51]. Furthermore, the occurrence of large pores is obvious in a fully-covered biofilm at an HRT of 4 h. The porous structure leads to a better substrate penetration into the deeper areas of the biofilm especially in a low substrate availability [20,52]. J. Guo et al. [53] concluded that porous biofilms are convenient for immobilizing of numerous microorganisms and perform well against the biofilm wash-out along with the effluent. To the best of our knowledge, no enough information is still available in the literature on the biofilm's morphology of Z-carriers, making comparison with the results of this study difficult. In general, the biofilm morphology, however, is apparently a function of many parameters. For instance, in the case of the biofilm formed by *Pseudomonas aeruginosa*, the biofilm structure can be slab or mushroom-like in shape, depending on the type of carbon source (citrate and glucose, respectively) [37]. Here, it seems that we have finally prepared a slab-like biofilm.

Images obtained from the confocal microscopy (Fig. 6), however, proves that we have finally prepared a thin biofilm (average thickness ~ 100 µm) with a high degree of viability even in deepest areas, stating a good penetration of the substrate and oxygen into these areas. In fact, to ensure the high substrate availability throughout the biofilm layers, thin and porous biofilms would be preferable, particularly in the case of low substrate availability [49]. Compared to thick biofilms, it has been reported that lower precipitates exist in thin biofilms, and on the another hand the biofilm sloughing and making an odorous biofilm occur rarely in this type of biofilm [19,20,35,52].

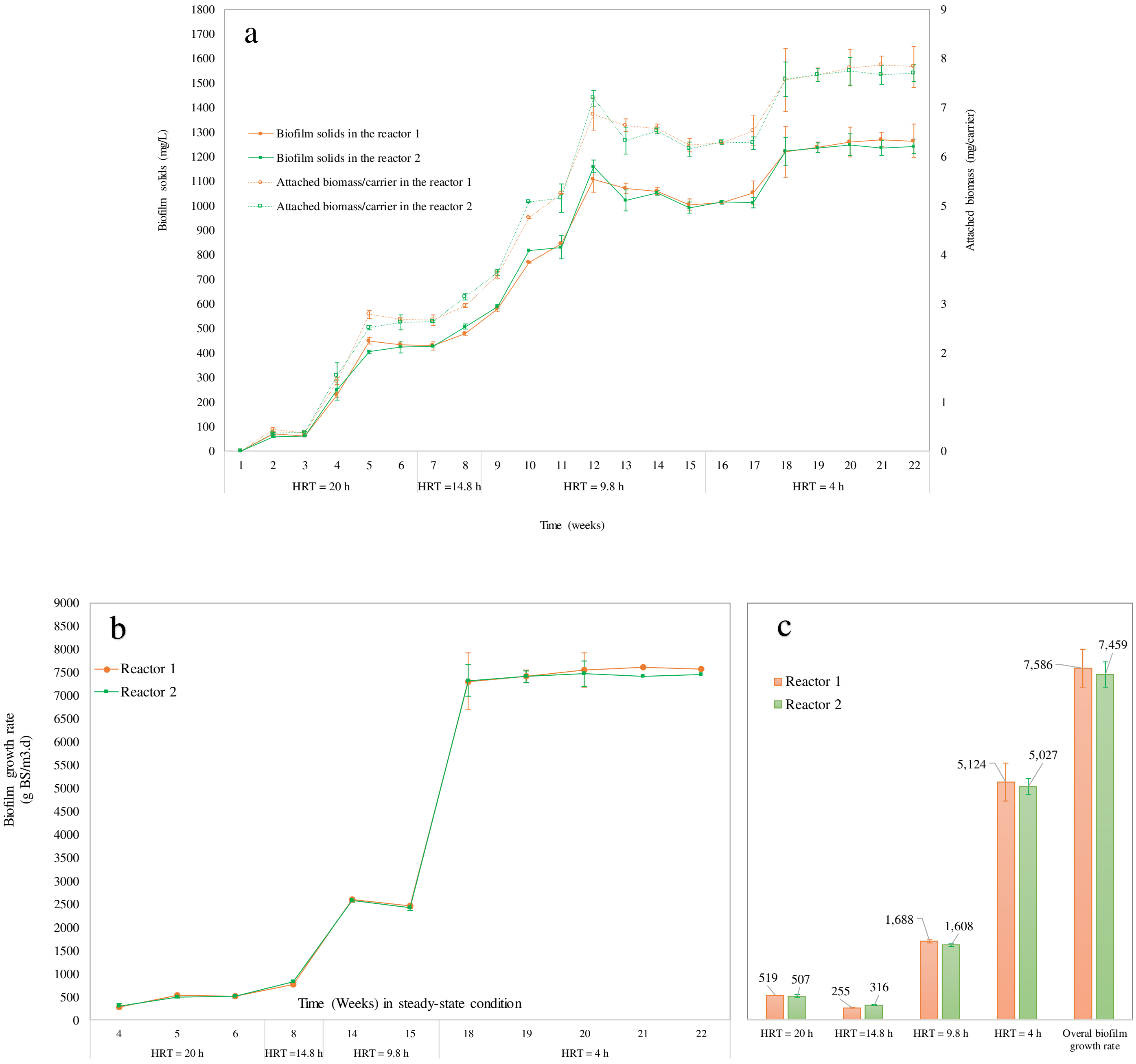


Fig. 3. (a): Gradual development of the biofilm on the surface of Z-carriers, (b): Overall, and (c): individual biofilm growth rate at each HRT during the biofilm formation at steady-state situation

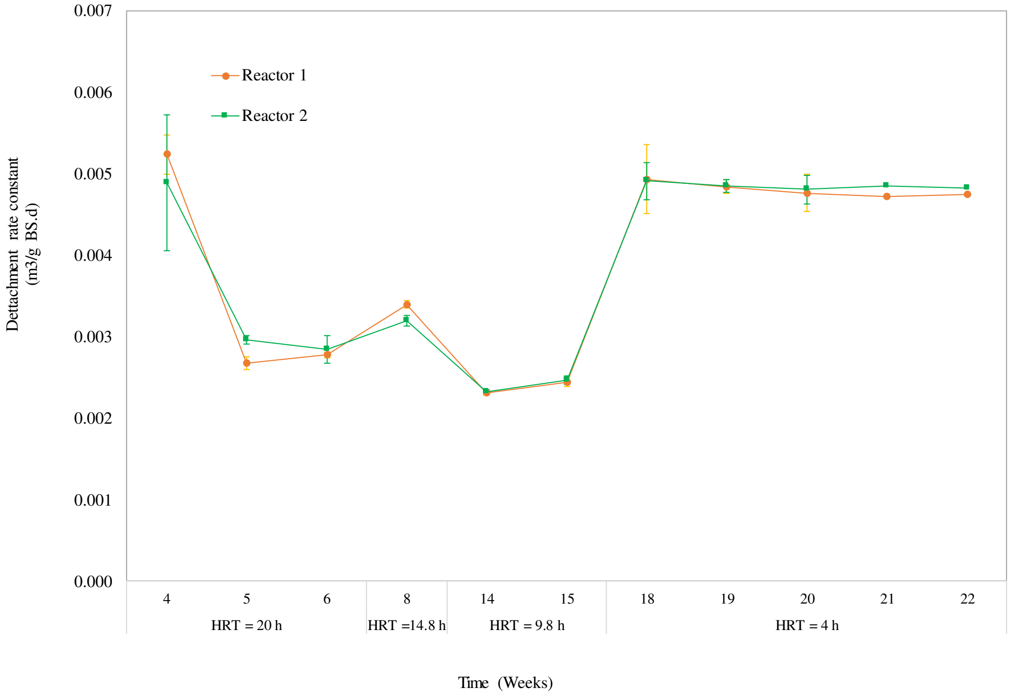


Fig. 4. k_{de} variations in different applied HRTs during the biofilm formation

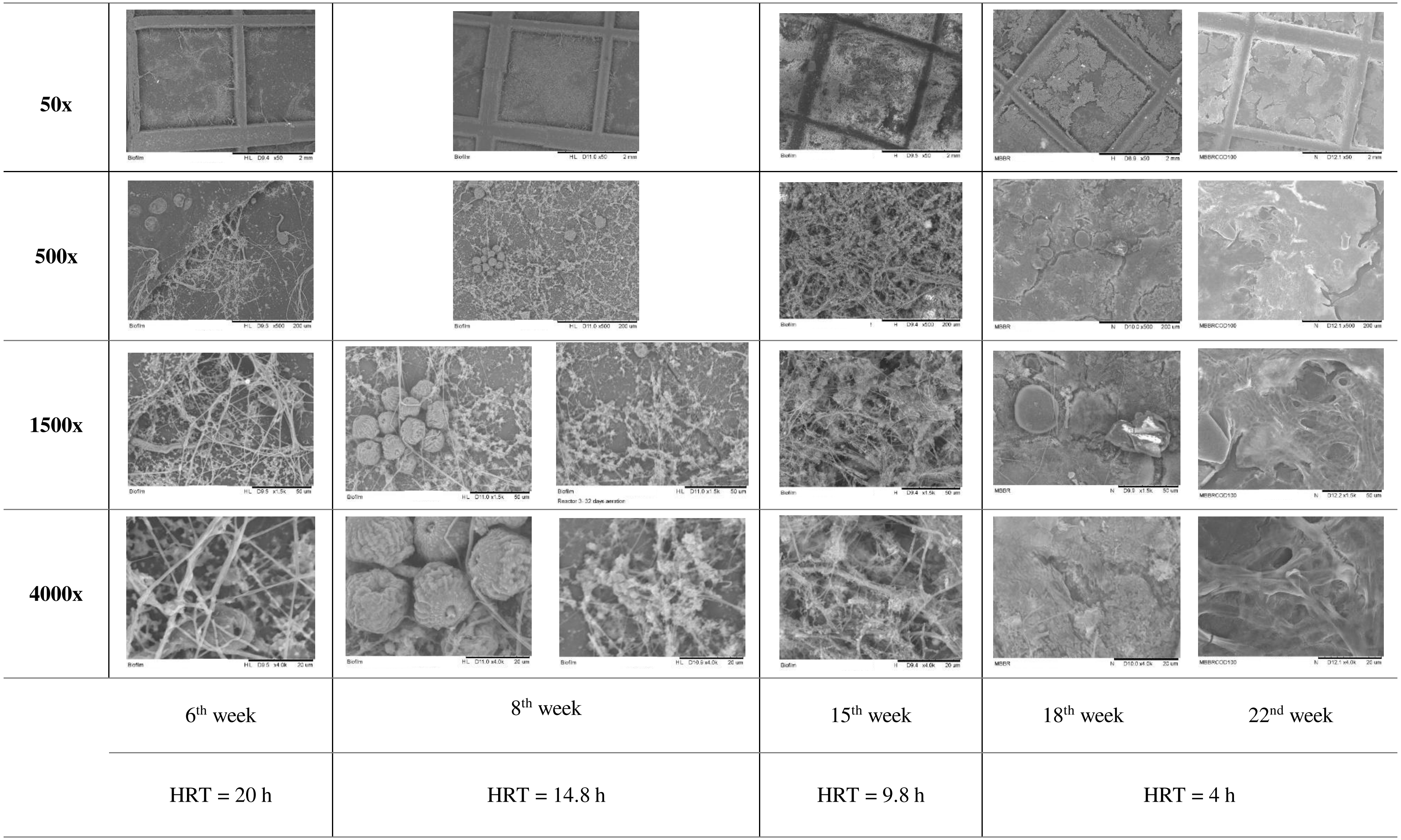


Fig. 5. Microscopic observation of the biofilm by the mini-SEM

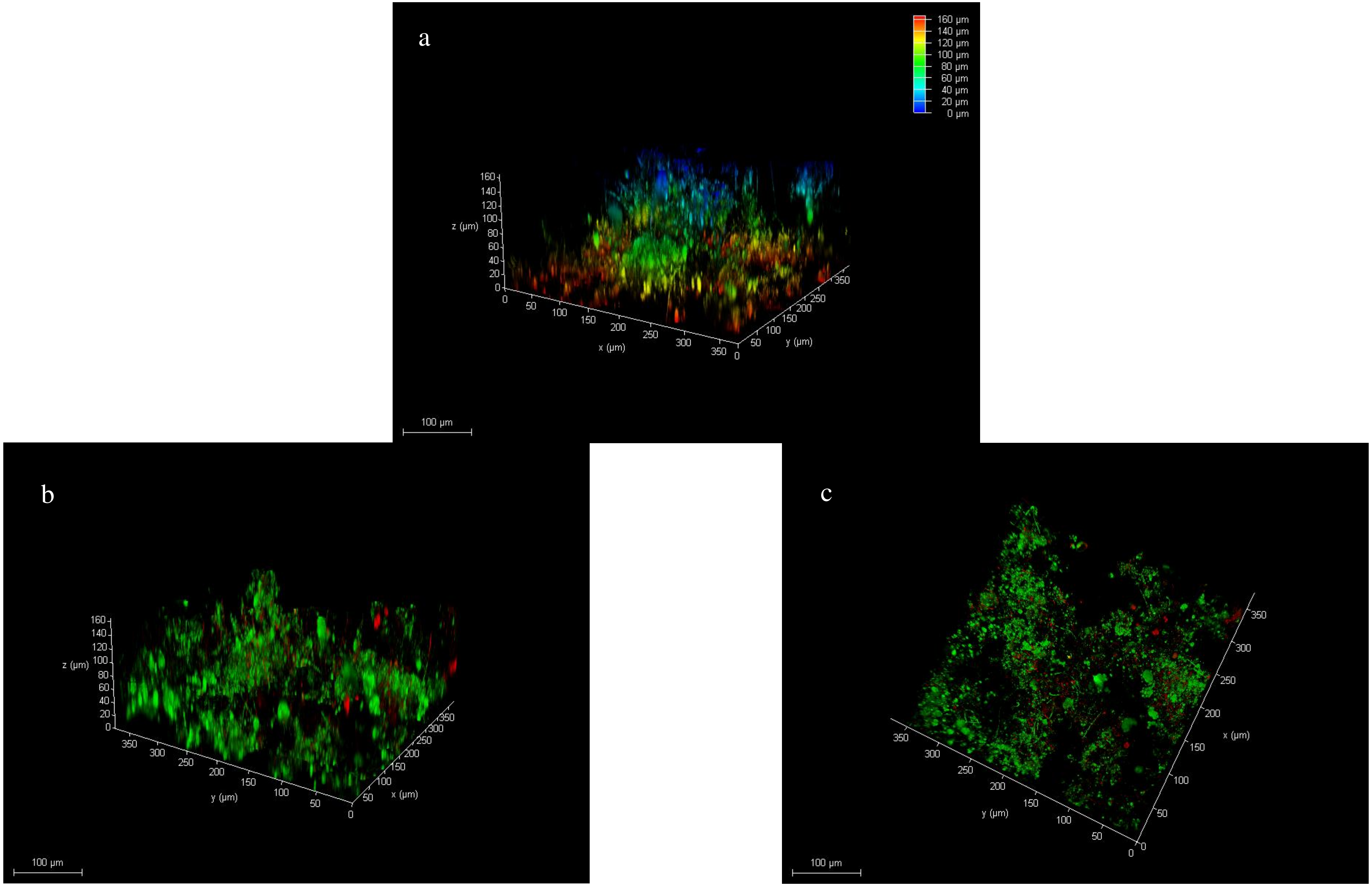


Fig. 6. Images of confocal microscopy to assess the thickness (a) and viability of biofilm (b: three dimensional profile, c: top view), at the HRT of 4 h (22nd week)

3.2. MBBR performance

3.2.1. Abiotic removal of MPs

3.2.1.1. Photodegradation

No MPs removal was occurred in flask 1 (Table 2) during the batch experiments performed in Erlenmeyer flasks, suggesting that neither the photodegradation nor the volatilization are not able to eliminate MPs in 2 h. Photodegradation consists of direct and indirect natural photolysis. Direct photolysis (direct absorption of light photons by the MPs) is found not affective in wastewater treatment plants because sunlight range is between 290 and 800 nm, while wavelengths for light absorption of many MPs are usually below 280 nm [54,55]. In the case of indirect photolysis, two different strategies are expressed in literature: (I) suspended solids and dissolved organic matters reduce the photodegradation efficiency by the light screening [56], and (II) when wastewater compounds (organic matters and carbonates) absorb sunlight form very reactive intermediates such as carbonate radical ($CO°_3^-$) and hydroxyl radical (°OH) which can somehow transform some types of photo-sensitive MPs [57] that we do not have them in this study.

3.2.1.2. Volatilization

Volatilization of MPs in conventional WWTPs is performed via surface volatilization and mostly air stripping [58]. Surface volatilization at the surface of the biological reactor is often not taken into account, although it is not negligible [59]. The fraction of compound volatilized in the aeration tank mainly depends on the flow of air getting in contact with wastewater and Henry's law constants (k_H) of MPs [60]. Taking into account the typical air flow rates used in CAS systems (5 – 15 m^3 air. m^{-3} wastewater according to Joss et al. [61]), and low Henry's law constants (k_H) of target MPs (4.73E-12, 3.39E-10, 4.7E-3, and 3.64E-011 atm.m^3.$mole^{-1}$ for Diclofenac, Naproxen, 4n-Nonylphenol and 17ß-Estradiol, respectively [62,63]), volatilization of MPs is generally negligible during the wastewater treatment process [64].

3.2.1.3. Sorption onto the bare carriers

With non-colonized carriers (flask 3, Table 2), MPs elimination was not observed due to the absence of biomass. Similarly, no sorption capacity for acidic pharmaceuticals was seen by Falås et al. [40] on the bare K1 AnoxKaldnes carriers. To our knowledge, except for the paper published by Y. Luo et al [41] who used a sponge-based carriers containing polar and non-polar functional groups in the structure, no research has been reported yet about the considerable sorption capability of bare carriers for MPs.

3.2.1.4. Sorption onto the biofilm & suspended biomass

Based on the results obtained from flasks 2 & 4, Fig. 7a is plotted to demonstrate that we can nearly attribute the abiotic removal to the only sorption. In general, two kinds of sorption profoundly occur in activated sludge systems: I) adsorption i.e. electrostatic interactions of the oppositely charged groups (positively charged groups of MPs with the negatively charged surfaces of the microorganisms and sludge), and II) absorption i.e. hydrophobic interactions between the aliphatic and aromatic groups of a compound and the lipophilic cell membrane of microorganisms [65–67]. A comprehensive study by Stevens-Garmon et al. [25] on the sorptive behavior of MPs onto the primary and secondary activated sludge indicates that positively-charged compounds such as Amitriptyline and Clozapine have the highest sorption potential as compared to the neutral and negatively-charged ones. Moreover, sorption onto the biofilm in a nitrifying MBBR was recognized significant only for positively charged MPs in the batch experiments of Torresi et al. [30]. In the current study, regarding the negative charge of Diclofenac and Naproxen, and uncharged situation of 4n-Nonylphenol and 17ß-Estradiol at neutral pH [68,69], no or a little amount of electrostatic interactions is expected due to the phenomenon of charge repulsion. Consequently, in this study, hydrophobic interactions are considered as the main responsible for the abiotic removal. To evaluate the hydrophobicity of MPs at any pH value, the parameter of $\log_D$ (logarithm of the octanol-water distribution coefficient) has been proposed [70] as compounds with $\log_D > 2.6$ are referred to as hydrophobic that prefer to accumulate in solid phases instead of being soluble in the aqueous phase, and hydrophilic when $\log_D \leq 2.6$ [71]. Here, Diclofenac and Naproxen are hydrophilic ($\log_D$: 1.77 and 0.34, respectively [6]), while 4n-Nonylphenol and 17ß-Estradiol ($\log_D$: 6.14 and 4.15, respectively [70]) are hydrophobic compounds.

As stated above, hydrophobic interactions are recognized to affect the sorption of MPs onto the both suspended and attached biomass in MBBR. To prove this hypothesis, relationship between the abiotic removal of MPs and their relevant $\log_D$ is plotted in Fig. 7b. From this figure, compounds of higher $\log_D$ are relatively better absorbed by the both suspended and attached biomass with the R-squared values > 0.90, as abiotic removals of 4n-Nonylphenol and then 17ß-Estradiol are the highest (15.00 ± 0.4% and 9.50 ± 2.12%, respectively), and for the hydrophilic compounds are the lowest (lower than 4%). These results are in a full agreement with the outcomes of Joss et al. [72] who concluded that for pharmaceuticals and fragrances having a $\log_D < 2.5$, the sorption onto secondary sludge can be deemed negligible.

Apart from the parameter of $\log_D$, sorption of MPs onto the biosolids depends on the solid-water partitioning coefficient (K_d) i.e. the ratio of the equilibrium concentration of the chemical on the solids to the corresponding equilibrium aqueous concentration [25,29]. Stevens-Garmon et al. [25] noticed that compounds with $K_d < 30$ $L.kg_{ss}^{-1}$ are compounds with a poor sorption potential on inactivated sludge [25]. Meanwhile, a mass balance prepared in a municipal WWTP by Joss et al. [72] proves that sorption onto secondary sludge is not relevant for compounds showing K_d value below 300 $L.kg_{ss}^{-1}$. Reported K_d values for Diclofenac (16 $L.kg_{ss}^{-1}$ [29], <30 $L.kg_{ss}^{-1}$ [25], and 32 $L.kg_{ss}^{-1}$ [73]) and Naproxen (<30 $L.kg_{ss}^{-1}$ [25] and 24 $L.kg_{ss}^{-1}$[73]) can logically justify very low sorption of these compounds onto the biosolids. This value has been reported up to 476 $L.kg_{ss}^{-1}$ [23] and 533-771 $L.kg_{ss}^{-1}$ for 17ß-Estradiol [25], and up to 850 $mg.kg_{ss}^{-1}$ [74] and 249.9 $mg.kg_{ss}^{-1}$ [75] for Nonylphenol that whereby, we see their higher sorption than the rest of compounds. For instance, according to the findings of Anderson et al.

[23], absorption of 17ß-Estradiol onto the suspended biomass was increased from 59% to 71% by increasing the MLSS of an aeration tank from 3 to 5 g.L^{-1} in an activated sludge system treating municipal wastewater. Furthermore, in the research of Bouki et al. [76], absorption of 4n-Nonylphenol onto the biomass (CAS system) was very fast, as this compound was removed by 90% in the first 60 minutes. They also did not observe any significant difference in absorptive behavior of live and dead biomass, and attributed this striking removal to the hydrophobic nature of both 4n-Nonylphenol and the biosolids. Apart from the type of biosolids (suspended or attached biomass) and the type of biological reactor, it seems that the uptake of MPs by live or non-living microbial biomass have a good potential for removal of hardly-degradable MPs from the wastewater. This scenario depends on the physico-chemical characteristics of the MPs and needs to be studied further.

Fig. 7a and Table 3 also reveal that the capability of suspended biomass is higher than the biofilm for absorption of all MPs. In the case of 17ß-Estradiol and 4n-Nonylphenol, a twofold absorption is observed by the suspended biomass compared to the biofilm. Moreover, Diclofenac and Naproxen have been absorbed by the biofilm below the 1%. Compared to the biofilm, we believe that better performance of the suspended biomass is due to its higher available surface area, providing a great deal of adsorptive sites for the uptake of target MPs. Since the surface of carriers becomes occupied by the on-growing biofilm, the available sorption sites of the colonized carriers decline by the passing of time, leading to the limited sorption capacity of the biofilm [41]. Some studies about particle size distribution (PSD) of the suspended solids [77–79] revealed that MBBR reactors contain smaller solids than activated sludge systems and membrane bioreactors (MBRs). In two parallel-operated MBRs one without carriers and one with carriers (both had the equal MLSS ≈ 5 g.L^{-1}), an average diameter of suspended solids without carriers was around 95 µm, whereas with carriers (Filling ratio:5%) an average diameter of them decreased to 68.3 µm after 72 hours of operation [78]. The reason of this occurrence is that circulating carriers are continuously shattering the suspended biomass and thereby higher accumulation of MPs in MBBRs' suspended biomass is expected than the above-mentioned treatment methods. It is noteworthy that PSD of MBBR reactors is a function of operational conditions, e.g. lowering HRT in MBBR reactors causes a shift in the average particle size of suspended solids towards smaller particles [77,79] that can affect the sorption capacity of MPs. Further studies are, however, required to substantiate this phenomenon. MPs desorption from the biosolids should be also taken into account when a saturation state is achieved.

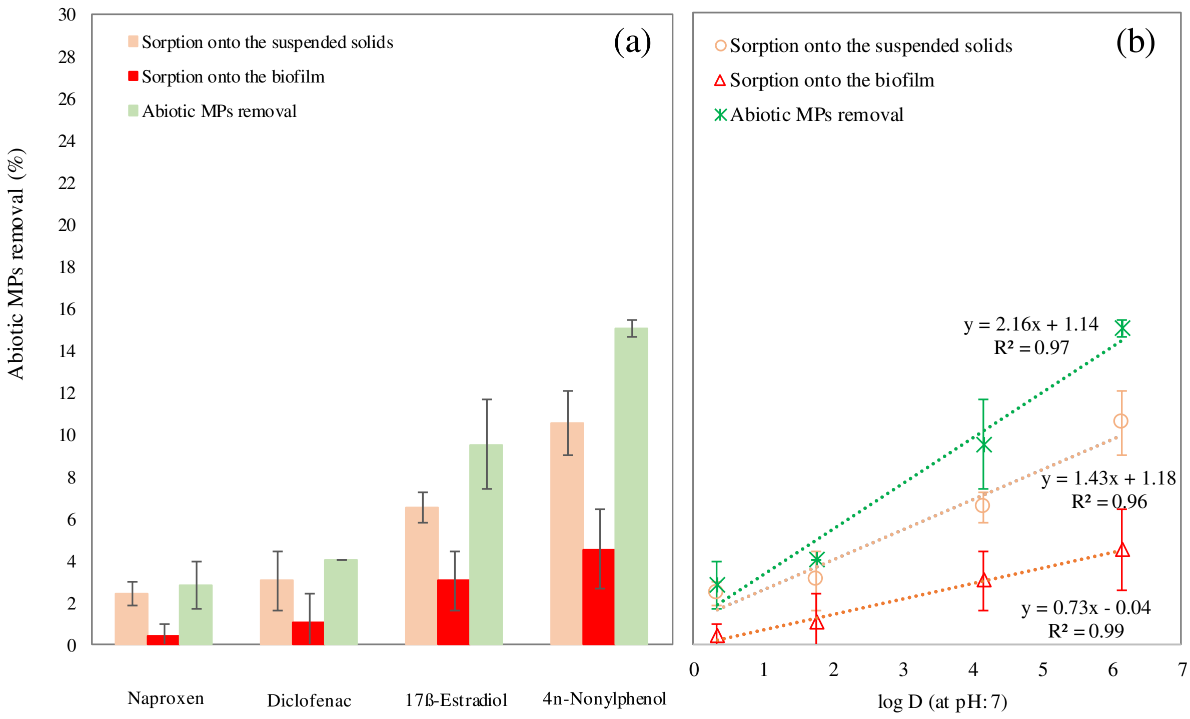


Fig. 7. The correlation between the MPs' hydrophobicity and their relevant abiotic removal

Table 3: k_{sor} values (L. g TSS^{-1}. d^{-1}) obtained in this study*

	Total value	related to the biofilm	related to the suspended biomass
Naproxen	0.0037 ± 0.0015	0.0007 ± 0.0005	0.0032 ± 0.0080
Diclofenac	0.0053 ± 0.0009	0.0019 ± 0.0003	0.0040 ± 0.0019
17ß-Estradiol	0.0135 ± 0.0033	0.0040 ± 0.0019	0.0089 ± 0.0010
4n-Nonylphenol	0.0226 ± 0.0007	0.0061 ± 0.0027	0.0151 ± 0.0020

*r_{sor} values (MPs sorption) are brought in Table 2S in supplementary data.

3.2.2. Overall removal of MPs

After biofilm formation, two MBBRs were continuously fed by synthetic secondary-treated wastewater (COD: 100 mg. L^{-1}) and operated with four HRTs (4, 6, 8 and 10 h) to assess the overall removal of COD and MPs. In general, as shown in Fig. 8, removal of 4n-Nonylphenol is the highest for all HRTs (below than LQ, i.e. 99.4%), followed by 17ß-Estradiol (61.1-94.2%), and then Naproxen (54-84%) and Diclofenac (45.2-76.8%). In order to make the results comparable with other studies in the literature, Fig. 3S and Table 3S in supplementary data were prepared. A glance at these data indicates that removal of Diclofenac and Naproxen is notably higher than other tertiary biological and hybrid reactors such as MBRs, but it is still somehow lower than tertiary membrane filtrations and AOPs. Interestingly, we can realize that removal of 4n-Nonylphenol and 17ß-Estradiol is nearly equal with tertiary membrane filtrations and AOPs. The importance of these results is that we have obtained removal rates in the levels of laborious and costly methods of membrane filtrations and AOPs by means of a biological pathway.

Fig. 8 also shows as HRT declines (or OLR increases), removal rates of Diclofenac and Naproxen increase while a converse behavior is observed for 17ß-Estradiol. These trends reflect that MPs removal deeply depends on the mechanism of MPs biodegradation. Hereafter, we will bring some explanations and/or hypotheses to interpret the results.

In the case of Diclofenac and Naproxen, this increment can be explained by an increased specific activity of the suspended and attached bacteria due to higher substrate availability in lower HRTs [80]. In this so-called co-metabolic mechanism, higher concentration of the substrate accelerates the biodegradation rate of MPs. During this mechanism, MPs are not used as a growth substrate but are biologically transformed, by side reactions catalyzed by unspecific enzymes or cofactors produced during the microbial conversion of the growth substrate [22,81]. Casas et al. [82] evaluated the ability of a staged MBBR (three identical reactors in series) on the removal of different pharmaceuticals (including X-ray contrast media, b-blockers, analgesics and antibiotics) from hospital wastewater. As a whole, the highest removal rate constants were found in the first reactor while the lowest were found in the third one. The authors noticed that the biodegradation of these pharmaceuticals occurred in parallel with the removal of COD and nitrogen that suggest a co-metabolic mechanism. Besides, in the research of Tang et al. [9] on a polishing MBBR, the removal rate constant of some pharmaceuticals such as Metoprolol and Iopromide was dramatically enhanced by adding humic acid salt (30 mg.L^{-1} dissolved organic carbon (DOC)), indicating the role of substrate availability in co-metabolic degradation of these MPs.

In contrast to co-metabolism, higher concentration of the substrate decelerates the biodegradation rate of some MPs in the scenario of competitive inhibition i.e., competition between the growth substrate and the pollutant to nonspecific enzyme active sites [22,83]. Here, removal of 17ß-Estradiol has obeyed this mechanism as though its highest removal was obtained in lowest organic loading rate. This finding is in accordance with the study of Joss et al. [84] who showed the substrate present in the raw wastewater competitively inhibits the degradation of Estrone and 17ß-Estradiol in CAS systems. These compounds were then mainly removed in activated sludge compartments with a low substrate loading.

Applying different OLRs did not affect 4n-Nonylphenol removal, leading to make the decision difficult about its removal mechanism only on the basis of a view on the Fig. 8 and Fig. 9a. According to the data presented in Table 4, a kind of descending order is observed for 4n-Nonylphenol's k_{biol} values when HRT increases. This manner probably reinforces the hypothesis that the co-metabolic mechanism could govern the removal of 4n-Nonylphenol. Tobajas et al. [85] found that co-metabolic biodegradation of 4-chlorophenol can be induced by adding carbon sources (phenol and glucose) in a batch test by *Comamonas testosterone*. However, regarding similarities between phenolic compounds of 4n-

Nonylphenol and 4-chlorophenol (both contain a single phenol ring and an electron donating group of –OH), we will make sure that 4n-Nonylphenol is biodegraded in a co-metabolic pathway.

During the mechanism of metabolism, microorganisms use MPs as growth substrates, along with other organic compounds. This mechanism leads to transformation of the MPs to smaller molecules, finally until their complete bio-mineralization to H_2O, CO_2, NH_4^+, etc [86]. Fischer and Majewsky [81] reported that co-metabolic and metabolic routes are closely connected and substitutable, since they are part of a metabolic network. In other word, a clear segregation between metabolic and co-metabolic reactions is hardly feasible in activated sludge systems as both reactions probably occur simultaneously because of the diversity of microorganisms present in the treatment system [22]. For instance, Çeçen et al. [87] noticed that both metabolic and co-metabolic mechanisms are involved in biodegradation of the chlorinated xenobiotics, depending on the microbial community of the treatment system. From the bibliographic review, it appears that there is a lack of comprehensive study about degradation mechanism of Diclofenac, Naproxen and 4n-Nonylphenol. Hence, in this study, we are not able to attribute their removal mechanism to the only co-metabolism, and we believe that contribution of metabolic reactions should be also considered in their elimination. Unlocking this not yet well-defined aspect of MPs degradation mechanism remains a challenge to researchers.

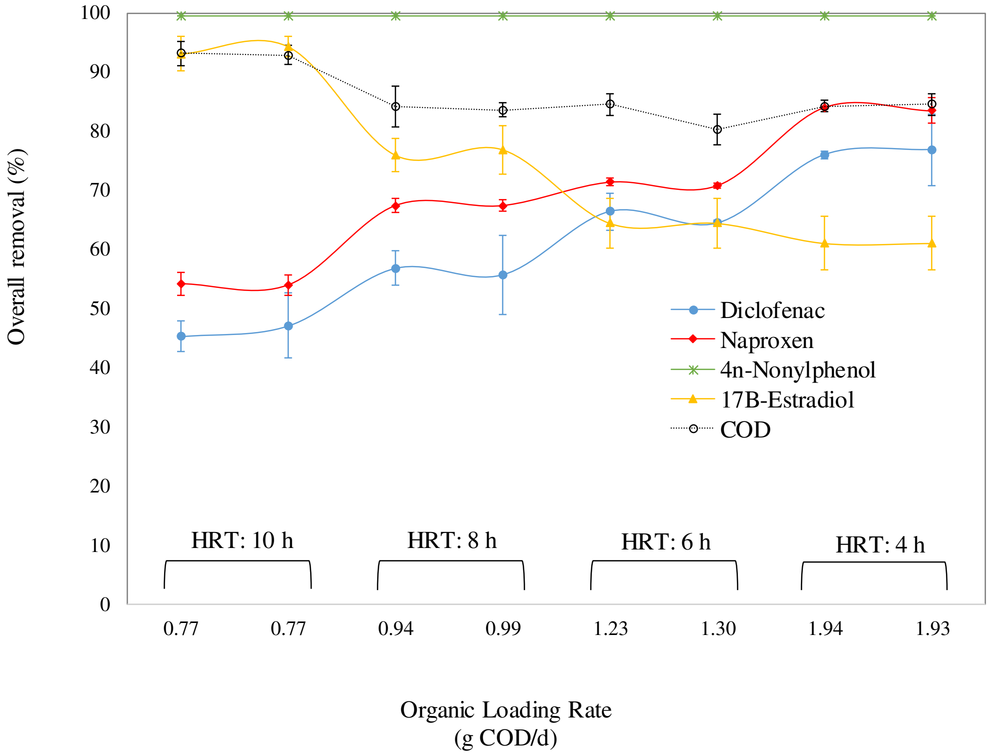


Fig. 8. Overall removal of MPs and COD in various OLRs

Table 4. k_{biol} values (L. gVSS^{-1}. d^{-1}) obtained in this study[1,2]

	Total value (both biofilm & suspended biomass)				related to the biofilm				related to the suspended biomass			
	HRT = 4 h	HRT = 6 h	HRT = 8 h	HRT = 10 h	HRT = 4 h	HRT = 6 h	HRT = 8 h	HRT = 10 h	HRT = 4 h	HRT = 6 h	HRT = 8 h	HRT = 10 h
Naproxen	10.88 ± 0.68	5.35 ± 0.22	3.46 ± 0.06	1.62 ± 0.09	6.79 ± 0.33	3.06 ± 0.18	1.78 ± 0.13	0.76 ± 0.21	3.23 ± 0.37	1.90 ± 0.04	1.69 ± 0.11	1.35 ± 0.30
Diclofenac	10.77 ± 2.15	4.11 ± 0.47	2.13 ± 0.32	1.16 ± 0.18	8.09 ± 0.84	3.89 ± 0.87	1.83 ± 0.25	0.77 ± 0.16	1.79 ± 0.57	1.08 ± 0.49	0.94 ± 0.15	0.89 ± 0.04
17ß-Estradiol	4.98 ± 0.82	3.78 ± 0.56	5.20 ± 0.95	6.91 ± 1.74	2.36 ± 0.85	2.03 ± 0.60	3.85 ± 0.83	6.10 ± 1.39	3.44 ± 0.49	2.12 ± 0.57	1.02 ± 0.15	0.95 ± 0.19
4n-Nonylphenol	-	-	-	-	1163.20 ± 23.45	809.89 ± 15.17	659.27 ± 66.02	587.21 ± 5.85	-	-	-	-

[1] As discussed in section 3.2.1, no MPs removal was seen by the air stripping. The mass flow of the air-stripped compound ($F_{stripped}$) was not therefore considered in Eq. (2).

[2] As 4n-Nonylphenol has declined up to LQ by the biofilm, no k_{biol} values have been reported here for the suspended biomass.

3.2.3. Contribution of the biofilm and suspended biomass in MPs removal

By the experimental method already explained in Section 2.4.3.1, Fig. 9 specifies individual contributions of the biofilm and suspended biomass in overall removal of MPs as a function of OLRs and HRTs.

According to Fig. 9a, when OLR increases we observe that role of the biofilm also increases in the overall removal of Diclofenac and Naproxen (up to around 54% and 51%, respectively). These findings are reinforced when the biofilm's k_{biol} values are under a downward trend in higher applied HRTs (Table 4). To bring an example about Naproxen, the biofilm's k_{biol} values decline from 6.79 to 0.76 L. gVSS^{-1}. d^{-1} by the increase of HRT from 4 to 10 h. Still, 4n-Nonylphenol removal is the highest (~99.4%) and the changes in the biofilm's k_{biol} values likely confirm its co-metabolic biodegradation. In the case of 17ß-Estradiol, as HRT is raised from 4 to 10 h, the biofilm's k_{biol} values grow from 2.4 to 6.1 L. gVSS^{-1}. d^{-1}, resulting in the increment of the removal from about 26% to 64% under the mechanism of competitive inhibition. Despite of our observations and mathematical calculations, we think there is still some doubts regarding the governance of "competitive inhibition" on the removal of 17ß-Estradiol, because of existence of a big difference between initial concentrations of 17ß-Estradiol (1 µg.L^{-1}) and carbon (COD: 100 mg. L^{-1}).

Compared to the efficiency of suspended biomass in MPs removal illustrated in Fig. 9b, it is apparent that the biofilm has wonderfully omitted recalcitrant compounds, as though the biofilm has reduced Diclofenac approximately two times more than the suspended biomass (~54% versus ~23%). In addition, Naproxen elimination by the biofilm is obtained about 20% higher than the suspended biomass. This outcome is in a good agreement with the study conducted by Falås et al. [88] who observed considerably higher MPs removal rates by the biofilm compared to the free biomass. In their study, the biofilm removed Diclofenac with k_{biol} of 1.3-1.7 L. gVSS^{-1}. d^{-1}, while its elimination by the suspended biomass was insignificant (k_{biol} < 0.1 L. gVSS^{-1}. d^{-1}) in a hybrid biofilm-activated sludge process treating municipal wastewater. As it can be seen in Table 4, the biofilm's k_{biol} values are higher than the suspended biomass's ones. The difference between the biofilm's and suspended biomass's k_{biol} values is more salient for the recalcitrant Diclofenac than the rest of compounds. For instance, a nearly fourfold biofilm's k_{biol} value is seen compared to its counterpart for Diclofenac at HRT: 4 h i.e. 8.09 ± 0.84 versus 1.79 ± 0.57 L. gVSS^{-1}. d^{-1}.

The main reason behind is that microbial community of the biofilm is too diverse [41,89] and this trait would possibly enable the biofilm to outperform the suspended biomass for removal of bio-refractory MPs. Additionally, regarding Fig. 4S in supplementary data, the amount of biofilm solids in the reactor increased from nearly 3 g at OLR = 0.77 g COD.d^{-1} to about 4 g at OLR = 1.94 g COD.d^{-1}. Hence, higher amounts of involved attached microbial strains, however, can be another explanation for enhancement of biotic and abiotic removal of persistent MPs in higher OLRs.

While CAS systems is usually dominated by the aerobic or facultative anaerobic heterotrophic bacteria [90], Biswas and Turner [89] indicated that MBBR reactors treating municipal sewage support the growth of both anaerobic and aerobic organisms inside the biofilm. They also found that the suspended biomass was dominated by aerobic members of the *Gammaproteobacteria* and *Betaproteobacteria*, while anaerobic *Clostridia* and aerobic *Deltaproteobacteria* (sulfate-reducing bacteria) overcame other strains in the biofilm. According to the previous microbiological studies on the biofilm of MBBR reactors, the presence of AOB and NOB bacteria [14], organisms associated with simultaneous nitrification and denitrification [91] and Anammox process [92], etc has been proved. Regardless of this fact that richness and evenness of the biofilm's microbial population is found very effectual in MPs removal [93], this widespread biodiversity is able to give a great potential to the biofilm for degradation of MPs. For instance, Torresi et al. [14], who worked on a nitrifying MBBR, concluded that although thin biofilms favored nitrification activity and the removal of some MPs, MBBR reactors based on thicker biofilms (400-500 μm attached on Z-Carriers) that contain more diverse strains should be considered to enhance the elimination of a broad spectrum of MPs. Conversely, a thin biofilm (~100 μm regarding the observation by the confocal microscopy (Fig. 6)) was finally resulted in the present work, whereby substrates diffusion into the biofilm is facilitated [35]. High removal of MPs by this thin biofilm probably disaffirms the finding of Torresi et al. [14] who reported a positive link between the MPs removal and the biofilm's thickness.

Fig. 9b reveals that contribution of the suspended biomass in MPs removal is not influenced by the changes in OLR. We also do not see a notable discrepancy in suspended biomass's k_{biol} values for each MPs at all HRTs, as shown in Table 4. Meanwhile, the amount of suspended biomass in the reactor has been nearly constant in all applied OLRs (~ 4.2 g, Fig. 4S in supplementary data). In this regard, we observe that Naproxen, 17ß-Estradiol and at the last place Diclofenac have been removed up to about 34%, 31% and 23%, respectively by the suspended biomass. As 4n-Nonylphenol is abated until below the LQ by the biofilm, we are not able to calculate its removal by the suspended biomass. Since the biodegradability of MPs intrinsically relies on the complexity of the structure [66], high removal of 4n-Nonylphenol is expected on the basis of its linear and monocyclic structure possessing electron donating group of -OH. Functional group of –OH embedded in the skeletons of Naproxen and 17ß-Estradiol is a good explanation for their removal until about one third of the initial concentration by the suspended biomass [94]. Persistency of Diclofenac against suspended biomass is mainly related to the existence of an electron withdrawing group named –COOH in the structure [94], and its complex pathway for biodegradation/biotransformation leads to see a high variation in elimination rates during activated sludge systems (between 20-50%) [95].

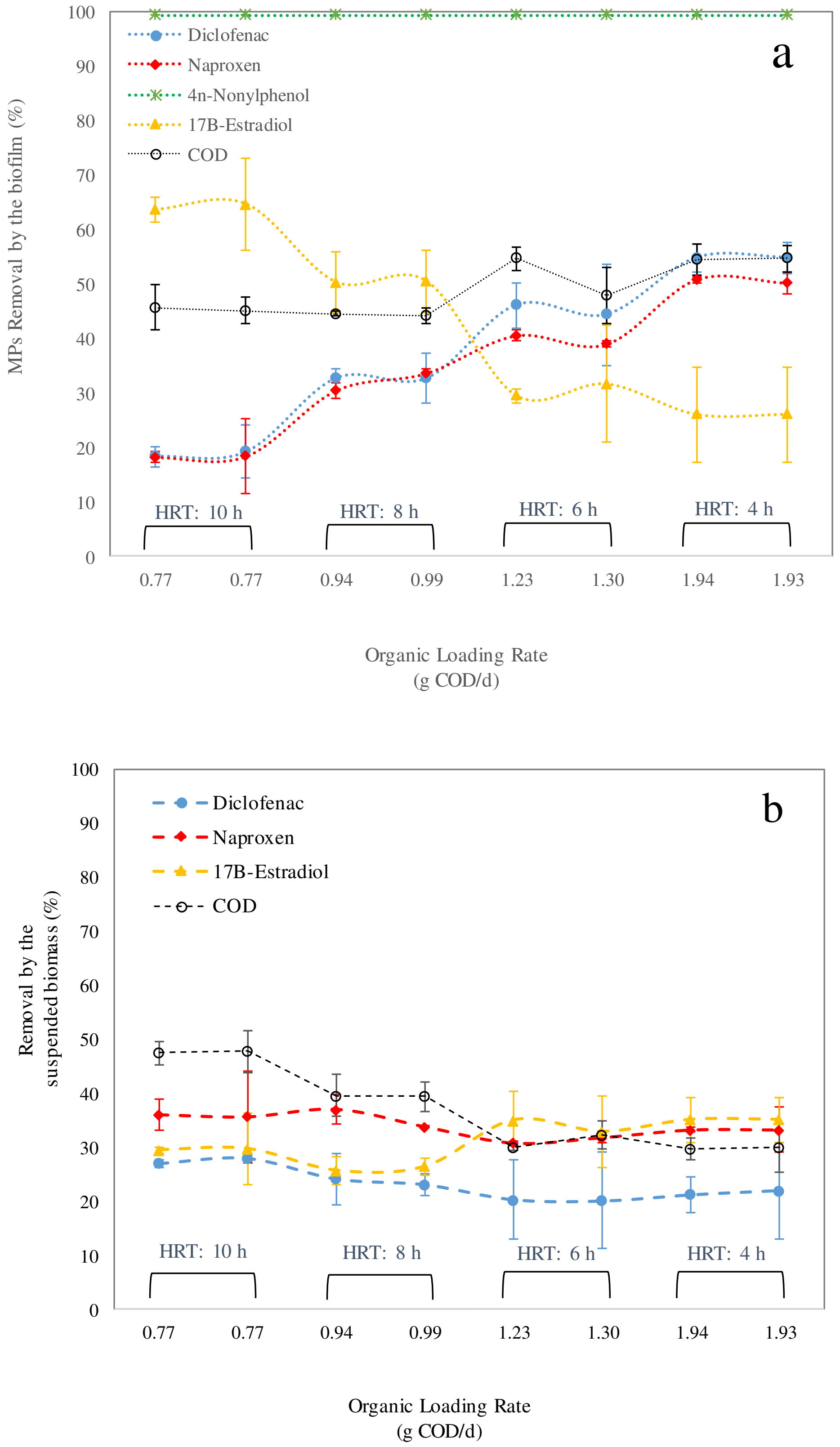


Fig. 9. Contribution of the biofilm (a) and suspended biomass (b) in overall removal of MPs

3.2.4. Abiotic and biotic distribution of MPs removal

Abiotic and biotic distribution of MPs removal is illustrated in Fig. 10. The main message of this figure is that the vast majority of MPs concentration has been mitigated by the biotic reactions, while abiotic mechanisms have no a significant role in MPs removal especially for recalcitrant compounds. Here, the abiotic removal is found to be around 4%, 2.8%, 9.5% and 15% for Diclofenac, Naproxen, 17ß-Estradiol and 4n-Nonylphenol, respectively. The low abiotic removal of these MPs were also reported between 0 and 5% in the secondary activated sludge systems [26], and from < 0.1% to 5.5% in a MBBR reactor treating a medium strength municipal wastewater [41].

When comparing biofilm and suspended biomass, we find that biofilm outperforms suspended biomass in the biotic removal of MPs. due to biodiversity of the biofilm as stated above. While a converse trend exists for the abiotic removal, where suspended biomass overcomes the biofilm because of the accessible surface area for the sorption behavior.

To elucidate the biotic removal further, we are able to refer to a simple classification scheme suggested by Joss et al. [61] who characterized the biological degradation of MPs using k_{biol} values. In this classification, compounds with $k_{biol} < 0.1$ L. gVSS^{-1}. d^{-1} are not removed to a significant extent (<20%), compounds with $k_{\mathrm{biol}} > 10$ L. g VSS^{-1}.d^{-1} are transformed by > 90%, and in-between a moderate removal is expected [61]. Table 4 indicates none of the target MPs has total $k_{biol} < 0.1$ L. gVSS^{-1}. d^{-1}. This parameter was obtained in the range of 1.6-10.9, 1.6-10.8 and 3.8-6.9 L. gVSS^{-1}. d^{-1} for Naproxen, Diclofenac and 17ß-Estradiol, respectively. Meanwhile, very high k_{biol} values for 4n-Nonylphenol are a good explanation for its fantastic biotic elimination. Total k_{biol} values of this study have been compared with what we have found in literature in Fig. 5S in supplementary data.

In the case of Diclofenac, k_{biol} values reported in a staged MBBR reactor treating hospital wastewater (0.62 L. gVSS^{-1}. d^{-1}) [82], 1.7 L. gVSS^{-1}. d^{-1} in a hybrid biofilm-activated sludge process treating municipal wastewater [88], 1.6-2.5 L. gVSS^{-1}. d^{-1} in a nitrifying MBBR [35], and 1.5-5.8 L. gVSS^{-1}.d^{-1} in a nitrifying MBBR treating an ammonium-rich secondary-treated wastewater [14]) are higher than most of the reported values for the CAS systems (< 0.1 L. g VSS^{-1}.d^{-1} [96]) and MBR reactors (< 0.1 L. gVSS^{-1}.d^{-1} [61]). The above-mentioned values are related to the secondary or tertiary nitrifying reactors and no k_{biol} value has been already reported for MBBR reactors treating a secondary-treated wastewater. A remarkable biotic removal of Diclofenac in this study (~ 72.8%) is probably linked to an admirable k_{biol} value for tertiary treatment systems where low amounts of carbon and nutrients exist.

Suárez et al. [96] calculated k_{biol} values for Naproxen and 17ß-Estradiol in a combined preanoxic-CAS up to 3.3 ± 2.8 and 32 ± 6 L. gVSS^{-1}. d^{-1}, respectively. Regarding the classification scheme proposed by Joss et al. [61] and a review paper published by Luo et al. [66], we see a moderate removal for Naproxen (40-70%) and a high removal for 17ß-Estradiol (> 70%) in CAS systems. So far, no work has been carried out to obtain k_{biol} values of these compounds in MBBR reactors. In the present study, about 80.6% and 84.7% of initial concentrations of Naproxen and 17ß-Estradiol have been declined, respectively by the biotic reactions, stating again achievement to high k_{biol} values in tertiary MBBRs. As illustrated in Fig. 5S in supplementary data, some researchers have found very high k_{biol} values for 17ß-Estradiol in simple CAS and nitrifying CAS systems even up to 350 L. gVSS^{-1}. d^{-1} [84]. Consequently, it seems that achieving a higher level of k_{biol} values is still doable in tertiary MBBRs by tuning the operational parameters such as HRT. In other words, we believe that applying higher HRTs (even more than 10h) can probably elevate k_{biol} values, leading to its supreme biotic removal. Unfortunately, we could not find 4n-Nonylphenol's k_{biol} in the literature but it has been highly removed in CAS even up to 99% [66]. Biodegradation of 4n-Nonylphenol until below than LQ is, however, attributed to the high k_{biol} values (587.2-1163.2 L. gVSS^{-1}. d^{-1}) obtained in this study.

Despite the fact that (I) MPs' k_{biol} values are not strongly affected by the SRT [39], and (II) the correlation between the SRT and elimination of target MPs is still not clear [72,95,96], some authors [14,40,82] have noted that possible high SRTs in MBBRs enable them to remove MPs more efficiently than other tertiary biological methods for the biotic removal of MPs (Fig. 3S and Table 3S in supplementary data). Longer SRTs allow bacterial population to become more diversified and more capable of degrading MPs either by direct metabolism or by co-metabolic degradation via enzymatic reactions [39]. On the other hand, low F/M ratio emerged by the high suspended and attached biomass and the relative shortage of biodegradable organic matter may force microorganisms to metabolize some MPs with the competitive inhibition mechanism [97]. We inevitably see that tertiary MBBRs support the main biodegradation mechanisms for the biotic removal of MPs, and the presence of the main substrates for microbial growth is generally neither a main trigger nor a strong inhibitor of MPs degradation.

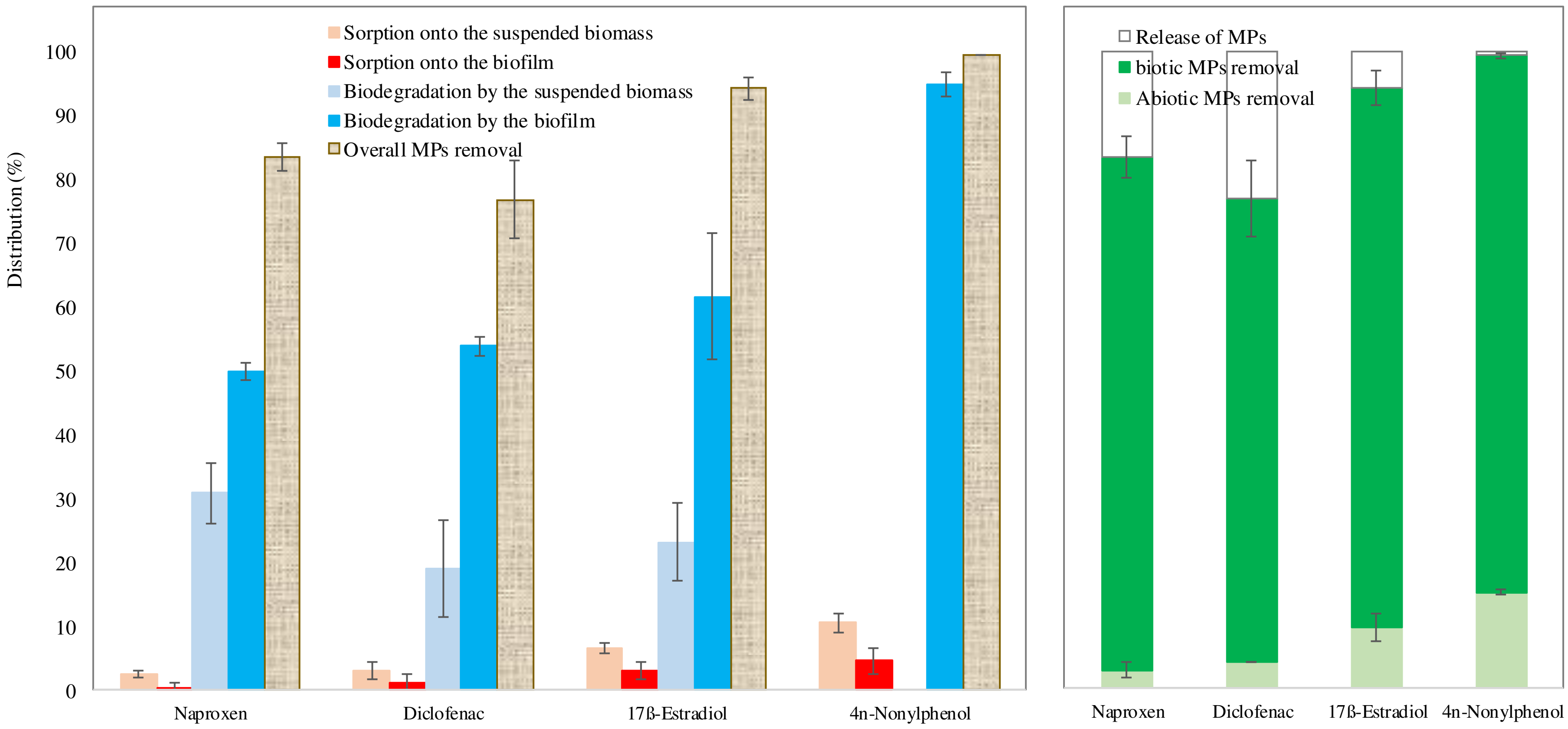


Fig. 10. Individual contribution of the biofilm and suspended biomass in abiotic and biotic removal of MPs

4. Conclusion

In the present work, we provided further insights into the key parameters involved in abiotic and biotic removal of MPs in tertiary MBBRs. No MPs abatement was observed by the both ways of photodegradation and air stripping, revealing that abiotic removal of MPs was completely attributed to the only sorption phenomenon. Compared to the percentages of the abiotic removal (~2.8-15%) that were strongly linked to the compounds' hydrophobicity, biotic removal of MPs was observed to be the principal removal mechanism for all compounds (~72.8-84.7%). Evaluating the effect of the changes in OLRs on the MPs removal and k_{biol} values proved that Diclofenac, Naproxen and 4n-Nonylphenol were mainly degraded by the co-metabolism mechanism, while competitive inhibition was the main mechanism involved in the removal of 17ß-Estradiol. Contribution of the biofilm was higher than the suspended biomass in biodegradation of all MPs (specially seen for Diclofenac), while MPs sorption onto the suspended biomass was occurred more than the biofilm.

As a future perspective, regarding the remarkable contribution of the biofilm in biodegradation of recalcitrant Diclofenac and Naproxen (~50%), the establishment of a mature biofilm bio-augmented by appropriate MPs-degrading microorganisms can be suggested for further optimization of MPs biodegradation.

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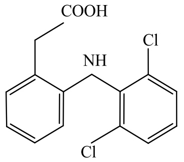
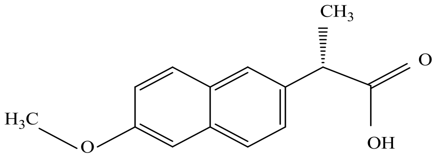
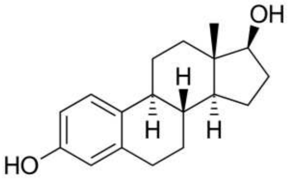
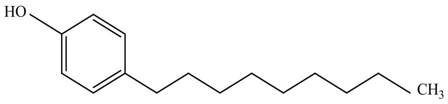
Table 1S. Physico-chemical characteristics and concentration of target MPs in the secondary-treated effluents of conventional WWTPs [98,6,99,100,66]

Compound	CAS number	Formula	Molecular Weight (g/mol)	Henry's law constant (atm.m^3.mol^{-1}) [62,63]	log K_{OW}	log D (pH:7)	pKa	Concentration of MPs in literature (µg/L) (min-average-max)	Concentration of MPs in the present study (µg/L)	Molecular structure
Diclofenac	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.15	4.73E12	4.548	1.77	4.18	0.035 - 0.477 - 1.72 [101] 0.040 - 0.679 - 2.448 [102] 0.21 - 0.34 - 0.62 [103] 0.013 – 0.024 – 0.049 [104] 0.044 – 0.173 – 0.329 [105] 0.006 – 0.179 – 0.496 [106] 0.131 – 0.263 – 0.424 [106] 0.006 – 0.220 – 0.431 [107] 0.15 – 0.41 – 1.1 [108]	0.5	
Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.26	3.39E10	3.18	0.34	4.3	0.017 – 0.934 – 2.62 [102] 0.09 – 0.13 – 0.28 [103] 0.037 – 0.111 – 0.166 [104] 0 – 0.0165 – 0.0918 [105] 0. 54 – 2.74 – 5.09 [109] 0.22 – 1.64 – 3.52 [109] 0.83 – 2.18 – 3.64 [109] 0.29 – 1.67 – 4.28 [109] 0.234 – 0.370 – 0.703 [106] 0.002 – 0.170 – 0.269 [106] 0.359 – 0.923 – 2.208 [107]	2.5	
17ß-Estradiol	50-28-2	$C_{18}H_{24}O_2$	272.38	4.7E3	4.13	4.15	10.27	<0.001 – 0.019 – 0.007 [110] 0.0005 – 0.0015 – 0.0029 [111] 0.0003 – 0.0009 – 0.0021 [111] 0.0007 – 0.0024 – 0.0035 [111] Average: 0.0025 [98] Average: 0.0036 [112] Average: 0.001 [113] 0 [114] 0 [104]	1	
4n-Nonylphenol	104-40-5	$C_{15}H_{24}O$	220.35	3.64E11	6.142	6.14	10.15	0.5 – 0.5 – 7.8 [110] 2.515 – 6.138 – 14.444 [115] 1.084 – 1.885 – 3.031 [115] Maximum: 7.8 [66] Average: 0.786 [116] Average: 7.19 [117] Average: 2 [118] Average: 1.42 [119]	7	

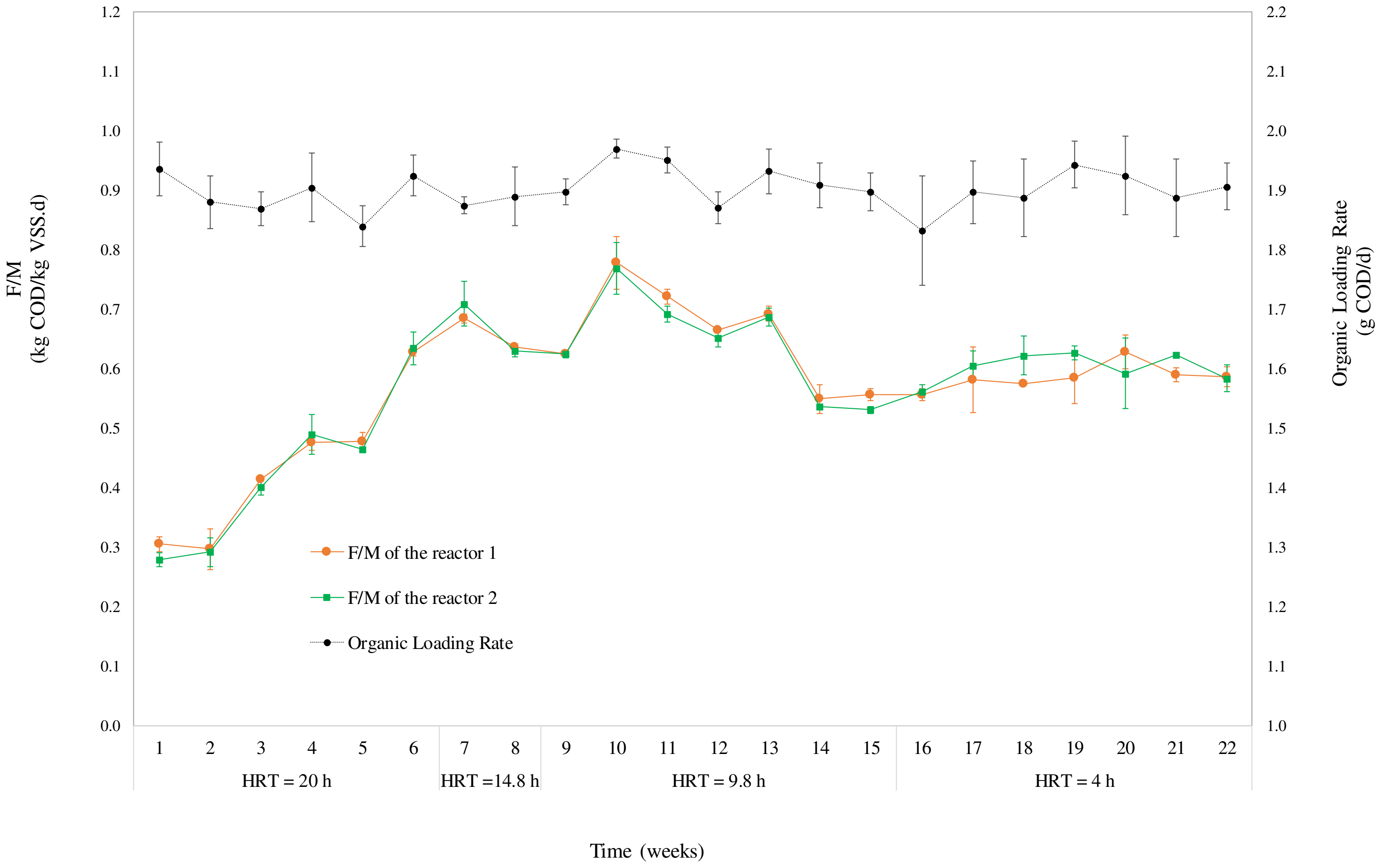


Fig.1S. Stepwise reduction of HRTs in a nearly constant OLR, and variations in the food to microorganisms (F/M) ratio during the start-up and biofilm formation

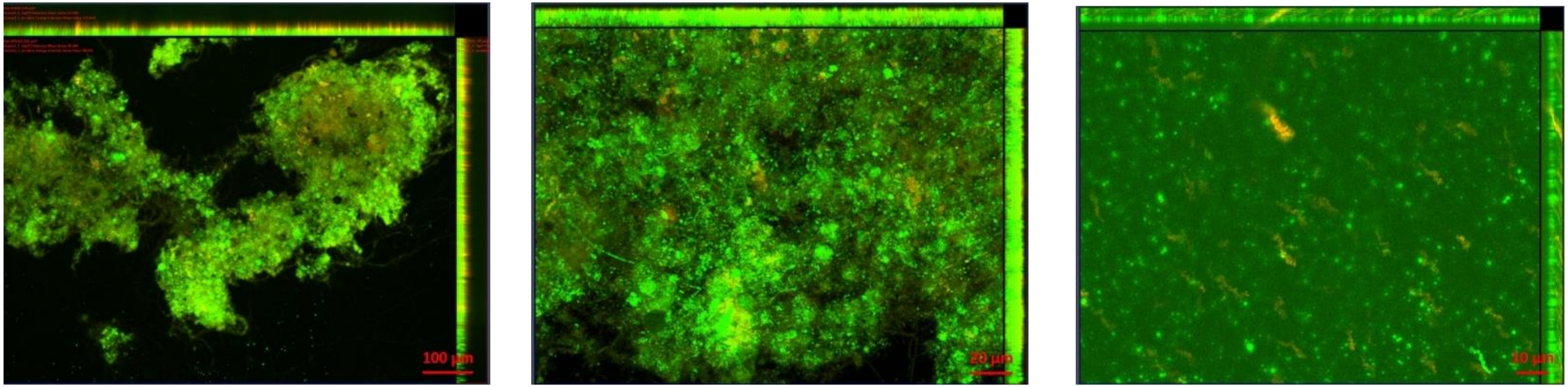


Fig. 2S. Assessment of the suspended solids' viability at an HRT of 4 h (in 20th week) using epifluorescence microscope (live cells are illuminated green and dead cells are illuminated red)

Table 2S. r_{sor} values corresponding to Eq. (4) (µg. L^{-1}. d^{-1})

	Total value	related to the biofilm	related to the suspended biomass
Naproxen	0.0058 ± 0.0024	0.0008 ± 0.0012	0.0050 ± 0.0012
Diclofenac	0.0017 ± 0.0001	0.0004 ± 0.0006	0.0013 ± 0.0006
17ß-Estradiol	0.0079 ± 0.0018	0.0025 ± 0.0012	0.0054 ± 0.0006
4n-Nonylphenol	0.0875 ± 0.0024	0.0263 ± 0.0112	0.0613 ± 0.0088

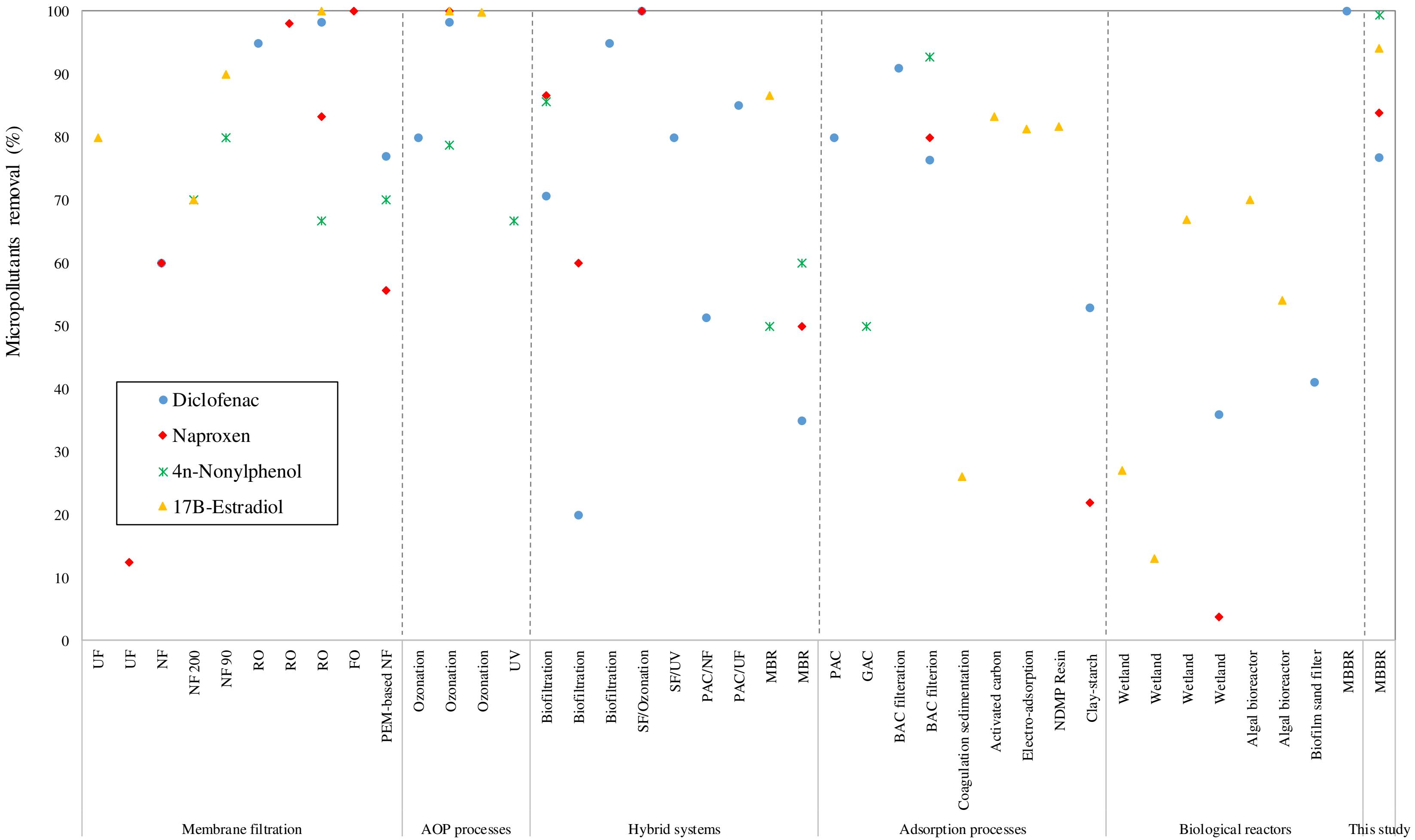


Fig. 3S. Comparison of overall MPs removal with other tertiary treatment methods found in literature (more details are given in Table 3S in supplementary data)

Table 3S. Comparison of overall MPs removal with other tertiary treatment methods found in literature

Tertiary treatment system		Description	Concentration of MPs (µg/L)	Overall MPs Removal (%)				
				Diclofenac	Naproxen	4n-Nonylphenol	17B-Estradiol	References
Membrane filtration	UF	PES flat-sheet, 100 kDa; TMP = 0.5 ± 0.01 bar	100 ng/L				80	[120]
	UF	a dead-end UF unit at an average flow-rate of 2.5 m^3/h	2.9 µg/L		12.4			[121]
	NF	Flat-sheet, area 3.5 m^2; TMP = 0.3 or 0.7 bar	0.5 - 1 µg/L	60	60			[122]
	NF 200	Operating flux: 13 $L/m^2.h$, 483 kPa	7-18 µg/L			70	70	[123]
	NF 90	Operating flux: 13 $L/m^2.h$, 345 kPa				80	90	
	RO	Filmtec TW30; TMP = 9.5–10.2 bar		95				
	RO	a low pressure gradient: (ΔP = 11 bar)., and constant feed flowrate: 2.4 m^3/h	2.9 µg/L		98.2			[121]
	RO	No detail is given about the RO membranes.	4n-Nonylphenol: 0.66 µg/L., Naproxen: 0.06 µg/L., Diclofenac: 0.63 µg/L	98.4	83.3	66.7	100	[124]
	FO	Hydration Technology Innovations (HTI, Albany, OR) FO membranes	10		100			[71]
	PEM-based NF	NF membranes made by layer by layer (LbL) assembly of weak polyelectrolytes	Diclofenac: 0.5 µg/L., Naproxen: 2.5 µg/L., 4n-Nonylphenol: 7 µg/L	77	55.6	70		[125]
AOP processes	Ozonation	Ozone dose: 2.8 ± 30%	2.6-5.8 µg/L	80				[126]
	Ozonation	No detail is given about the ozonation.	4n-Nonylphenol: 0.66 µg/L., Naproxen: 0.06 µg/L., Diclofenac: 0.63 µg/L	98.4	100	78.8	100	[124]
	Ozonation	Ozone dose: 5-40 mg/L., contact time: 20 min	4.68 ± 0.89 ng/L				99.99	[127]
	UV	No detail is given about the UV.	6 µg/L			66.7		[118]

Hybrid systems	Biofiltration	The plastic media was used for this experiment. The length, diameter, density and the internal surface area of the used plastic media are 3 mm, 5 mm, 0.42–0.46 g/cm^3 and 305 m^2/m^3, respectively.	Diclofenac: 1700 ng/L, Naproxen: 1500 ng/L., 4n-Nonylphenol: 1400 ng/L	70.59	86.67	85.71		[128]
	Biofiltration	Granular anthracite media: 0.84-1 mm	2	20	60			[42]
	Biofiltration	Aerated biofilters (MnOx ore (Aqua-mandix®) and natural zeolite) with manganese feeding (20 mg/L).	4	95				[7]
	SF/Ozonation	Ozone dose: 0.79 ± 0.02 g O^3/g DOC	Diclofenac: 1200 ng/L, Naproxen: 250 ng/L	100	100			[129]
	SF/UV	Three media in the filter: quartz sand, FiltraliteH and LECA., The intensity of UV light: 500 mJ/cm^2	0.3-1.5 µg/L	80				[130]
	PAC/NF	PAC concentration: 10-100 mg/L, 1.5 mm capillary Nanofiltration NF50 M10 from Norit X-Flow with TMP: 1.5 - 4 bar	10 ng/L - 10 µg/L	51.4				[131]
	PAC/UF	PAC concentration: 20 mg/L, PES-UF membrane: permeability: 80-200 L/(m2.h.bar) and water flux: 23 L/(m^2.h)	1.3 - 9.1 µg/L	85				[132]
	MBR	The hollow fibre polyvinylidene fluoride membrane modules (nominal pore size: 0.04 µm, total membrane area: 182.9 m^2)	4n-Nonylphenol: 4.2-12.6 ng/L, 17B-Estradiol: 144.3 ng/L			50	86.7	[133]
	MBR	No detail is given about the MBR.	4n-Nonylphenol: 0.66 µg/L, Naproxen: 0.06 µg/L, Diclofenac: 0.63 µg/L	35	50	60		[124]
Adsorption processes	PAC	PAC concentration: 10 ± 8% mg/L	2.6-5.8 µg/L	80				[126]
	GAC	No detail is given about the GAC	15 - 402 ng/L			50		[134]
	BAC filteration	Media: GAC; media height: 80 cm; diameter: 22.5 cm; EBCT: 18 min	1 µg/L	91				[135]
	BAC filterion	The surface area, total pore volume and micropore volume of the activated carbon are 800 BET m^2/g, 0.865 cm^3/g and 0.354 cm^3/g, respectively.	Diclofenac: 1700 ng/L, Naproxen: 1500 ng/L, 4n-Nonylphenol: 1400 ng/L	76.5	80	92.9		[128]

	Coagulation sedimentation	The coagulation sedimentation process:18 mg/L polyaluminium + 9 mg/L polyacrylamide	4.68 ± 0.89 ng/L				26.07	[127]
	Activated carbon	Dose: of 20-160 mg/L, the response time: 30 h	4.68 ± 0.89 ng/L				83.33	
	Electro-adsorption	1.8 V of applied potential, 2 mm of plate distance, and 15 mL/min of flow rate for 10-100 min.					81.41	
	NDMP Resin	Resin: Nan da magnetic polyacrylic anion exchange resin (NDMP)., The retention time: 1 h					81.83	
	Clay-starch	Clay dosage: 0-60 mg/L., Nalco Starch EX10704 doage: 20 mg/L	Diclofenac: 30.6 ng/L, Naproxen: 12.8 ng/L	53.00	22			[136]
Biological reactors	Wetland	Subsurface flow (SSF) wetland	32.80- 55.54 ng/L				27	[137]
	Wetland	Floating aquatic plant (FAP) wetland					13	
	Wetland	The combination of wetland and ground water flow-through system	180 ng/L				67	[138]
	Wetland	a free water surface wetlands located in Oxelösund in Sweden	Diclofenac: 0.48 µg/L, Naproxen: 0.064 µg/L	36.00	3.7			[139]
	Algal bioreactor	algal strain: Scenedesmus dimorphus	5 µg/L				70	[140]
	Algal bioreactor	algae genera: Anabaena cylindrica, Chlorococcus, Spirulina platensis, Chlorella, Scenedesmus quadricauda, and Anaebena var	1 µg/L				54	[141]
	Biofilm sand filter	Media (quartz sand: 0.210–0.297 mm particle size)., HRT: 0.012 m^3/h	0.24 ± 0.047 µg/L	41.00				[31]
	MBBR	polishing MBBRs, filling ratio: 50% (AnoxKaldnes K5 carriers), HRT: 4 h	3-20 µg/L	100.00				[10]
This study	MBBR	Moving Bed Biofilm Reactor (MBBR)	Diclofenac: 0.5 µg/L, Naproxen: 2.5 µg/L, 4n-Nonylphenol: 7 µg/L, 17-B-Estradiol: 1 µg/L	76.84	83.99	99.43	94.24	This study

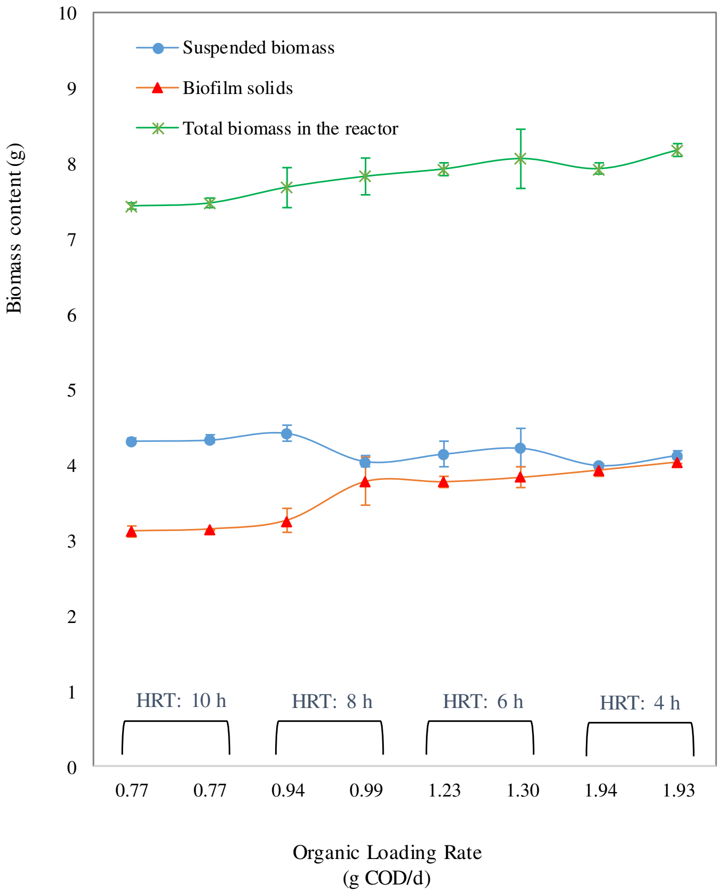


Fig. 4S. Variation of the suspended, attached and total biomass content at different OLRs

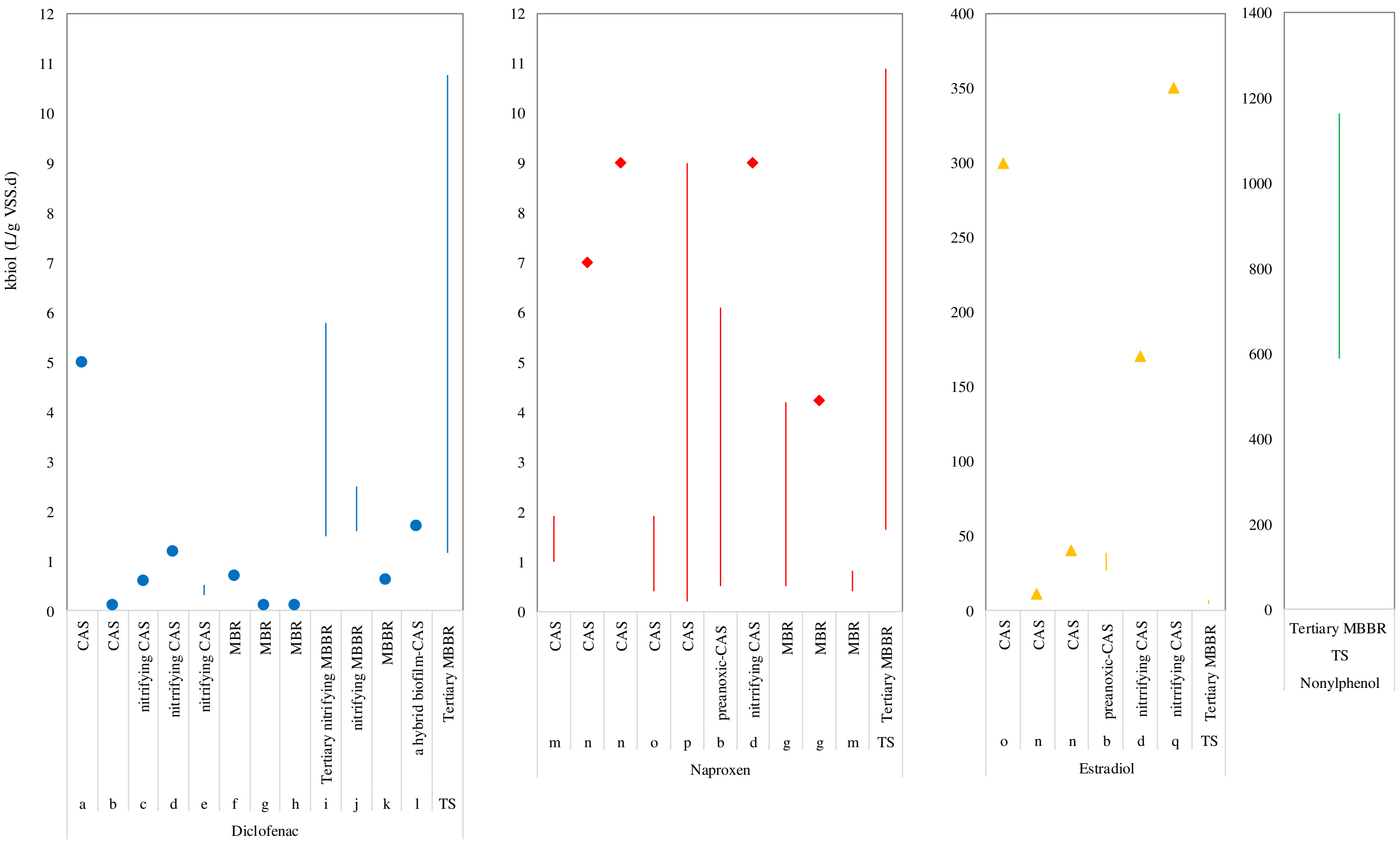


Fig. 5S. Comparison of k_{biol} values of this study and the literature

References: [a][142], [b][96], [c][143], [d][144], [e][95], [f][145], [g][46], [h][61], [i][14], [j][35], [k][82], [l][88], [m][73], [n][45], [o][146], [p][26], [q][84], **TS**: This Study

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CHAPTER (III)

The influence of bioaugmentation on the performance of tertiary moving bed biofilm reactors (MBBRs) for micropollutants removal

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Abstract

Microbial biofilms are recently recognized as a natural medium for immobilization of micropollutant (MP)-degrading microbes, leading to an enhancement in MPs removal from wastewater. The present study aims at answering whether bioaugmentation of tertiary moving bed biofilm reactors (MBBRs) receiving a secondary-treated municipal wastewater could successfully increase MPs removal. After start-up, biofilm formation and well adaptation of the biomass to target MPs, *Pseudomonas fluorescens* was inoculated into two out of three tertiary MBBRs with a novel protocol., and its abundance in the biofilm and liquid phase was monitored by DNA extraction and qPCR throughout the continuous operation. Although a gradual reduction was observed in the abundance of *P. fluorescens* with time, bioaugmented MBBRs (bMBBRs) showed relatively higher pseudo-first order biodegradation constants (k_{biol}) than the control MBBR (cMBBR) for all target MPs. According to the batch experiments, neither the photodegradation nor the volatilization could remove MPs, indicating that abiotic removal of MPs could be only ascribed to the sorption onto the biosolids. When comparing two major pathways of biodegradation and sorption, we found that the biodegradation strongly outperformed its counterpart for the removal of all MPs, in particular for the bMBBRs, whereby MPs sorption was nearly negligible (0.4-3.9%) against a great biotic removal i.e. 84.5, 90.4 and 95.5% for Diclofenac, Naproxen and 4n-Nonylphenol, respectively. Compared to the bMBBRs, a higher abiotic removal (2.8-15%) along with an around 10% lower biotic removal was seen in the cMBBR, that still looks very high. High efficiency of the cMBBR for MPs removal is probably attributed to the well-adapted biofilm. Even though further research is still needed to optimize the process of bioaugmentation, bMBBR potentially appears a promising technology to achieve enhanced removal of MPs.

1. Introduction

The presence of different categories of micropollutants (MPs) in the aquatic environment was proven to have adverse effects on living organisms, raising concern about their insufficient removal during the conventional wastewater treatment [1]. Along with environmental standards legalized by, for example, the European Union (the Directives 2008/105/EC [2] and 2013/39/EU [3], and the Decision 2015/495/EU [4]), implantation of additional treatment steps (i.e. tertiary treatment) is so far proposed to overcome this ever-growing anxiety.

Among various tertiary biological treatment technologies examined (such as membrane bioreactors [5], bio-filters [6], algal bioreactors [7] and wetlands [8]), biofilm reactors, especially moving bed biofilm reactors (MBBRs), are recently seen proficient in MPs removal [9,10]. In such reactors where high solids retention times (SRTs) are achievable in low hydraulic retention times (HRTs), high biomass concentration and the presence of slow-growing species, both resulting from high SRTs, lead to an achievement to high removal efficiencies for a broad range of MPs [11]. For instance, Escolà Casas et al. [12] investigated a pilot plant consisting of a series of one activated sludge reactor, two Hybas™ (VeoliaWater Technology) reactors, and a polishing MBBR during 10 months of continuous operation. Removal of organic matter and nitrification mainly occurred in the first reactor. When the removal rate constants were normalized to biomass amount, the last reactor (polishing MBBR) appeared to have the most effective biomass in respect to removing MPs. They concluded that the polishing MBBR combines a fast growing biomass with a low SRT in free activated sludge flocs, and a slow-growing biomass with a high SRT on the biofilm attached to the MBBR carriers [12]. Meanwhile, Longer SRTs allow bacterial population to become more diversified and more capable of degrading MPs either by direct metabolism or by co-metabolic degradation via enzymatic reactions [13]. On the other hand, low food to microorganism (F/M) ratio emerged by the high suspended and attached biomass and the relative shortage of biodegradable organic matter may force microorganisms to metabolize some MPs with the competitive inhibition mechanism [14].

A glance through the publications indicates that working on tertiary MBBRs is still stood on the beginning steps. Tang et al. [9] investigated the effect of humic acid, as a model for complex organic substrate, on the biodegradation of 22 pharmaceuticals by a tertiary MBBR. From the results of the batch incubations of MBBR carriers, the biodegradation rate constants of ten of those compounds (e.g. Metoprolol and Iopromide) were increasing with increased humic acid concentrations. At the highest humic acid concentration (30 mgC. L^{-1}), the biodegradation rate constants were four times higher than the biodegradation rate constants without added humic acid. They concluded that the presence of complex substrate stimulates degradation of some MPs via a co-metabolism mechanism. Also, biodegradation improvement of some compounds such as Carbamazepine and Ibuprofen was not observed by adding humic acid [9]. In their next study [10], the authors ran a tertiary MBBR in the continuous mode with a novel strategy. To overcome that effluent contains insufficient organic matter to sustain enough biomass, the reactor was intermittently fed by raw wastewater. By this method, the removal of the majority of pharmaceuticals such as Diclofenac, Metoprolol and Atenolol was dramatically enhanced [10].

Over the last two decades, bioaugmentation of conventional activated sludge (CAS) systems has been often used to speed up the start-up process, to protect the existing microbial community against adverse effects, to compensate of organic or hydraulic overloading, and to eliminate the refractory compounds [15–17]. However, due to the intricacy of the practical operational conditions, full-scale application of the CAS systems bioaugmented by specialized microorganisms has been rarely reported [18]. In recent years, some researchers have focused on the effectiveness of bacterial/fungal bioaugmentation for MPs elimination. For example, in the study of Nguyen et al. [19] who added white rot fungus *Trametes Versicolor* in a non-sterile lab-scale membrane bioreactor (MBR) for purifying a malt-based synthetic wastewater, a mixed culture of fungi and bacteria gradually developed in the reactor. In their study, white-rot fungal enzyme (laccase), coupled with a redox mediator (1-hydroxy benzotriazol), could degrade 51% Diclofenac, 70% Triclosan, 99% Naproxen and 80% Atrazine. In many experiences, a major obstacle to fruitful bioaugmentation is the usual insufficient establishment of the desired functions within the microbial community due to the wash-out of inoculated microbes. Therefore, immobilization of bacterial/fungal strains has been lately proposed as a novel strategy for preventing wash-out of the inoculated microorganisms [20]. This technique can also lead to a better survival rate by shielding cells under stressed environmental conditions, usually enabling a faster and more efficient biodegradation as compared to the suspended biomass [21]. Bacterial biofilms are recognized as a natural medium for this kind of immobilization process [20]. To date, many attempts for bioaugmentation of biofilm reactors have failed [22]. For instance, in the study of Feakin et al. [23], two bacterial strains of *Rhodococcus rhodochrous* and *Acinetobacter junii* capable of biodegrading Atrazine and Simazine (1-10 μg. L^{-1}) were inoculated into a fixed-bed reactor pre-filled with silanized glass wool and granular activated carbon (GAC). The reactors (one as a control and the other one as a bioaugmented reactor), continuously operated at an empty bed contact time of 20 min, did not show a noticeable biodegradation rate i.e. the removal rate ranged from 19.5 to 32% of each herbicide for both inoculated and non-inoculated reactors [23].

In a novel strategy lately used for bioaugmentation of biofilm reactors, immobilizing the specific-pollutant degrading strains into the biofilm is mediated by biofilm-forming bacteria. A handful of studies have shown that this strategy might be an efficient approach for colonization of the degraders into the biofilm. For instance, bioaugmentation of sequencing batch biofilm reactors by bacterial strains of *Comamonas testosteroni* and *Bacillus cereus* and their impact on reactor bacterial communities was investigated by Cheng et al. [20]. The reactors, filled by sphere-like porous PVC carriers, were firstly inoculated with activated sludge and continuously fed by a synthetic wastewater containing 100-500 mg. L^{-1} 3,5-dinitrobenzoic acid. After the start-up stage, the reactors were inoculated by *Bacillus cereus* G5 as a biofilm-forming bacteria and *Comamonas testosteroni* A3 as a 3,5 dinitrobenzoic acid (DNB)-degrading bacteria, and continuously operated at a HRT of 24 h. In the bioaugmented reactor, the removal efficiency of 3,5-dinitrobenzoic acid achieved up to 83% after 28 days of operation, while this value was reported by 75.9% after 33 days of operation in non-bioaugmented reactor. Although the difference between removal efficiencies was low, but the bioaugmented reactor exhibited obvious resistance to shock loading with 3,5-dinitrobenzoic acid. Microbial diversity of the reactors was also explored. *C. testosteroni* A3 was predominant in the bioaugmented reactor, indicating the effect of *B. cereus* G5 in promoting immobilization of *C. testosteroni* A3 cells in the biofilm. They finally concluded that those microbial strains, e.g. *B. cereus* G5, which can stimulate the self-immobilization of the degrading bacteria offer an innovative method for immobilization of the degraders in bioaugmented biofilm reactors [20]. The same strategy was also used by Chunyan Li et al. [24], whereby a unique biofilm consisting of three bacterial strains with a high biofilm-forming capability (*Bacillus subtilis* E2, E3, and N4) and an acetonitrile-degrading bacteria (*Rhodococcus rhodochrous* BX2) were established for acetonitrile-containing wastewater treatment in MBBR reactors. Activated sludge was initially used for starting-up the reactors, and then the above strains were inoculated into the reactors. Continuous operation of reactors lasted for 30 days at HRT of 24h. The bioaugmented MBBR exhibited strong resistance to Acetonitrile loading shock and completely depleted the initial concentration of Acetonitrile (800 mg. L^{-1}). The immobilization of *R. rhodochrous* BX2 cells in the biofilm was also confirmed by PCR–DGGE method. Similar to Cheng et al. [20], they revealed that biofilm-forming bacteria can promote the immobilization of contaminant-degrading bacteria in the biofilms and can subsequently improve the degradation of contaminants in wastewater [24]. Even to be more cost-effective and less laborious that this strategy, Dvorak et al. [25] used only one strain for bioaugmentation of full-scale MBBRs treating an industrial wastewater containing Aniline and Cyanide. They used *Rhodococcus erythropolis* CCM that has a proven ability to catabolize a wide range of compounds and metabolize harmful environmental pollutants. Furthermore, this strain has a good biofilm-forming ability and have a high resistance to extreme conditions (e.g. salinity 2–3% and temperatures of 10–38 °C). Over a long operation time of 5 years, the removal rates of Aniline and Cyanide were obtained up to 75-99% and more than 88%, respectively [25]. From our literature review, no report has been so far published in terms of MPs removal by this strategy.

Even though the attempts to use “bioaugmentation of biofilm reactors” did not hitherto show reliable results to improve MP biodegradation, but this area of research remains fascinating and potentially promising [22]. According to our literature review, there is yet no research on the subject of tertiary MPs removal using bioaugmented biofilm reactors operating in the continuous mode. In the present study, the removal of several MPs including two analgesic and anti-inflammatory pharmaceutical compounds (Diclofenac, Naproxen) and one endocrine disrupting compound (4n-Nonylphenol) was investigated. We aimed at determining whether bacterial bioaugmentation of tertiary MBBRs could successfully enhance MPs removal from conventionally-treated municipal wastewater. The bacterial strain used for bioaugmentation was “*Pseudomonas fluorescens”* that has a proven capability in both aspects of the biofilm formation, and in metabolizing the industrial pollutants. The potency of tertiary bioaugmented MBBRs for MPs removal has not been evaluated so far, probably converting this study to a prerequisite for future researches.

2. Materials and methods

2.1. *Chemicals*

The main supplier of all analytical-grade MPs, with the physico-chemical properties given in our previous study [26], was Sigma-Aldrich. All chemical compounds including all salts ($CaCl_2.2H_2O$, NaCl, K_2HPO_4, $MgSO_4.7H_2O$, $NaHCO_3$, $KMnO_4$, NaOAc, NaN_3, allylthiourea, peptone, meat extract, sucrose), acetone, methanol, hexamethyldisilazane (HMDS), and glutaraldehyde were also purchased from Sigma-Aldrich.

2.2. MPs-bearing synthetic wastewater

The protocol of “OECD Guideline for Testing of Chemicals” [27,28] was used to prepare synthetic secondary-treated municipal wastewater. Mother stock solutions of MPs were separately prepared in high-pure methanol with concentration of 1 g.L^{-1}, stored in 15-mL amber glass bottles and kept in a freezer (-18°C). Daughter stock solutions of each MP were then prepared separately in Milli-Q water from their individual mother stock solutions. An appropriate amount of each MP was subsequently added to the synthetic wastewater to reach to the target concentration of MPs in the reactor’s influent. As discussed in our previous study [26], final concentrations of Diclofenac, Naproxen, and 4n-Nonylphenol were 0.5, 2.5, and 7 µg.L^{-1}, respectively, based on available data in literature about concentration of target MPs in effluents of conventional municipal WWTPs.

2.3. COD, TN, and $P\text{-}PO_4^{3-}$ measurements

After filtration of samples by 0.70 µm glass fiber filters (VWR, 516-0348, France), HACH LANGE kits (LCI 500 for COD, LCK 341 for TN, and LCK 341 for $P\text{-}PO_4^{3}$) along with DR3900 Benchtop VIS Spectrophotometer equipped with HT200S oven (HACH LANGE, Germany) were used for measurements. The parameters were measured in duplicate and the average values and standard deviations are reported.

2.4. MPs analysis

Samples collected from the inlet and outlet of the reactors were firstly filtered using 0.70 µm glass fiber filters (VWR, 516-0348, France) in order to remove big particles. Each sample that had a volume of 250 mL was then immediately stored in amber-glass bottles and finally kept in freezer (-18°C). Afterwards, samples were shipped to the LaDrôme laboratory (France) in a freeze box for analysis under the analyzing license of COFRAC-ESSAIS. A multi detection procedure including Gas Chromatography (coupled with ECD/NPD mass spectrometry) and Liquid Chromatography (along with DAD, fluorescence, tandem mass spectrometry) was applied for all MPs with Limit of Quantification (LQ) of 0.01 µg/L for Diclofenac and Naproxen., and 0.04 µg/L for 4n-Nonylphenol. Removal values R were calculated according to the Eq. (1), where S_i and S_e are MP concentration in the inlet and outlet of the reactors, respectively. Each measurement was performed in duplicate and the average of values with standard deviation are reported.

$$R = \left(1 - \frac{S_e}{S_i}\right) \times 100 \tag{1}$$

2.5. Determination of biomass concentration

2.5.1. Suspended biomass

Mixed liquor suspended solids (MLSS) were measured by filtering through a paper filter (VWR, 516-0348, France) with 0.70 µm pore size followed by drying overnight at 105 °C (Memmert Oven) and the final weight determination. Meanwhile, overnight heating under the temperature of 550 °C in a furnace

(Salvis Lab Thermocenter, TC40) was applied in order to measure mixed liquor volatile suspended solids (MLVSS) [12].

2.5.2. Biofilm solids

Four carriers from each reactor were placed on an aluminum-wrapped cup, dried overnight at 105 °C (Memmert Oven), and weighed. Dried carriers were then washed in 3 M NaOH solution to detach the whole biofilm, and cleaned with demineralized water to rinse excess NaOH solution. Samples were dried again at 105 °C overnight and weighed. Finally, the biofilm solids were calculated as the weight difference before and after washing of carriers [12]. The biomass per area was calculated knowing that each carrier (Z-400 carriers) has a protective surface area (PSA) of 2194 mm^2 [29].

2.6. Biofilm morphology

For the microscopic observation of the biofilm, firstly, each biofilm-coated carrier was gently cut into small pieces (each piece: 6 mm × 6 mm). Each piece was initially fixed with 2 mL of 4% glutaraldehyde, 1 mL of phosphate buffer (pH: 7.4) and 1 mL of demineralized water for 20 minutes, and then washed 2 times in 1 mL of phosphate buffer, 2 mL of 0.4 M sucrose and 1 mL of demineralized water for 15 minutes. In the step of dehydration, sample was immersed in 2-mL acetone-water solution (50%:50%) for 5 minutes, 2-mL acetone-water solution (70%:30%) for 5 minutes, and 2-mL acetone-HMDS solution (50%:50%) for 5 minutes. Finally, the sample was dried overnight under the evaporation of 2 mL HMDS solution. For the metallization, dried sample was coated with 10-nm gold for 60 seconds via a compact sputter coater (The Scancoat Six, EDWARDS) according to the protocol of manufacture. Metallized pieces were then observed by a mini-scanning electron microscope (SEM) (TM 3000 tabletop, HITACHI) at different magnifications.

2.7. Configuration, start-up and operation of the MBBRs

2.7.1. Biofilm carriers

Saddle-formed Z-400 carriers were provided from AnoxKaldnes company (Lund, Sweden). In general, Z-Carriers are seen less prone to the scaling phenomenon, as the formed biofilm is shown to be filled by lower amounts of inorganic precipitates [30]. Also, biofilm expands on the outside of the Z-carriers instead of inside voids, and the exposed biofilm is covered on the entire surface of the carrier. Each carrier had a 30 mm diameter, 2194 mm^2/carrier PSA, and compartment size of 2.3 mm × 2.3 mm [29]. Before starting the operation, Z-400 carriers were rinsed by 1 mg.L^{-1} $KMnO_4$ for overnight [31] in order to increase the surface roughness, leading to provide more available surface for the bacterial attachment [32].

2.7.2. MBBRs set-up

Three identical-sized glass MBBRs equipped with a feed container, an adjustable peristaltic pump (Minipuls 3, GILSON), a rotameter-based system, air distribution nozzles and other belongings were operated in parallel mode at ambient temperature. The effective volume of each reactor was 3.1 L.

During both batch and continuous running of the reactors, dissolved oxygen (DO) was maintained between 4 and 5 mg. L^{-1} (Honeywell DO probe). No mixing agitators were employed in the reactors. Coarse-bubble air provided from the bottom of each reactor was sufficient to provide a proper circulation of all carriers inside the reactors and also to maintain the required DO for the biomass growth.

2.7.3. Start-up & operation

Activated sludge (1.5 L, 4.74 g MLSS. L^{-1}), got from a municipal WWTP with a conventional CAS (Toulouse, France), was added into each MBBR already filled by pre-rinsed Z-400 carriers (filling ratio: 40%) and synthetic wastewater (COD: 500 mg. L^{-1}). After the process of acclimation for 24 h, continuous running of the reactors was started and continued as described in Fig. 1S & 2S in supplementary data. During Phase 1, organic loading rate (OLR) was kept constant at 1.9 g COD. d^{-1}, while HRT and influent COD were gradually reduced from 20 to 4 h, and 500 to 100 mg. L^{-1}, respectively. Operating the reactors at each applied HRT was continued until achieving the steady-state condition (i.e. COD removal > 80%). For the purpose of the biomass adaptation to MPs, MBBRs were also fed by MPs-bearing wastewater from the ninth week of operation. After reaching the steady-state condition at the last step (i.e. HRT: 4 h, influent COD: 100 mg. L^{-1}), the biofilm solids attached on the surface of each carrier was around 7.9 mg. From this time forward, in Phase 2, we started to inoculate two out of three MBBRs by pre-adapted microbial strains (i.e. bioaugmentation) by a procedure described in Section 2.9. The remained (control) MBBR (cMBBR) was continuously operated (at HRT: 4 h, and influent COD: 100 mg. L^{-1}) without any further inoculation to be compared with bioaugmented MBBRs (bMBBRs). All reactors were operated in non-sterile condition at ambient temperature.

2.7.4. Distributional removal of MPs

2.7.4.1. Overall removal of MPs

When the steady-state condition was seen, the samples from the inlet and outlet of the reactors were collected for MPs measurements. This gave us the “overall removal of MPs”, which is the sum of the abiotic and biotic removal of MPs. In the present study, biotic removal was obtained from the difference observed between the overall and abiotic removal.

2.7.4.2. Abiotic removal of MPs

Abiotic removal comprises all non-alive removal mechanisms including photodegradation, volatilization, and sorption onto the biosolids [33]. To calculate each parameter, batch experiments were performed in six pre-autoclaved and sealed 1-L erlenmeyer flasks as illustrated in Fig. 3S in supplementary data. Similar to the properties of the MBBRs’ influent, pre-autoclaved synthetic wastewater with COD: 100 mg. L^{-1} containing MPs with initial concentrations of 0.5, 2.5, and 7 µg. L^{-1} for Diclofenac, Naproxen, and 4n-Nonylphenol, respectively, was equally distributed into the all flasks (each flask: 500 mL). This wastewater also contained 500 mg.L^{-1} sodium azide (NaN_3) and 5 mg.L^{-1}

allylthiourea with aim at suppressing aerobic microbial activity and inhibiting nitrification, respectively [31,34]. No suspended biomass or biofilm-coated carriers were added to the 1st and 2nd flasks. By considering the filling ratio of 40%, 82 biofilm-coated (colonized) carriers were put in the 5th and 6th flasks that corresponds to the biofilm solids of 1300 mg. L^{-1} at each flask. The same concentration for the suspended biomass was also selected for the 3rd and 4th flasks. All flasks were incubated (TR-250 incubator shaker, Novotron HT) in batch mode at 120 rpm and 20°C. The experiment lasted for 2 h and homogenous samples were collected at regular intervals for MPs analysis, assuming that an equilibrium state was achieved [35,36].

2.7.4.3. Contribution of the biofilm and suspended biomass in overall removal of MPs

On the issue of MPs removal in MBBRs, to date, individual contribution of the biofilm and suspended biomass has been rarely studied. Colonized carriers were transferred into another clean MBBR until reaching the filling ratio of 40%. This reactor, pre-filled by a pre-autoclaved MPs-bearing secondary-treated wastewater, was then immediately operated in continuous mode at HRT: 4 h and ambient temperature. A pristine synthetic wastewater with the properties exactly like what was already used (COD: 100 mg. L^{-1}, Diclofenac: 0.5 µg. L^{-1}, Naproxen: 2.5 µg. L^{-1}, and 4n-Nonylphenol: 7 µg. L^{-1}) was utilized for feeding the reactor. The continuous operation lasted for two days at HRT: 4 h, and samples were collected for MPs analysis as soon as a stable COD removal was observed. “Overall removal of MPs by the suspended biomass” was calculated as the difference between the “overall removal (section 2.7.4.1)” and “overall removal by the biofilm” obtained here. The above procedure was separately carried out for the both cMBBR and bMBBRs.

2.7.4.4. Pseudo-first order degradation kinetics

By using the pseudo-first order kinetics as expressed as Eq. (2), the biotransformation of MPs was determined [33,37,38].

$$k_{biol} = \frac{F_{biod}}{X_S . S . V} \tag{2}$$

Where, k_{biol} is pseudo-first order degradation constant (L. g^{-1}. d^{-1}), S is soluble compound concentration (µg. L^{-1}), and V is the volume of the reactor (L). In the present work, in addition to the total k_{biol} (calculated for the both biofilm and suspended biomass), k_{biol} was separately calculated for the biofilm and suspended biomass. For the total k_{biol}, X_S is sum of the volatile suspended solids and the volatile biofilm solids (g. L^{-1}) (the ratio of volatile biofilm solids/biofilm solids was assumed: 0.7). Furthermore, X_S is the volatile biofilm solids for the biofilm’s k_{biol} (g VBS. L^{-1}), while is the volatile suspended solids for the k_{biol} related to the suspended biomass (g VSS. L^{-1}). Parameter of F_{biod}, mass flow of the biotransformed compound (µg. d^{-1}), was calculated by Eq. (3).

$$F_{biod} = F_{inf} - \left(F_{eff} + F_{stripped} + F_{sor}\right) \tag{3}$$

Where, F_{inf}, F_{eff}, $F_{stripped}$ and F_{sor} indicate the mass flows of MPs in the influent, effluent, air-stripped compound, and sorbed onto the suspended and/or attached biomass, respectively (µg. d^{-1}). As we calculated k_{biol} at steady-state condition, F_{sor} was not considered in Eq. (3) (constant F_{sor} with time). The item of $F_{stripped}$ was calculated according to the Eq. (4).

$$F_{stripped} = Q.H.q.S \tag{4}$$

Where, Q is the feed flow rate (L. d^{-1}), H is Henry's law constant (dimensionless), and q is the air supply per unit of wastewater (L_{air}. L^{-1} influent).

2.8. Pre-evaluation of candidate bacterial strain for bioaugmentation

Usual properties required for the candidate microbial strain/consortium are given in Section S1 in supplementary data. In this study, dormant-state pure culture of *P. fluorescens* (kept in physiological salt solution, and properties given in Table 1S in supplementary data), was provided form Biovitis Company (France). Pure cultures were kept in fridge (4°C) for further use.

Before starting the process of bioaugmentation, the effect of adding *P. fluorescens* on the pre-formed biofilm was evaluated in a series of batch experiments as illustrated in Fig.4S and Table 2S in supplementary data. The idea was adding *P. fluorescens* by 10% of the total biofilm solids present in each erlenmeyer flask, the same with our strategy for the process of bioaugmentation (i.e. inoculation rate: 10%). In brief, in order to reactive the cellular metabolism, a volume from the pure culture was firstly centrifuged at 5000 rpm and 4°C for 20 min, using a mini centrifuge machine (Fisher Scientific, the USA). Under sterile condition, the pellets were then re-suspended in pre-autoclaved synthetic wastewater, already prepared with COD of 1000 mg. L^{-1} [28]. The cultures were finally incubated for 18 h at 100 rpm and 20°C (TR-250 incubator shaker, Novotron HT) followed by adding the biofilm-coated carriers. Incubation of all flasks at 100 rpm and 20°C was continued for 48 h with monitoring of COD, MLSS, biofilm solids, pH and biofilm morphology. All parameters were measured in duplicate and the average of values with standard deviation are reported.

2.9. Bioaugmentation of the MBBRs

2.9.1. The protocol of bioaugmentation

Main steps of the bioaugmentation process, including i) reactivation of the dormant cells, ii) adaptation of the biomass to MPs, and iii) reactors inoculation, are described in Fig. 1 and Table 1. In short, dormant cells were initially centrifuged at 5000 rpm and 4°C for 20 min. Re-suspending the pellets in bare MPs-synthetic wastewater (COD: 1000 mg. L^{-1}) followed by incubating at 100 rpm and 20°C for 18 h were subsequently performed to wake the biomass' metabolism up.

For adapting the biomass to target MPs, a high concentration of MPs was selected (four-fold higher than the influent: 2, 10, and 28 µg.L^{-1} for Diclofenac, Naproxen, and 4n-Nonylphenol, respectively). Afterwards, still under sterile condition, further incubation was employed for 24 h at 100 rpm and 20°C for the purpose of acclimation of biomass to MPs. To avoid entry of concentrated MPs into the MBBRs on one hand, and in order to wash the biomass from the medium on the other hand, adapted strains were centrifuged at 5000 rpm and 4°C for 20 min. Resulted pellets were then re-suspended in a small volume of synthetic wastewater (50 mL, COD: 1000 mg. L^{-1}), and kept in fridge (6°C) for the maximum of three days.

As can be seen in Table 1, the process of bioaugmentation lasted for 14 weeks, including eight weeks of the batch- and six weeks of the continuous-mode of operation, under non-sterile condition and at ambient temperature. During inoculating the bMBBRs, the rate of inoculation was kept constant. it was hypothesized that adding the pre-adapted strains by the amount of 10% of the total biofilm solids present in each MBBR, for six weeks in a row, would be sufficient for implanting the strains into the biofilm's microbial community. Also, i) the reactors were operated in batch mode in order to avoid the washout of biomass, and ii) both feeding and inoculating the reactors were performed at the same time and twice per week. For the first four weeks, the reactors were fed by a synthetic wastewater with COD: 1000 mg. L^{-1}. This pattern was applied with the aim at providing enough food for the added strains in order to prevent potentially unwanted competitions with other indigenous microbes present in the reactors. From fifth week onwards, influent COD were gradually declined to get close to the COD of secondary-treated wastewater. "Stepwise reduction of the COD" and in general "low influent COD" was assumed to stimulate the immobilization of added strains instead of being in suspended phase. Continuous running of the reactors was started-off from the ninth week at HRT: 4 h, and lasted for six weeks. Steady-state condition (COD > 80%) was observed in twelfth week till the end of work, whereby samples were also collected for MPs analysis. In parallel, cMBBR was also operated at HRT: 4 h and influent COD: 100 mg. L^{-1}.

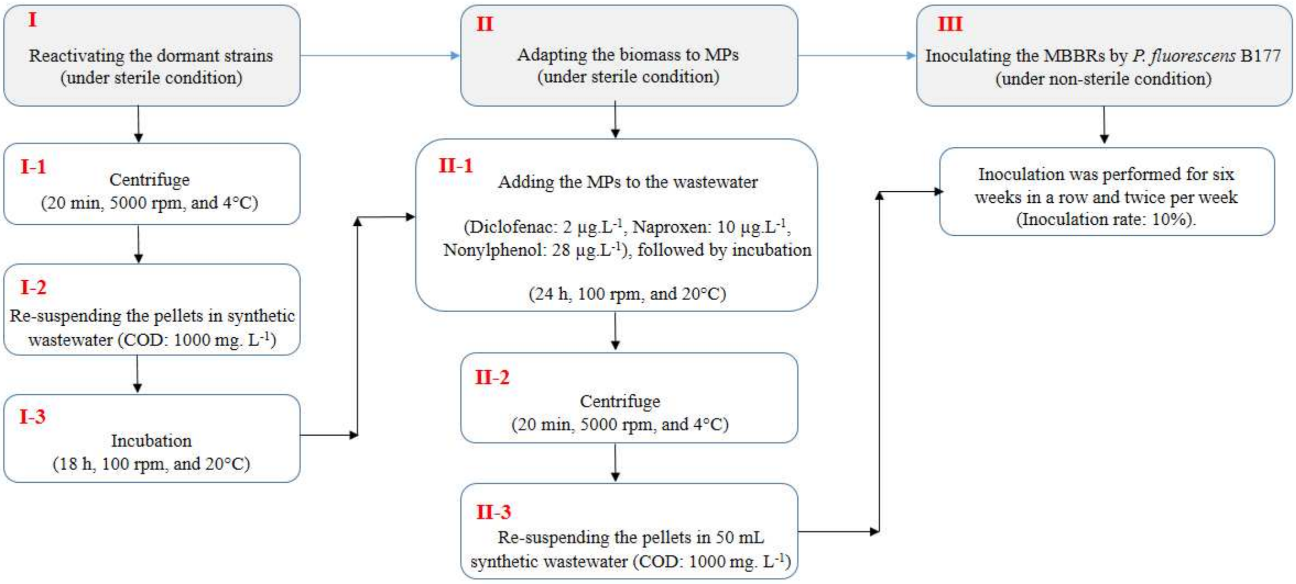

Fig. 1. Main steps of the bioaugmentation process, from the strains reactivation to the final inoculation.

Table 1. Strategy used for inoculating the pre-adapted strains into the bMBBRs

Running mode		Batch mode						Continuous mode	
Operating weeks		1 & 2	3 & 4	5	6	7	8	9 - 11	12-14
Influent COD (mg. L^{-1})		1000		750	500	250	100	100	
		(Feeding: twice per week)							
Inoculation rate*	percent of the biofilm solids (%)	10				No inoculation			
	concentration of added strain in each MBBR (mg. L^{-1})	127** (Inoculation: twice per week)							
***Volume taken form the dormant-state pure culture of *P. fluorescens* (mL)		113							
HRT (h)		-						4	
COD removal (%)		-						65-77%	79-82%**** (Steady-state condition)

Notes:

*This term is also named as "bioaugmentation dosage".

**This value is calculated based on the concentration of the biofilm solids in each MBBR (500 carriers × each had 7.9 mg of biofilm solids = 1274 mg.L^{-1}) as well as the rate of inoculation. The idea was adding the pre-adapted strain by the amount of 10% of the biofilm solids present in the reactor i.e. 10% × 1274 mg.L^{-1} ≈ 127 mg. L^{-1}.

***The value was calculated according to the biomass concentration of the dormant strains, stored in physiological salt solutions (Table 1S in supplementary data).

****Regarding the steady-state condition (COD removal > 80%), samples were collected from the inlet and outlet of the reactors for MPs analysis from the thirteenth week.

2.9.2. DNA extraction & quantitative polymerase chain reaction assay (qPCR)

To monitor the implantation of the pre-adapted strains into the biofilm, throughout the whole process of bioaugmentation described in Table 1, one carrier was grabbed from each MBBR twice per week, put into a plastic pocket (Fig. 5Sa in supplementary data) and stored in freezer (-18°C) to be analyzed later. In addition, 50 mL of the mixed liquid, weekly collected from each reactor, was centrifuged at 5000 rpm and 4°C for 20 min. The resulted pellets were then transferred into eppendorf tubes (Fig. 5Sb in supplementary data) and kept in the same freezer until the DNA extraction.

Before starting the analysis process, frozen samples were initially defrosted. The collected biomass was subject to DNA extraction using the PowerSoil® DNA isolation kit (MoBio Laboratories, Inc.) following manufacturer's instructions. The extracted DNA was then frozen for the further steps. After defrosting the extracted DNA, concentration and purity of the extracts were measured by Nanodrop spectrometry (Nanodrop2000, Thermo Fisher Scientific). Next, the abundance of total bacteria as well as *P. fluorescens* were estimated using a qPCR cycler (Bio-Rad® CFX96 Real-Time-System). For the detection of total bacteria, 16S rRNA bacterial gene primers bac1055YF (5'-ATGGYTGTCGTCAGCT-3') and bac1392R (5'- ACGGGCGGTGTGTAC-3') were used [39]. Primers targeting the DNA gyrase gen named P.fluo-255F (5'- TGTTACCGGTGATTTTACGCAG-3') and P.fluo-409R (5'- CATGCTGGTGCGCTCCA-3') were employed to detect *P. fluorescens* according to the modified protocol of Filteau et al. [40]. Both thermo-cycling protocols are reported in Fig. 6S & 7S in supplementary data.

3. Results and discussion

This section is divided into three main parts. In the first part, a brief description about the biofilm development is given. The second part deals with the effects of *P. fluorescens* on the biofilm (in batch experiments), to avoid any subsequent adverse effects on the biofilm in bMBBRs. Properties and performance of the cMBBR and bMBBRs are then studied in the third part in terms of microbial implantation into the reactor as well as MPs removal.

3.1. Biofilm development (Phase 1)

Operating parameters and performance of the MBBRs during the start-up, biofilm formation and adaptation (phase 1) are summarized in Table 3S in supplementary data. Gradual development of the biofilm is also shown in Fig. 2. At the end of Phase 1, average concentration of the biofilm solids reached to around 1274 mg. L^{-1}, calculated from multiplying the average mass of the attached biomass on each carrier (7.9 mg) by the number of carriers present in each MBBR (500 carriers) over the reactor volume (3.1 L). A kind of an invariable biofilm growth was observed throughout the last five weeks, indicating the occurrence of a balance between the attachment and detachment of the biomass from the colonized carriers [30,41]. Next to this, high COD removal efficiencies (> 80%) were observed for more than one month of continuous operation at HRT: 4 h. Despite the fact that the definition of steady-state condition is still controversial for biofilm reactors [42], stable behavior of the reactors in terms of the biofilm growth and COD removal convinced us to terminate the first phase followed by starting the Phase 2.

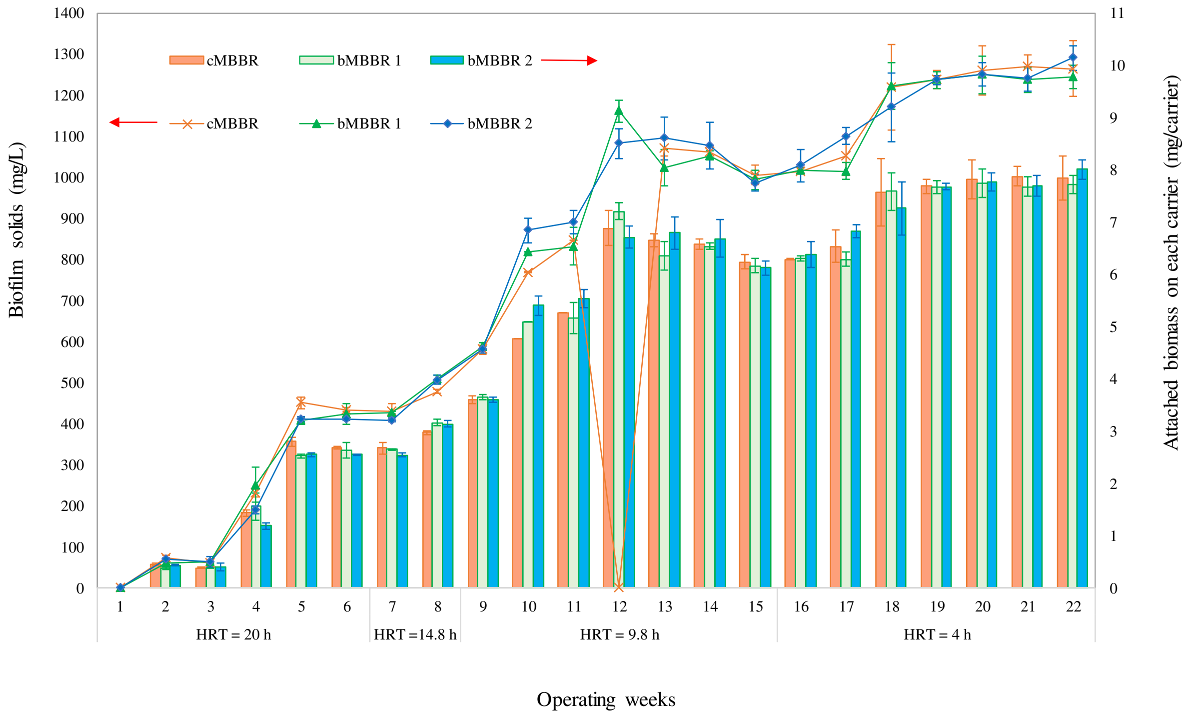

Fig. 2. Gradual development of the biofilm throughout Phase 1 (all MBBRs were operated at the same condition described in Section 2.7.3 and Table 3S in supplementary data)

3.2. Pre-evaluation of candidate bacterial strain

For the purpose of assessing the suitability of *P. fluorescens* for bioaugmentation, a series of batch experiments explained in Section 2.8 and Fig. 4S in supplementary data was performed in order to avoid any adverse effect on the pre-formed biofilm in bMBBRs. The results are plotted in Fig. 3 with respect to the changes in the biofilm solids, MLSS, and COD during the incubation period. The effect of adding *P. fluorescens* on the pre-formed biofilm is also visualized in Fig. 8S in supplementary data.

In non-inoculated flasks, a gentle reduction followed by a return to its initial value is seen for the biofilm solids (Fig. 3a). Temporary reduction of the biofilm solids is probably attributed to the exposure of the attached biomass to a high COD value, leading to increasing the biofilm detachment rate. A rapid increase in MLSS concentration can be a good proof for this assumption (Fig. 3b). Afterwards, the biofilm solids increased a little and then remained nearly constant, indicating an achievement to a balance between the attachment and detachment rate of the biofilm. This, beside the fast growth in MLSS concentration, were observed along with a sharp depletion in COD (Fig. 3c). A similar trend was also occurred in the flasks inoculated by *P. fluorescens*, with this difference that the biofilm detachment rate was a little bit higher than the non-inoculated flasks (Fig. 3a). In addition to the above assumption, sudden exposure of the biofilm to the the newly-introduced microbial strains probably leads to an increase in the biofilm detachment rate. Meanwhile, a steeper slope in MLSS and COD concentrations was seen compared to the non-inoculated flasks. This piece of evidence likely reveals that the competition between an exogenous microbial strain of *P. fluorescens* and autochthonous microbial community did not cause detrimental impacts on the growth of *P. fluorescens*. On the other hand, introducing the *P. fluorescens* to the system had not a devastating impact on the pre-formed biofilm.

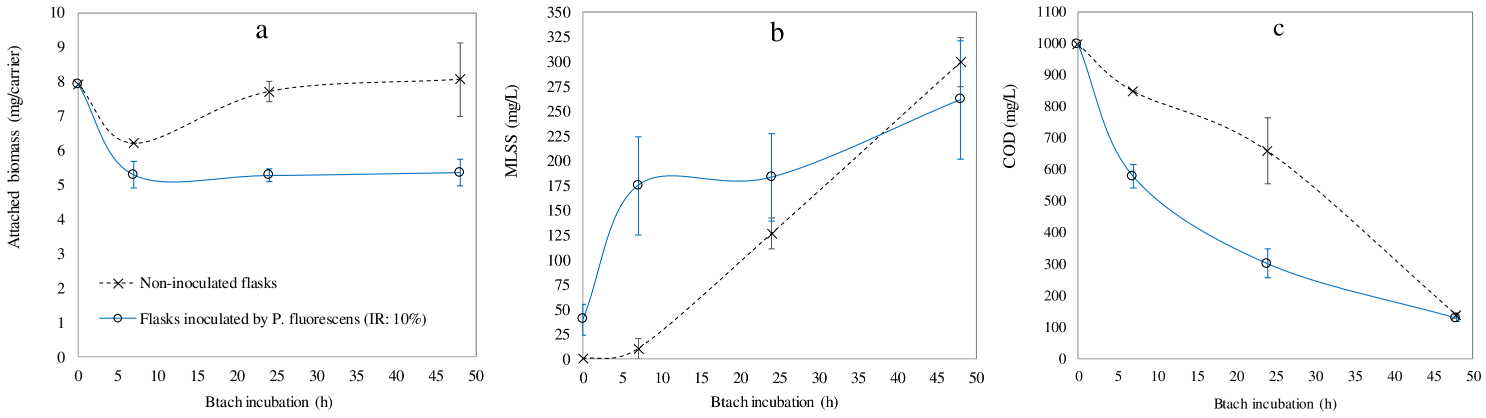

Fig. 3. Pre-evaluating the effects of adding *P. fluorescens* on the pre-formed biofilm (IR: inoculation rate)

3.3. MBBRs operation & performance (Phase 2)

The second phase of the MBBRs operation was immediately started with the circumstances already described in Fig. 1S and 2S in supplementary data, Fig. 1 and Table 1. At this phase, parameters of the biofilm solids, MLSS, COD removal, and the population of total bacteria and *P. fluorescens* were weekly monitored. Regarding MPs, parameters of overall removal, overall removal by the biofilm, and overall removal by the suspended biomass were measured when steady-state condition happened in MBBRs running in continuous mode (Section 2.7.4.3). Abiotic removal of MPs by the biofilm and suspended biomass was then obtained from the batch experiments already explained in Section 2.7.4.2 and Fig. 3S in supplementary data. Subtracting the overall removal from the abiotic removal resulted in achieving the biotic removal of MPs for the biofilm and suspended biomass.

3.3.1. Detailed monitoring of Phase 2

An overview on the variations of the biofilm solids, MLSS and COD removal, seen in Phase 2, is given in Fig. 4. Inoculating the bMBBRs with pre-adapted *P. fluorescens* at influent COD: 1000 mg. L^{-1} was accompanied with a sharp increase in the concentration of MLSS up to 5616 mg. L^{-1}. At this step, an initial growth of the biofilm (by 3610 mg. L^{-1}) followed by a zig-zag trend was seen (Fig. 4b). Since the influent COD was reduced to 500 mg. L^{-1}, a gentle drop in MLSS as well as a rapid slump in biofilm solids were observed up to 5354 and 2213 mg. L^{-1}, respectively (Fig. 4c). Stopping the inoculation along with reducing the COD to 100 mg. L^{-1} did not remarkably affect the MLSS, while an unstable amount of the biofilm solids was again observed (Fig. 4d).

By now (Fig. 4b-d), in general, although suspended biomass was in a nearly constant concentration, no balance was occurred between the biofilm attachment and detachment, probably due to the shift in the equilibrium between the microbes in the liquid and solid phase. Learnt from the zig-zag trend of the biofilm solids, even though an initial attachment of the suspended biomass strikingly happened on the pre-formed biofilm, but the durability of this attachment was short. What might be the reason for the rapid biomass detachment is ongoing entrance of the exogenous strains into the reactor, persuading the attached biomass to be detached in order to compete with those strains on the available substrate.

Immediately after starting the continuous operation at HRT: 4 h and influent COD: 100 mg. L^{-1}, a sudden drop in the MLSS (to 1860 mg. L^{-1}) as well as a negligible change in the biofilm solids (~1870 mg. L^{-1}) were certainly drawn our attention (Fig. 4e). Loss of the MLSS indicates that our manual sludge recycling process has been unable to avoid the phenomenon of biomass washout. Unchanged amount of the biofilm solids is probably a sign for the capability of the biofilm for shielding the attached microorganisms against the sudden changes happen in the reactor [21].

In view of the biosolids concentration, we see a slight downward trend until reaching to a roughly stable amounts of the biosolids in the reactors for three weeks in a row (Fig. 4f) i.e. 1510 mg. L^{-1} for the MLSS and 1083 mg. L^{-1} for the biofilm solids. Besides, a noticeable removal for COD (> 80%) was observed throughout the last operating weeks. At this so-called steady-state situation, samples were collected for MPs measurements.

From the time we stopped the inoculation i.e. from the 7th week onwards, no zig-zag trend was noticed in the biofilm solids. At this period, regardless of a small drop in the biofilm solids, our above hypothesis about the impact of newly-introduced strains on the fast detachment of newly-attached biomass seems logical. Further studies, however, which also incorporate operating parameters, are required to substantiate this hypothesis.

Interestingly, the last step of Phase 2 (Fig. 4f) was almost similar to what was observed in the cMBBR, operated under the same condition with bMBBRs. This makes our comparison feasible as the both types of MBBRs approximately contained equal amounts of the suspended and attached biomass at steady-state condition.

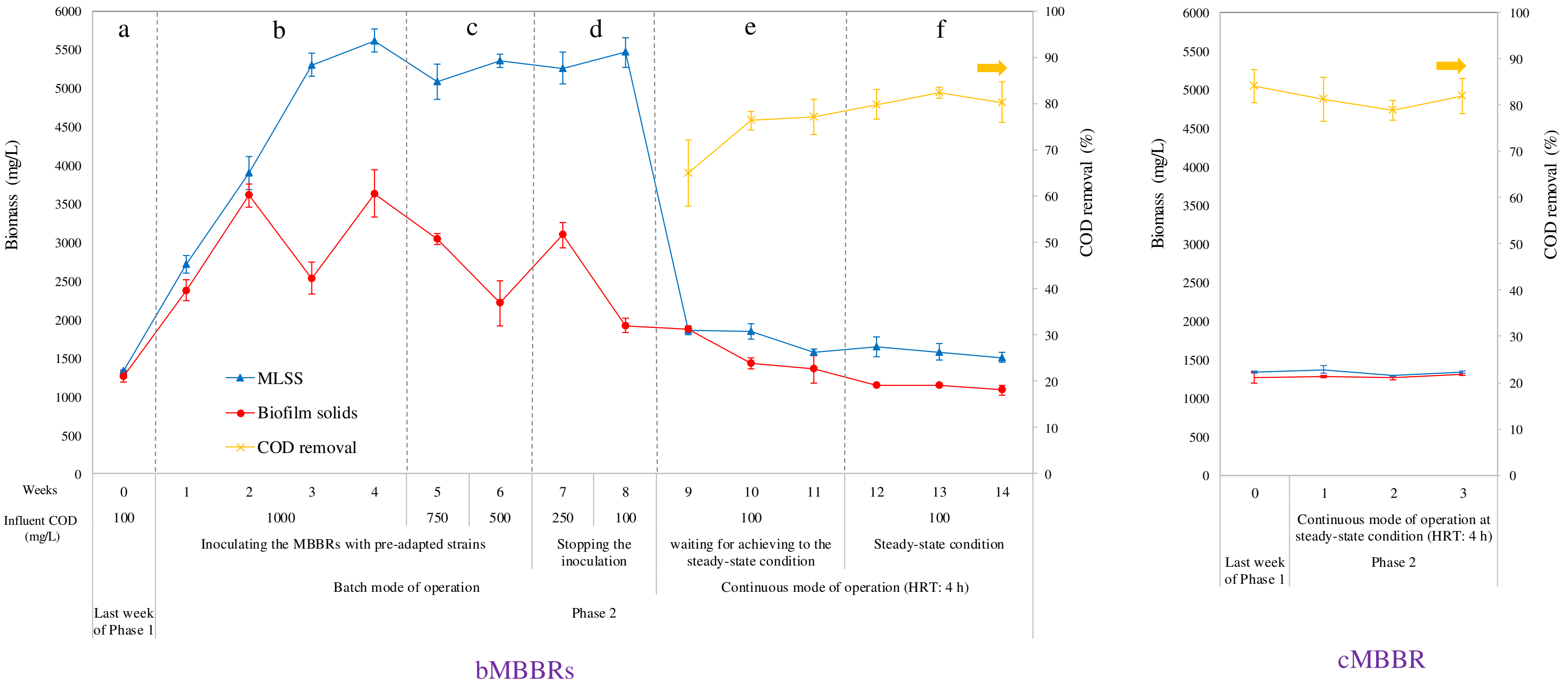

Fig. 4. Detailed monitoring of Phase 2, considering the biofilm solids, MLSS and COD removal

To investigate the implantation and maintenance of *P. fluorescens* into the biofilm and liquid phase, we quantified the relative abundance of *P. fluorescens* and also total bacteria throughout the whole process of bioaugmentation using DNA extraction and qPCR already explained in Section 2.9.2. The results are brought in Fig. 5. Once the reactors were faced with the inoculation (i.e. the 1st week), the abundance of *P. fluorescens* in the biofilm was observed by around $10^{8.35}$ cells/reactor. This evidence demonstrates great potential of *P. fluorescens* for penetrating into the microbial community of the biofilm interestingly after its immediate entrance into the reactor. Its population did not significantly change after the resumption of inoculation (Fig. 5b), and remained nearly constant even after reducing the COD to 250 mg. L^{-1} (Fig. 5c,d). By contrast, the abundance of *P. fluorescens* in the liquid phase declined to a high extent by reducing the COD (Fig. 5c). Therefore, by preventing wash-out, the strategy of immobilization can lead to a better preservation of the degraders under stressed environmental conditions (here is the shortage of carbon and nutrients) as compared to the suspended biomass [43].

By looking at the population variation of *P. fluorescens* in Fig. 5e, we realise that primary steps of the continuous operation encountered with a moderate decrement in the biofilm's population simultaneously with a light increase in the liquid phase. Taking this into account that reactors' inoculation had been already stopped at the end of the 6th week, it seems that detachment of pre-attached *P. fluorescens* has increased its population in the liquid phase. This observation presumably denies our previous assumption about preference of bacteria to be in attached form instead of being in liquid phase in low amounts of COD (section 2.9.1). Although "low influent COD" (here is 100 mg. L^{-1}) could not stimulate the immobilization of inoculated strains into the biofilm, we do believe that further studies with more operating considerations should be done to establish a precise conclusion.

Outstanding drops were found for the abundance of *P. fluorescens* and total bacteria in the last part of Phase 2 (Fig. 5f), stating that neither the biofilm nor the liquid phase could retain the majority of *P. fluorescens* cells inside. Taking this into account, invariable amount of the biofilm solids and MLSS (Fig. 4f) shows a kind of reduction in the survival rate of the *P. fluorescens* and the indigenous bacteria.

The present research aimed at answering whether bioaugmentation of tertiary MBBRs receiving a nutrient-poor feed can be considered as a long-lasting process. Results obtained herein indicate that the chosen operating conditions are not convenient for surviving the inoculated strains, probably due to the insufficient nutrients. Therefore, enhancing the survival and maintenance of the implanted strain can be an impressive area of research for future studies on bMBBRs. As a hypothetical outlook, periodical addition of the carbon and nutrients (i.e. biostimulation) [21,44] or intermittent feeding of the reactors by raw wastewater [10] can be probably defined as strategies for enhancing the survival rate of inoculated microbes. In this regard, the competition between the autochthonous and inoculated microbes must be heeded, since autochthonous microbes probably surpass the inoculated ones for the consumption of easily-biodegradable substrate, whereby inoculated strains won't be able to establish themselves in the system again. Nevertheless, the above strategies are only assumptions that should be clarified in future studies. In addition, choosing the right inoculation rate (bioaugmentation dosage) that definitely needs further studies would be another remedial approach [45]. Also, we recommend to use membranes inside or at the effluent side of tertiary bMBBRs in order to prevent the biomass wash-out [46].

Regarding the occurrence of a high COD removal (Fig. 4f) and still the existence of *P. fluorescens* in the system, we will compare bMBBRs and cMBBR in terms of the MPs removal in the following sections. Here, the abiotic aspect of MPs removal is firstly discussed. We report on the rest of aspects later on.

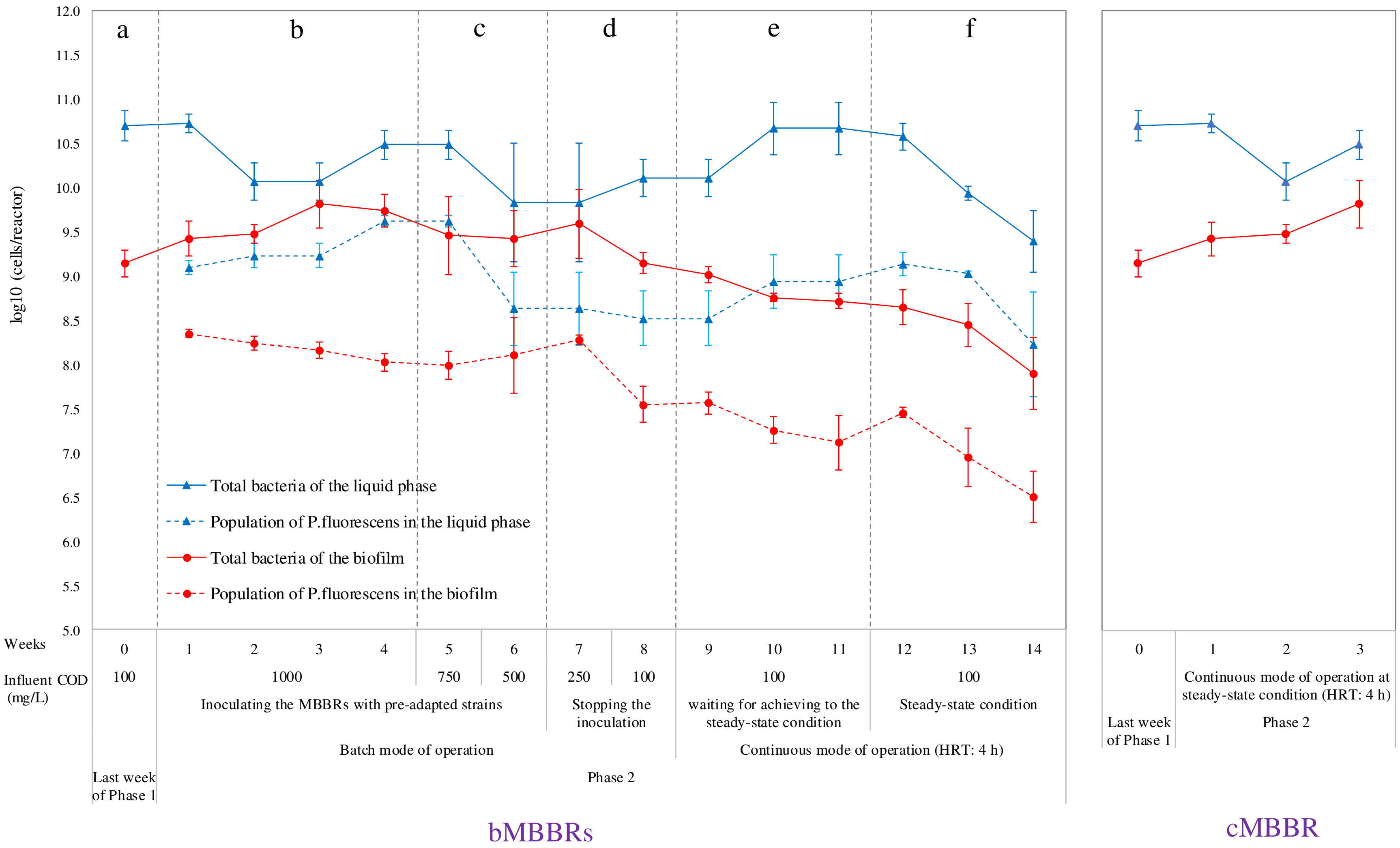

Fig. 5. Detailed monitoring of Phase 2, considering the abundance of *P. fluorescens* and total bacteria
(Solid lines show the abundance of total bacteria, while dashed lines indicate the abundance of *P. fluorescens*)

3.3.2. Abiotic removal of MPs

MPs may be biotically and abiotically transformed in the WWTPs with various degrees. The importance of abiotic mechanisms is not lower than the role of biotic ones for MPs removal [47]. Knowing well enough about the abiotic reactions prevents researchers to under or overestimate the role of biotic factors. Regarding the significance of abiotic factors, reported observations of "negative removal" can be sometimes ascribed to the desorption of MPs from the biosolids and suspended particulate matters [48].

In the 1st and 2nd flasks (Fig. 3S in supplementary data), no significant change was observed in the concentration of all MPs after 2 hours of incubation. This shows that, under our experimental conditions, either the photodegradation or the volatilization and also the sorption onto the non-colonized carriers do not play a key role in MPs removal. Recent studies suggest that direct and indirect natural photolysis may act as a driver for the removal of some photo-sensitive MPs, where a high surface-to-volume ratio is available for sunlight irradiation (like wetlands and polishing lagoons) [49,50]. In the current study, limiting factors for the MPs photodegradation are i) the low surface-to-volume ratio, and ii) the high turbidity of the wastewater [51]. Also, it has been shown that wavelengths for light absorption of many MPs are usually below the 280 nm which is far from the sunlight's wavelength (290-800 nm) [52,53]. The transfer of a MP from the dissolved to the gaseous phase by volatilization depends essentially on the Henry's law constant of the MP and on the operating conditions of the process (i.e. aeration, agitation, temperature and atmospheric pressure) [54]. Byrns [55] concluded that if Henry's law constants was lower than the threshold value of 99E-05^5 atm.m^3.mol^{-1}, volatilization was not significant. Therefore, with respect to the low Henry constants (Diclofenac: 4.73E-012 [56], Naproxen: 3.39E-010 [56], and 4n-Nonylphenol: 3.64E-011 atm.m^3.mol^{-1} [57]), it can be concluded that volatilization is in general not relevant for the removal of our target MPs [56]. Meanwhile, Suarez et al. [58] who reviewed the fate of MPs in WWTPs reported that losses due to the volatilization are completely negligible for pharmaceuticals and estrogens.

By abandoning the above parameters from the list involved in abiotic removal, the breakdown of the MPs sorption onto the both types of biosolids is shown in Fig. 6, according to the results observed in the rest of flasks. MPs sorption onto the biosolids influences the MPs bioavailability [59], and corresponds to the occasional negative mass balance of MPs, where MPs desorption from the suspended or attached biomass occurs during the treatment process [60]. In the MBBRs, continuous circulation of the carriers leads to the breakage of the suspended biomass to the smaller size solids than the CAS [61–63]. Smaller particles provide higher available sorption sites for the uptake of MPs. On the other hand, growing the biofilm is accompanied with reducing the biofilm's sorption sites [64]. As a result, here, higher sorption of MPs onto the suspended biomass is probably referred to the higher available sorption sites, as compared to the biofilm.

The affinity between the biosolids and the MPs is remarkably under the control of electrostatic interactions (i.e. adsorption) and hydrophobic interactions (i.e. absorption) [48,65,66]. By the probable reason of the repulsive forces between the MPs and the biosolids (considering the negative charge of Diclofenac and Naproxen at neutral pH [67] and the negative charge of the biosolids [68]), hydrophobic interactions seem to be more influential than the electrostatic interactions for MPs sorption onto the biosolids. For the sorption of uncharged 4n-Nonylphenol [67], the role of electrostatic interactions appears to be more paler than the rest of charged MPs. The relationship between the MPs sorption, nearly constituting the abiotic removal of MPs, and their relevant hydrophobicity is also plotted in Fig. 6. In the current study, the parameter of log$_D$ was used to predict the MPs hydrophobicity. Log$_D$ is defined as the ratio between the ionized and unionized form of the solute at a specific pH value (here, pH was adjusted at 7) [69]. Compounds with $\log_D > 2.6$ are referred as hydrophobic, and hydrophilic when $\log_D \leq 2.6$ [70]. Hence, Diclofenac and Naproxen are recognized as hydrophilic compounds (log$_D$: 1.77 and 0.34, respectively [71]), while 4n-Nonylphenol (log$_D$: 6.14 [69]) is considered as a hydrophobic molecule. In this matter, a linear increase (R^2: 0.82) between the MPs sorption and their relevant log$_D$ was observed for the cMBBR. Meanwhile, no strong relation (R^2: 0.57) was found for the bMBBRs. It might be careless to draw a conclusion at this point that the process of bioaugmentation reduces the biosolids hydrophobicity. Paying attention to the abiotic removal of the hydrophobic 4n-Nonylphenol makes this assumption even stronger, as this compound was abiotically removed by 15% and 3.9% by the cMBBR and bMBBRs, respectively. From our bibliographic review, no conclusive explanation could be found to explain the observed behavior.

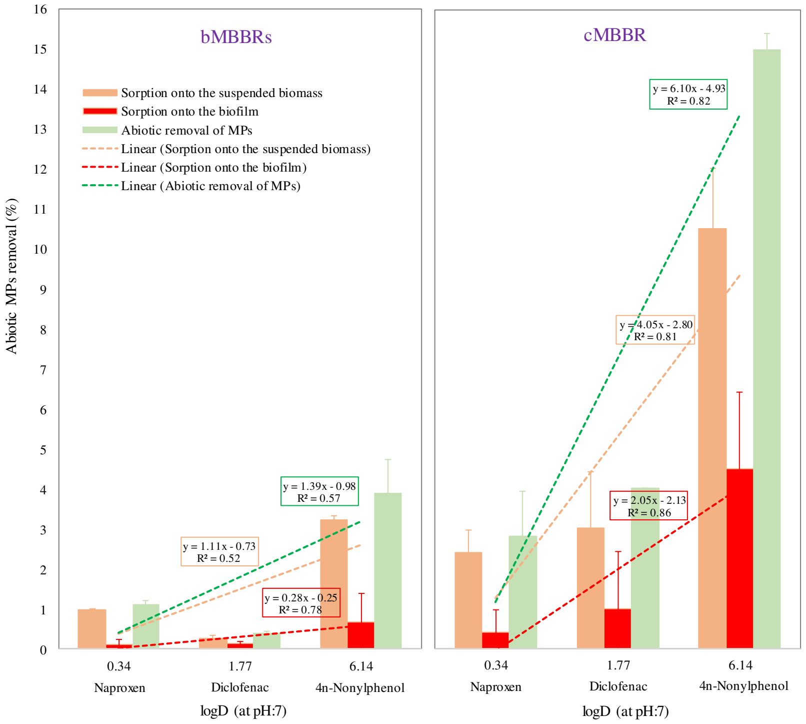

Fig. 6. The correlation between the abiotic removal of MPs and their relevant hydrophobicity

3.3.3. *Biotic removal of MPs*

In steady-state condition detected in the last three weeks of Phase 2 (Fig. 4f), samples were collected for overall removal of MPs. As shown in Fig. 7, overall removal of bMBBRs was observed up to 84.9%, 91.5%, and 99.4% (below the LQ) for Diclofenac, Naproxen and 4n-Nonylphenol, respectively. Compared to the bMBBRs, the control reactor showed a similar behavior for 4n-Nonylphenol (99.4%), and a little lower removal efficiency for the rest of MPs i.e. 76.8% for Diclofenac and 83.4% for Naproxen.

To go through the details, individual role of the biofilm and suspended biomass was determined by the procedure given in Section 2.7.4.3. For each parts of the biofilm and suspended biomass, biotic removal of MPs was then calculated by subtracting the overall removal from the abiotic removal. Considering this point that 4n-Nonylphenol was abated until below the LQ by the biofilm, we could not obtain individual role of the suspended biomass in its removal by the above procedure. Fig. 7 indicates that the biofilm was more effective than the suspended biomass for the biotic removal of all MPs, interestingly for the recalcitrant Diclofenac i.e. 59.6% versus 24.9% for the bMBBRs, and 53.9% against 19% for the cMBBR. The results are supported by the k_{biol} values, shown in Fig. 8. As stated, the biofilm's k_{biol} values are higher than the relevant values for the suspended biomass. For instance, in the case of bMBBRs, k_{biol} values of Diclofenac are seen by 12.02 L. g VBS^{-1}. d^{-1} and 1.90 L. gVSS^{-1}. d^{-1} for the biofilm and suspended biomass, respectively. Higher MPs removal by the biofilm was also reported by Falås et al. [72] who studied the performance of a hybrid biofilm-activated sludge process treating municipal wastewater. In their study, Diclofenac was removed by the biofilm with k_{biol} of 1.3-1.7 L. gVSS^{-1}. d^{-1}, while its elimination by the suspended biomass was insignificant, and with k_{biol} of < 0.1 L. gVSS^{-1}. d^{-1}. Thus, the biofilm appears to possess a better biodegradation capacity, possibly due to more diverse microbial community of the biofilm compared to the suspended biomass [64].

In Fig. 8, total k_{biol} values are also given. According to a simple classification scheme suggested by Joss et al. [33] who characterized the biological degradation of 35 MPs using k_{biol} values in nutrient-removing CAS systems (Fig. 9S in supplementary data), MPs with k_{biol} < 0.1 L. gVSS^{-1}. d^{-1} are not removed to a significant extent (<20%), while compounds with k_{biol} >10 L. g VSS^{-1}. d^{-1} are transformed by > 90%, and in-between a moderate removal is expected [33]. This classification is compatible with total k_{biol} values of Diclofenac and Naproxen in the present work (>10 L. g VSS^{-1}. d^{-1}), where quite high overall removals were observed for the both cMBBR and bMBBRs. Observed k_{biol} values for Diclofenac are higher than other values reported for tertiary treatment of municipal wastewater. For instance, it was reported by around 0.1 L. gVSS^{-1}. d^{-1} in an one-stage MBBR which was a polishing process after treatment with CAS combined with Hybas^{TM} MBBR [12]. By the way, k_{biol} values of Diclofenac was reported between 1.5 and 5.8 L. gVSS^{-1}. d^{-1} by Torresi et al. [31] who operated a nitrifying MBBR treating an ammonium-rich secondary-treated wastewater [31]. So far, no work has been carried out to obtain k_{biol} values of Naproxen and 4n-Nonylphenol in tertiary treatment systems and MBBR reactors.

Although both biodegradation and sorption are recognized as two dominant mechanisms for MPs removal in WWTPs (Fig. 10S in supplementary data) [73], MPs removal efficiencies vary depending on the operating conditions, such as HRT, SRT, F/M and temperature; even though the influence of these parameters is not always clearly understood [54]. When comparing two major pathways of biodegradation and sorption in Fig. 7, we find that the biodegradation strongly outperformed its counterpart for the removal of all MPs, in particular for bMBBRs where abiotic MPs removal was nearly negligible (0.4-3.9%) against a wonderful biotic removal (i.e. 84.5, 90.4 and 95.5% for Diclofenac, Naproxen and 4n-Nonylphenol, respectively). In line with the review paper of Verlicchi et al. [74], sorption onto the secondary activated sludge was reported up to maximum 5% for most of the analgesic and anti-inflammatory pharmaceuticals, beta-blockers, and steroid hormones which was too much lower than the role of biodegradation in MPs removal (even up to 100%). Here, compared to the bMBBRs, a relatively lower biotic removal (around 10%) was seen in the cMBBR (i.e. 72.8, 80.6 and 84.4% arranged in the above order) that still appears very high. Correspondingly, k_{biol} values of MPs resulted in the bMBBRs overcame the relevant values seen in the cMBBR. This, regarding Fig. 8, shows the positive impact of the bioaugmentation on the biodegradation potential of the biofilm followed by the suspended biomass.

Astonishing performance of the cMBBR is probably attributed to the profitable adaptation process, performed during the Phase 1 for all MBBRs. Without such an adaptation process, the gap between the efficiency of bMBBRs and cMBBR will be likely higher than what was observed. This might be an indication that the autochthonous microbial community is able to well adapt itself with recalcitrant compounds when the feed is suffering from enough growth substrates (i.e. secondary-treated wastewater).

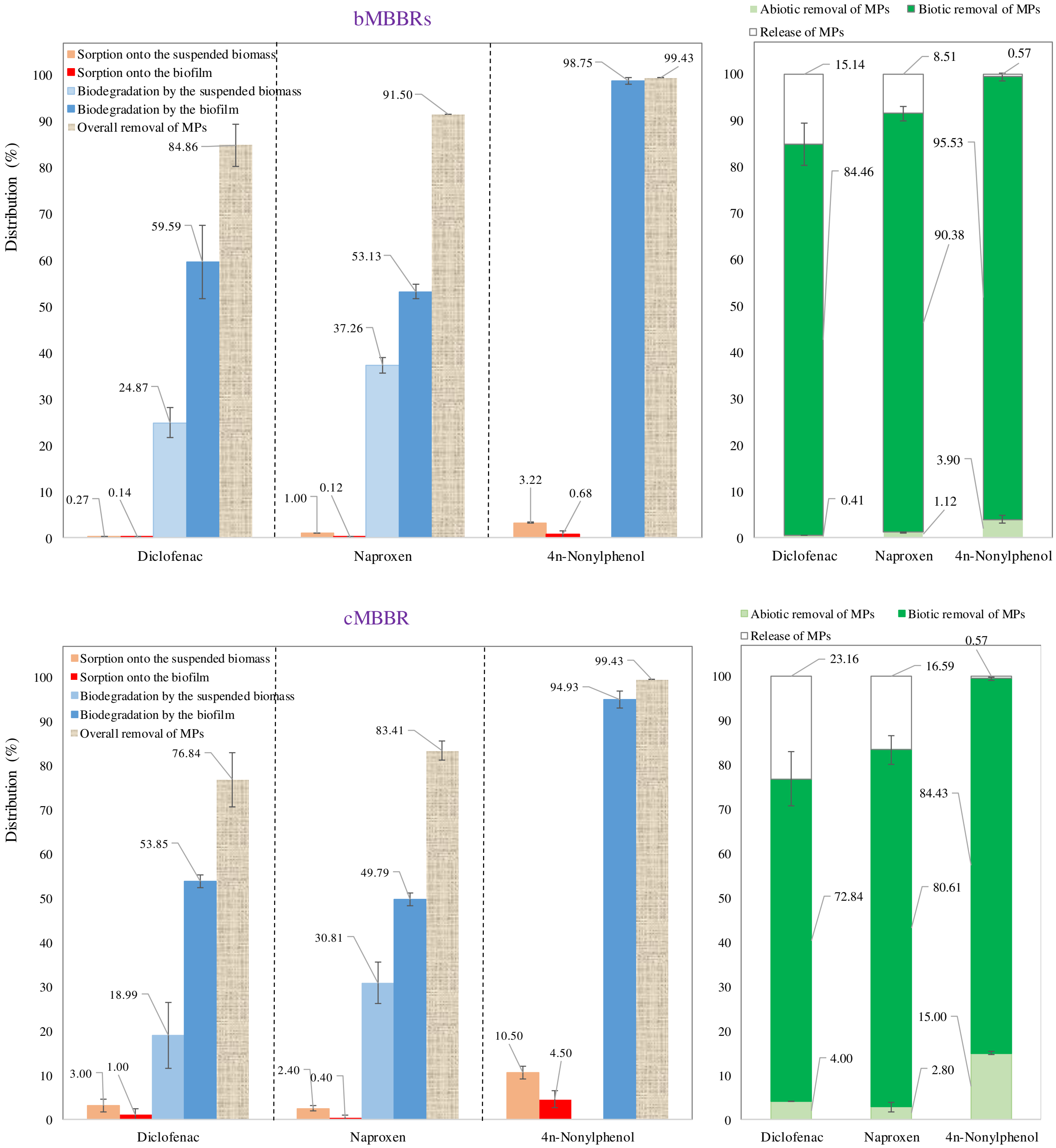

Fig. 7. Individual contribution of the biofilm and suspended biomass in abiotic and biotic removal of MPs (the above graph deals with the bMBBRs, while the below graph corresponds to the cMBBR)

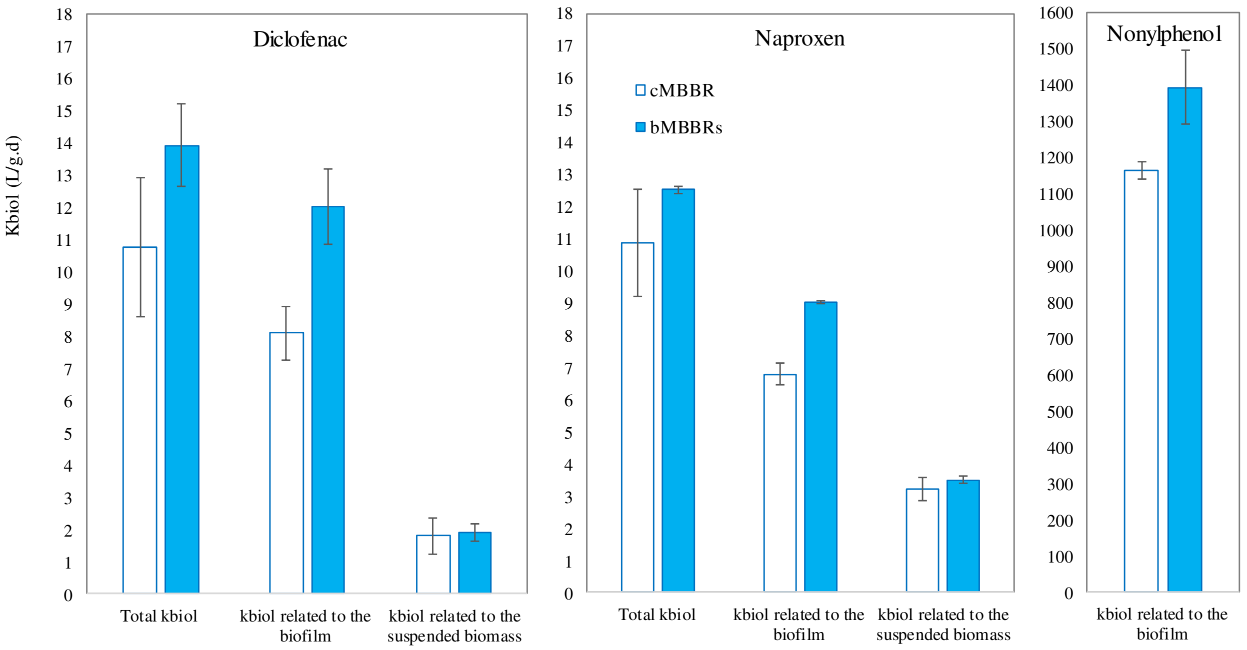

Fig. 8. k_{biol} values of MPs in bMBBRs and cMBBR[1,2,3]

[1] As discussed in Section 3.3.2, no MPs removal was seen by the volatilization. The mass flow of the air-stripped compound ($F_{stripped}$) was not therefore considered in Eq. (3).

[2] As 4n-Nonylphenol has declined up to LQ by the biofilm, no k_{biol} values have been reported here for the suspended biomass.

3.3.4. Challenges ahead of the bMBBRs

Bioaugmentation of tertiary MBBRs, like any bio-technological technique, needs some feats of engineering and expertise. Full-scale application of this process initially depends on the commercialization of the inocula that might be otherwise costly and time-consuming to be produced [75]. Another challenge is the survival of inocula during the wastewater treatment. Augmented microorganisms are added to cooperate with autochthones or to replace them, so survival of the cells is the bottleneck to success [76]. In this study, a nutrient-poor feed (secondary-treated wastewater) could not sufficiently sustain the survival of *P. fluorescens* in the biofilm. Even though a very promising removal was seen for all MPs in bMMBRs (Fig. 7), ongoing downward trend of the abundance of *P. fluorescens* (Fig. 5) will be eventually led to the bioaugmentation failure. To avoid it, further in-depth research is still required to achieve an efficient and long-lasting process. For instance, studying the impact of complementary processes (e.g. biostimulation [21,44] or using encapsulating agents that would protect and nourish the inoculum [75]) on the performance of tertiary bMBBRs would be proposed. Regardless of the fact that such processes definitely add the intricacy and cost to the process, they potentially appear an impressive strategy to establish a durable bMBBR. The right selection of microbial strain along with applying a proper inoculation rate should be also taken into account in future studies, a subject that has been rarely studied in the literature.

4. Conclusion

On the issue of MPs removal from conventionally-treated wastewater, achievement to better-performing tertiary MBBRs by the approach of bioaugmentation was the main goal of the present study. While bMBBRs showed high k_{biol} values accompanied with very promising removals for all MPs, implanted strain (*P. fluorescens*) into the biofilm and suspended biomass faded off with time. Hence, future studies must be focused on enhancing the survival and maintenance of the implanted strain. Under identical operating conditions, high level of removals was also seen in the cMBBR, with only a little discrepancy from the bMBBRs. This finding in the cMBBR might be ascribed to the well-performed adaptation process, something that was done for all MBBRs before starting the process of bioaugmentation. Otherwise, if no adaptation process is done, the gap between the efficiency of cMBBR and bMBBRs is likely expected to be higher than what we observed. With a more emphasis on the importance of biomass adaptation, the bleeding-edge technology of bMBBR still needs much more detailed studies for a wide implementation at full-scale applications.

Acknowledgments

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Supplementary data of Chapter (III)

Evaluating the influence of bioaugmentation on the performance of tertiary moving bed biofilm reactors (MBBRs) for micropollutants removal

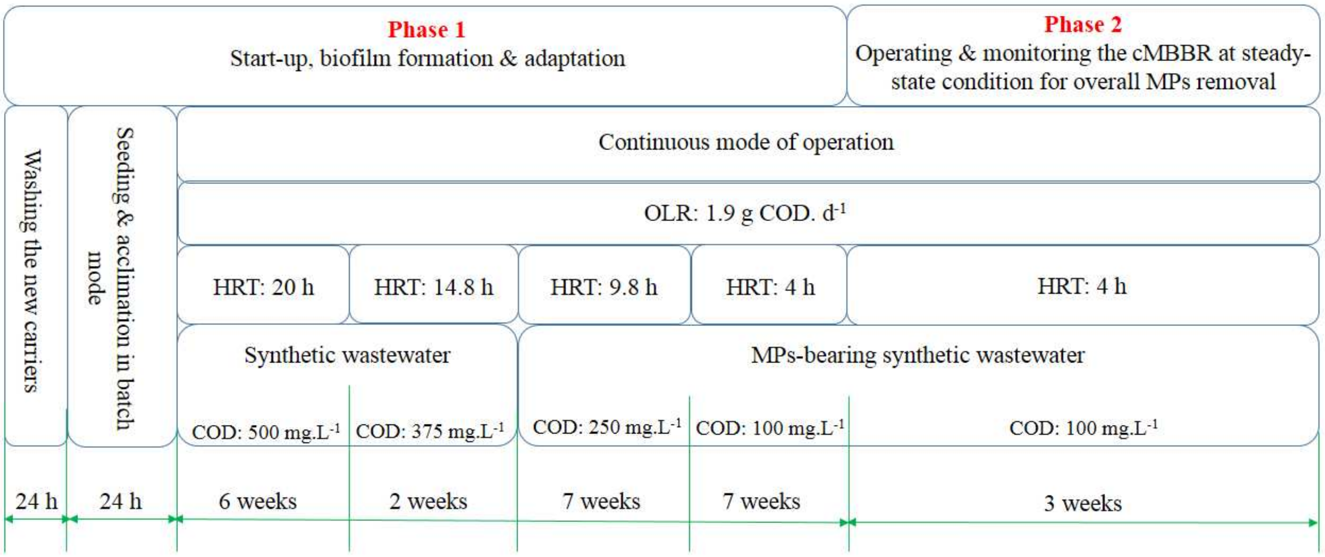


Fig. 1S. Start-up and operation of the control MBBR (cMBBR)

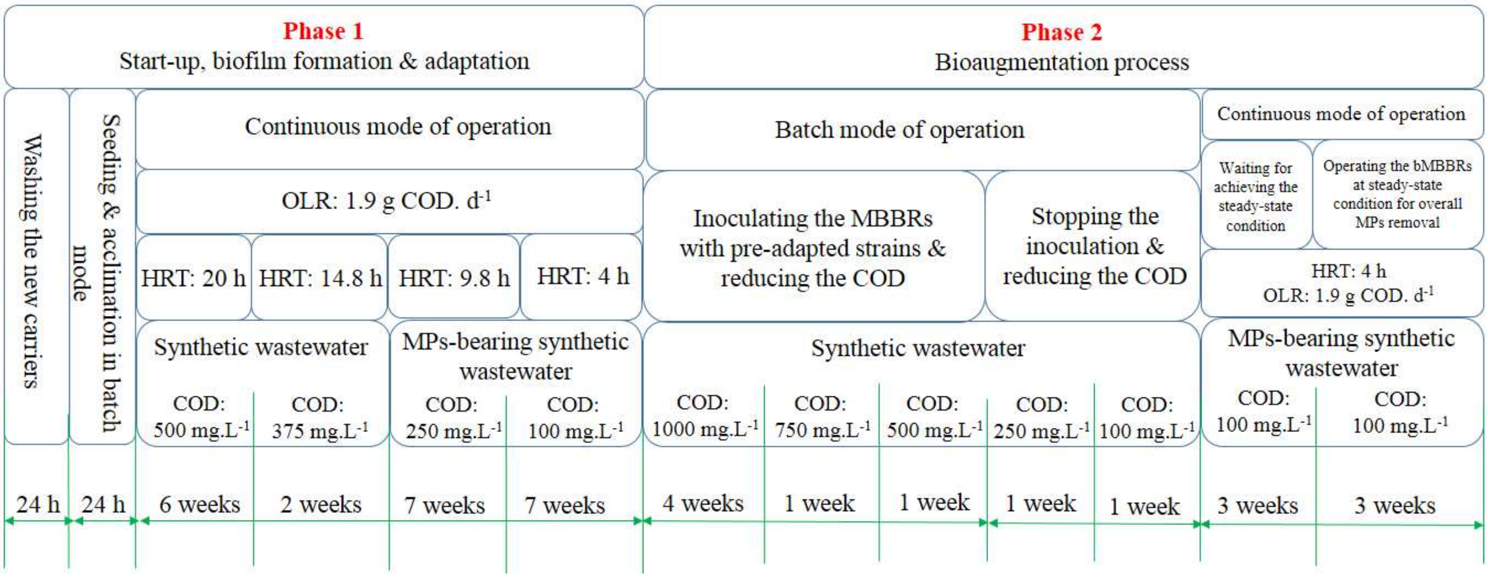


Fig. 2S. Start-up and operation of the bioaugmented MBBRs (bMBBRs)

(more details about the bioaugmentation are given in Section 2.9, Fig. 1 and Table 1.

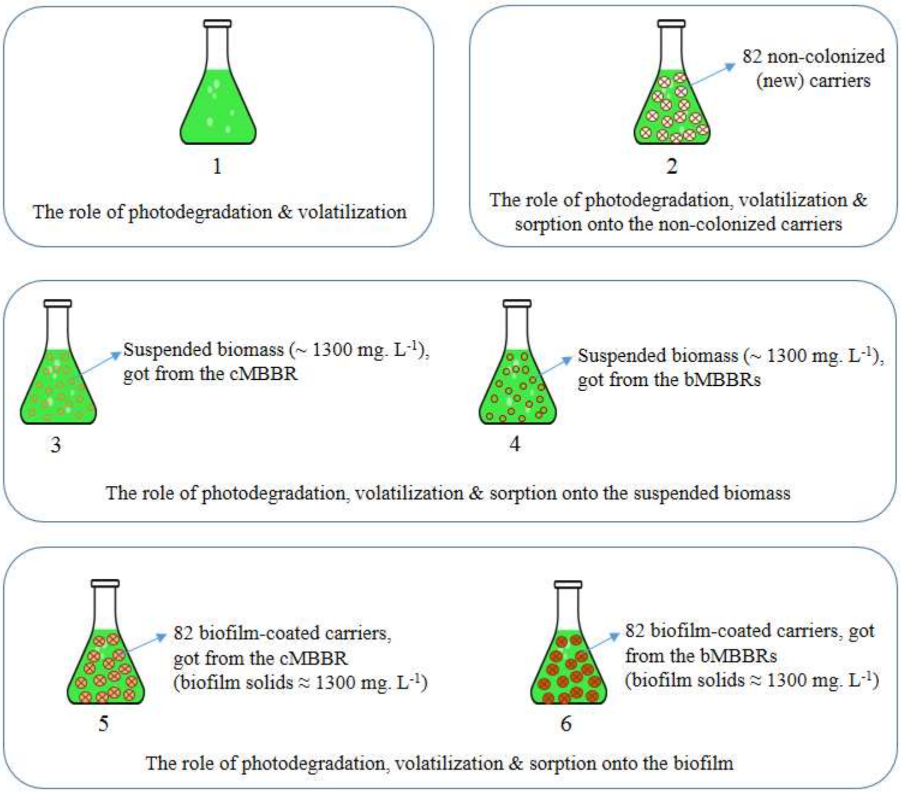


Fig. 3S. Experimental design for assessing the abiotic removal of MPs

Section S1: candidate microbial strain/consortium for bioaugmentation

The initial screening/selection step of the microorganism should be based on the metabolic potential of the microorganism, and also on essential features that enable the cells to be functionally active and persistent under the desired environmental conditions [1]. As reported by Yu and Mohn [2], candidate microorganisms should meet at least three main criteria: firstly, to be catabolically able to degrade the pollutant, even in the presence of other potentially inhibitory pollutants; secondly, they must persist and be competitive after their introduction into the bioreactor; and thirdly, they should be compatible with the indigenous microbial communities [2]. In addition, they should not be closely related to human pathogens e.g. *Pseudomonas aeruginosa* [3]. When a microbial consortium is going to be added into the biofilm reactors, the capability of biofilm formation should be also taken into account as the biofilm-forming microbes can stimulate the immobilization of pollutant-degrading strains into the biofilms and can subsequently improve the biodegradation of contaminants in wastewater [4]. If a bacterial or fungal strain is only used for bioaugmentation, it’s biofilm-forming capability must be accompanied with its proven ability in pollutants biodegradation [5].

P. fluorescens, a gram-negative and rod-shaped bacterium, has a great potential for adhesion on different surfaces such as glass, stainless steel [6] and plastic tubes [7]. Experimentally, Naik et al. [8] treated municipal wastewater efficiently by means of *In vitro* biofilm formation of this strain on polystyrene plates. Moreover, in a research about bioremediation of soil from pesticides, Lakshmi et al. [9] enriched sandy loam soil by *P. fluorescens* up to 50 mg.kg^{-1} of soil. The degradation of pesticide Chlorpyrifos was 43% and 89% after 10 and 30 days, respectively.

Table 1S. Properties of candidate microbes for bioaugmentation of tertiary MBBRs

	Formulation (CFU.mL^{-1})	Biomass (g.L^{-1})	Total COD (mg. L^{-1})	Dissolved COD (mg. L^{-1})	pH
Pseudomonas fluorescens	1x10^{8}	3.5	274	179	5.9

Table 2S. Arrangement of batch experiments for evaluating the effects of adding *P. fluorescens* on the pre-formed biofilm[1]

	Biomass concentration of pure culture (g. L^{-1})	Volume taken form the pure culture (mL)	Inoculation rate (%)[2]	Concentration of the biofilm solids at each flask (mg.L^{-1})[3]	Concentration of added strain in each flask (mg. L^{-1})	Number of biofilm-coated carriers in each flask
P. fluorescens	3.5	4.74	10%	331.8	33.18	21

[1] All batch experiments were performed in 1-L autoclaved Erlenmeyer flasks containing 500 mL of pre-autoclaved synthetics wastewater with COD of 1000 mg. L^{-1}.

[2] The inoculation rate was assumed by 10% of the biofilm solids present.

[3] This value is calculated based on the amount of attached biomass on each carrier (≈ 7.9 mg. L^{-1}).

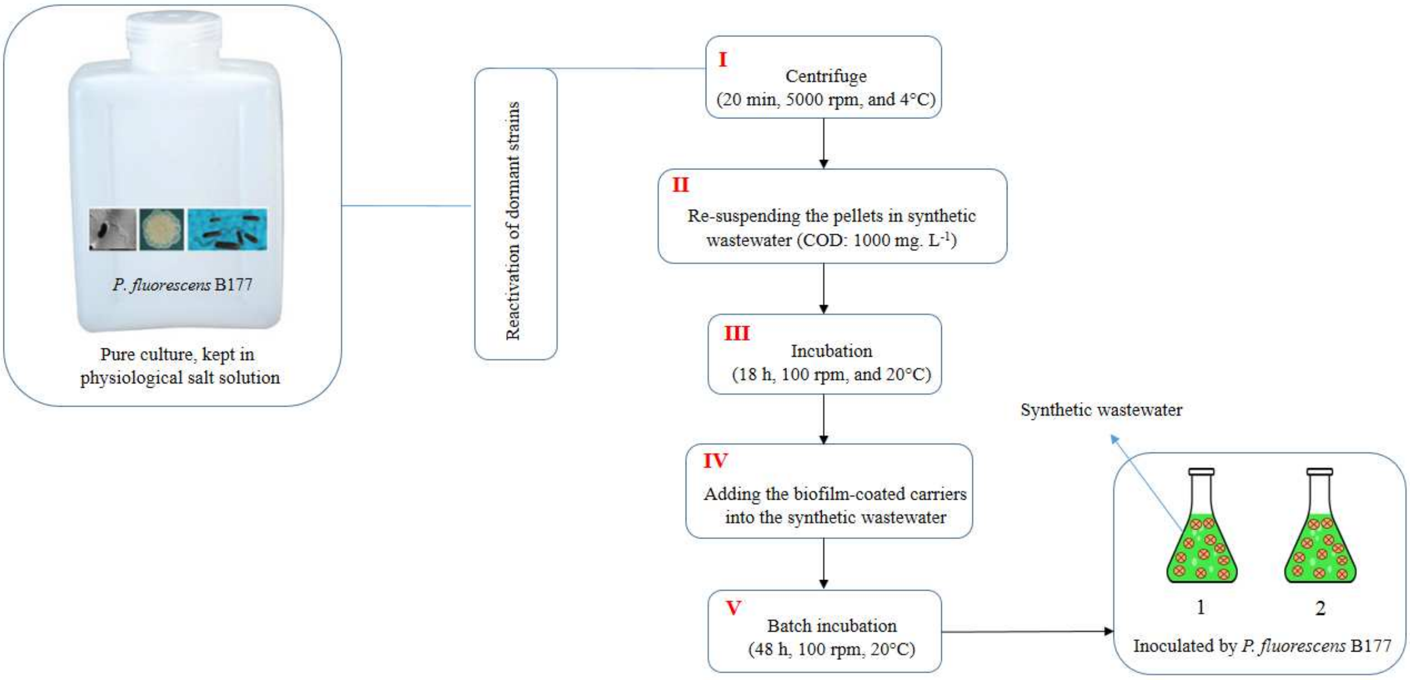
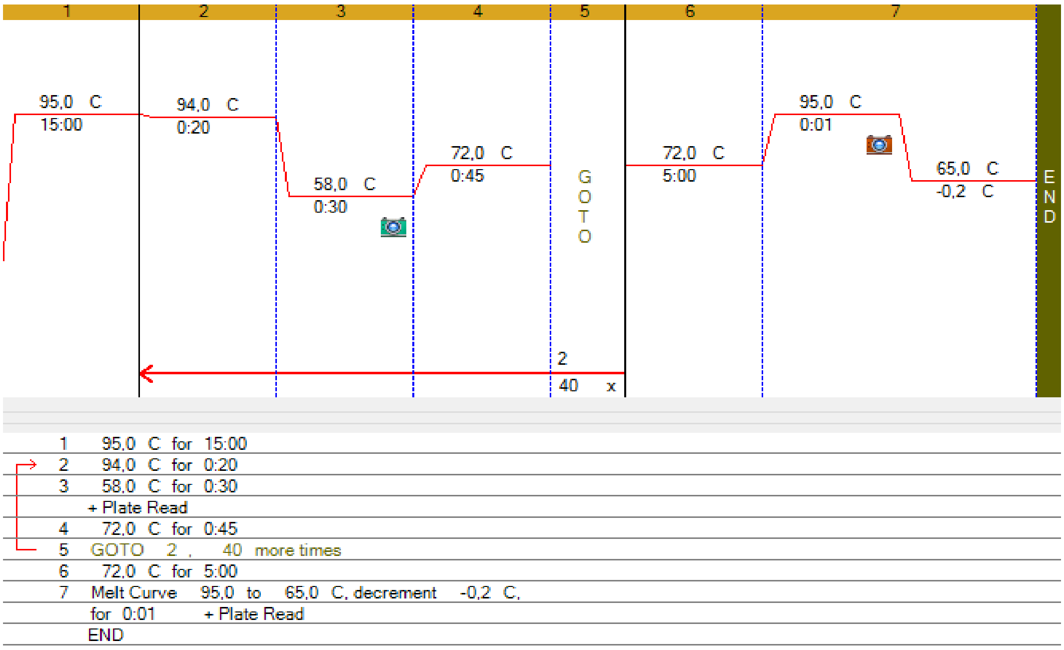


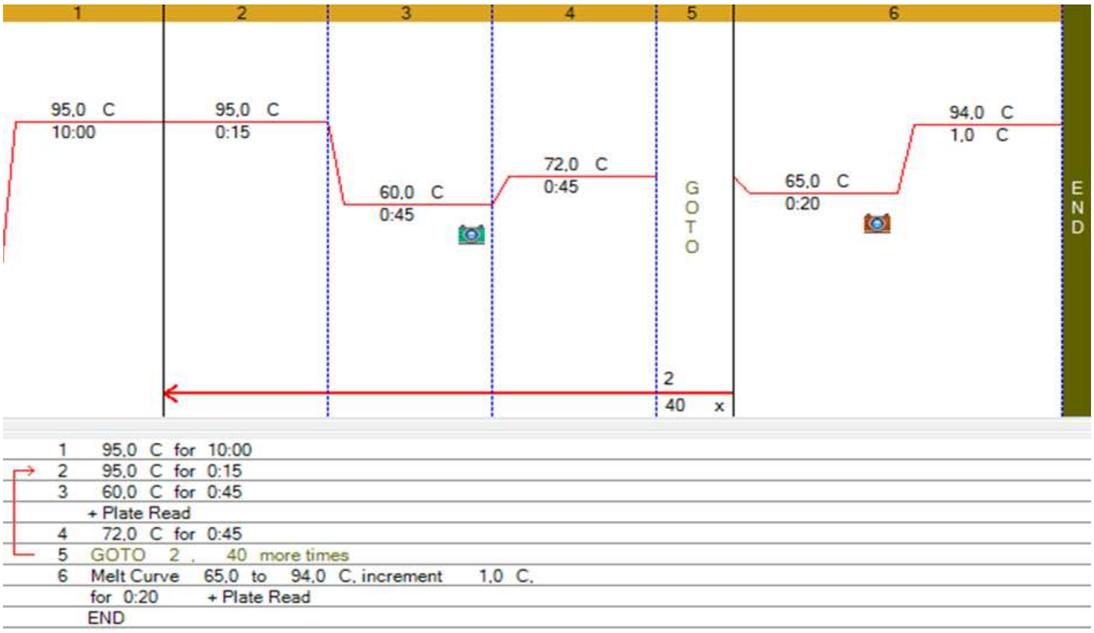
Fig. 4S. Experimental design for pre-evaluating the effects of adding *P. fluorescens* on the pre-formed biofilm

Fig. 5S. Prepared samples of the biofilm-coated carrier (a), and pellets produced from the centrifuge of the mixed liquor (b) for DNA extraction



```
1    95,0  C  for  15:00
2    94,0  C  for  0:20
3    58,0  C  for  0:30
   + Plate Read
4    72,0  C  for  0:45
5   GOTO   2 ,   40  more times
6    72,0  C  for  5:00
7   Melt Curve   95,0  to   65,0  C, decrement   -0,2  C,
    for  0:01        + Plate Read
   END
```

Fig. 6S. qPCR thermo-cycling protocol for the analysis of total bacteria



```
1    95,0  C  for  10:00
2    95,0  C  for  0:15
3    60,0  C  for  0:45
   + Plate Read
4    72,0  C  for  0:45
5   GOTO   2 ,   40  more times
6   Melt Curve   65,0  to   94,0  C, increment   1,0  C,
    for  0:20        + Plate Read
   END
```

Fig. 7S. Modified qPCR thermo-cycling protocol for the analysis of *P. fluorescens*

Table 3S. Operating parameters and performance of the MBBRs at Phase 1 (Start-up, biofilm formation & adaptation)

(details of Phase 1 are given in Fig. 1S & 2S in supplementary data)

		Suspended biomass			Attached biomass		Food to Microorganism (F/M) (kg COD.kg VSS^{-1}.d^{-1})	OLR (g COD.d^{-1})	COD	
		MLSS (mg. L^{-1})	MLVSS (mg. L^{-1})	MLVSS/MLSS	Biofilm solids (mg. L^{-1})	Attached biomass (mg/carrier)			Inlet COD (mg. L^{-1})	COD removal (%)*
HRT: 20 h	cMBBR	1908 ± 433	1514 ± 426	0.79 ± 0.06	207 ± 185	1.28 ± 1.16	0.43 ± 0.12	1.89 ± 0.05	508 ± 12	90.06 ± 0.56
	bMBBR 1	1918 ± 462	1558 ± 482	0.80 ± 0.07	199 ± 170	1.24 ± 1.11	0.43 ± 0.13			89.37 ± 1.12
	bMBBR 2	1945 ± 515	1581 ± 502	0.80 ± 0.06	189 ± 173	1.18 ± 1.07	0.42 ± 0.16			88.11 ± 2.13
HRT: 14.8 h	cMBBR	1346 ± 82	920 ± 47	0.68 ± 0.01	453 ± 30	2.81 ± 0.10	0.66 ± 0.03	1.88 ± 0.03	374 ± 6	85.45 ± 1.29
	bMBBR 1	1364 ± 75	910 ± 72	0.67 ± 0.03	467 ± 48	2.89 ± 0.30	0.67 ± 0.05			87.20 ± 0.31
	bMBBR 2	1332 ± 96	929 ± 40	0.70 ± 0.04	456 ± 56	2.83 ± 0.35	0.65 ± 0.03			83.11 ± 0.69
HRT: 9.8 h	cMBBR	1425 ± 106	957 ± 113	0.67 ± 0.07	920 ± 189	5.70 ± 1.17	0.66 ± 0.08	1.92 ± 0.04	253 ± 5	84.79 ± 1.16
	bMBBR 1	1412 ± 110	979 ± 122	0.69 ± 0.07	924 ± 185	5.73 ± 1.15	0.64 ± 0.05			85.00 ± 2.29
	bMBBR 2	1388 ± 107	909 ± 84	0.66 ± 0.04	940 ± 171	5.83 ± 1.12	0.69 ± 0.07			86.14 ± 2.10
HRT: 4 h	cMBBR	1342 ± 57	1046 ± 44	0.78 ± 0.05	1188 ± 112	7.37 ± 0.69	0.59 ± 0.03	1.90 ± 0.06	102 ± 3	80.02 ± 1.48
	bMBBR 1	1322 ± 37	1018 ± 45	0.77 ± 0.06	1174 ± 108	7.28 ± 0.66	0.60 ± 0.09			79.23 ± 4.02
	bMBBR 2	1377 ± 32	1070 ± 65	0.78 ± 0.08	1188 ± 95	7.35 ± 0.59	0.57 ± 0.04			81.47 ± 3.77

*at steady-state condition.

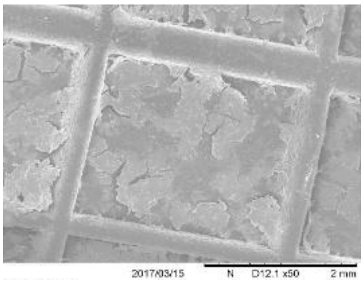
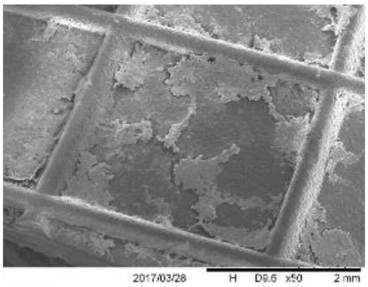
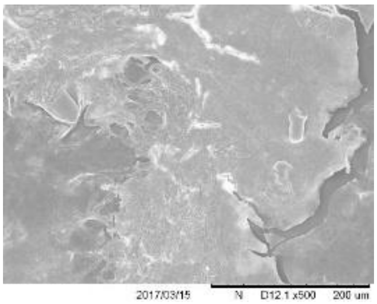
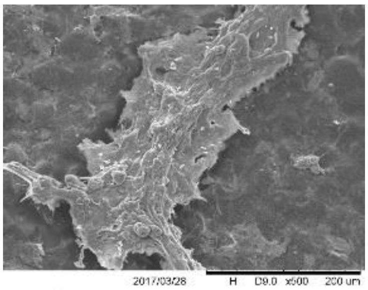
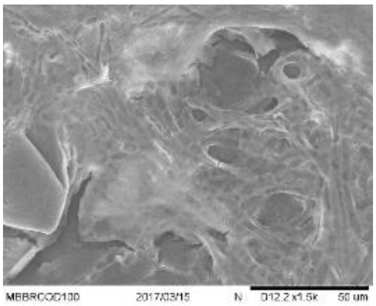
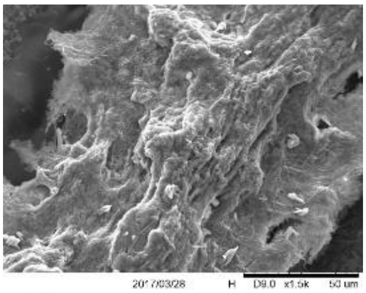
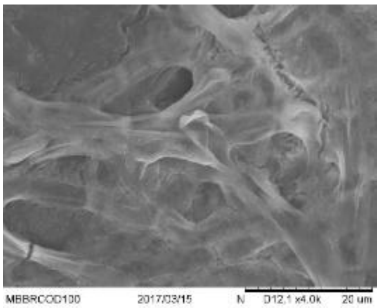
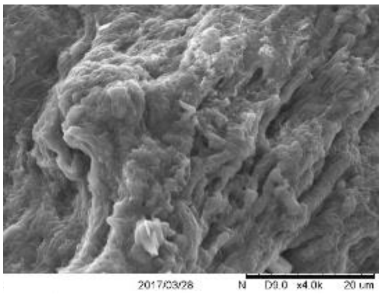
50x		
500x		
1500x		
4000x		
	Control flask	Flask inoculated by *P. fluorescens*

Fig. 8S. Microscopic observation of the biofilm (after the batch incubation) by SEM, for the purpose of pre-evaluating the addition of *P. fluorescens* on the pre-formed biofilm

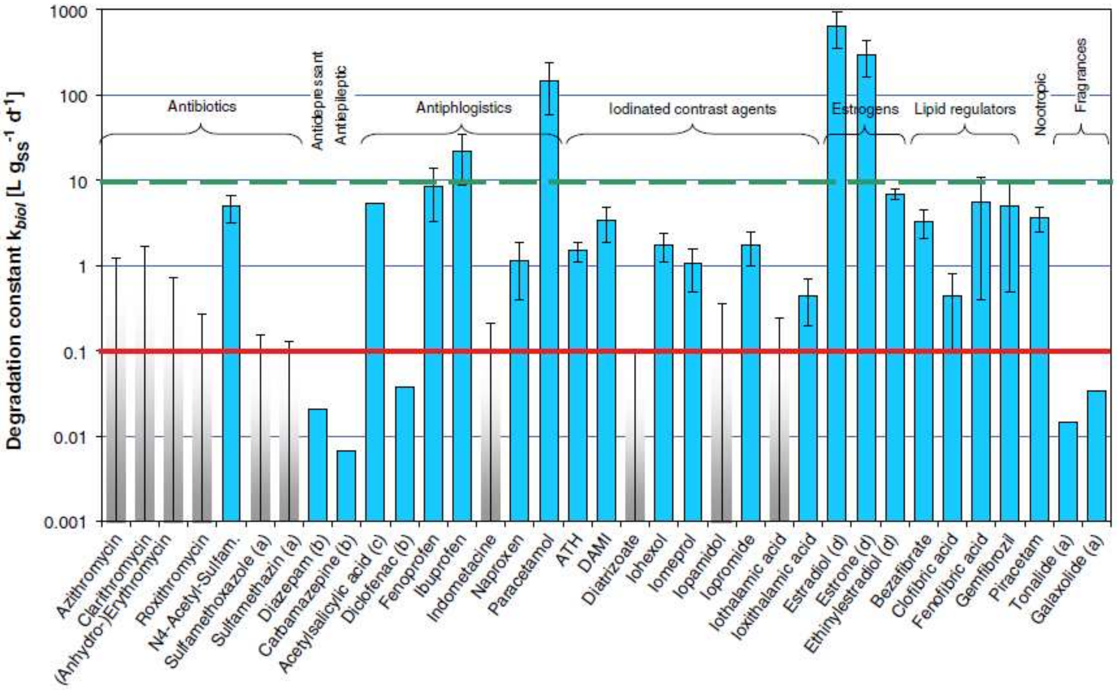
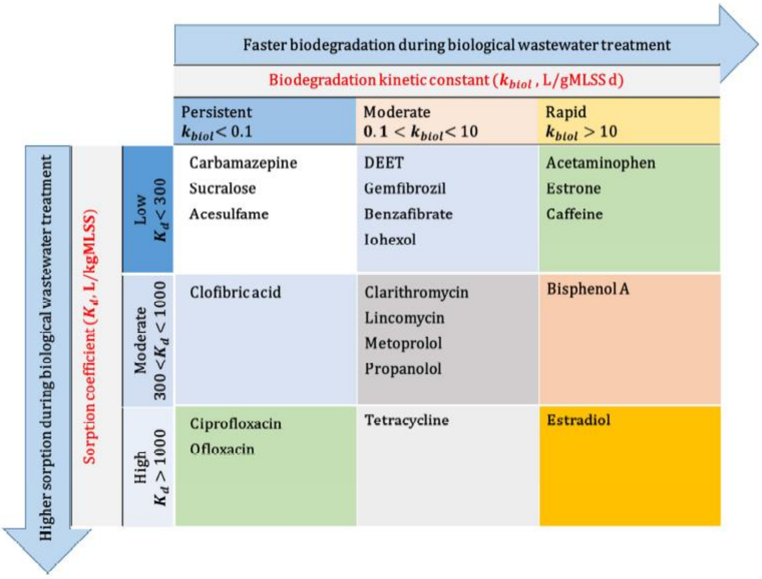


Fig. 9S. k_{biol} values of several MPs obtained in nutrient-removing CAS systems (adapted from Joss et al. [10])



	Persistent $k_{biol} < 0.1$	Moderate $0.1 < k_{biol} < 10$	Rapid $k_{biol} > 10$
Low $K_d < 300$	Carbamazepine Sucralose Acesulfame	DEET Gemfibrozil Benzafibrate Iohexol	Acetaminophen Estrone Caffeine
Moderate $300 < K_d < 1000$	Clofibric acid	Clarithromycin Lincomycin Metoprolol Propanolol	Bisphenol A
High $K_d > 1000$	Ciprofloxacin Ofloxacin	Tetracycline	Estradiol

Fig. 10S. The role of biodegradation and sorption in MPs removal (adapted from Tran et al. [11])

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CHAPTER (IV)

Tertiary removal of micropollutants using weak polyelectrolyte multilayer (PEM)-based NF membranes

This chapter has been published as:

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Table of Contents

Abstract

Nanofiltration (NF) is seen as a very promising technology to remove micropollutants (MPs) from wastewater. Unfortunately, this process tends to produce a highly saline concentrate stream, as commercial NF membranes retain both the MPs and most of the ions. The high salinity makes subsequent degradation of the MPs in a bio-reactor very difficult. The main goal of this study is to prepare and study a NF membrane that combines a low salt rejection with a high MPs rejection for the treatment of secondary-treated municipal wastewater. This membrane was prepared using layer by layer (LbL) deposition of the weak polycation poly(allylamine hydrochloride) (PAH), and the weak polyanion poly(acrylic acid) (PAA), on the surface of a hollow fiber dense ultrafiltration (UF) membrane. The ionic strength of the coating solutions was varied and properties of the formed polyelectrolyte multilayers (PEMs), such as hydration, hydrophilicity, hydraulic resistance and ions retention were studied. Subsequently we tested the apparent and steady state rejection of MPs from synthetic wastewater under cross-flow conditions. The synthetic wastewater contained the MPs Diclofenac, Naproxen, Ibuprofen and 4n-Nonylphenol, all under relevant concentrations (0.5-40 μg/L, depending on the MP). PEMs prepared at lower ionic strength showed a lower hydration and consequently a better retention of MPs than PEMs prepared at higher ionic strengths. A strong relationship between the apparent rejection of MPs and their hydrophobicity was observed, likely due to adsorption of the more hydrophobic MPs to the membrane surface. Once saturated (steady state), the molecular size of the MPs showed the best correlation with their rejection, indicating rejection on the basis of size exclusion. In contrast to available commercial NF membranes with both high salt and MP rejection, we have prepared an unique membrane with a very low NaCl retention (around 17%) combined with a very promising removal of MPs, with Diclofenac, Naproxen, Ibuprofen and 4n-Nonylphenol being removed up to 77%, 56%, 44% and 70% respectively. This membrane would allow the treatment of secondary treated municipal wastewater, strongly reducing the load of MPs, without producing a highly saline concentrate stream.

1. Introduction

Over the last few years, a great concern has been highlighted regarding the occurrence of micropollutants (MPs) in aquatic resources and the subsequent effects on humans and the environment [1]. In addition to the 45 priority substances on the European Watch List (Directive, 2013) [2], an additional watch list of 10 priority substances that should be monitored within the European Union was recently included in Decision 495/2015/EU [3] indicating the growing attention to this issue. In this regard, effluents of wastewater treatment plants have been recognized as the main entry point of these compounds into the aquatic environment [4]. Conventional treatment methods do not lead to sufficient removal of MPs, and adding additional steps during wastewater treatment is seen as the most promising way to reduce the release of these compounds into surface waters [5]. To date, identification of technically and economically feasible advanced wastewater treatment options for the elimination of MPs from secondary-treated effluent is ongoing. Adsorption processes, advanced oxidation processes (AOPs) and membrane filtration are important examples of such technologies. Among these options, listed in Table 1, membrane technologies such as nanofiltration (NF) and reverse osmosis (RO) have attracted a great interest because of high removal rates (> 90%) of low molecular weight MPs, excellent quality of treated effluent, modularity and the ability to integrate with other systems. On the other hand, fouling is often a real problem for these membrane processes [6]. A lower energy consumption and higher permeate fluxes for NF membranes in comparison to RO membranes have encouraged the use of NF membranes for several commercial purposes, such as wastewater reclamation, water softening, and desalination [7,8]. Also for MPs removal, NF membranes are seen as a more cost effective alternative to RO membranes.

A major drawback of these pressurized membranes is the production of a waste stream (concentrate) which typically has a volume of up to 10–20% of the original wastewater volume [9]. This stream is rich in dissolved organic compounds, heavy metals and inorganic salts of Na^+, Cl^-, Ca^{2+}, Mg^{2+} and SO_4^{2}, and also contains the removed organic MPs [10]. Since the discharge of untreated concentrate poses a significant risk to the environment, increasing attention has been paid to this issue in recent years. Today, various methods exist for the disposal and management of concentrate produced from membrane plants such as discharge to surface water, wastewater treatment plants and deep wells, land application, and evaporation ponds. The removal of specific compounds from this unwanted stream may be performed by using activated sludge systems which are more cost-effective compared to other treatment options such as oxidation processes, adsorption or ion exchange [11–13]. The biological treatment of the concentrate stream strongly depends on its chemical composition which is often influenced by the membrane recovery rates (or expressed as the volume reduction factor) [11,14]. Azaïs et al. [14] investigated the chemical composition of the concentrate stream produced from NF90 membranes, treating secondary-treated wastewater, at different volume reduction factors (from 2 to 10). They reported the average composition of the NF concentrates: conductivity from 2 to 5.1 mS. cm^{-1}, dissolved organic compound (DOC) from 12 to 48 mg. L^{-1}, chemical oxygen demand (COD) from 49 to 180 mg. L^{-1}, and MPs concentrations multiplied by a factor of 3-7 compared to those encountered in the secondary-treated wastewater. From this bibliographic review, there is still a lack of knowledge on the favorable concentration of MPs for their efficient biotic removal during the concentrate's biological treatment. Apart from that, the main limitation in biological treatment of the concentrate is its high salinity (> 1%) which is harmful to the bacteria because the increased osmotic pressure damages bacterial cell walls (plasmolysis of the organisms at high salt concentrations) [9]. More information about detrimental levels of the salinity on the performance of activated sludge reactors is given in Section S1 in supplementary data. Therefore, in the present work, we propose to make use of NF membranes with a much lower rejection of salts than most of the commercial NF membranes, with the aim to achieve easy and feasible biological treatment of the generated concentrate stream. For this purpose, one requires NF membranes with a low ion rejection (< 30%) and a high rejection of MPs (>80%), a membrane that is currently not commercially available.

Recently, the development of better performing NF membranes has been an important on-going challenge, especially because a higher flux normally goes hand-in-hand with lower selectivity and *vice versa*. To achieve membranes with a high flux combined with a high selectivity, it is required to establish a thin and defectless separation membrane on top of a highly permeable and mechanically robust support [15]. To prepare such promising membranes, some techniques have been developed for membrane surface modification such as grafting and interfacial polymerization [16,17]. Since these processes are laborious, costly and rely on environmentally unfriendly solvents [18], the method chosen for this study is a polyelectrolyte layer by layer (LbL) deposition technique. In this approach, a substrate is alternatively exposed to polyanions and polycations to build polyelectrolyte multilayers (PEMs) of a controllable thickness [15]. Nowadays, the LbL adsorption of polyelectrolytes (PEs) is performed by some developed methods like dip-coating [19], spray coating [20] and spin coating [21] to make polyelectrolyte multilayer membranes. Indeed, PEM based membranes can be considered as functionalized membranes with a strong potential for application in, for example, desalination [22], Heavy metals removal [23], alcohol/water separation [24], filtration of sludge supernatant [25] and recently in MPs removal [26,27]. In addition to the electrostatic interactions present in PEMs [28,29], other interactions such as hydrophobic interactions [30], hydrogen bonding [31] and chemical crosslinking [7] can play a role. As such, the choice of convenient PEs is the distinguished parameter that it affects all above-mentioned driving forces.

Apart from the choice of PEs, it has been demonstrated that multiple parameters such as pH, ionic strength, and charge density, can influence the LbL process and the resulting PEMs [32–34]. This versatility makes it possible to prepare PEM based membranes that are really optimized for a certain application. The application of PEMs-based membranes has been recently investigated in MPs removal by some researchers [26,27]. For the first time, Joris de Grooth et al. [26] obtained excellent retentions for both positively and negatively charged MPs in NF Membranes made by Polycation/Polyzwitterion/Polyanion Multilayers. Unfortunately, neutral and small micropollutants were hardly retained. Then, in the research of Ilyas et al.[27], a PEM based NF membrane made by LbL assembly of weak PEs was developed with interesting properties for the removal of MPs from wastewater effluents. The membrane combined a low ion rejection, with a good MP rejection (60-80%) even for small and neutral MPs, providing for the first time a membrane that could remove MPs without producing a highly saline waste stream. This membrane was only studied under ideal conditions and for unrealistically high MP concentrations (mg/mL). The performance under conditions relevant for wastewater treatment still needs to be studied.

In the present study, we aim to study the membrane developed by Ilyas et al. [27] under realistic conditions for municipal wastewater treatment, studying the ion rejection, and the rejection of relevant MPs within a complex water composition. Furthermore, we have continued to optimize the membrane performance by studying the impact of ionic strength on the properties of the formed PEMs in the case of salts and MPs retention. The polymers used here are two weak oppositely-charged PEs, with physical structures illustrated in Fig. 1, named Poly(allyl amine) hydrochloride (PAH) containing a primary amine (– NH_3^+) (weak cationic) and poly (acrylic acid) (PAA) with a weak anionic carboxylic acid group. The PEM based active separation layers were coated onto Hollow fiber dense UF membranes by LbL adsorption.

The removal of relevant MPs including three analgesic and anti-inflammatory pharmaceutical compounds (Diclofenac, Naproxen and Ibuprofen) and one endocrine disrupting compound (4n-Nonylphenol) from secondary-treated municipal wastewater was studied. The main objective of this study was to demonstrate the possibility to prepare LbL-made NF membranes with a high rejection of MPs and a low retention of salts from secondary-treated municipal wastewater. This strategy would lead to make membrane processes with a low-saline concentrate stream which is more convenient for the biological treatment in activated sludge systems.

Fig. 1. Molecular structure of PAA and PAH used in this study [35]

Table 1. The most-frequently used treatment technologies for removal of MPs from secondary-treated municipal wastewater

Category of tertiary treatment	Subcategory	Advantages	Disadvantages/limitations	References
Advanced oxidation processes (AOPs)	Ozonation	Remarkable capability for removing most of the pharmaceuticals and industrial chemicals	As O_3 is a highly selective oxidant, ozonation often cannot ensure the effective removal of ozone-refractory compounds such as Ibuprofen.	[36]
		It has been successfully applied in many full-scale applications in reasonable ozone dosages.	Ozonation produces carcinogenic bromate from bromide that exists in secondary-treated effluents.	[36,37]
	Fenton oxidation	This kind of system is attractive because it uses low-cost reagents, iron is abundant and a non toxic element and hydrogen peroxide is easy to handle and environmentally safe.	In this process, the low pH value often required in order to avoid iron precipitation that takes place at higher pH values. This process is not convenient for high volumes of wastewater in full-scale applications.	[2,38]
	Heterogeneous photocatalysis with TiO_2	The principle of this methodology involves the activation of a semiconductor (typically TiO_2 due to its high stability, good performance and low cost) by artificial or sunlight.	The need of post-separation and recovery of the catalyst particles from the reaction mixture in aqueous slurry systems can be problematic.	[38]
			The relatively narrow light-response range of TiO_2 is one of the challenges in this process. This process is not convenient for high volumes of wastewater in full-scale applications.	
	photolysis under ultraviolet (UV) irradiation	Photo-sensitive compounds can be easily degraded with this method.	UV irradiation is a high-efficient process just for effluents containing photo-sensitive compounds. This process is not convenient for high volumes of wastewater in full-scale applications.	[38]
			The addition of H_2O_2 to UV is more efficient in removing MPs than UV alone, but UV/H_2O_2 is a viable solution for the transformation of organic MPs with low O_3 and ◦OH reactivity.	
	Ultrasound irradiation (Sonolysis)	It is a relatively new process and therefore, has unsurprisingly received less attention than other AOPs. But it seems that this process is economically more cost-effective.	There are very few studies and consequently rare experience about sonolysis of the effluent MPs.	[39]
Adsorption processes	Adsorption processes with activated carbon	It has been identified as powerful and easily adjustable technology to remove MPs.	This process should be followed by a final polishing step (sand filtration or UF membranes) to retain adsorbed contaminants and spent activated carbon. So higher energy requirements of UF membrane and the relatively high carbon dosage (up to 20 mg/L) necessary to achieve the required MPs removal.	[5]
		Large-scale trials have not only demonstrated excellent removal (>80%) of a broad range of micropollutants, but also contributed to reducing the effluent toxicity.	In the case of "granular activated carbon", a regeneration process of the spent carbon is required, while spent "powdered activated carbon" must be incinerated or dumped after filtration process.	
Membrane filtration	RO and NF membranes	These processes have attracted a great interest because of higher removal rate of low molecular weight PSs, excellent quality of effluent, modularity and ability to integrate with other systems despite their fouling problems.	High quantities of cations, anions, sulfate, MPs, etc. in the concentrate produced in NF and RO processes compel wastewater managers and decision makers to treat it with complicated processes specially in the case of full-scale applications.	[6,40,41]
			High energy consumption (about 4.7 and 3.4 kWh/m^3), high capital (334.3 and 338.2 \$/m^3/d) and operational costs (0.72 and 0.57 \$/m^3) of RO and NF membranes, respectively, and their problematic fouling issues may preclude membrane treatment as an option.	

2. Experimental

2.1. Chemicals

All chemicals used in this study including MPs (listed in Table 2 with their physical and chemical properties)., two weak PEs (PAH with Mw = 15,000 $g.mol^{-1}$ and PAA with Mw = 15,000 $g.mol^{-1}$)., $NaNO_3$ as a background electrolyte., all salts ($CaCl_2$, $CaCl_2.2H_2O$, Na_2SO_4, NaCl, K_2HPO_4, $MgSO_4.7H_2O$)., peptone., meat extract and urea) were obtained from Sigma–Aldrich. The concentration of PAH and PAA in PE solutions were always 100 $mg.L^{-1}$ with pH of 6 for both PEs and they were prepared in two ionic strengths of 5 and 50 mM $NaNO_3$. By the way, for evaluating salt rejection, concentration of all salts in feed solution of all membranes were adjusted at 5 mM ($CaCl_2$: 554.9, Na_2SO_4: 710.2, and NaCl: 292.2 mg. L^{-1}). Furthermore, Milli Q water (18.2 MΩ cm) was used to prepare PE and salts solutions, rinse and measure parameters including membranes permeability and resistance.

The hydrophobicity of MPs is expressed as the log D (logarithm of the octanol-water distribution coefficient), or the log Kow (logarithm of the octanol-water partition coefficient). However, log D appears to be a better hydrophobicity indicator than log Kow and can be used to evaluate the hydrophobicity of MPs at any pH value [42]. In this regard, compounds with $\log D > 2.6$ are referred to as hydrophobic that prefer to accumulate in solid phases instead of being soluble in the aqueous phase, and hydrophilic when $\log D \leq 2.6$ [43]. Hence, according to the values presented in Table 2 for log D, 4n-Nonylphenol is classified as hydrophobic, in contrast with the rest of MPs, and is therefore expected to adsorb to the surface of hydrophobic membrane surfaces by hydrophobic interactions.

Minimum projection area (MPA), calculated from the van der Waals radius, is defined as the smallest two-dimensional projection area of a three-dimensional molecule. By projecting the molecule on an arbitrary plane, two-dimensional projection area can be calculated and the process is repeated until the minimum of the projection area is obtained (Fig. 1S in supplementary data) [44,45].

2.2. Synthetic wastewater

Synthetic secondary-treated municipal wastewater was prepared according to the OECD protocol [46,47]. In order to make it, firstly, a mother stock solution was made in 1 L of tap water containing 160 mg peptone, 110 mg meat extract, 30 mg urea, 28 mg K_2HPO_4, 7 mg NaCl, 4 mg $CaCl_2.2H_2O$ and 2 mg $MgSO_4.7H_2O$[46,47]. Then the daughter stock solution was made in an effective volume of 5 L. This synthetic wastewater contained 50 ± 2 mg. L^{-1} of COD, 10 ± 1 mg. L^{-1} of total nitrogen (TN) and 1 ± 0.1 mg P-PO_4^{3-}. L^{-1}. Moreover, daughter stock solutions of each target MP were prepared separately in Milli-Q water from their individual mother stock solutions, prepared in methanol at a concentration of 1 $g.L^{-1}$. Regarding the review paper published by Lue et al. [48], and also on the basis of available data in literature about the concentration of target MPs in effluents of municipal wastewater treatment plants treated with conventional activated sludge systems (Fig.2), final concentrations of Diclofenac, Naproxen, Ibuprofen and 4n-Nonylphenol in feed solution were considered 0.5, 2.5, 40 and 7 $\mu g.L^{-1}$, respectively. To avoid possible bacterial biodegradation and photodegradation, mother stock solutions of MPs were stored in amber glass bottles and kept in freezer (-18°C) while synthetic wastewater and daughter stock solutions of MPs were prepared immediately before starting the filtration process in aluminum-wrapped glass containers.

2.3.COD, TN, and P-PO_4^{3-} measurements

Samples were firstly filtered through 0.45 μm glass fiber filters (Sartorius, Göttingen, Germany). Then, the analysis process were done using HACH LANGE kits for COD, TN, and *P-*PO_4^{3}, along with DR3900 Benchtop VIS Spectrophotometer equipped with HT200S oven (HACH LANGE, Germany). These parameters were measured in duplicate and the average values were presented.

2.4.Membrane characteristics

Hollow fiber dense UF membranes (Hollow Fibre Silica (HFS)) with a molecular weight cutoff of 10 kDa and an inner diameter of 0.79 mm prepared from poly(ether sulfone) with a sulfonated poly(ether sulfone) separation layer (SPES) were kindly provided by Pentair X-Flow (The Netherlands). This membrane is designed for inside-out filtration. The presence of the anionic SO_3^- group on the sulfonated polymer backbone allows for a good adhesion of PEMs.

2.5.Preparation of PEMs via Dip-coating

Dip-coating involves the sequential immersion of a given substrate into solutions with oppositely charged polyelectrolyte solutions, typically with one or more rinsing steps in between. By this simple procedure, transport of the polymer to the substrate surface is mainly based on diffusion. As we immerse the hollow fiber support membrane completely in the coating solution, PEs deposition is not limited to the inner surface of the membrane only and the whole porous structure can be coated by the PEs [49]. In this study, hollow fibers and silicon wafers were coated according to the protocol described by Joris de Grooth et al. [50]. Considering the negatively charged surface of these membranes (zeta potential of -25 mV in 5 mM KCl [51]), the first applied polyelectrolyte should have an opposite charge, here PAH. In this study, we have used silicon wafers in order to follow the growth and thickness of adsorbed PEs which are difficult parameters to be monitored in coated HFS membranes.

Before coating, wetting of 20-cm hollow fibers were done in 15 wt.% ethanol in water overnight. Then wet fibers were rinsed with deionized water three times followed by three times rinsing in the background electrolyte solution ($NaNO_3$). The used silica wafers were effectively cleaned by a 10-minute plasma treatment using a low-pressure Plasma Etcher (Femto model) purchased from Diener Electronics, leading to a reproducible negative charge at the surface of all wafers.

Afterwards, fibers/wafers were completely immersed in a 0.1 $g \cdot L^{-1}$ polycation solution (PAH) with a pH of 6 and ionic strengths of 5 or 50 mM $NaNO_3$ at room temperature. After 30 minutes, to remove polymer chains that are loosely attached to the pre-adsorbed polymer layer, fibers/wafers were rinsed in two separate solutions containing only $NaNO_3$ with an ionic strength similar to that of the coating solution for 15 min per solution. The rearrangement of the polymer chains that occurs during the rinsing step, leads to increased stability and improved thickness control [52]. Then to form the first bilayer of PAH/PAA, fibers/wafers were dipped for 30 minutes in 0.1 $g{\cdot}L^{-1}$ polyanion solution (PAA) with pH of 6 and two ionic strengths of 5 or 50 mM $NaNO_3$ and rinsed again in two separate background solutions exactly as before. This procedure was repeated up to the formation of 13 layers of PEs i.e. $(PAH/PAA)_6$-PAH. After each step of coating, three samples of fibers/wafers were picked up for future experiments.

To avoid pore collapse, coated fibers were kept in glycerol/water (15wt.%/85wt.%) solution for at least 4 h and dried overnight under ambient conditions. These coated fibers were subsequentlly potted in single fiber plastic modules of 15 cm in length, with a hole in middle and two heads potted with an epoxy resin. Before filtration, these modules were put in deionized water overnight to help opening of blocked pores.

2.6. Spectroscopic ellipsometry (hydration measurement)

Ellipsometry is a very sensitive optical technique based on detecting the changes in polarization state of a light beam upon reflection from the sample of interest [53]. In the present work, dry and wet thicknesses of deposited multilayers on the surface of plasma-treated silicon wafers were measured using an in-situ Rotating Compensator Spectroscopic Ellipsometer (M-2000X, J. A. Woollam Co, Inc.) operated in a wavelength range from 370 – 920 nm at incident angles of 65, 70 and 75°. Thickness measurements were calculated using the Cauchy model for ellipsometric parameters (Δ and ψ) and refractive index (n) was taken from independent measurements using a standard laboratory refractometer (Carl Zeiss). Finally, data obtained on three parts of each wafer were reported as a mean dry thickness ± standard deviation [54], and subsequently hydration ratio (swelling degree) was determined using Eq. (1) by means of resulted wet thickness of multilayers [55].

$$Hydration\ ratio = \frac{d_{swollen} - d_{dry}}{d_{dry}} \tag{1}$$

Where, $d_{swollen}$ is the wet thickness of multilayers measured in the presence of milli-Q water in nm, and d_{dry} is dry thickness of multilayers in nm.

2.7. Contact Angle

In order to measure the hydrophilicity of coated fibers/wafers, optical contact angle measurements were performed on an OCA15 plus instrument (Dataphysics Inc.) using a sessile drop method. Sessile drops of 2 µl and 0.4 µl deionized water for coated wafers and fibers, respectively were used to measure the contact angle. The small droplets were essential to be able to obtain a reliable contact angle from the hollow fibers. The hollow fiber surface is curved, but for such a small droplet the effect of curvature can be neglected when determining the contact angle. These measurements were carried out four times for each sample (at 20 °C), and the average and standard deviation are reported. The measurement was carried out five seconds after the bubble was placed on the surface of the wafers/fibers. We evaluated the hydrophilicity of coated wafers before and after immersion in the feed solution (synthetic wastewater containing target MPs) for 48 hours, and coated fibers before and after filtration of the feed solution (see 2.8). Immersed silicon wafers were dried with nitrogen gas, and the fouled fibers were dried for 24 h at room temperature (20 °C) before the measurements.

2.8.Membrane performance

2.8.1. Water permeability & hydraulic resistance

To evaluate the water permeability and thereby the resistance of coated membranes, a lab-scale filtration system with dead-end mode was used. The pure water flux was measured at 20 °C with demineralized water at a trans-membrane pressure (TMP) of 1.5 bar (Eq. (2)). Then from the water flux, the membrane resistance was obtained using Eq. (3).

$$J = \frac{Q}{A_{mem}} \qquad (2)$$

$$R = \frac{\Delta P}{\mu \times J} \qquad (3)$$

Here, J is water flux in $m^3/m^2.s$, Q is volume flow in m^3/s, A_{mem} is membrane area in m^2, μ is the dynamic viscosity of the feed in Pa.s, and ΔP is the TMP in Pa. From each deposited layer of polymer, at least two modules were tested and the average of the permeability and resistance with standard deviation are reported.

2.8.2. Salts and MPs retention

For salts and MPs retention measurements, another lab-scale filtration set-up was used in a cross-flow mode at a TMP of 1.5 bar. The cross-flow velocity of the feed solution through the fibers was set at 4.5 $m.s^{-1}$ in order to reduce the effect of concentration polarization. This corresponds to a Reynolds number of approximately 3500, and is in the turbulent regime. We run the filtration set-up at extremely low recovery. That means that the concentration effect would be very small. In the case of wastewater filtration for MPs retention, membrane compaction was carried out at 1.5 bar for 2 hours using demineralized water prior to feeding the filtration set-up with wastewater. Subsequently, permeate samples of the first 24 hours of the filtration process were collected to measure the apparent rejection. Then a filtration duration of 48 hours was applied in order to provide sufficient membrane saturation to ensure steady state rejections, and a sample was taken after this time. Kimura et al., [56] observed quasi-saturation of the membranes after about a 20-hour filtration of hydrophobic compounds at low concentration (~100 ppb). To avoid overestimation of compounds rejection, they proposed longer filtration times in order to reach adequate membrane saturation whenever low concentrations of solutes exists in the water.

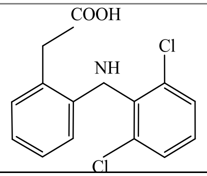
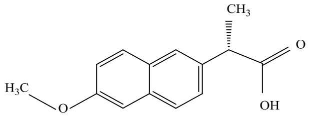
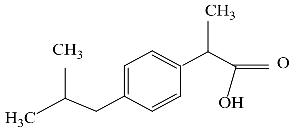
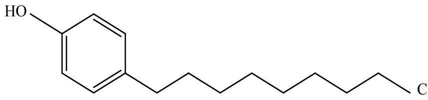
Concentration values of all salts were measured with a Cond 3210 conductivity meter purchased from Wissenschaftlich-Technische Werkstätten GmbH. Each measurement was performed in triplet and the average of values with standard deviation is reported just for twelfth and thirteenth layers of polymer. Retention (Re) in % was calculated using Eq. (4).

$$Re = \left(1 - \frac{C_P}{C_F}\right) \times 100 \tag{4}$$

Where, C_P and C_F are solutes concentrations of permeate and feed solution, respectively.

For MPs analysis, samples of feed and permeate streams (duplicate samples) of the NF installation were shipped to the LaDrôme laboratory (in France) in a freeze box for analysis within 24 h under the analyzing license of COFRAC-ESSAIS. A multi detection procedure including Gas Chromatography (coupled with ECD/NPD mass spectrometry) and Liquid Chromatography (along with DAD, fluorescence, tandem mass spectrometry) was applied for all MPs with Limit of Quantification (LQ) of 0.01 µg/L for Diclofenac, Naproxen and Ibuprofen, and 0.04 µg/L for 4n-Nonylphenol. Then, as mentioned in Eq. (4), apparent (R_{app}) and steady-state rejection (R_{ste}) of MPs were determined.

Table 2. Physico-chemical characteristics of target MPs in this study [6,42,45,48,57–59]

Compound	CAS number	Formula	Molecular Weight (g.mol^{-1})	Solubility in water at 25°C (mg.L^{-1})	Vapor pressure (mm Hg), at 25°C	Boiling point (°C)	log K_{OW}	log D (pH:7)	pKa	Minimum Projection Area (Å^2)	Molar volume (cm^3/mol)	Molecular dimension Length × Width ×Height (nm)	Molecular structure
Diclofenac	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.15	2.4	1.59E-7	412 ± 45	4.548	1.77	4.18	43.3	182	0.829× 0.354 × 0.767	
Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.26	16	3.01E-7	404 ±20	3.18	0.34	4.3	34.8	192.2	1.37 × 0.78 × 0.75	
Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.28	21	1.39E-4	320 ± 11	3.97	0.77	4.47	35.4	200.3	1.39 × 0.73 × 0.55	
4n-Nonylphenol	104-40-5	$C_{15}H_{24}O$	220.35	6.35	8.53E-5	331 ± 11	6.142	6.14	10.15	NA	279.8	1.179 × 0.354 × 0.519	

NA: not available in literature

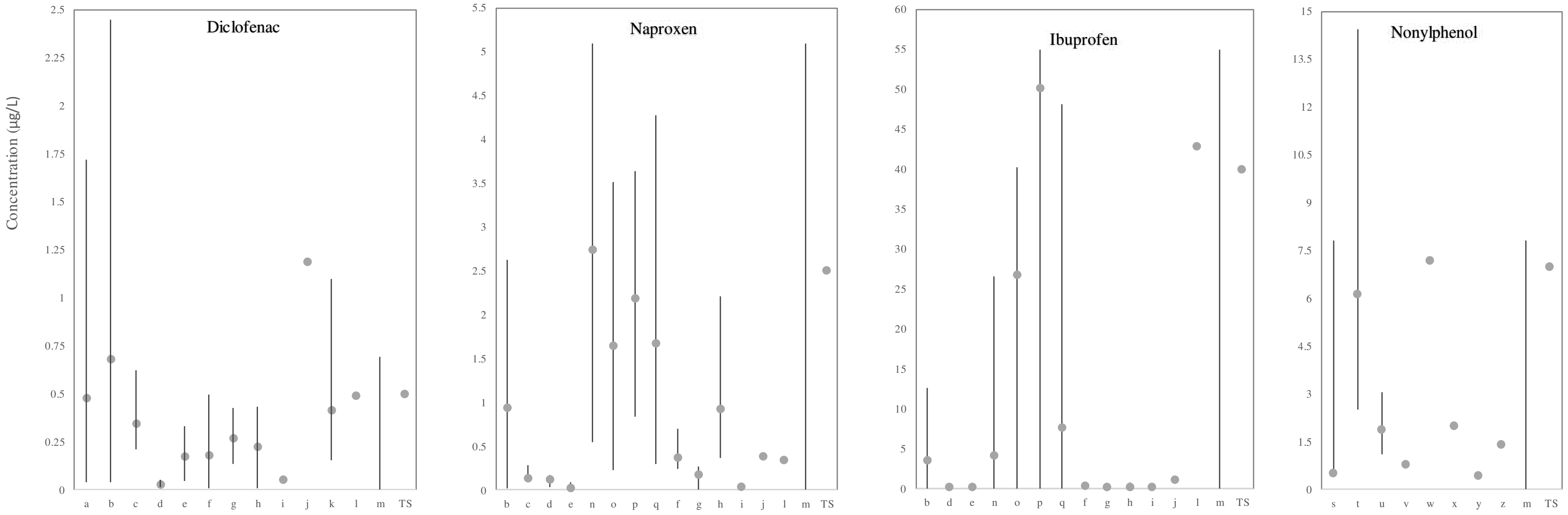


Fig. 2. Concentration range of target MPs in secondary-treated effluent of conventional wastewater treatment plants s found in literature

(TS: This study, References: ^{a}[60], ^{b}[61], ^{c}[62], ^{d}[63], ^{e}[64], $^{f-g}$[65], ^{h}[66], ^{i}[67], ^{j}[4], ^{k}[68], ^{l}[69], ^{m}[48], $^{n-o-p-q}$[70], ^{s}[71], $^{t-u}$[72], ^{v}[73], ^{w}[74], ^{x}[75], ^{y}[76], ^{z}[77])

3. Results and discussion

3.1.The hydraulic resistance of PEM based membranes

The hydraulic resistance of the PEM based membranes, prepared at an ionic strength of 50 mM $NaNO_3$, were measured for each deposited layer to observe the transition from the pore dominated regime to the layer dominated regime [49]. As it can be seen in Fig. 3, the hydraulic resistance generally increases after an additional coating step, in line with the increasing PEM layer thickness. Initially, the smaller increment in hydraulic resistance from bare fiber until the fourth deposited layer (part a) indicates that firstly pores become narrower. Then, the much sharper increase is observed between layers 4 to 9 (part b), indicating the pores becoming fully filled with the PEM layer. After that, the resistance increases much slower again (part c), an increase simply related to the increasing thickness of the PEM coating. The sharp transition between layer 4 and 9 is a first clear indication of a transition from a pore dominated to a layer dominated regime. More evidence comes from the observed zig-zag behavior, which is related to the so-called odd-even effect. The final layer in a PEM can determine the degree of swelling of the whole layer, with PAH terminated layers being more swollen than PAA terminated layers. The change in swelling with different terminating layers leads to the zig-zag behavior. Initially, the resistance upon PAH adsorption (layer 3) shows a strong increase, which goes down when PAA is absorbed (layer 4). But for thicker layers (layer 12) PAA adsorption leads to an increase in resistance, while we see lower resistance for the 13th layer. This behavior (the flipping of the odd–even effect) also reflects a shift from the pore dominated regime to the layer dominated regime. In the pore dominated regime, the pores of the membrane are coated with the PEM, and an increase in swelling of that multilayer will result in a pore size decline and subsequently a reduced membrane permeability. While in the layer dominated regime, a dense layer is formed on top of the membrane and swelling of the layer leads to a more permeable layer and consequently a lower resistance [49]. From the observed behavior, we can be certain that we are well within the layer dominated regime, and that any separation will be dominated by the PEM coating, rather than the original membrane pores.

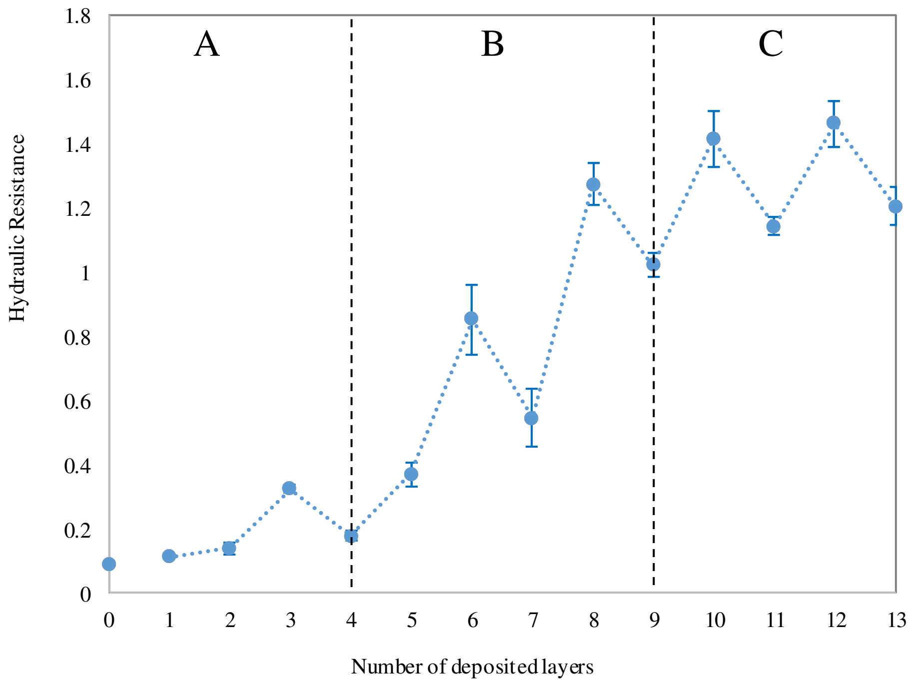


Fig. 3. Changes in hydraulic resistance of virgin and coated membrane (×10^{14} m^{-1}) after deposition of each additional monolayer for PAH/PAA) multilayers prepared in ionic strength of 50 mM $NaNO_3$

3.2.The influence of ionic strength on the PEMs performance

To compare the properties of coated membranes at different ionic strengths, PEMs were also prepared at the lower ionic strength of 5 mM of $NaNO_3$. Lowering the ionic strength used for PEM preparation is known to lead to denser PEM layers, with better separation properties and lower permeabilities [50], but it has not been investigated for this type of polyelectrolyte system (PAH/PAA). Fig. 2S in supplementary data compares pure water permeabilities of the PEMs-based membranes made in this study with the common commercial UF, NF and RO membranes. In this figure, we show that permeability of our membranes is lower than UF and most of NF membranes, while it is mostly close to RO membranes. To compare the PEM growth under different conditions, ellipsometric thicknesses of PEMs on model surfaces along with hydraulic resistances of the prepared membranes were obtained.

Fig. 4 compares the dry thicknesses of adsorbed multilayers in two ionic strengths. After 13 layers, the PEM prepared at the lower ionic strength is about 2.3 times thinner than its counterpart. When polyelectrolyte assembly takes place at a low ionic strength, the polymer chains are more extended, resulting in a thinner film. Increasing the ionic strength results in the coiling of the chains, which become less extended but increase the volume of a multilayer [78].The hydration of a PEM is a very important parameter to predict membrane performance, as it shows how open the layer structure is. The hydration ratio of PEMs consisting of 12 and 13 layers was determined from the measured wet and dry ellipsometric thicknesses as shown in Fig. 5. From this data, it is evident that PEMs prepared under lower ionic strength have a lower hydration, and therefore the layers will be expected to act as a denser membrane. This is also observed from the measured hydraulic resistance (Fig. 6). While the layers prepared under higher ionic strength are about 2.3 times thicker, the resistance is only 1.5 or 1.25 times higher. As the resistance linearly scales with the thickness of a layer, this must mean that the PEMs prepared at 5 mM are denser and are expected to have a better separation performance.

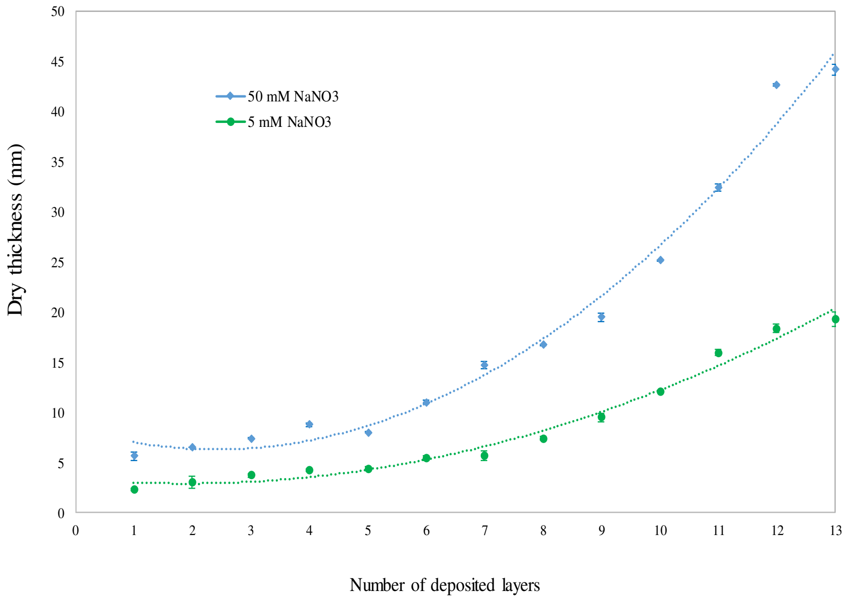


Fig. 4. Comparison of ellipsometric dry thicknesses of each deposited layer in two ionic strengths of 5 and 50 mM $NaNO_3$

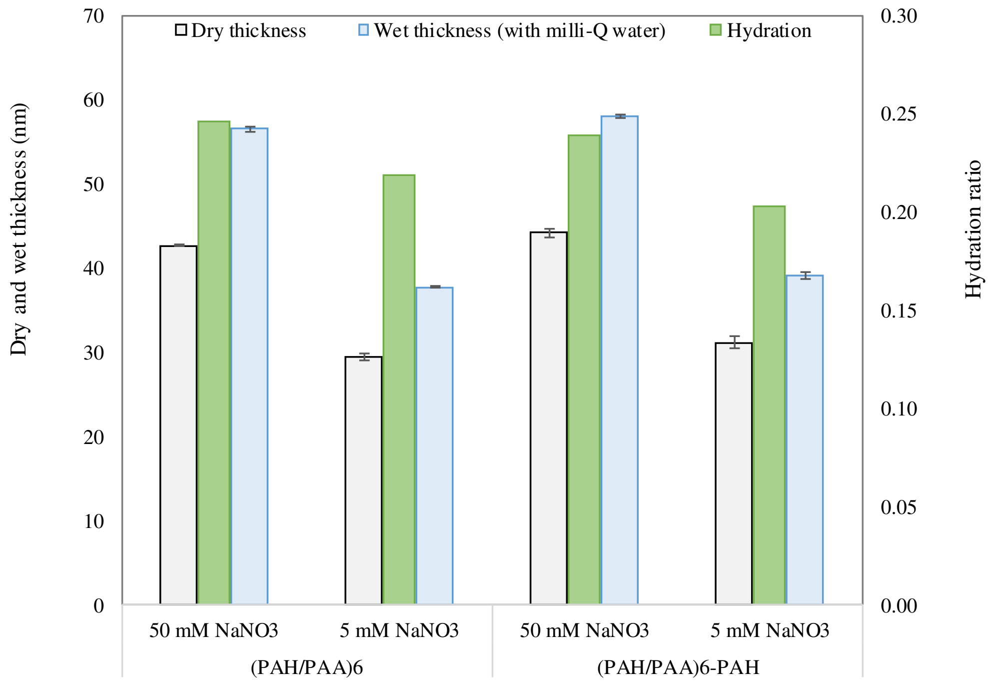


Fig. 5. Hydration, dry and wet thicknesses of membranes coated with $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers in two ionic strengths of 5 and 50 mM $NaNO_3$

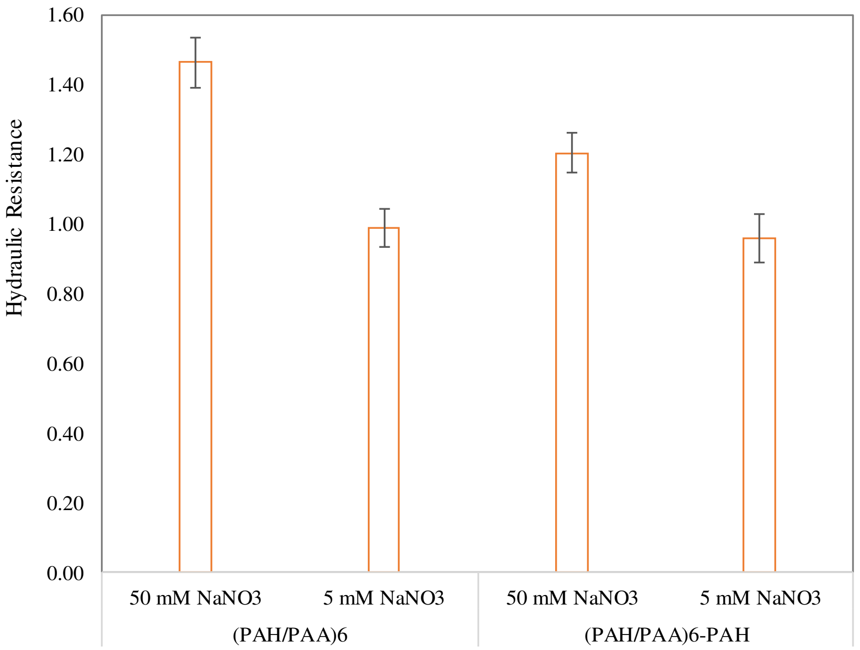


Fig. 6. Hydraulic resistance of membranes ($\times10^{14}m^{-1}$) coated with $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers in two ionic strengths of 5 and 50 mM $NaNO_3$

3.3.Contact angle of PEMs

In Fig. 3S in supplementary data, we clearly show variations in the water contact angle among both positively and negatively-charged PEMs with two ionic strengths of 5 and 50 mM $NaNO_3$. A decrease in contact angle was obtained after deposition of PEs (For instance 42.2 ± 1.6° and 42.4 ± 1.5° for silicon wafers and fibers, respectively coated with $(PAH/PAA)_6$ multilayers in 5 mM of $NaNO_3$) compared to bare HFS fiber that had a contact angle of 67.3 ± 0.3°. This phenomenon indicates that multilayers adsorption imparts hydrophilicity to the membrane surface. This finding is in accordance with study performed by Fadhillah F. et al., [35] who verified PSF membrane with PAH/PAA multilayers where the decrease in contact angle was resulted after 60 bilayers (35.48 ± 6.38°) compared to bare PSF substrate with a contact angle of 79.8°. Membranes with hydrophilic surfaces are less susceptible to fouling and their fouling is often reversible [79]. This is due to membrane hydration by water molecules which act as a barrier for potential foulants. Furthermore, these water soluble PEs form loops and tails which increase surface charge density. This rise in surface charge density contributes in the hydrophilicity of the membrane [80].There was a small amount of increase in the hydrophilicity of coated silicon wafers after a 48-hour immersion in synthetic wastewater containing target MPs. This reduction in contact angle did not change after re-immersing them in milli-Q water for another 48 hours, indicating that this change is irreversible. In a similar trend, contact angles of coated fibers declined a little after filtration of feed solution e.g. contact angles of clean and fouled fibers were 42.4 ± 1.5° and 36.3 ± 0.9°, respectively for $(PAH/PAA)_6$ multilayers coated with ionic strength of 5 mM $NaNO_3$. To the best of our knowledge, no literature data are available on contact angle changes after MPs rejection by NF membranes fabricated with PEMs, making comparison with the results of this study difficult.

3.4. Salts retention

PEM-based membranes, fabricated by the LbL assembly of PEs on hollow fiber support membranes, have been employed for ion rejection applications such as water softening or desalination [8]. In the category of NF membranes prepared with this method, membranes with high rejections of divalent ions and typically still significant rejections of monovalent ions have been studied [18]. Typically such membranes have two separation mechanisms (i) sieving in the case of species bigger than the membrane pore size and (ii) electric repulsion due to Donnan and dielectric effects in the case of charged species [81]. In the present work, the ion rejections were measured for three different ion pairs, namely NaCl, $CaCl_2$ and Na_2SO_4 at a concentration of 5 mM for all compounds. The results are presented in Fig. 7. For the both negatively and positively-charged membranes, the highest retention is obtained for the ion pair with the large SO_4^{2-} ion and a lower rejection is found for Ca^{2+} and Cl^- (the size order of the used ions is: $SO_4^{2-} > Ca^{2+} > Cl^- > Na^+$ [82]). On the other side, a higher SO_4^{2-} rejection is seen in negatively-charged membranes compared to the PAH-terminated membranes. This trend is also observed in the case of Ca^{2+} rejection, but with a lower difference between PAA and PAH-terminated membranes. This behavior, next to the fact that a little difference is observed between the membranes prepared at two ionic strengths, indicates that size exclusion followed by charge repulsion are the main mechanisms involved in salts retention by these membranes.

Fig. 7 also indicates that fibers coated with lower ionic strength have a somewhat higher salt rejection than membranes coated at higher ionic strength. For instance, Na_2SO_4 rejections of $(PAH/PAA)_6$

multilayers for ionic strengths of 5 and 50 mM $NaNO_3$ are 64.7 ± 3.5% and 59.0 ± 0.9%, respectively. This behavior comes from this fact that PEMs prepared under lower ionic strength have a more compact structure (lower hydration ratio illustrated in Fig. 5) with less open multilayers leading to better retention. The most important result shown in this figure, however, is that we have prepared a NF membrane with a very low ionic rejection, similar to the results of Ilyas et al. [27]. As mentioned, a low ion rejection would be highly beneficial; as such membranes would not create a brine waste stream. Still the low ion rejection is only relevant, if the MPs rejection of these membranes under conditions relevant to wastewater treatment, is high enough.

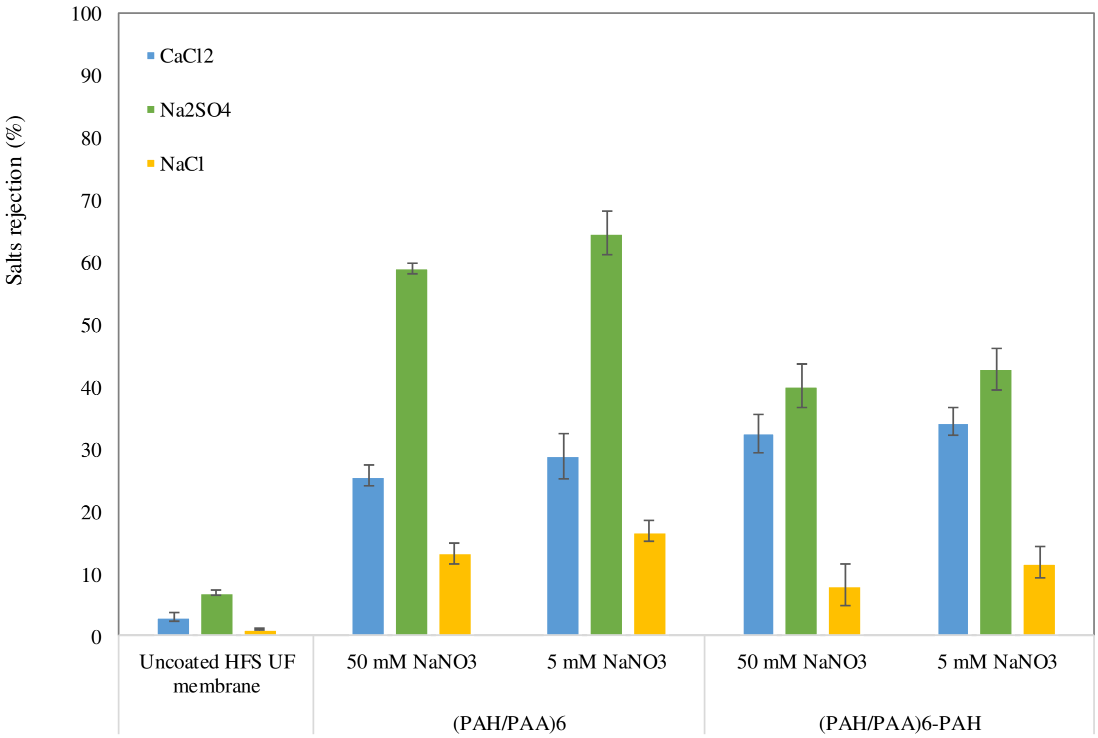


Fig. 7. Single salt rejection of HFS membranes coated with $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers in two ionic strengths under cross-flow filtration, at turbulent regime (Reynold number > 3500) and TMP of 1.5 bar.

3.5.MPs rejection

The apparent and steady-state retention of MPs from synthetic secondary-treated wastewater was examined under filtration circumstances similar to those for the salts rejection tests. Then, relationships between physicochemical properties of MPs and their rejections were evaluated.

3.5.1. Apparent MPs rejection

In Fig. 8a, we report on the apparent rejection of our four target MPs for PAA and PAH terminated PEM membranes, prepared at 5 and 50 mM $NaNO_3$. The apparent rejection of the hydrophobic 4n-Nonylphenol is the highest for all cases, followed by Diclofenac and then Ibuprofen and Naproxen. Of the membranes, the PAA terminated membranes perform better than the PAH terminated membranes.

This effect was also observed by Ilyas et al. [27] and was attributed to PAA terminated layers being more dense in nature. Another way to densify the membrane is by lowering the ionic strength of preparation, as also discussed in section 3.2. In apparent rejection, adsorption of MPs to the membrane can significantly affect the results. That means that affinity between the membrane and the MPs can be a crucial parameter. We investigated the connection between the rejection and some of the molecular properties of the MPs (Fig. 9 and Fig 4S in supplementary data). In this matter, a linear increase ($R^2 \geq 0.9$) between hydrophobicity (log D) and apparent rejection of all MPs was observed (Fig. 9). Additionally, no strong relation was found between the apparent rejection of MPs and their correspondent molecular weight and molecular sizes (molecular volume and molar volume) (Fig. 4S in supplementary data). This gives a strong indication that affinity dominates the apparent rejection, with more hydrophobic MPs adsorbing more to the membrane surface. This can be due to the PEM layer, but more likely the adsorption takes place to the more hydrophobic PES support membrane.

3.5.2. Steady-state MPs rejection

In comparison with apparent rejection, the steady state rejections are lower for all investigated membranes (Fig 8b). After reaching to steady-state condition, the membrane does not take up any MPs by adsorption, and other rejection mechanisms become dominant. This reduction is the most severe for the hydrophobic 4n-Nonylphenol (e.g. from 90.7 ± 0.1% to 70.1 ± 2.3% for 5mM of $NaNO_3$ and $(PAH/PAA)_6$ multilayers), and is less notable for hydrophilic compounds. Consequently, in line with the findings of V. Yangali-Quintanilla et al. [59], we are not able to consider hydrophobic adsorption of MPs into the membrane surface as a long term rejection mechanism because diffusion through the membrane occurs over the time causing retention decadence after saturation of the membrane [59].

When comparing our prepared membranes, we again find that the membrane prepared at 5mM and terminated with PAA outperforms the other membranes, although the effect is relatively small. The separation layer of this membrane is less hydrated compared to the others. In Fig. 8b we also show the rejection performance of the both $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers prepared under two ionic strengths is in accordance with those observed for the salt rejection. On one hand, the rejection performance of the membranes prepared at 5mM $NaNO_3$ is still somewhat higher for all MPs as a result of lower hydration compared with its counterpart described in subclause 3.2. For instance, rejection of Diclofenac for $(PAH/PAA)_6$ multilayers was 76.9 ± 1.1% versus 65.8 ± 1.2% for 5 and 50 mM $NaNO_3$, respectively. On the other side, in the case of the PAA-terminated PEMs, rejection mechanism of charge repulsion observed for negatively-charged MPs as though these negative-surface membranes showed about 32, 24 and 20% of higher retention for Diclofenac, Naproxen and Ibuprofen, respectively than PAH-terminated PEMs for ionic strength of 5 mM $NaNO_3$. This evidence is what we saw in the case of SO_4^{-2} rejection by negatively-charged membrane. The higher rejection even also occurred for neutral 4n-Nonylphenol probably as a result of more-dense surface of PAA-terminated PEMs compared with PAH-terminated ones. As there is no charge involved in the rejection of 4n-Nonylphenol, we believe that its steady-state rejection is fundamentally based on size exclusion and still hydrophobic adsorption. Jermann D. et al., [83] indicated that Ibuprofen (up to 25%) and Estradiol (up to 80%) can be removed in hydrophobic UF membranes via adsorption onto membrane polymers, as well as interaction with natural organic matter in wastewater. Furthermore, it seems that long-shaped molecular geometry of 4n-Nonylphenol should be also taken into account in the retention adequacy since it can easily pass through the membrane's pores.

Relationship between steady-state rejection of MPs and their relevant molecular weights (Fig. 10) represent that compounds of larger molecular weights are relatively better rejected even though the R-squared values of these linear curves are not gratifying. Meanwhile, as shown in Fig. 5S in supplementary data, parameters of log D, molecular and molar volume did not show striking correlation with steady-state rejection of all MPs. These results are in full agreement with the outcomes of Van der Bruggen et al. [84] who concluded that molecular weight can be a convenient representative of NF performance for retention of a series of organic molecules (molecular weight of 32 to 697 $g.mol^{-1}$ and stokes diameter of 0.51 to 2.65 nm) compared with other molecular sizes.

In addition, as plotted in Fig. 11, we could also find a good correlation ($R^2 \geq 0.70$ - 0.97) between the steady-state rejection of charged MPs with their relevant MPA. Although the MPA was found as a better surrogate parameter in comparison to molecular weight, we do believe that much more research needs to be done to understand the MPs rejection by LbL-made NF membranes. In the case of commercial membranes, Takahiro Fujioka et al.[85] reported that the rejection of charged MPs is high (over 90%) by hollow fiber cellulose triacetate RO membranes when the MPA of the compounds is over 35 $Å^2$ like this study. Conversely, there was not a strong correlation between the rejection of charged MPs and their MPA by the ceramic NF membranes in the observations of Takahiro Fujioka et al. [44]. Kiso et al. [86], who investigated the effect of molecular shape on rejection of uncharged organic compounds, concluded that molecular width is a major factor controlling solute permeation in NF membranes. Similarly, Madsen and Søgaard [87] obtained the best relationships between the pesticides rejection by NF membranes and their molecular width. Hence, it seems that spatial dimensions that determine the movement and rotation of the molecules outperform the molecular weights in the rejection behavior of the membranes. Having a look at the 4n-Nonylphenol's molecular shape (Table 2) shows the long-shaped geometry of this molecule should be taken into account in the retention adequacy since it could easily pass through the membrane's pores.

3.5.3. Comparison of LbL-made NF membranes with commercial NF membranes in salts and MPs removal

When we now combine the data from Figs. 7 and 8b, we find that we have indeed prepared a membrane (PAA-terminated PEMs, prepared at 5 mM $NaNO_3$) with a very reasonable removal of MPs (around 45-80%) under relevant conditions for wastewater treatment, with and a very low ionic rejections (nearly

17% NaCl). It becomes clear how unique this membrane is when we compare our results to commercial NF membranes that have been applied to MPs removal. In Fig. 12, we compare the rejection of target MPs and NaCl simultaneously from commercial NF membranes found in literature and our best LbL-made NF membranes. More details about the type of feed, membrane and operational conditions are given in Table 1S in supplementary data. This data shows clearly that commercial NF membranes reject both MPs and salts to a great extent while the membranes prepared in this study rejected salts only slightly and MPs considerably. For example, commercial NF membranes could retain NaCl and Diclofenac up to 70-90% and 99-100%, respectively while these rejections have occurred by 16.8 ± 1.6% and 76.9 ± 1.1%, respectively for our PEMs. Thus, a big advantage of our LbL-made NF membrane is that it could be used for MPs removal without producing a salty concentrate. Compared to the commercial membranes, that have been optimized towards high Donnan and Di-electric exclusion, we believe that size exclusion is the dominant mechanism for MP removal with our LbL based membranes. Still, the exact separation mechanism will need to be studied in much more detail in the future. We strongly expect that with further optimization, for example by coating at even lower ionic strengths, that even higher MPs removals can be attained at still low NaCl rejections. This makes this type of membrane very interesting for use as a tertiary treatment step for wastewater treatment plants, of which the concentrate can be treated in a bioreactor as discussed in the introduction. Moreover, as the salt balance of the effluent will not be changed dramatically after passing through these PEMs-based membranes, the effluent could be used for the irrigation of agricultural crops that are sensitive to salinity balance of the water used [88,89].

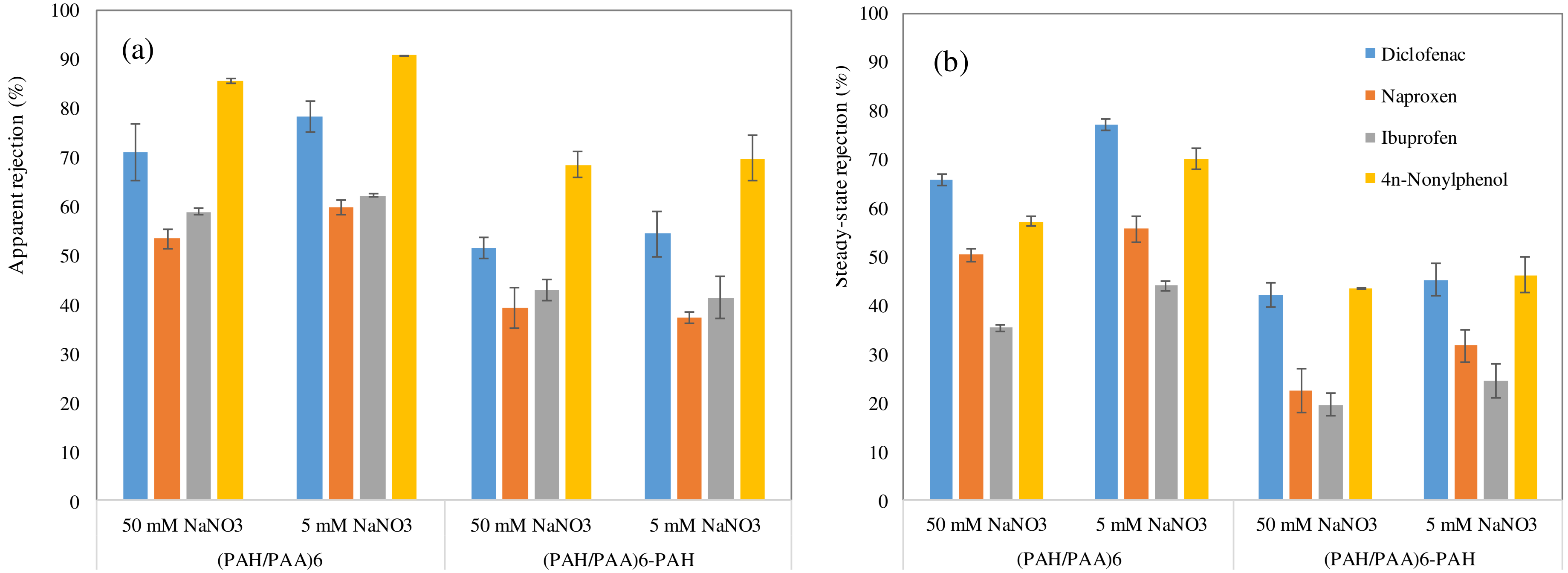


Fig. 8. Apparent (a) and steady-state rejection (b) of MPs in membranes coated with $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers (pH: 6/6 for both PEs) in two ionic strengths of 5 and 50 mM $NaNO_3$

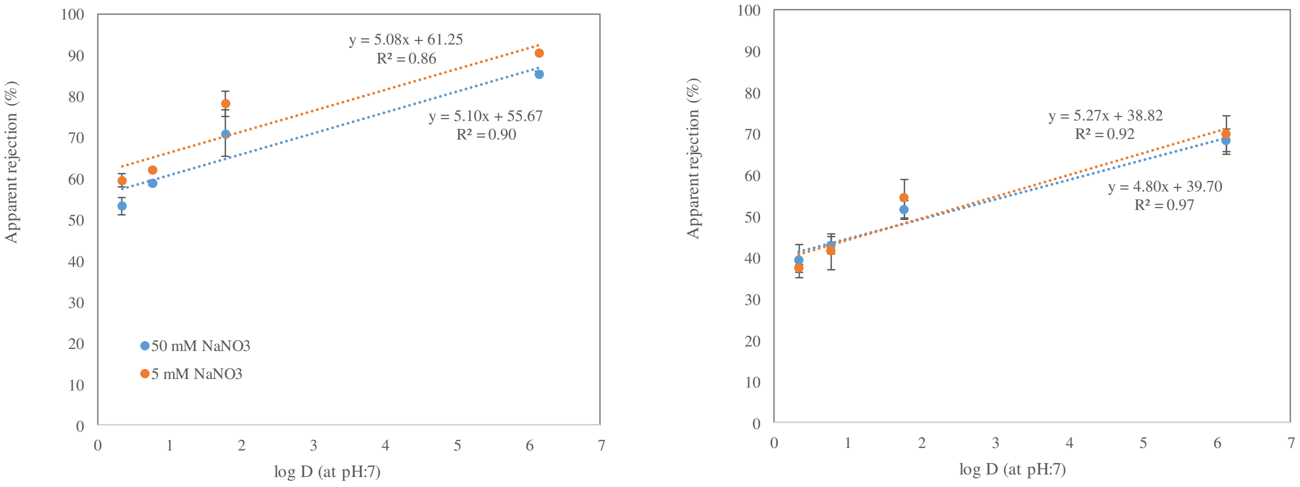


Fig. 9. The correlation between apparent rejection and hydrophobicity of MPs (Left and right figures are related to $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers, respectively).

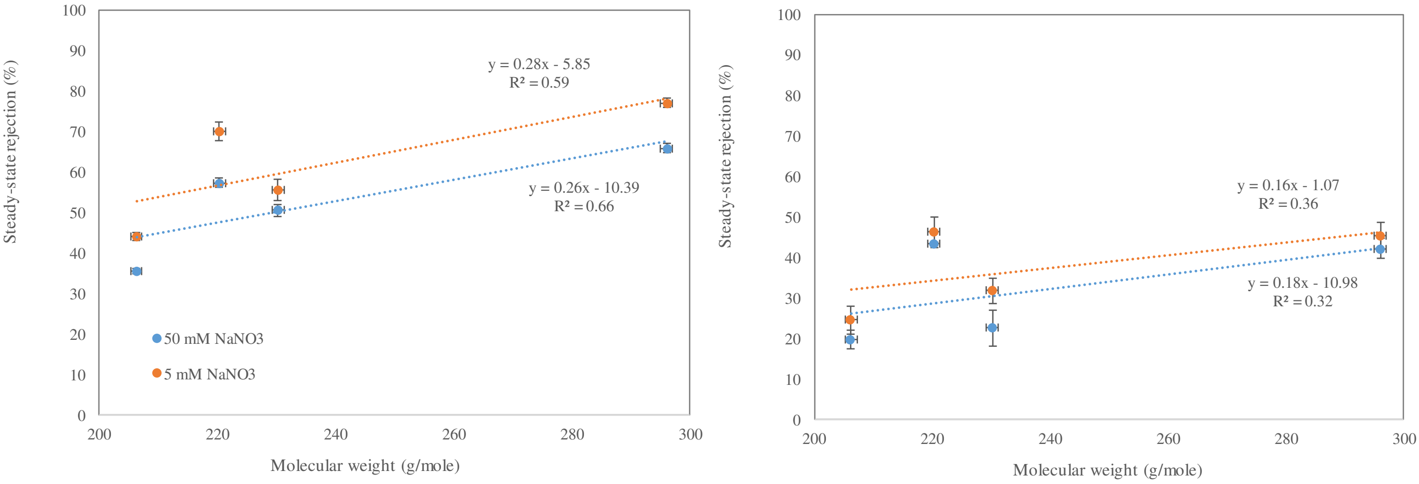


Fig. 10. The correlation between steady-state rejection and molecular weight of MPs (Left and right figures are related to $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers, respectively).

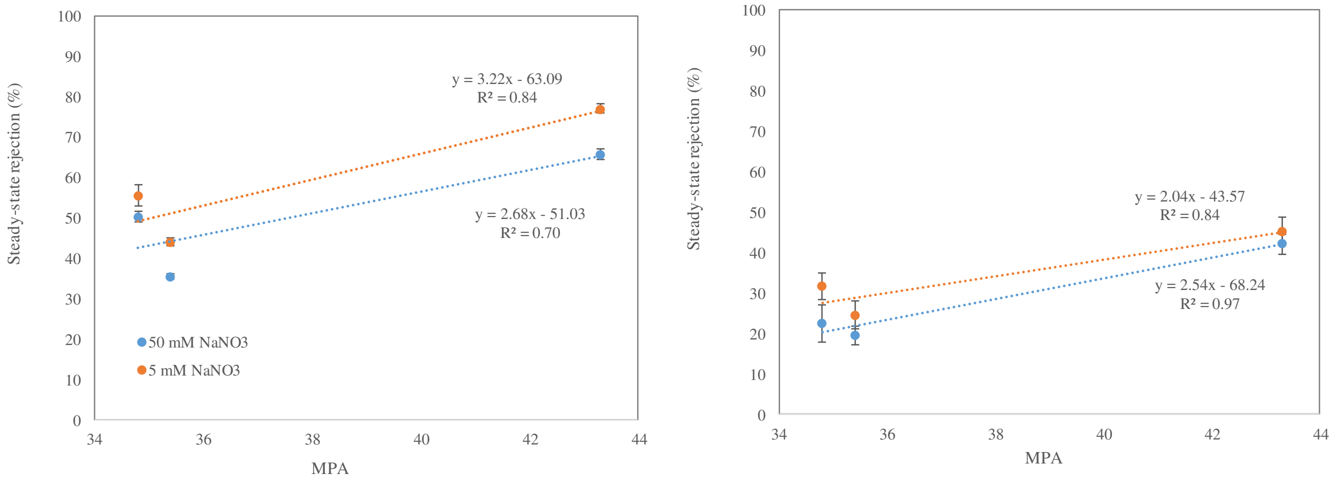


Fig. 11. The correlation between steady-state rejection and MPA ($Å^2$) of charged MPs (Left and right figures are related to $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers, respectively).

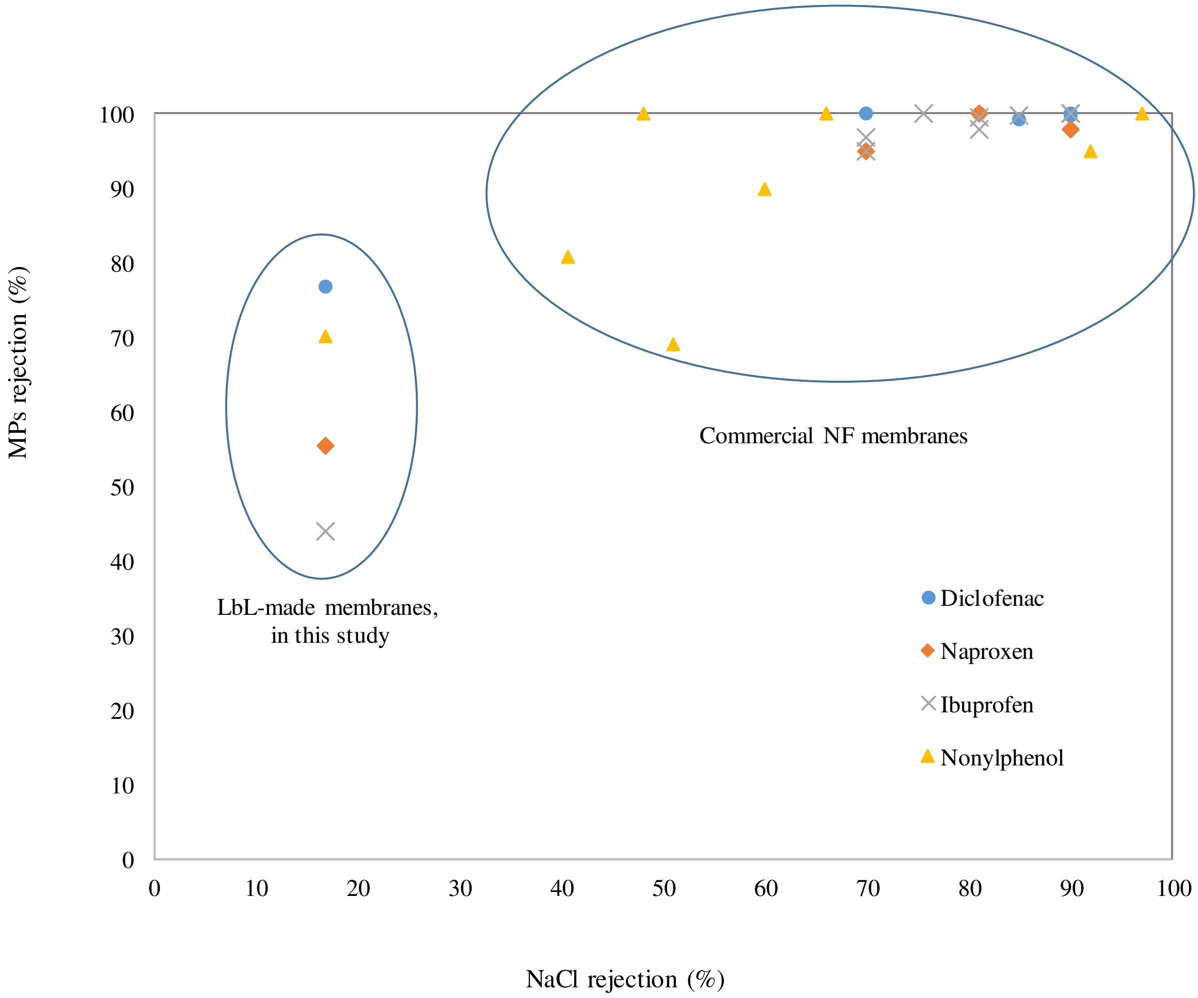


Fig. 12. Simultaneous rejection of target MPs and NaCl using commercial NF membranes found in literature (Table 1S in supplementary data), and LbL-based NF membranes made with (PAH/PAA)$_6$ multilayers prepared in ionic strength of 5 mM $NaNO_3$.

4. Conclusion

The scientific community is currently faced with the important challenge of MPs accumulation in aquatic environments. For this reason, various tertiary treatment methods are proposed to efficiently remove MPs from the wastewater effluent. In the present work, we provide further insights into the key parameters involved in apparent and steady-state rejections of MPs by NF membranes made with LbL adsorption of weak PEs on the surface of hollow fiber UF membrane. In addition, the effect of ionic strengths on the properties of PEMs was studied as this parameter determines the charge compensation of the PEs in the multilayer [49] and thereby the hydration and the effective pore size of the membrane. Here, we prove that PEMs prepared in lower ionic strength and terminated with PAA are more efficient in salts and MPs removal as they were found to be thinner and less open. We also demonstrate that it is possible to achieve good MPs rejections at realistic wastewater treatment conditions, combined with low ionic rejections. Lower rejection of salts will be much more favorable for biological treatment of the retentate stream. In addition, these membranes do not significantly disturb the salinity balance of the effluent, making the filtered effluent much more appropriate for use, for example, irrigation water. Considering these capabilities, low ion retentions and high MPs retentions would possibly enable these membranes to outperform currently available commercial NF membranes for MPs removal from municipals wastewater effluents.

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Section S1: Detrimental levels of the salinity on the performance of activated sludge reactors

High salinity of the concentrate stream produced in NF membranes will lead to some difficulties in biological treatment processes. In this type of treatment, conflicting reports on the influence of salt (NaCl) on the performance of biological treatment processes exist.

High salinity effluents are those with salt concentrations above 1% (10 g/L NaCl) [1]. Increased salt concentrations influence physico-chemical and microbiological parameters, thereby hampering biological wastewater treatment [2]. High salinity can cause high osmotic stress or the inhibition of the reaction pathways in the organic degradation process. In addition, high salt content induces cell lysis, which causes increased effluent solids [3]. Most of the microorganisms involved in wastewater treatment process are non-halophilic, and can tolerate salt concentrations up to 10 g/L without applying any pre-acclimatization step [4]. When an acclimatization step is applied, microorganisms are able to tolerate NaCl concentrations as high as 30 g/L [5]. Here, we bring a snapshot of what researchers have found about detrimental levels of the salinity on the biological performance.

The effect of high salinity on the performance of trickling filters and rotating biological contactors was investigated by Kargi and Uygur [6]. The results indicated that the efficiency of COD removal decrease significantly up to 50% with the increase in salt content above 2% (20 g/L NaCl).

Kargi and Dincer [7], worked on the issue of salinity effects on the nitrification in conventional activated sludge systems, reported that a critical salinity concentration of approximately 1–2% (w/w) exists at which the mechanism governing bacteria aggregation and stability of sludge flocs changes. Moreover, as the concentration of salinity exceeds than 1-2%, the tendency of bacteria aggregation decreases and this interrupts in the formation process of sludge flocs [8].

It has been proved that microorganisms, involved in the nitrification and denitrification processes, are able to degrade a wide range of MPs [9]. Nitrification and denitrification processes are also susceptible to inhibition by the salinity. For instance, in the study of Kargi and Dincer [7], *Nitrobacter* was more adversely affected by high salinity levels (above 2%) than *Nitrosomonas*, resulting in the accumulation of nitrite in the effluent. Furthermore, Panswad and Anan [10] obtained a 15% reduction in the total nitrogen removal and also a 21% reduction in the COD removal in a nitrifying activated sludge system, when the wastewater's NaCl concentration was increased from 20 g/L to 30 g/L. The efficiency of activated sludge systems in micropollutants removal can be consequently interrupted by the high salinity.

During the activated sludge process, Micropollutants degradation can be dramatically enhanced via extracted enzymes from microorganisms [11]. The shock effects of different salinities on a non-halophilic activated sludge were examined by Linaric et al. [2]. In their study, the presence of only 10 g/L NaCl caused a 50% reduction in the enzymatic activity of Amylase, Lipase and Protease. As a result, efficacious enzymatic degradation of micropollutants will be damaged in high levels of salinity.

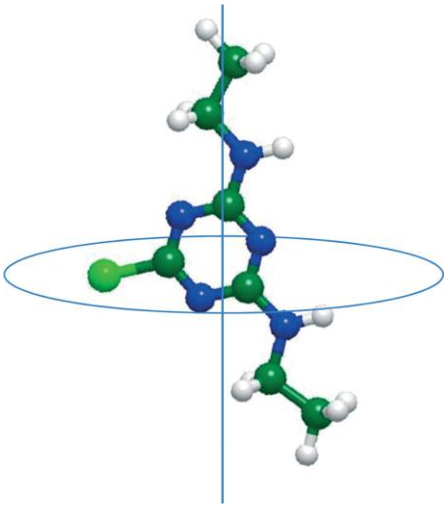

Fig. 1S. Schematic figure of the minimum projection area. The line perpendicular to the circular disk represents the center axis of the minimum projection area (adapted from [12,13]).

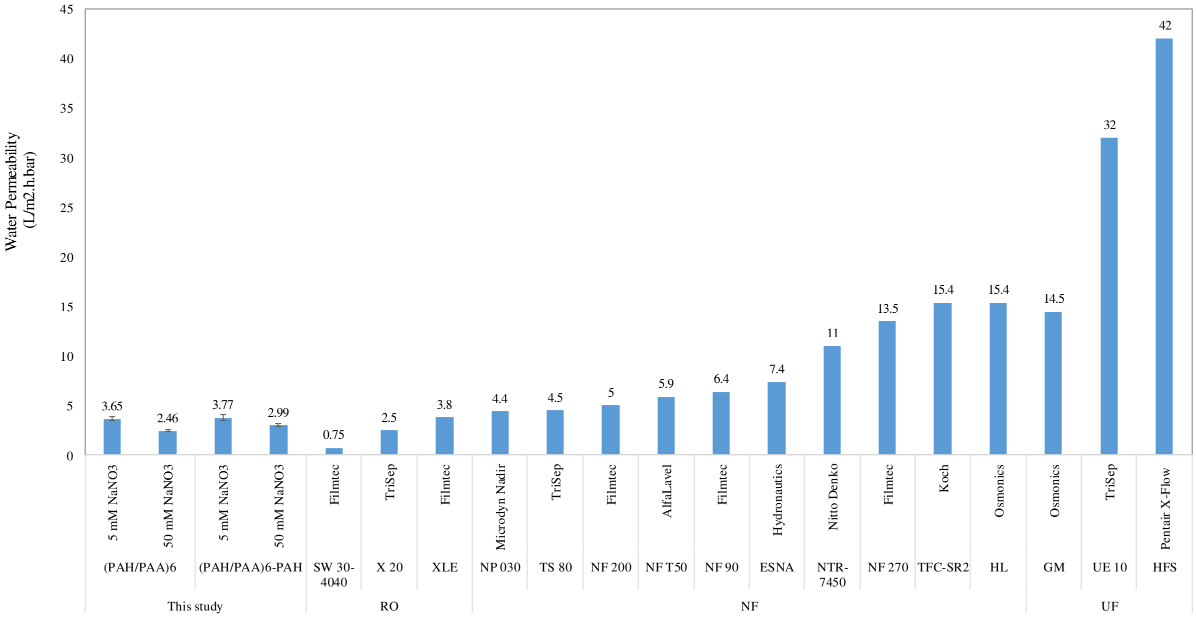

Fig. 2S. Comparison of pure water permeability of the coated HFS membranes in this study with the commercial UF, NF and RO membranes found in literature [14–19]

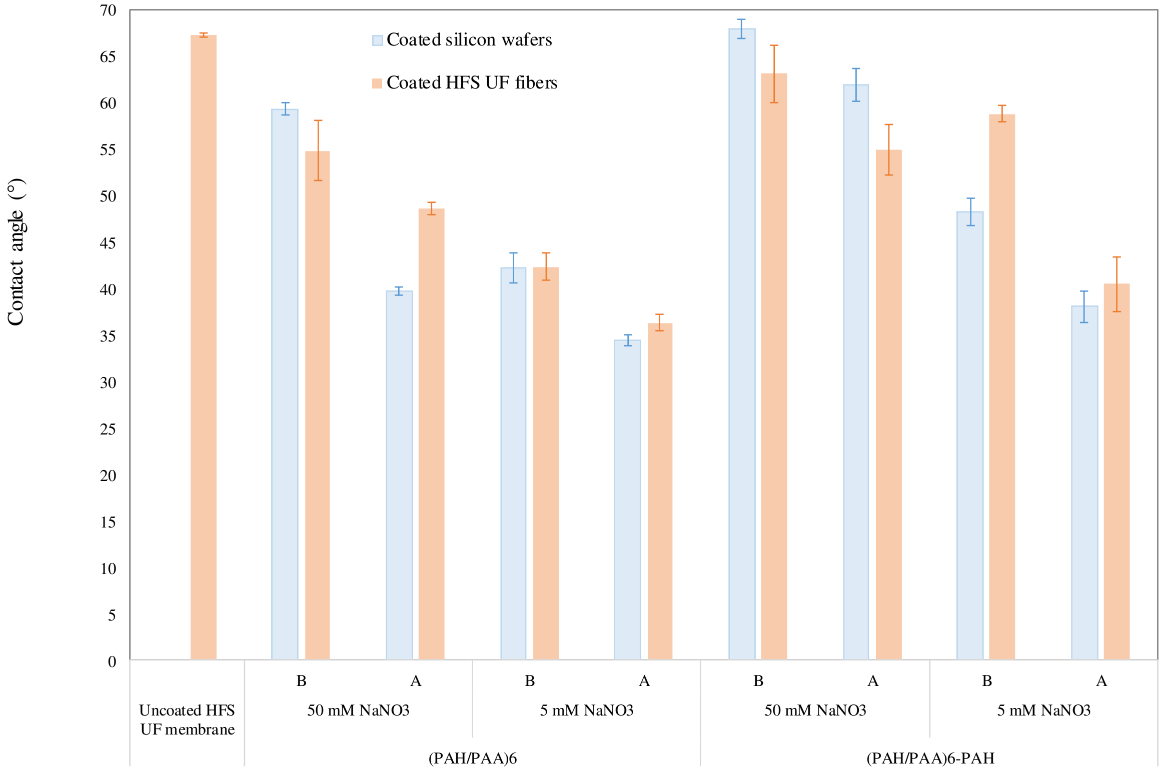

Fig. 3S. Hydrophilicity changes of wafers/membranes coated with (PAH/PAA)$_6$ and (PAH/PAA)$_6$-PAH multilayers (pH: 6/6 for both PEs) in two ionic strengths of 5 and 50 mM $NaNO_3$, monitored with contact angle measurement.

(For silicon wafers: B: before immersing and A: after immersing of coated wafers in feed solution containing MPs., and for membranes: B: before filtration process and A: after filtration process of feed solution containing MPs by coated membranes)

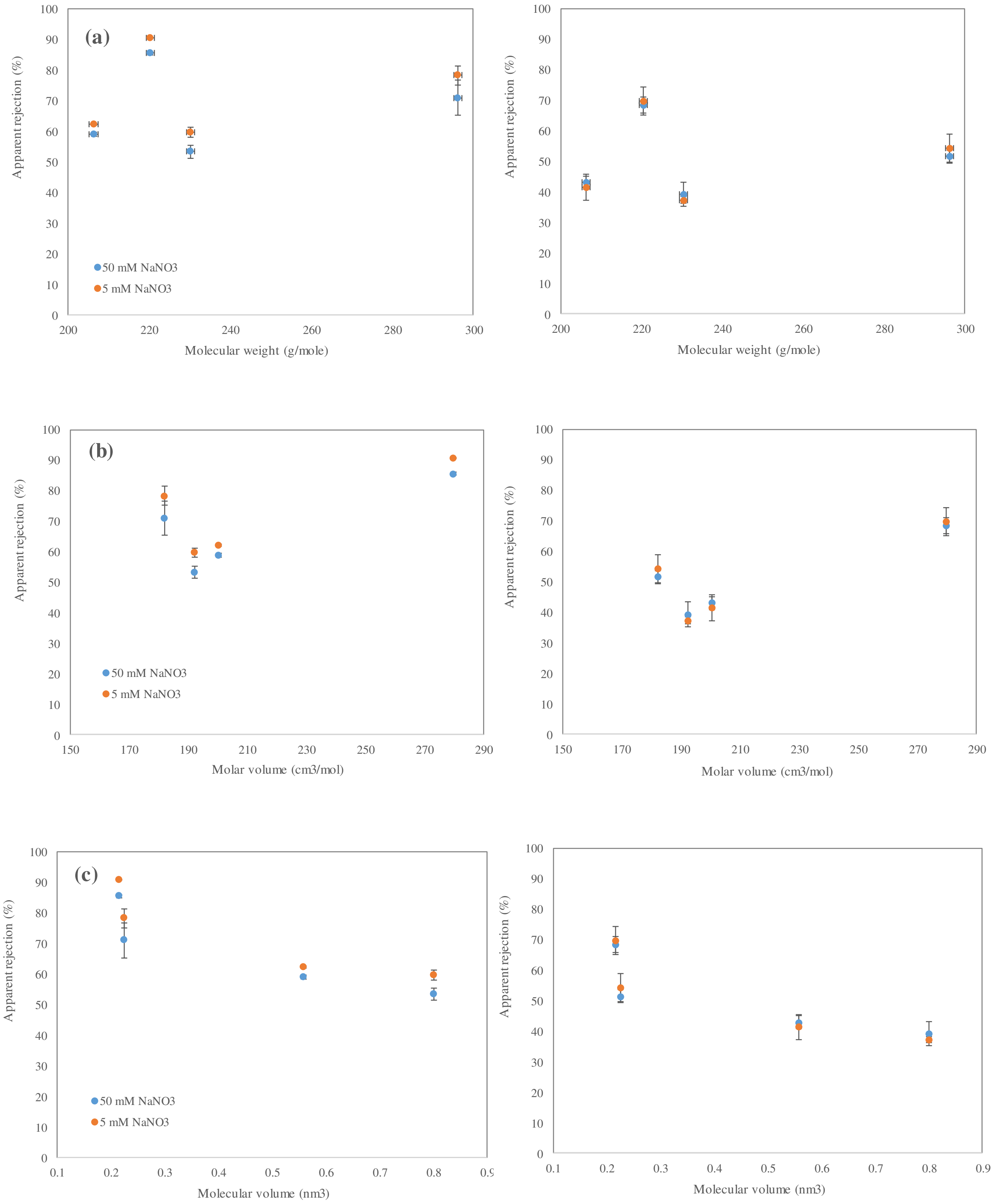

Fig. 4S. The weak correlation between apparent rejection and MPs properties: (a) molecular weight, (b) molar volume and (c) molecular volume (Left and right figures are related to $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers, respectively).

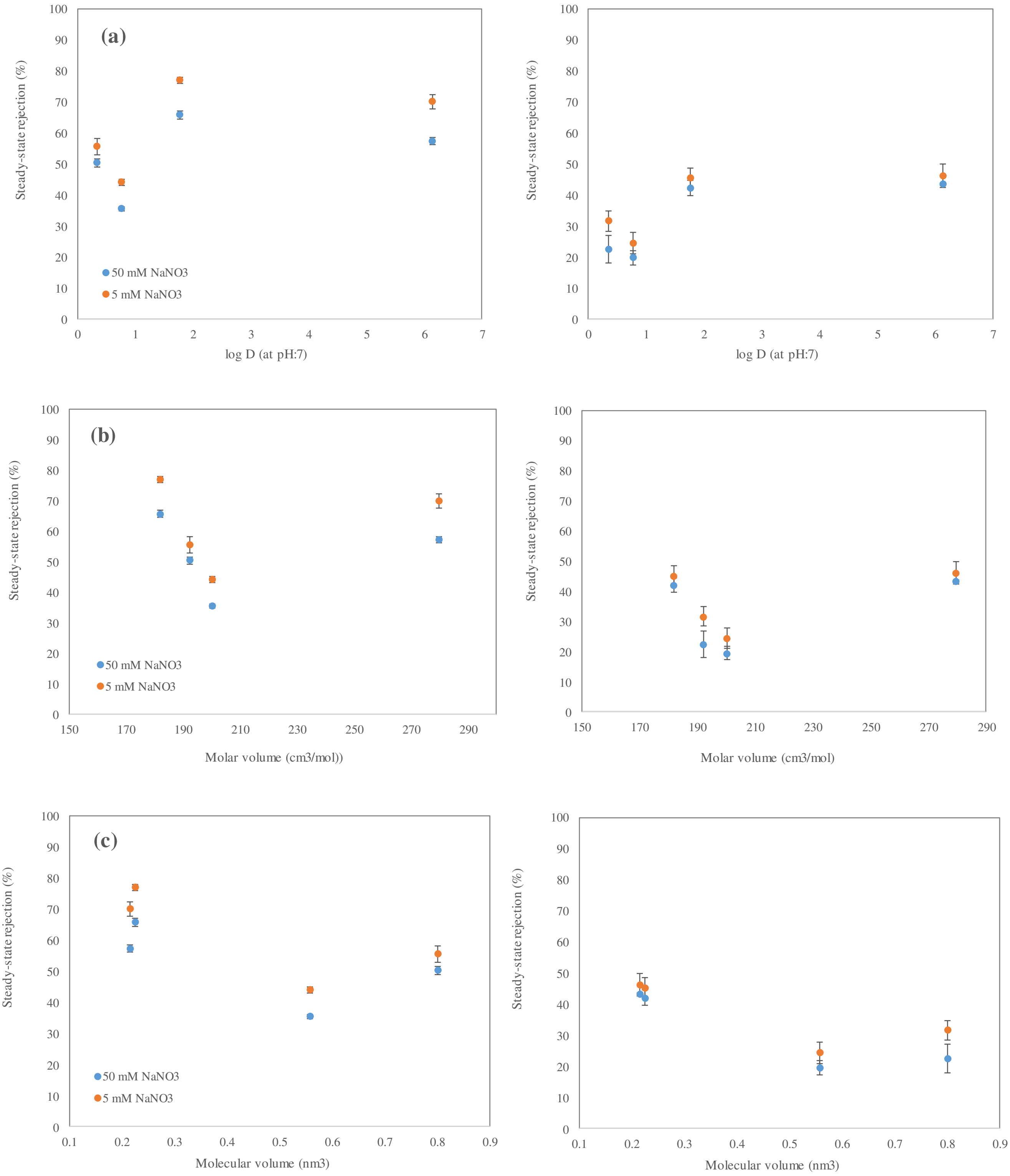

Fig. 5S. The weak correlation between steady-state rejection and MPs properties: (a) log D, (b) molar volume and (c) molecular volume (Left and right figures are related to $(PAH/PAA)_6$ and $(PAH/PAA)_6$-PAH multilayers, respectively).

Table 1S. Rejection of target MPs and salts using available high-efficient commercial NF membranes found in literature

Compound	Type of Feed solution	Aim of the study	Brand name and operation of commercial NF	Concentration in Feed solution (µg/L)	Rejection rate (%)	Salts rejection (%)	References
Diclofenac	River water	Influence of electrostatic interactions on the MPs rejection with NF	TS-80 (TMP: 5 bar, cross-flow velocity: 0.2 m/s)	5	99% at both 10 and 80% recovery	MgSO$_4$: 99%	[20]
	Groundwater	Investigation of MPs removal in a full-scale drinking water treatment plant fed with groundwater	NF90 (TMP: 6 kg/cm^2, Operating Flux: 22.9 L.m^{-2}.h^{-1})	0.05	99.90%	NaCl: 85-95%., and MgSO$_4$: 97%	[21]
	River water	Impact of different types of pretreatments on membrane fouling in rejection of MPs	TS-80 (Feed pressure: 5 bar, cross-flow velocity: 0.2 m/s)	2	89.2% for clean and 89.9% for fouled membrane with river water., 96.3% for fouled membrane with river water pretreated with a fluidized anionic ion exchange., and 93.2% for river water pretreated with UF.	MgSO$_4$: 99%	[22]
			Desal HL (Feed pressure: 5 bar, cross-flow velocity: 0.2 m/s)		86.8% for clean and 91.5% for fouled membrane with river water., 94.7% for fouled membrane with river water pretreated with a fluidized anionic ion exchange., and 91.8% for river water pretreated with UF.	MgSO$_4$: 98%	
	Municipal wastewater pre-treated with membrane bioreactor	Trace contaminant control and fouling mitigation in NF for municipal wastewater reclamation	NE 90, Woongjin Chemical Corporation (Retentate flux: 500 mL/min, Permeate pressure 413.7 kPa)	0.135	97%	Mg^{+2}: 94%., Ca^{+2}: 94%	[23]
	Municipal wastewater pre-treated with membrane bioreactor	Removal of organic matters and MPS using a hybrid MBR-NF system	NE 40, Woongjin Chemical Corporation (cross flow velocities: 6 µm/s)	0.138	86.1%	Mg^{+2}: 44.1%., Ca^{+2}: 46.3%	[24]
	MPs cocktail,dissolved in mother methanol stock solution	A comparison between ceramic and polymeric membranes for MPs removal	NF 90 (Cross-flow velocity: 0.43 m/s., permeate flux: 20 L/m^2 h).	50	around 100%	NaCl: 81%	[12]
	Synthetic secondary-treated municipal wastewater containing MPs	Investigation of MPs removal mechanisms using NF membranes	NF 90 (Pure-water permeability: 2.49 L/m^2 d kPa., J$_o$/K: 1.3., applied feed pressure: 414 kPa)	0.3	around 100%	NaCl: 90%	[25]
			NF 200 (Pure-water permeability: 1.20 L/m2 d kPa., J$_o$/K: 1.3., applied feed pressure: 345 kPa)			NaCl: 70%	
	Cocktail of MPs dissolved in synthetic secondary-treated wastewater	Tertiary treatment of negatively-charged MPs using LbL-made NF membrane	Surface-modified HFS UF membrane (TMP: 1.5 bar, Cross-flow velocity: 4.5 m/s)	0.5	76.98% ± 1.12 for NF membranes made by (PAH/PAA)$_6$ multilayers in pH: 6/6 for both PEs and ionic strength of 5 mM NaNO$_3$		Present study
Naproxen	River water	Impact of different types of pretreatments on membrane fouling in rejection of MPs	TS-80 (Feed pressure: 5 bar, cross-flow velocity: 0.2 m/s)	2	88.7% for clean and 88.7% for fould membrane with river water., 95.1% for fould membrane with river water pretreated with a fluidized anionic ion exchange., and 92.9% for river water pretreated with UF.	MgSO$_4$: 99%	[22]
			Desal HL (Feed pressure: 5 bar, cross-flow velocity: 0.2 m/s)		77.6% for clean and 87.8% for fould membrane with river water., 92.5% for fould membrane with river water pretreated with a fluidized anionic ion exchange., and 98.6% for river water pretreated with UF.	MgSO$_4$: 98%	

	Municipal wastewater pre-treated with membrane bioreactor	Trace contaminant control and fouling mitigation in NF for municipal wastewater reclamation	NE 90, Woongjin Chemical Corporation (Retentate flux: 500 mL/min, Permeate pressure 413.7 kPa)	0.38	78%	Mg^{+2}: 94%., Ca^{+2}: 94%	[23]
	Municipal wastewater pre-treated with membrane bioreactor	Removal of organic matters and MPS using a hybrid MBR-NF system	NE 40, Woongjin Chemical Corporation (cross flow velocities: 6 µm/s)	0.082	44.3	Mg^{+2}: 44.1%., Ca^{+2}: 46.3%	[24]
	MPs cocktail,dissolved in mother methanol stock solution	Comparison of clean and fould membranes in rejection of MPs	NF 90 (Cross-flow velocity: 0.38 - 0.50 cm/s, TMP: 276 - 482 kPa)	6,5 - 65	99% in clean and 96,5% in fould membrane (at recovery of 8%)	$MgSO_4$: 98% for clean and fouled membranes	[26]
			NF 200 (Cross-flow velocity: 0.38 - 0.50 cm/s, TMP: 276 - 482 kPa)		93,9% in clean and 79,7% in fould membrane (at recovery of 8%)	$MgSO_4$: 96% for clean and fouled membranes	
	MPs cocktail,dissolved in mother methanol stock solution	A comparison between ceramic and polymeric membranes for MPs removal	NF 90 (Cross-flow velocity: 0.43 m/s., permeate flux: 20 L/m2 h).	50	around 100%	NaCl: 81%	[12]
	Synthetic secondary-treated municipal wastewater containing MPs	Investigation of MPs removal mechanisms using NF membranes	NF 90 (Pure-water permeability: 2.49 L/m^2 d kPa., J_o/K: 1.3., applied feed pressure: 414 kPa)	0.3	98%	NaCl: 90%	[25]
			NF 200 (Pure-water permeability: 1.20 L/m2 d kPa., J_o/K: 1.3., applied feed pressure: 345 kPa)		95%	NaCl: 70%	
	Cocktail of MPs dissolved in synthetic secondary-treated wastewater	Tertiary treatment of negatively-charged MPs using LbL-made NF membrane	Surface-modified HFS UF membrane (TMP: 1.5 bar, Cross-flow velocity: 4.5 m/s)	2.5 µg/L	55.58% ± 2.63 for NF membranes made by $(PAH/PAA)_6$ multilayers in pH: 6/6 for both PEs and ionic strength of 5 mM $NaNO_3$		Present study
Ibuprofen	River water	Influence of electrostatic interactions on the MPs rejection with NF	TS-80 (TMP: 5 bar, cross-flow velocity: 0.2 m/s)	30	99% at 10 % recovery., 53% at 80 % recovery	$MgSO_4$: 99%	[20]
	River water	Impact of different types of pretreatments on membrane fouling in rejection of MPs	TS-80 (Feed pressure: 5 bar, cross-flow velocity: 0.2 m/s)	2	88.9% for clean and 92.1% for fould membrane with river water., 97.1% for fould membrane with river water pretreated with a fluidized anionic ion exchange., and 93.5% for river water pretreated with UF.	$MgSO_4$: 99%	[22]
			Desal HL (Feed pressure: 5 bar, cross-flow velocity: 0.2 m/s)		83.9% for clean and 90.2% for fould membrane with river water., 95.1% for fould membrane with river water pretreated with a fluidized anionic ion exchange., and 90.7% for river water pretreated with UF.	$MgSO_4$: 98%	
	MPs cocktail,dissolved in mother methanol stock solution	The role of membrane pore size and pH on the NF of MPs	NF90 (Cross-flow velocity: 30.4 cm/s, Permeate flux : 15 µm/s)	750	99.9% in pH values of 5, 7 and 9.	NaCl: 85%	[18]
			NF270 (Cross-flow velocity: 30.4 cm/s, Permeate flux : 15 µm/s)		89.6% in pH: 5., 98.5% in pH: 7 and 99.1% in pH: 9	NaCL: 40%	

		TFC-SR2 (Cross-flow velocity: 30.4 cm/s, Permeate flux : 15 µm/s)		36.2% in pH: 5., 64.4% in pH: 7 and 82.3% in pH: 9	NaCl: 9.8%	
MPs cocktail,dissolved in mother methanol stock solution	Comparison of clean and fouled membranes in rejection of MPs	NF 90 (Cross-flow velocity: 0.38 - 0.50 cm/s, TMP: 276 - 482 kPa)	6,5 - 65	99% in clean and 97,1% in fould membrane (at recovery of 8%)	$MgSO_4$: 98% for clean and fouled membranes	[26]
		NF 200 (Cross-flow velocity: 0.38 - 0.50 cm/s, TMP: 276 - 482 kPa)		99,8% in clean and 87,5% in fould membrane (at recovery of 8%)	$MgSO_4$: 96% for clean and fouled membranes	
Municipal wastewater pre-treated with membrane bioreactor	Removal of organic matters and MPS using a hybrid MBR-NF system	NE 40, 70 and 90 Woongjin Chemical Corporation (cross flow velocities: 6, 8 and 10.9 µm/s, respectively)	NE 40: 0.11., NE: 70: 0.07., NE 90: 0.05.	NE 40: 39.1%., NE 70: 27.3%., and NE 90: 96.9%.	Mg+2: 44.1%., Ca+2: 46.3%	[24]
MPs cocktail,dissolved in mother methanol stock solution	A comparison between ceramic and polymeric membranes for MPs removal	NF 90 (Cross-flow velocity: 0.43 m/s., permeate flux: 20 L/m^2 h).	50	around 98%	NaCl: 81%	[12]
MPs cocktail,dissolved in mother methanol stock solution	Pharmaceutical Retention Mechanisms by NF Membranes	NF 90 and NF 270 (Crossflow velocity: 30.4 cm/s, Permeate flux: 15 µm/s, temperature: 20 °C).	500	NF 90: around 100%., NF 270: around 98% (Both on solution pH: 7)	NaCl: around 90% at pH: 7 for NF 90., and around 50% for NF 270 at pH: 7	[27]
Natural water spiked with MPs	Investigation of NF membranes combined with advanced tertiary treatments for removal of MPs from natural waters	NF90 – 2540 (maximum pressure of 41 bar, maximum flow rate of 1.4 m^3/h)	13.9 – 15.3	94-97%	NaCl: 70%	[28]
Natural water spiked with MPs	Investigation of NF membranes combined with photo-Fenton treatment for removal of MPs from natural waters	NF90 – 2540 (maximum pressure of 41 bar, maximum flow rate of 1.4 m^3/h)	100	100%	Cl^-: 68-83%., SO_4^{-2}: 96-97%., Ca^{+2}: 93-94%., Mg^{+2}: 91-97%.	[29]
Secondary-treated municipal wastewater	Removal of pharmaceuticals from municipal wastewater by NF and solar photo-Fenton process.	NF90 – 2540 (maximum pressure of 41 bar, maximum flow rate of 1.4 m^3/h)	15	99-100%	Cl-: 75-87%., SO_4^{-2}: 99-100%., Ca^{+2}: 96-99%., Mg^{+2}: 97-98%.	[30]
Synthetic secondary-treated municipal wastewater containing MPs	Investigation of MPs removal mechanisms using NF membranes	NF 90 (Pure-water permeability: 2.49 L/m^2 d kPa., J_o/K: 1.3., applied feed pressure: 414 kPa)	0.3	around 100%	NaCl: 90%	[25]
		NF 200 (Pure-water permeability: 1.20 L/m2 d kPa., J_o/K: 1.3., applied feed pressure: 345 kPa)		95%	NaCl: 70%	
Cocktail of MPs dissolved in synthetic secondary-treated wastewater	Tertiary treatment of negatively-charged MPs using LbL-made NF membrane	Surface-modified HFS UF membrane (TMP: 1.5 bar, Cross-flow velocity: 4.5 m/s)	40 µg/L	44.04% ± 0.98 for NF membranes made by $(PAH/PAA)_6$ multilayers in pH: 6/6 for both PEs and ionic strength of 5 mM $NaNO_3$		Present study

Nonylphenol	MPs cocktail,dissolved in mother methanol stock solution	Assessment of the adsorption properties of the Alkylphenols on the membrane polymer in NF	NTR-729HF (applied pressure: 1 MPa)	1000	around 95%	NaCl : 92%	[31]
			NTR-7250 (applied pressure: 1 MPa)		around 90%	NaCl : 60%	
			NTR-7450 (applied pressure: 1 MPa)		around 69%	NaCl : 51%	
			NTR-7410 (applied pressure: 0.5MPa)		around 57%	NaCl : 15%	
	River water	NF rejection of natural organic matters, inoculated with Endocrine Disrupters	NF90 (at feed circulation flowrate of 0.6 L/min, and operating pressure of 30 bar)	359	100%	NaCl : 97%., Na_2SO_4 : 99%	[32]
			NF200 (at feed circulation flowrate of 0.6 L/min, and operating pressure of 30 bar)		100%	NaCl : 66%., Na_2SO_4 : 98%	
			NF270 (at feed circulation flowrate of 0.6 L/min, and operating pressure of 30 bar)		100%	NaCl : 48%., Na_2SO_4 : 94%	
	MPs cocktail,dissolved in mother methanol stock solution	Investigation of factors driving rejection of MPs in Nanofiltration	DS–5–DK tight NF (TMP: 2 MPa., solution filtered at 20°C)	40	80 ± 9.1%	NaCl: 40.6%	[33]
	Cocktail of MPs dissolved in synthetic secondary-treated wastewater	Tertiary treatment of negatively-charged MPs using LbL-made NF membrane	Surface-modified HFS UF membrane (TMP: 1.5 bar, Cross-flow velocity: 4.5 m/s)	7 µg/L	70.06% ± 2.31 for NF membranes made by $(PAH/PAA)_6$ multilayers in pH: 6/6 for both PEs and ionic strength of 5 mM $NaNO_3$		Present study

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CHAPTER (V)

Enhanced rejection of micropollutants in annealed polyelectrolyte multilayer (PEM)-based nanofiltration membranes

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S. Mehran Abtahi, Lisendra Marbelia, Abaynesh Yihdego Gebreyohannes, Pejman Ahmadiannamini, Claire Joannis Cassan, Claire Albasi, Wiebe M. de Vos, Ivo F.J. Vankelecom; “Micropollutant rejection of annealed polyelectrolyte multilayer based nanofiltration membranes for treatment of conventionally-treated municipal wastewater”., 2018.

Table of Contents

Abstract

The ever-increasing concentrations of micropollutants (MPs) found at the outlet of conventional wastewater treatments plants, is a serious environmental concern. Polyelectrolyte multilayer (PEM)-based nanofiltration (NF) membranes are seen as an attractive approach for MPs removal from wastewater effluents. In this work, PEMs of poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) were coated in a layer by layer (LbL) fashion on the surface of a polyacrylonitrile ultrafiltration support to obtain PEM-based NF membranes. The impact of PEM post-treatment, by applying salt and thermal annealing, was then investigated in terms of swelling, hydrophilicity, permeability, and ion rejection. While thermal annealing produced a more compact structure of PEM, it did not improve the ion rejection. Among the different salt concentrations examined for the salt-annealing process, the highest ion rejection was observed for $(PAH/PAA)_{15}$ membranes annealed in 100 mM $NaNO_3$, interestingly without any decrease in the water permeability. This membrane was studied for the rejection of four MPs including Diclofenac, Naproxen, 4n-Nonylphenol and Ibuprofen from synthetic secondary-treated wastewater, over a filtration time of 54 h. At an early stage of filtration, the membrane became more hydrophobic and a good correlation was found between the compounds hydrophobicity and their rejection. As the filtration continued until the membrane saturation, an increase in membranes hydrophilicity was observed. Hence, in the latter stage of filtration, the role of hydrophobic interactions faded-off and the role of molecular and spatial dimensions emerged instead in MPs rejection. To test the suitability of the membranes for the ease of cleaning and repeated use, the sacrificial PEMs and foulants were completely removed, followed by re-coating of PEMs on the cleaned membrane. The higher MPs rejection observed in salt-annealed membranes compared to the non-annealed counterparts (52-82% against 43-69%), accompanied with still low ion rejection, confirm the high potential of PEM post-treatment to achieve better performing PEM-based NF membranes.

1. Introduction

Micropollutants (MPs) are usually defined as “chemical compounds present at extremely low concentrations i.e. from $ng.L^{-1}$ to $\mu g.L^{-1}$ in the aquatic environment, and which, despite their low concentrations, can generate adverse effects for living organisms” [1]. Sources of MPs in the environment are diverse and many of those originate from mass-produced materials and commodities [2]. Today’s wastewater treatment plants were never designed to remove MPs from municipal wastewater, and as a consequence, MP accumulation in water bodies is increasing [3]. Over the last few years, this has created concerns due to their potentially harmful effects on the aquatic environment towards humans. This has persuaded researchers to develop, replace or improve the traditional wastewater treatment processes with novel process concepts [4]. Moreover, environmental regulations have been prepared to establish a framework for a water protection policy, for example within the EU. The first list of the EU’s environmental quality standards was published in 2008 under the Directive 2008/105/EC [5]. Five years later, the Directive 2013/39/EU was launched to update the previous documents [6]. This directive suggested the monitoring of 49 priority substances and 4 metals, and also proposed the first European Watch List which was then published in the Decision 2015/495/EU of 20 March 2015 [7]. This list comprises 17 organic compounds, named "contaminants of emerging concern (CECs)", unregulated pollutants, for which Union-wide monitoring data needs to be gathered for the purpose of supporting future prioritization exercises [8,9]. In addition to these compounds, there are many organic compounds that are still not listed in the European environmental regulations. According to the review paper of Sousa et al. [9], 28 organic MPs not listed in the European legislation, were found at concentrations above 500 ng. L^{-1}. Therefore, more research about occurrence and fate is needed for many of these emerging compounds.

Frequently used options to remove MPs from municipal wastewater effluents are: advanced oxidation processes [10,11], adsorption processes [3,12], and membrane filtrations [13]. Of these options, the high-pressure membrane processes nanofiltration (NF) and reverse osmosis (RO) are of great interest because of their higher removal rate, modularity and the possibility to integrate them with other systems [14]. For several applications, such as wastewater reclamation, the high energy consumption, high capital investments and operational costs of RO membranes has led to the preferred use of NF membranes over RO membranes [13,15]. In the last decade, the development of better performing NF membranes by surface modification techniques like grafting and interfacial polymerization is seen [16,17]. However, a more facile method for membrane modification, based on the self-assembly of oppositely-charged polyelectrolytes, has recently attracted a considerable attention [18]. In this so-called layer by layer (LbL) approach, the membrane is alternatively exposed to polycations and polyanions, to build polyelectrolyte multilayers (PEMs) of a controllable thickness [19]. Parameters, such as ionic strength, pH, charge density, and the type of polyelectrolytes, influence the LbL process and determine the final properties of the resulting PEMs [20–22]. Apart from that, the stability of the PEMs should be taken into account. For example, some PEMs are commonly highly swollen in water or even removed at higher salt concentration [23–26]. It has been demonstrated that thermal annealing (i.e. exposing the PEMs to heat for a defined period of time) of these weaker PEMs is able to lead to improved stability and robustness [23,27]. Heating of multilayers up to >200 °C caused an amidization reaction between the COO^- groups of poly (acrylic acid) (PAA) and the NH_3^+ groups of poly (allyl amine) hydrochloride (PAH) to form amide (NHCO) cross-links that rigidify the multilayers [28]. Despite the PEMs' stability through covalent crosslinks, the best arrangement of the multilayers would not be as separated layers but as complexes, where there is a maximal compensation between the negative and positive charges. PEMs' re-arrangement into denser complexes, could also provide more stability. Thermal annealing increases mobility of the polyelectrolytes allowing them to re-arrange in the films to find more convenient conformations [27]. In addition, post-treatment of the multilayers in salt solution, i.e. salt annealing, also brings significant variation of the multilayer structure [29]. The films can be annealed when they are immersed in salt solutions of higher concentrations [30]. According to Izumrudov and Sukhishvili [31], the stability of the multilayers composed of two polyacids poly(methacrylic acid) (PMAA) and PAA increased after annealing the PEMs in NaCl solutions [31]. Salt annealing enhances the mobility of polyelectrolyte chains that are otherwise “frozen” in place via numerous ion pairs cross-links [32]. Indeed, the salt ions compete with the polyelectrolyte ionic groups for binding sites. This competition can lead to dissociation of the polyelectrolyte ion pairs, and thus should increase the mobility of dissociated polyelectrolyte chains [33].

One of the major disadvantages of NF and RO based membrane processes is the production of a “concentrate” stream containing all retained compounds [34]. So far, some achievements have been reported for the treatment of membrane concentrates (mainly using advanced oxidation processes and adsorption with activated carbon [35,36]). These methods however have only been examined at laboratory or pilot-plant scales. Additionally, the high cost of these post-treatment processes can inhibit their wider implementation [37,38]. Thus, biological treatment of the concentrate has been lately taken into account by some scientists [39,40]. The main obstacle for a biological treatment of MP containing concentrates is their high salinities, i.e. above 1% (10 g.L^{-1} NaCl), that can cause high osmotic stress for the involved microorganisms or the inhibition of the reaction pathways in the organic degradation process [41,42]. Indeed, the efficiency of MPs biodegradation drastically declines due to the high salt content of the concentrate steam [43–45]. In view of this, our recent studies focused on the application of LbL-made NF membranes for tertiary treatment of municipal wastewater [46,47]. In these studies, two weak oppositely-charged polyelectrolytes, PAH and PAA (Fig. 1S in supplementary data) were coated onto hollow fiber dense ultrafiltration (UF) membranes by dip-coating [19]. In contrast to available commercial NF membranes that combine high salts and MPs rejection, a unique membrane with a low salt rejection (~17% for NaCl) and a very promising removal of MPs (~44 to 77%) was obtained [46]. This membrane could thus remove MPs without producing a highly saline concentrate stream that would otherwise disrupt its biological treatment. Moreover, it does not considerably change the salt balance of the effluent, making it an ideal effluent for the irrigation of agricultural crops that are sensitive to the salinity balance of the water used [48,49].

The aim of this investigation is to study the impact of thermal and salt-annealing processes on weak PEM-based membranes in terms of MPs removal from secondary-treated wastewater. PEMs composed of PAH and PAA were coated on the surface of flat-sheet polyacrylonitrile (PAN) UF membranes. The PEMs were then post-treated by thermal and/or salt annealing, and were carefully characterized before and after annealing by hydration ratio, hydrophobicity, permeability and ion rejection. Afterwards, the rejection behavior of the best membrane for the removal of four MPs (including 4n-Nonylphenol (listed in the Directive 2008/105/EC [5] and 2013/39/EU [6]), Diclofenac (listed in the Decision 2015/495/EU [7]), Naproxen and Ibuprofen (both not listed in the European legislations [9])) from synthetic secondary-treated wastewater was studied over the filtration time. As severe fouling would always be a large problem in the MP removal from wastewater, we additionally show that these membranes can be easily cleaned using a sacrificial layer approach. The fouled membranes were cleaned by a cleaning solution to release both the foulants and the sacrificial PEMs coating. The re-deposition of the same PEMs on the pre-rinsed membranes was subsequently performed.

2. Experimental

2.1.Chemicals

The polymer PAN (Mw = 150,000 Da) was obtained from Scientific Polymer Product Inc., USA. The solvent, dimethyl sulfoxide (DMSO) was purchased from Acros Organics, Belgium. Other chemicals including two weak polyelectrolytes (PAH with Mw = 15,000 $g.mol^{-1}$ and PAA with Mw = 15,000 $g.mol^{-1}$), all salts ($CaCl_2.2H_2O$, Na_2SO_4, NaCl, K_2HPO_4, $MgSO_4.7H_2O$, $NaNO_3$), peptone, meat extract and urea were obtained from Sigma–Aldrich. The main supplier of all analytical-grade MPs, with the physico-chemical properties given in our previous study [46], was also Sigma-Aldrich.

2.2. Synthetic wastewater

Synthetic secondary-treated municipal wastewater was prepared according to the "OECD Guideline for Testing of Chemicals" [50,51]. This media contained 50 ± 2 mg. L^{-1} of chemical oxygen demand (COD), 10 ± 1 $mg.L^{-1}$ of total nitrogen (TN) and 1 ± 0.1 mg $P\text{-}PO_4^{3-}.L^{-1}$. Mother stock solutions of MPs were separately prepared in highly pure methanol at a concentration of 1 $g.L^{-1}$, stored in 15-mL amber glass bottles and kept in a freezer (-18°C). Daughter stock solutions of each MP were then prepared separately in Milli-Q water from their individual mother stock solutions. An appropriate amount of each MP was subsequently added to the synthetic wastewater to reach to the target concentration of MPs in the feed. Here, as discussed in our previous study [46], the final concentrations of Diclofenac, Naproxen, Ibuprofen and 4n-Nonylphenol were 0.5, 2.5, 40 and 7 µg/L, respectively, based on available data in literature about concentration of target MPs in effluents of conventional municipal WWTPs.

2.3. COD, TN, and $P\text{-}PO_4^{3-}$ measurements

Feed samples were initially filtered through 0.70 µm glass fiber filters (VWR, 516-0348, France). The analysis was later carried out by means of HACH LANGE kits (LCI 500 for COD, LCK 341 for TN, LCK 304 for NH_3-N, and LCK 341 for $P\text{-}PO_4^{3}$) along with a DR3900 Benchtop VIS Spectrophotometer equipped with a HT200S oven (HACH LANGE, Germany). These parameters were measured in duplicate and the average values are reported.

2.4. Preparation of hydrolyzed PAN (PAN-H) membranes

According to the protocol described by Xianfeng Li et al. [52], PAN-H flat sheet membranes were prepared via the phase inversion method. In short, 15 wt% PAN was dissolved in DMSO overnight at ambient temperature. It was then degassed for 3h and the bubble-free solution was cast on the smooth surface of a non-woven polypropylene/polyethylene (PP/PE) support (Novatexx 2471, Freudenberg, Germany) by an automated casting machine (Automatic Film Applicator, Braive Instruments) at 2.25

cm.s^{-1} casting speed to form a 250 µm thick wet film. The solvent was allowed to evaporate for 60 S prior to immersing the film in demineralized water (as a non-solvent solution) for ~15 min. In order to provide the surface with a negative charge, membrane hydrolysis was performed i.e. PAN films were immersed in 10 wt% NaOH at 50°C for 40 min while stirring at 100 rpm. Under alkaline condition, part of the -CN groups are converted into COO^-. The resulting PAN-H membranes were then washed with tap water to remove the remaining NaOH, and were stirred overnight in demineralized water at ambient temperature, and finally stored in demineralized water for further use.

2.5.Attenuated Total Reflectance (ATR)-Fourier Transform Infrared Spectroscopy (FTIR)

ATR-FTIR was used to determine the functional groups present at the membrane surface, by collecting an infrared spectrum in the range 370-4000 cm^{-1} [53]. This method was used to confirm the hydrolysis of the PAN support into a negatively-charged membrane support (PAN-H). ATR-FTIR spectra of membranes were acquired using a spectrometer (Varian 670-IR, Varian Inc., USA) in absorbance mode. Two coupons per membrane were air-dried overnight prior to the measurements to minimize the effect of water. From each coupon, three points were selected and the average of absorbance values are reported.

2.6. Preparation of PEM-based membranes/silicon wafers

LbL deposition of oppositely-charged weak polyelectrolytes was performed by dip-coating [19]. The PAN-H membranes were first put into the background electrolyte solution (50 mM $NaNO_3$) for 15 min, in order to wash the pores [53]. Buildup of PEMs was then carried out by means of an automated dip-coating machine (HTML, Belgium) comprising four compartments: the 1st and 3rd compartments are for both polyelectrolytes and the 2nd and the 4th for rinsing solutions [54]. In a sequencial manner, PAN-H membranes were entirely immersed in a 0.1 g·L^{-1} polycation solution (PAH) containing 5 mM $NaNO_3$ at pH 6 and at ambient temperature. After 30 min, membranes were put in a rinsing solution containing only $NaNO_3$ with an ionic strength and a pH similar to that of the coating solution for 15 min to remove any loosely bound polymer chains. To form the first bilayer of PAH/PAA, the membranes were dipped for 30 min in a 0.1 g·L^{-1} polyanion solution (PAA) at pH 6 and an ionic strength of 5 mM $NaNO_3$ and rinsed again in a separate rinsing solution exactly as before. This pattern was repeated until the formation of the desired number of polycation/polyanion bilayers i.e. $(PAH/PAA)_n$ [55]. Selected PEM-based membranes were separately annealed in solutions of 50, 100 and 150 mM $NaNO_3$ for 20 h at room temperature [32,56]. The thermal annealing process was conducted by heating of some of the membranes to 60°C for 5 h [57] in order to impose chemical crosslinking between the amine group and the carboxylic acid of the PAH and PAA polyelectrolytes, respectively [58].

In order to measure the dry and wet thicknesses of adsorbed polyelectrolytes (section 2.7), the same deposition technique was also applied on the surface of silicon wafers, pre-treated by a 10-min plasma treatment using a low-pressure Plasma Etcher (JLS designs Ltd, UK), leading to a reproducible negative charge at the surface of all wafers. After coating, all samples were dried under a nitrogen stream prior to further measurements.

2.7. *Spectroscopic ellipsometry*

Dry and wet thicknesses of deposited multilayers on the surface of the plasma-treated silicon wafers were measured using an *in-situ* Rotating Compensator Spectroscopic Ellipsometer (M-2000X, J. A. Woollam Co, Inc.) operated in a wavelength range from 246–1000 nm at incident angle of 70°. The Cauchy model was used to fit to the ellipsometric parameters (Δ and ψ). The refractive index (n) was taken from independent measurements using a standard laboratory refractometer (Carl Zeiss). Data obtained on three parts of each wafer were reported as a mean dry thickness ± standard deviation. By using Milli-Q water, and a Woollam wet cell, the wet thickness of the multilayers was also measured three times for each wafer. By dry and wet thicknesses, the hydration ratio was determined by Eq. (1) [59,60], and denotes the fraction of water in the layer.

$$Hydration\,ratio = \frac{d_{swollen} - d_{dry}}{d_{swollen}} \tag{1}$$

2.8. *Contact Angle*

Optical contact angle measurements were performed using a Krüss goniometer (Drop Shape Analyzer DSA 10 Mk2) in order to investigate the membranes hydrophilicity. Sessile drops of 2 µl deionized water was used to measure the contact angle. These measurements were carried out at three locations per membrane coupon and the average and standard deviation are reported. The measurement was carried out five seconds after the bubble was placed on the surface of the membranes. The membranes' hydrophilicity was evaluated before, during and after filtration of the MP-bearing synthetic effluent. Clean and fouled membranes were dried for 24 h at room temperature (20°C) before the contact angle measurements.

2.9. *Membrane performance*

The performance of the PEM-based membranes was tested using a high-throughput dead-end filtration system (HTML, Belgium) containing 16 filtration cells with 3.14 cm^2 membrane area each. The system was pressurized with nitrogen (2 bar), and the feed solution was constantly stirred at 600 rpm to minimize concentration polarization. Before filtration tests, membranes were initially equilibrated by filtering deionized water until the permeate stream would remain constant.

2.9.1. *Water and solute permeability*

In order to calculate the permeate flux J, Eq. (2) was used, where V is the permeate flowrate ($L.h^{-1}$), A is the membrane area (m^2), t is the permeation time (h), and P is the applied pressure (bar). From each type of membranes, two coupons were selected and the average permeability with standard deviations are reported.

$$J = \frac{V}{A.t.P} \tag{2}$$

2.9.2. *Salts retention*

The concentrations of NaCl, Na_2SO_4 and Na_3PO_4 in the feed solutions were adjusted to 0.1 g. L^{-1} of each in mixed-salt solutions. To determine the anion concentrations, an ion chromatograph machine (Metrohm 883 Basic IC Plus, USA) equipped with an anion separation column (Metrosep A Supp 5 - 100/4.0, Metrohm, USA) and software MaglCnet 3.1 was used. The sample loop was 20 µL and a conductivity based detector was used. The chemical suppression was performed with 100 mM H_2SO_4 and a mobile phase of 5 mM Na_2CO_3/5 mM $NaHCO_3$ was applied at a flow rate of 1.0 ml.min^{-1}. Furthermore, single-salt solutions containing 0.1 g. L^{-1} of $CaCl_2$ were also prepared. The concentration of $CaCl_2$ was measured with a conductivity meter (Consort C3010, Belgium). Finally, the retention value R was calculated according to Eq. (3), where C_p and C_f are the solute concentration in the permeate and feed, respectively. Each measurement was performed in duplicate and the average values with standard deviations are reported.

$$R = \left(1 - \frac{C_p}{C_f}\right) \times 100 \tag{3}$$

2.9.3. *MPs retention and analysis*

In the case of wastewater filtration for MP retention, membrane compaction was first performed at 2 bar for 2 h using demineralized water. Subsequently, the MPs-bearing synthetic effluent was filtrated for 54 h in order to provide sufficient membrane saturation to ensure steady state rejections. During the filtration, permeate samples were collected after 2, 4, 7, 23, 27, 31, 46, 50 and 54 h.

For MP analysis, samples were shipped to the LaDrôme laboratory (France) in a freeze box for analysis within 24 h under the analyzing license of cofrac ESSAIS. A multi-detection procedure including Gas Chromatography (coupled with ECD/NPD mass spectrometry) and Liquid Chromatography (along with DAD, fluorescence, tandem mass spectrometry) was applied for all MPs with Limit of Quantification (LQ) of 0.01 µg/L for Diclofenac, Naproxen and Ibuprofen, and 0.04 µg/L for 4n-Nonylphenol. Each measurement was performed in duplicate and the average of rejections with standard deviations are reported.

2.10. *Cleaning protocol of the fouled membrane*

After filtration of MP-bearing wastewater for 54 h, a modified cleaning protocol adapted from Ilyas et al. [61] and Fujioka et al. [62] was applied in order to remove both the sacrificial PEMs and foulants. Ilyas et al. [61] have already concluded that (PAH/PAA) multilayers can act as sacrificial coatings allowing them to be easily cleaned. The fouled membrane was first rinsed with the rinsing solution (3 M $NaNO_3$, pH:3) in a dead-end mode at a low pressure (2 bar) for 180 min. Membrane samples were subsequently stored in a 50-mL glass beaker filled with the rinsing solution. This beaker was then immediately put in a simple water bath (at ~30°C) for overnight. The membrane was then washed with Milli-Q water to remove residual cleaning solution. Removal of the PEMs and foulants was investigated by comparing the permeability before and after rinsing to see if the permeability could be restored to that of the pristine uncoated membrane. Finally, re-deposition of the same multilayer of (PAH/PAA) was manually performed on the cleaned membrane and permeability was again measured. (Because of the small size of the membrane coupons already used for the filtration, we were not able to use the dip-coating machine. That is why coupons were re-coated by using beakers filled with polyelectrolyte and rinsing solutions under identical conditions as for the dip-coating machine).

3. Results and discussion

In first part of this section, the PEMs and the PEM-based membranes are characterized using ellipsometric measurements, ATR-FTIR analysis and the contact angle. The second part deals with the performance of the PEM-based membranes, in terms of the permeability, salt and MP retention and cleanability.

As described in the experimental section, PEMs were deposited on the surface of PAN-H membranes to form $(PAH/PAA)_{15}$ and $(PAH/PAA)_{15}$-PAH multilayers to ensure that the separating membrane is dense and free of defects. In addition, these PEMs were coated on the surface of plasma-treated silicon wafers with the same preparation method. Afterwards, post-treatment of the PEM-based membranes/wafers was immediately performed. According to these procedures, four categories of membranes/wafers were finally produced and tested: i) non-annealed, ii) thermally-annealed, iii) salt-annealed, and iv) salt and thermally-annealed PEMs.

3.1. Properties of PEMs

3.1.1. Ellipsometric measurements

The thickness and water content of PEMs are important parameters particularly when the membrane surface modification is combined with other post-treatments [57]. In this study, the hydration ratio of PEMs deposited on the surface of plasma-treated silicon wafers were obtained using dry and wet ellipsometric thicknesses. Both the dry and wet thickness of the multilayers generally increased after additional coating steps, while a decreasing hydration ratio is observed (Fig. 1a). The build-up of these multilayers, prepared at an ionic strength of 5 mM $NaNO_3$ and pH 6 for both polyelectrolytes, follows a typical linear growth pattern, which was also found in previous study [46]. These results were then compared with salt and/or thermally annealed multilayes (Fig. 1b to Fig. 1d). By applying thermal annealing (Fig. 1b), the wet and dry thickness of $(PAH/PAA)_{15}$ multilayers decreased, but the wet thickness to a much larger extend, indicating that PEMs became more compact and less hydrated by thermal annealing. Upon salt annealing at various salt concentrations presented in Fig. 1c, the dry thickness remained nearly unaffected by increasing the salt concentration, while a slight increase in wet thickness was observed. Our data also indicate that the PEMs became more hydrated after annealing in salt solutions, the degree of which depends on the salt concentration [63]. To interpret this behavior, the type of dominant charge compensation of the multilayers should be taken into account. Schlenoff et al. [64,65] distinguished two kinds of charge compensation within the PEMs. When the charges of the polyelectrolyte are balanced by the oppositely charged polyelectrolyte, this is called “intrinsic charge compensation”. While, when the polyelectrolyte charges are balanced by counterions, this is called “extrinsic charge compensation” [64,65]. At low ionic strength that PEMs were made (i.e. 5 mM $NaNO_3$), the charge compensation of the polyelectrolytes is dominated by electrostatic interactions between the oppositely-charged polyelectrolytes (i.e. intrinsic charge compensation). This resulted in thin and dense multilayers with a relatively low mobility of the polymer chains. Upon post-treatment of PEMs at high ionic strengths (i.e. salt annealing), charge compensation by counterions is favored, shifting the equilibrium towards extrinsic charge compensation [64]. The transition from intrinsic to extrinsic charge compensation is accompanied by more hydrated multilayers [66]. As illustrated in Fig. 1d, applying both salt and thermal annealing substantially reduced the PEMs’ wet thickness. The lowest hydration ratio was found in salt and thermally-annealed $(PAH/PAA)_{15}$ multilayers. It seems that the thermal annealing step dominates the change in properties.

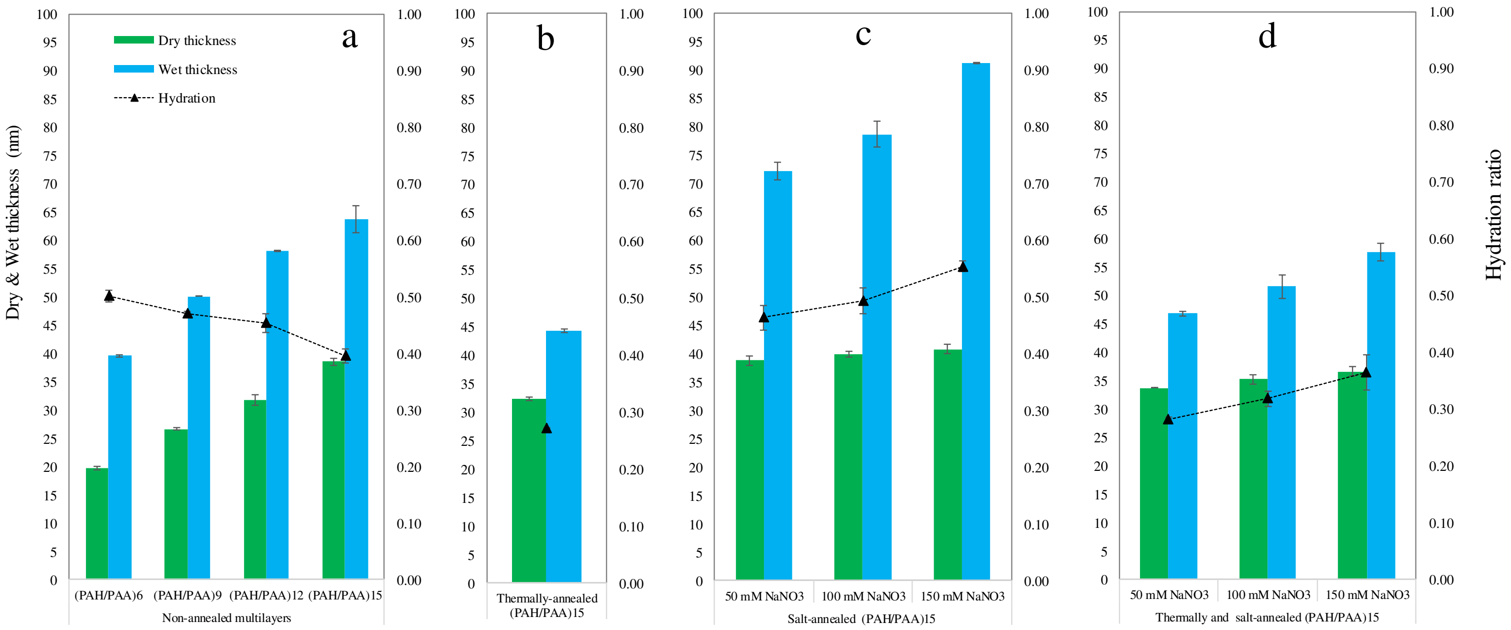

Fig. 1. Ellipsometric measurements of the coated silicon wafers of: a) non-annealed, b) thermally-annealed, c) salt annealed and d) themally and salt-annealed PEMs.

3.1.2. ATR-FTIR

To provide charge to the PAN membrane, a hydrolysis step was performed and checked with ATR-FTIR. As shown in Fig. 2S in Supplementary data, the hydrolysis with alkaline solution is mainly based on the conversion of nitrile groups (C≡N) on the PAN membrane surface first into amide groups ($CONH_2$), and then into carboxylate (COO^-) groups [67]. Fig 3S in Supplementary data gives the ATR-FTIR spectra of the PAN and PAN-H membranes. The PAN membrane shows three main peaks at

1460, 2245 and 2362 cm^{-1}. These peaks correspond to the stretching vibrations of the CN groups of the PAN membrane support. After the hydrolysis, most of the CN groups were converted to COO- groups, as demonstrated by the disappearance of peaks at 2245 and 2362 cm^{-1}. Additionally, a prominent peak at 1508 cm^{-1} can now be noticed, corresponding to the carbonyl (-C=O) bond in the COO^- groups [68]. No $CONH_2$ group peak (1570 cm^{-1}) [69] was present on the FTIR spectra. This indicates the preference for the COO^- groups over the $CONH_2$ groups. These results demonstrate the successful hydrolysis of the PAN membrane into a negatively-charged membrane.

3.1.3. Contact angle of PEM-based-membranes

Water contact angles of both non-annealed and annealed PEM-based membranes are shown in Fig. 2. The unmodified PAN membrane exhibited an average contact angle of 70.2°, which is in good agreement with other studies [53,70] indicating that the substrate is somewhat hydrophobic. The contact angle significantly declined to around 22° when the membrane was hydrolyzed (Fig. 2a). This is due to the polar character of the carboxylate (COO^-) surface groups, thus facilitating hydrogen bonding with the water molecules [71]. For the multilayers, the PAA-terminated membrane is more hydrophilic as compared to the PAH-terminated membrane, likely because of the large excess of carboxylic groups after deposition [72]. The coating of $(PAH/PAA)_{15}$ multilayers on the PAN-H membrane support led to a contact angle of 41.3° (Fig. 2b). This value corresponds to a hydrophilic surface. Membrane coatings with PAH/PAA multilayers usually produce hydrophilic membranes [73]. This is seen as an advantage for wastewater purification, where fouling problems have been always a challenge. Generally, a hydrophilic membrane surface fouls less due to the hydration of surface which suppresses the adsorption of organic substances [74]. The contact angle increased to 66.3°, which shows a nearly hydrophobic surface after thermal annealing of $(PAH/PAA)_{15}$ multilayers (Fig. 2c). Diamanti et al. [27] observed a similar trend when they investigated the impact of thermal annealing on the wettability of alginate poly-L-lysine polyelectrolyte multilayers. This behavior was attributed to the restructuring of the PEMs from stratified multilayers to the formation of complexes between the oppositely charged polyelectrolytes [27]. Multilayers annealed at 150 mM $NaNO_3$, however, became more hydrophobic than those annealed at lower salt concentrations (Fig. 2d). Salt annealing enhances the mobility of polyelectrolyte chains in the structure of multilayers [32,33]. As a result, the multilayers and the top layer become more mixed, and the excess charge of the top layer declines, leading to an increase in hydrophobicity. Upon applying both thermal and salt annealing, the hydrophobicity of $(PAH/PAA)_{15}$ multilayers increased from 41.3° to 65°. In this case, only minor hydrophobicity changes were observed when multilayers were exposed to the various salt concentrations (Fig. 2e). This variation in wetting behavior is likely a consequence of the multilayers' re-arrangement, which needs to be studied further in detail.

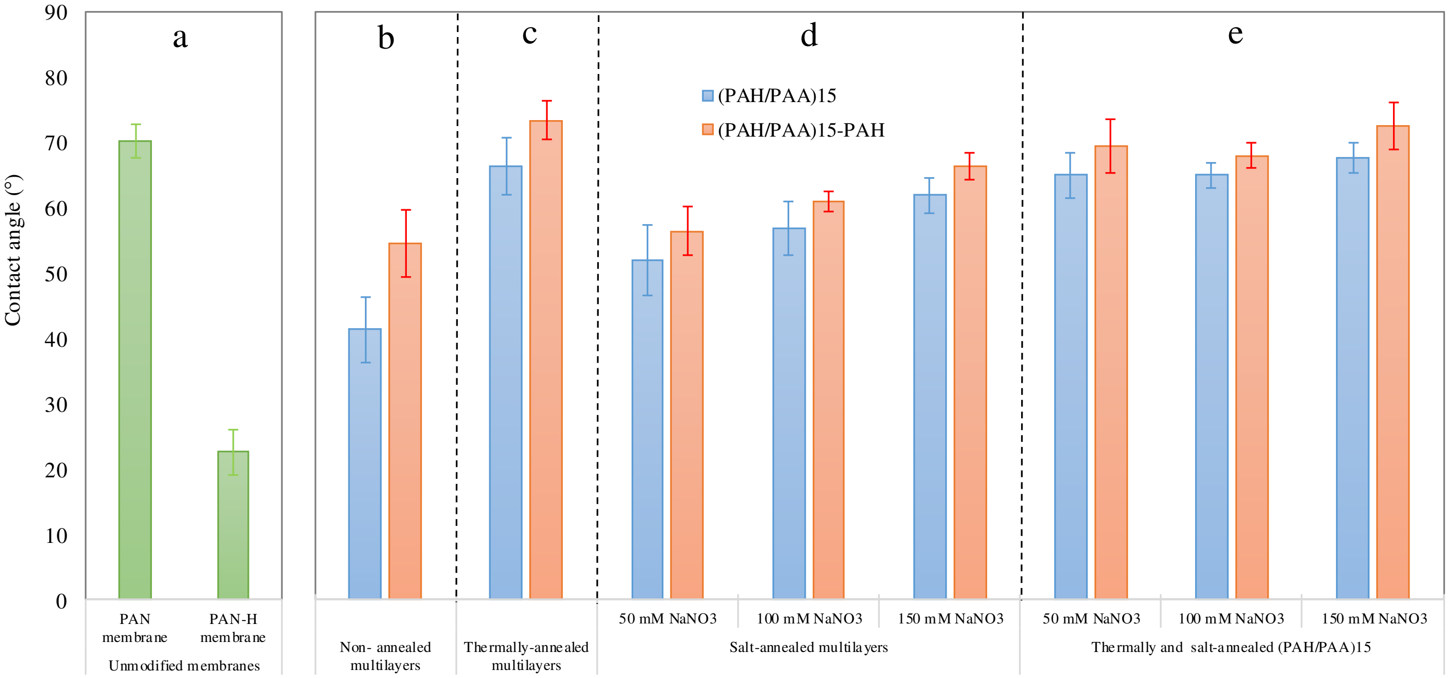

Fig. 2. Contact angle values of the PEM-based membranes: a) uncoated, b) non-annealed, c) thermally-annealed, d) salt annealed, and e) themally and salt-annealed membranes.

3.2. Performance of PEM-based membranes

3.2.1. Permeability

Fig. 3 shows the pure water permeability of bare and coated PAN-H membranes. The permeability of the bare PAN-H membranes was 724 L.m^{-2}.h^{-1}.bar^{-1} (Fig.3a). This value depends on the preparation condition and also the concentration of PAN in the casting solution [75]. For example, Hernalsteens [53] reported a pure water permeability of 890 L.m^{-2}.h^{-1}.bar^{-1} for PAN-H membranes prepared under similar conditions with this paper but at 13 wt% PAN concentration. By increasing the number of coated layers, the membrane permeability went down to 10.2 and 14.1 for (PAH/PAA)$_{15}$ and (PAH/PAA)$_{15}$-PAH membranes, respectively (Fig.3b,c). These permeabilities are comparable to reported values for commercial NF membranes (4.5-15.5 L.m^{-2}.h^{-1}.bar^{-1}) [13], and did not significantly decline by further coating. The permeability's downward trend, shown in Fig.3, is also in agreement with the ellipsometry data of multilayers growth (Fig.1a) and indicates that the addition of material on the membrane surface firstly decreases the membrane pore size (pore dominated regime) (Fig.3b) and secondly comprises a thin film on top of the porous support (layer dominated regime) (Fig.3c), leading to a decline in water permeability [76]. The swelling degree of the PEMs can also change the membrane permeability, whereby an increase in swelling leads to thicker but less dense polymer layers. PAH-terminated PEMs are more swollen than PAA-terminated layers [61]. Hence, in the case of thicker layers (Fig.3c), the membrane permeability increased when PAH layers are coated and decreased again when PAA layers are applied. This zig-zag behavior (the flipping of the odd–even effect [76]) confirms that we are well within the layer dominated regime, and that any solute separation will be dominated by the PEM coating, rather than the original membrane pores.

Fig. 4 compares the permeability of the annealed and non-annealed PEM-based membranes. In general, there was no significant difference between them, but a glance at Fig. 4c,d demonstrates that an increase in swelling of the multilayer (Fig. 1c,d) leads to a more open layer and thus a higher permeability.

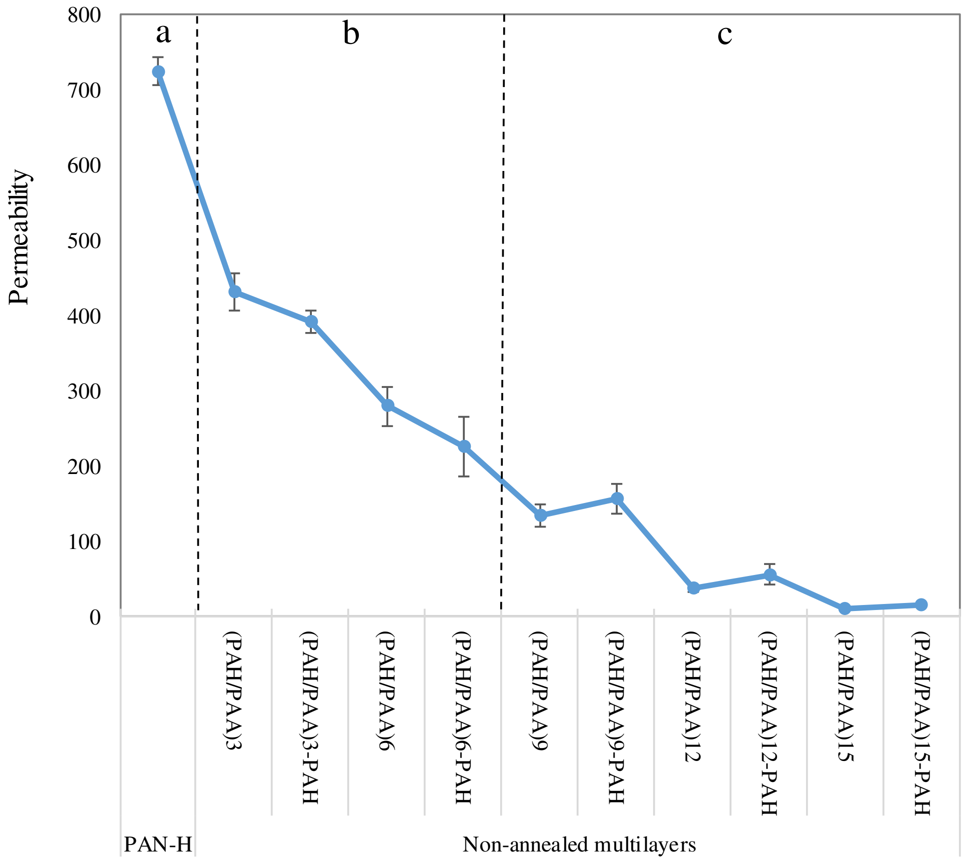

Fig. 3. Changes in the pure water permeability (L.m^{-2}.h^{-1}.bar^{-1}) of the bare and coated membrane after deposition of (PAH/PAA) multilayers.

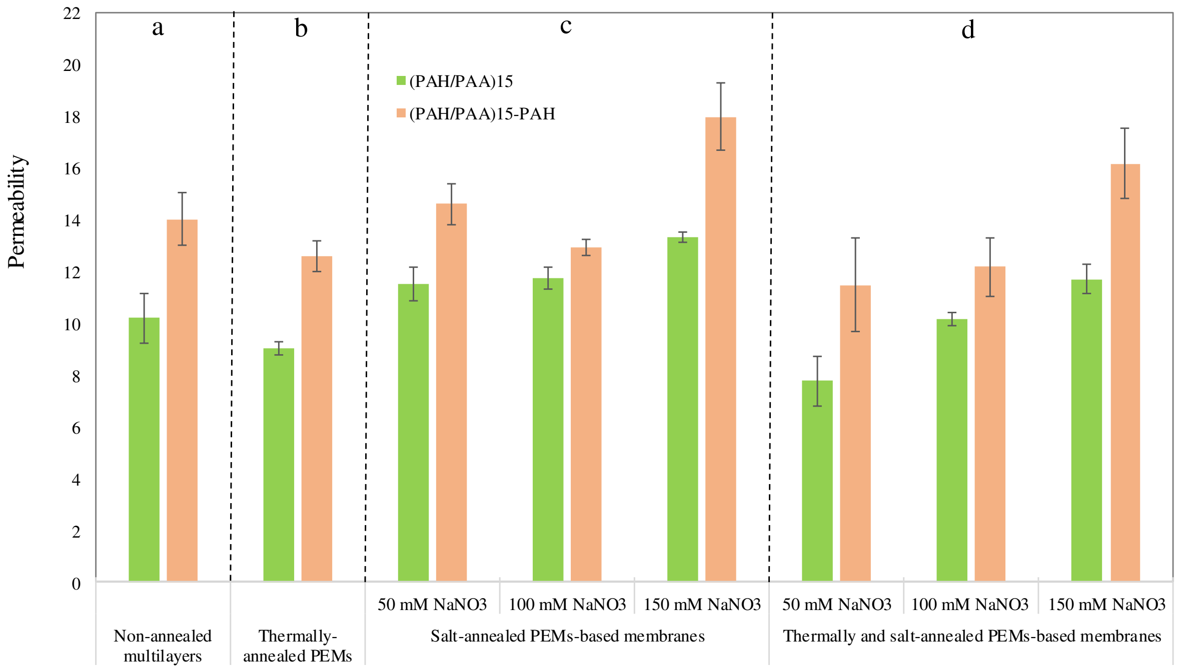

Fig. 4. Pure water permeability ($L.m^{-2}.h^{-1}.bar^{-1}$) of a) non-annealed, b) thermally-annealed, c) salt annealed, and d) themally and salt-annealed PEM-based membranes.

3.2.2. Salts rejection

PEM-based NF membranes are attractive for the separation of ions with different charges as well as for the size-selective separation of ions with the same charge [18]. As summarized in Table 1S in supplementary data, these membranes are recognized to have high rejections for divalent ions with low to moderate removal of monovalent ions, in particular when weak polyelectrolytes exist in the backbone of multilayers. In addition to size exclusion, charge exclusion plays a big role in solute rejection as the divalent ions are more charged than the monovalent resulting in a stronger repulsion [77,78].

To examine ion retention, filtration was performed using mixed-salt solutions containing NaCl, Na_2SO_4 and Na_3PO_4 (0.1 g. L^{-1} each) and also single-salt solutions containing $CaCl_2$ (0.1 g. L^{-1}). As the form of phosphate ion depends on the pH of the feed, the phosphate ions are present as HPO_4^{2-} (Fig.4S in Supplementary data) [79]. Salt retentions of the annealed and non-annealed $(PAH/PAA)_{15}$ and $(PAH/PAA)_{15}$-PAH membranes are shown in Fig.5 and Fig.6, respectively. For all membranes, in addition to the role of charge repulsion, the highest retentions were obtained for the large HPO_4^{2-}, followed by SO_4^{2-} and then Ca^{+2} and Cl^- at the last place, indicating that size exclusion plays an important role in their rejection. Fig.5a and Fig.6a show as the number of PAH/PAA bilayers were gradually elevated from 6 to 15, a considerable increment in salt rejection was noticed e.g. from 18.3% to 49.8% for HPO_4^{2-} retained by the PAA-terminated membranes. With more layers, less defects are present and the layer hydration is also lower.

Fig. 5b,d and Fig. 6b,d confirm that salts rejection did not improve with thermal annealing. Although thermal annealing could lead to denser multilayers (Fig. 1b,d) which would inevitably enhance the role of the size exclusion, it apparently reduces the charge of the top layer, leading to a reduction in the role of charge repulsion in salts rejection.

Regarding Fig. 5c & 6c, salt-annealed membranes performed better than non-annealed and thermally-annealed membranes for the purpose of ions retention. The highest salts retention was obtained for $(PAH/PAA)_{15}$ multilayers annealed in 100 mM $NaNO_3$. This membrane retained Na_3PO_4, Na_2SO_4, $CaCl_2$ and NaCl up to 69.8%, 57.5%, 37.8% and 25.3%, respectively. As discussed in section 3.1.1, the shift from intrinsic to extrinsic charge compensation due to the salt annealing, probably leads to an enhancement of the charge density of the PEMs. Then, the higher charge density of the layers results in membranes with better rejection properties for ions [80]. Furthermore, we see that the role of so-called "terminating layer's charge" is less pronounced in non-annealed membranes (compare Fig. 5c to Fig. 6c), while it is more apparent in salt-annealed membranes. For instance, on one hand, we do not see a substantial difference in rejection of the negative SO_4^{-2} or positive Ca^{+2} by both non-annealed negatively and positively-terminated membranes. On the other hand, retention of HPO_4^{-2} was observed by 69.8% for the salt-annealed PAA-terminated membranes (100 mM $NaNO_3$) compared to 54.7% for the salt-annealed PAH-terminated membranes. A converse behavior was observed for retention of Ca^{+2} i.e. 37.8% versus 48.4% for the salt-annealed PAA and PAH-terminated membranes, respectively. Consequently, for the non-annealed membranes, ion rejection is predominantly based on the ion size. While, in the case of salt-annealed counterparts, ion rejection is determined by the ion size followed by the surface charge of the multilayers.

While the focus so far has been on explaining the observed salt retentions, it should also be clear that $(PAH/PAA)_{15}$ membranes annealed in 100 mM $NaNO_3$ have good salt retention properties, while retaining a relatively high flux. For this membrane, a rejection of 69.8% for HPO_4^{2-} was obtained with a permeability of 11.8 $L.m^{-2}.h^{-1}.bar^{-1}$, while its non-annealed counterpart showed retention and permeability of 54.7% and 10.2 $L.m^{-2}.h^{-1}.bar^{-1}$, respectively. This shows the potential of PEMs annealing to design NF membranes and control their performance. The membrane with the highest ionic rejection was then tested for its MP removal.

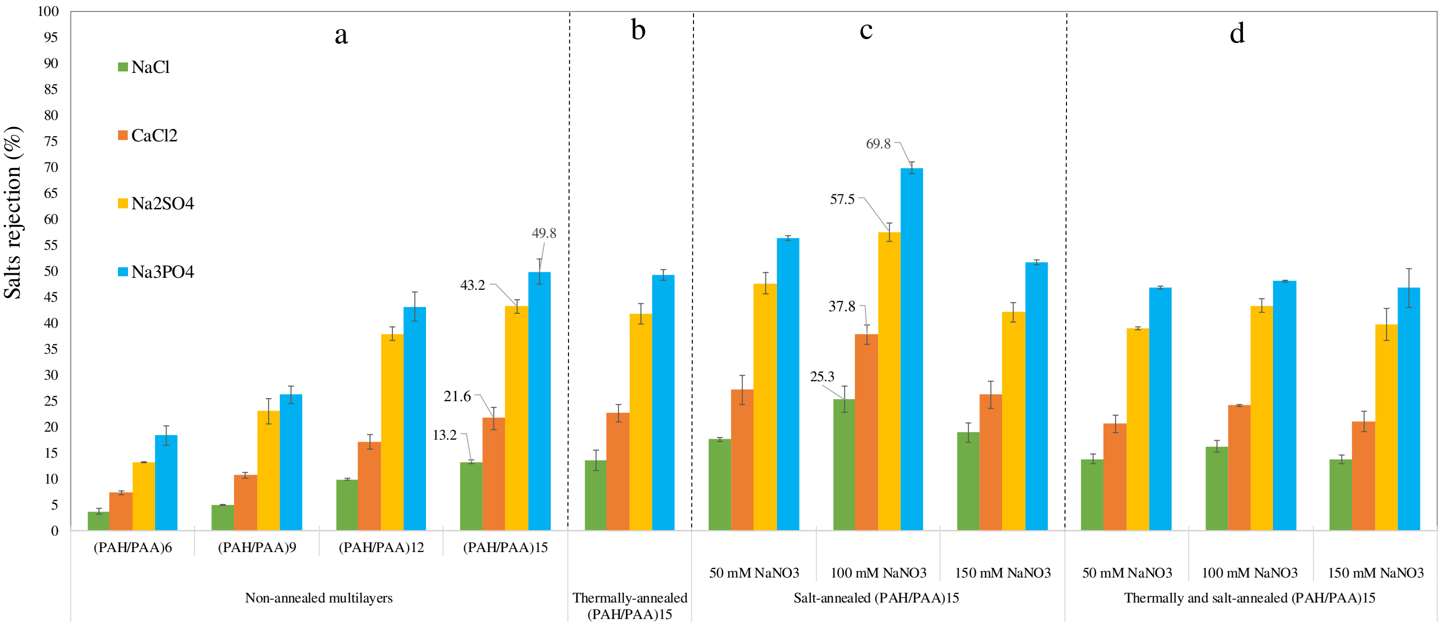

Fig. 5. Salts retention and selectivity values of the non-annealed and annealed $(PAH/PAA)_{15}$ membranes

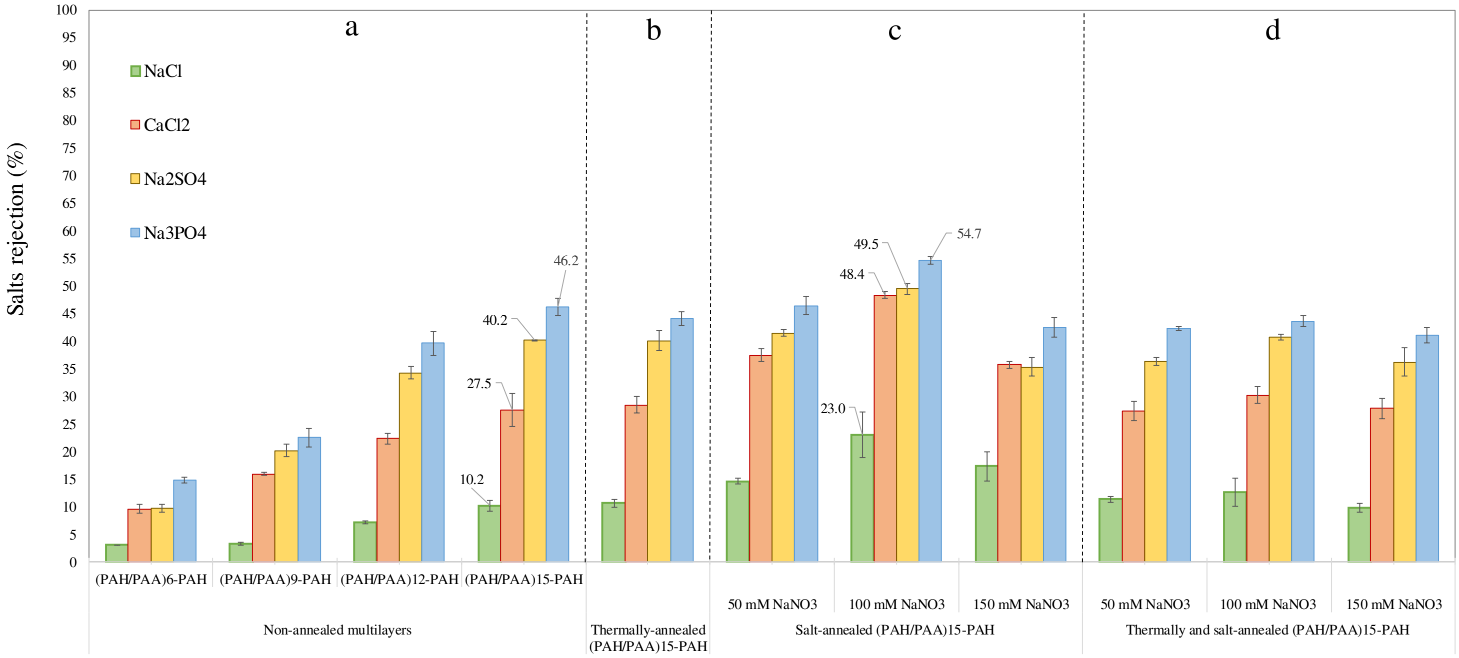

Fig. 6. Salts retention and selectivity values of the non-annealed and annealed $(PAH/PAA)_{15}$-PAH membranes

3.3.3. MPs rejection

3.3.3.1. An overview of the MPs rejection by the NF membranes & general rejection mechanisms

Table 2S in Supplementary data presents some recent research data concerning the effectiveness of NF membranes in eliminating target MPs. To date, the rejection of uncharged MPs by NF membranes is considered to be predominantly caused by size exclusion, while charged molecules are also rejected by electrostatic interactions with the charged membranes [81]. In this study, Diclofenac, Naproxen, and Ibuprofen are MPs with negative charge, while 4n-Nonylphenol is an uncharged compound at neutral pH [82].

Often, molecular weight is used to reflect molecular size. However, it does not truly reflect the size [83]. Consequently, spatial dimensions of MPs such as molecular width [84,85] and minimum projection area (MPA) [62,86] are also used to study the rejection behavior of NF membranes. MPA, as calculated from the van der Waals radius, is defined as the smallest two-dimensional projection area of a three-dimensional molecule. By projecting the molecule on an arbitrary plane, the two-dimensional projection area can be calculated and the process is repeated until the minimum projection area is obtained (Fig. 5S in Supplementary Data) [62].

When wastewater is used as feed solution, the existing interactions between the molecules and membranes may be influenced by the effluent organic matter. Then the separation mechanism of MPs cannot simply be attributed to the sieving effect and surface charge. In this case, hydrophobic interactions that take place between the fouled membrane surface and solutes can become dominant [87]. Regarding the hydrophilic or hydrophobic character of MPs, the octanol-water partition coefficient (K_{ow}) can be used as an indicator of hydrophobicity. Here, a pH-corrected value of log K_{ow}, known as log D, has been employed to predict the MPs' hydrophobicity. It can be defined as the K_{ow} ratio between the ionized and unionized form of the solute at a specific pH value (here the pH is adjusted at 7) [88]. Compounds with log D>2.6 are referred as hydrophobic, and hydrophilic when log D $\leq$ 2.6 [89]. Hence, in the present work, using a synthetic wastewater effluent with neutral pH, Diclofenac, Naproxen and Ibuprofen are recognized as hydrophilic compounds ($\log_D$: 1.77, 0.34 and 1.44, respectively [13]), while 4n-Nonylphenol ($\log_D$: 6.14 [88]) is considered as a hydrophobic molecule.

3.3.3.2. MPs rejection by non-annealed and salt-annealed PEM-based membranes

Higher ion rejection combined with a high flux, already shown in Fig. 4c and Fig. 5c, make the "salt-annealed $(PAH/PAA)_{15}$ membrane (annealed in 100 mM $NaNO_3$)" promising for the separation of MPs. According to the suggestions of Kimura et al. [90] and Yangali-Quintanilla et al. [83], and considering the very low concentrations of MPs in the effluent (0.5-40 $\mu g.L^{-1}$), a long filtration duration of 54 hours was applied to avoid overestimation of MP rejection (Fig. 7). First, the MP rejection increases over time (Fig. 7a,b). The apparent rejection of the hydrophobic 4n-Nonylphenol was the highest, followed by Diclofenac and then Ibuprofen and Naproxen. At that stage, there was no significant difference between the non-annealed and annealed membranes in MP rejection. At 31h (Fig. 7c), we see a sudden reduction in retention of all MPs, the most severe for the hydrophobic 4n-Nonylphenol (e.g. from 95.9% to 69.1% for the salt-annealed membranes). After that, a nearly stable retention of MPs was observed until the end of filtration process (Fig. 7d), whereby the steady-state rejection of Diclofenac, Naproxen, 4n-Nonylphenol and Ibuprofen were up to 81.5%, 66.6%, 61.7%, and 51.6%, respectively, for the salt-annealed membranes. Except for 4n-Nonylphenol, annealed membranes show better MP retention, compared to the non-annealed membranes. This is in a good agreement with the improved salt rejection observed in the previous section. It shows clearly that salt annealing can improve the rejection of specific organic compounds in PEM-based NF membranes.

To describe the rejection behavior seen in Fig. 7, contact angle values of pristine and fouled membranes were plotted in Fig. 8. After filtration of MPs-bearing solution, contact angles of salt-annealed membranes increased first to 68.9° (part *a* of Fig.7) and then to 81.8° (part *b* of Fig.7). Membrane fouling has thus first imparted hydrophobicity to the membrane surface. Bellona et al. [91] also found that both NF-270 and TFC-SR2 membranes rapidly became more hydrophobic, when different organic foulants were accumulated on the membrane surface. Two hypotheses might explain the high retention of all MPs observed in Fig. 7b: i) the high removal efficiency of 4n-Nonylphenol can be related to its hydrophobic interactions with the hydrophobic foulant layer formed on the membrane surface [90], and ii) the foulant layer could act as a second barrier for the separation process [87], thereby rejection of hydrophilic Diclofenac, Naproxen and Ibuprofen has slightly increased. As a result, the foulants increased the adsorption capacity of the membrane for the both hydrophobic and hydrophilic compounds, and thus, the rejection of all target MPs was higher when the membrane was fouled in part *b* of Fig.7. As it can be seen in Fig. 6S in Supplementary Data, a linear increase ($R^2 \approx 0.82$-0.91) between the hydrophobicity (log D) and apparent rejection of all MPs is observed for both parts *a* and *b* of Fig.7. This high correlation can confirm that compound hydrophobicity plays an important role in the early stage of MPs rejection, especially for the highly hydrophobic compounds.

The rejection behavior observed in Fig.7c,d probably is correlated with the changes in the contact angle (Fig. 8) and also density of the surface charge. Contact angles of non-annealed and salt-annealed membranes eventually declined to 50.4° and 47.9° (part *d* of the Fig.7), respectively, indicating that the membrane eventually became more hydrophilic compared to the previous steps. Higher hydrophilicity generated by the fouling layer on the membrane may allow a higher amount of MPs to partition through the membrane, and ultimately, decreased the rejection [89]. For the charged compounds, the negative charge of the membrane is greater when fouled, increasing eventually the electrostatic repulsion between the negative charge of membrane surface and the negative charge of the compound [92]. Linares et al. [89] studied the performance of Forward Osmosis (FO) process for the removal of selected MPs and concluded that when the FO membrane was fouled, the hydrophilic nature of foulants caused the hydrophilic ionic compounds (such as Ibuprofen and Naproxen) to be rejected more effectively due to higher negative charge of the fouled membrane. For the rejection of uncharged compound of 4n-Nonylphenol (Fig.7d), No significant deference between non-annealed and salt-annealed membrane was observed. This outcome makes a sense that our previously discussed hypothesis about the effect of salt annealing on the change of the charge compensation from intrinsic to extrinsic that enhances the charge density is likely plausible.

As seen in Fig.7, as filtration time progresses, MPs rejection is likely to decline after the membrane is saturated upon long term operation. In other words, in line with Yangali-Quintanilla et al. [83], hydrophobic adsorption of MPs to the membrane was significant only in the first steps of wastewater filtration and it is less effective over time compared to the other rejection mechanisms. Regarding the importance of molecular dimensions and other physico-chemical properties in solutes rejection, the correlation between the steady-state rejection of MPs and such parameters is discussed in Section 1S in Supplementary Data.

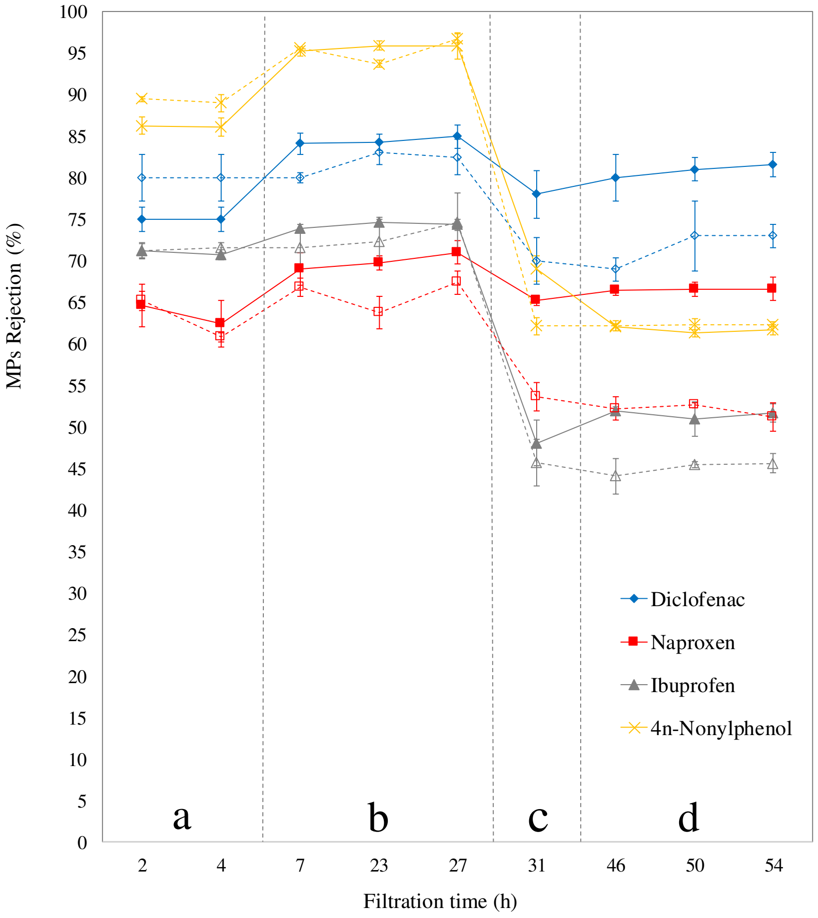

Fig. 7. Evolution of the MPs rejection over the filtration time (solid lines are related to the salt-annealed $(PAH/PAA)_{15}$ membranes, while dashed lines indicate the performance of non-annealed $(PAH/PAA)_{15}$ membranes)

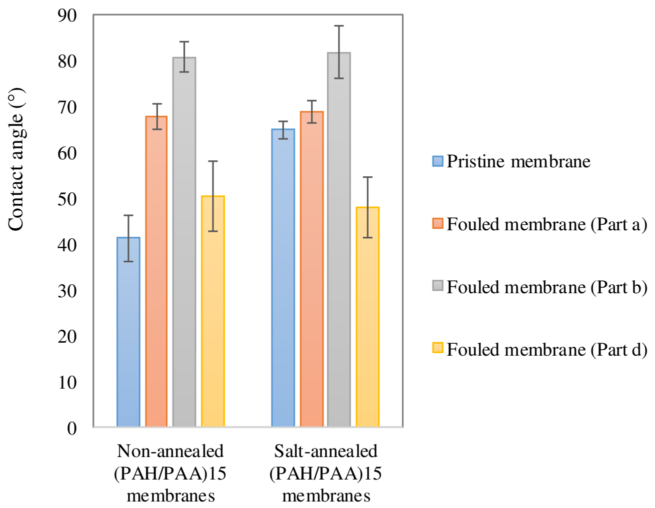

Fig.8. Contact angle values of the pristine and fouled membranes at different time-steps of the filtration period (the above-mentioned parts in this figure correspond to the parts of the Fig.7)

3.3.3.3. Comparison with other tertiary treatment technologies

In order to compare our results with studies in the literature, Fig.9 and Table 3S in Supplementary data have been prepared. Working on tertiary MPs removal is in its early days and it appears that there is a lack of comprehensive study in the literature. Considering this data, high MPs retentions would possibly enable these membranes to outperform current biological tertiary treatment methods (especially for the recalcitrant Diclofenac and Naproxen), and to compete with the available commercial NF and RO membranes for MPs removal from municipal effluents. Another priority of this membrane over the available commercial pressures-driven membranes is its lower salts rejection. Low salts rejection leads to the production of a concentrate stream with a low level of salinity. The biological treatment of the low-saline concentrate, will be more feasible in activated sludge-based reactors than the saline concentrates produced from commercial membranes [34,93–95]. Detrimental levels of the salinity on the performance of activated sludge reactors is discussed in our previous study [46]. Although the process of salt annealing slightly increases ion rejection (Fig. 5&6), its rate of rejection is still too lower than what we see for both tight and loose NF membranes. For example, the rejections of NaCl and $MgSO_4$ by a tight NF90 membrane are reported up to 85-95% and 97-100%, respectively [96]. In the research of Levchenko and Freger [97] who studied the performance of NF membranes in salts rejection from secondary-treated municipal wastewater, loose NF270 membranes rejected NaCl, $MgCl_2$ and Na_2SO_4 up to around 60, 65 and 100%, respectively, while our salt-annealed membrane retained NaCl, $CaCl_2$ and Na_2SO_4 by around 25, 37 and 57%, respectively. This capability would be beneficial: i) when the concentrate stream is going to be treated by activated sludge-based reactors, and ii) when MPs removal is highly needed without or with a small change in salt balance of the effluent, to allow use for agricultural irrigation.

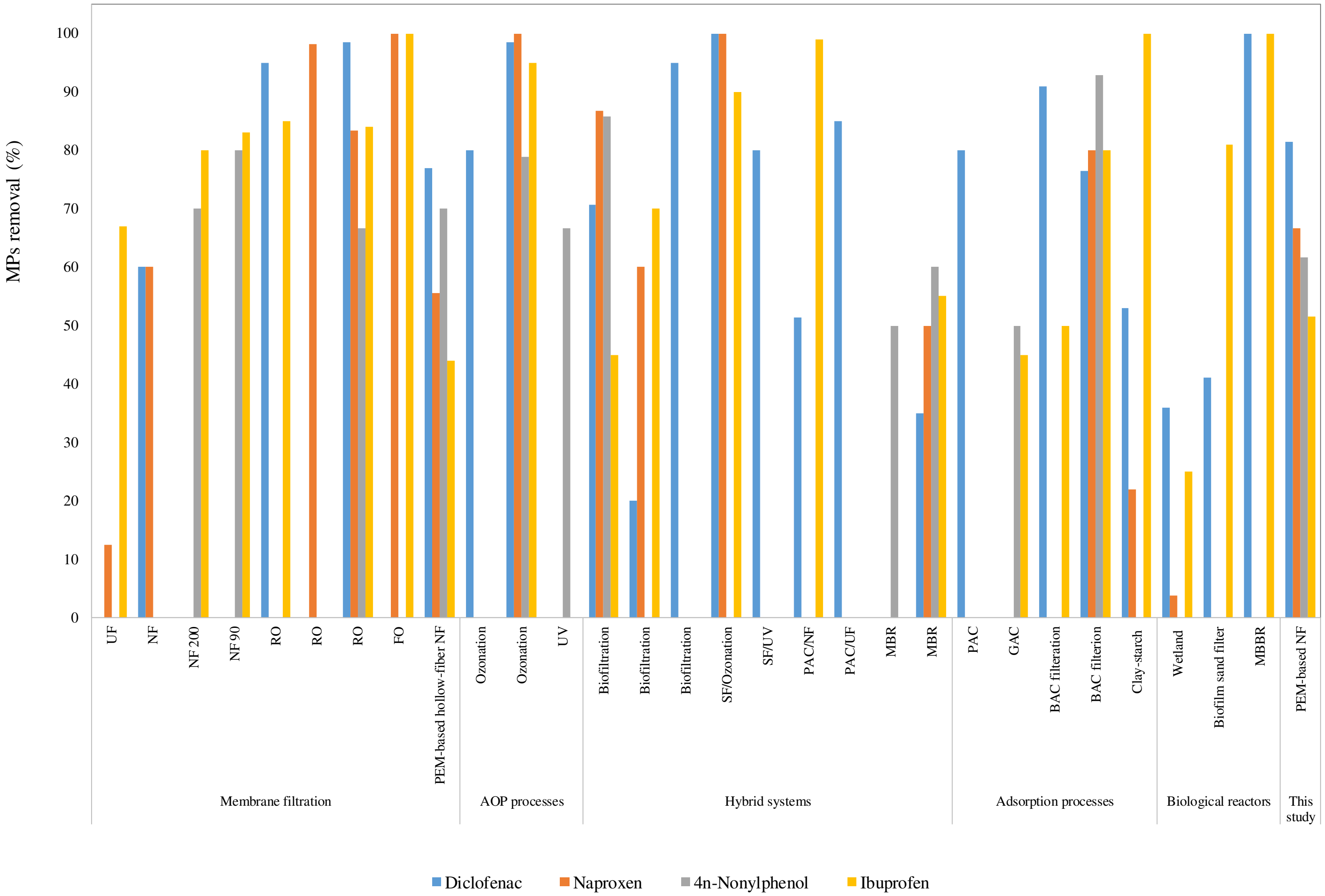

Fig. 9. Comparison of the steady-state rejection of MPs in this study with other tertiary treatment methods from the literature (More details are given in Table 3S in Supplementary data)

(Abbreviations: AOP: advanced oxidation process, SF: sand filter, PAC: powdered activated carbon, GAC: granular activated carbon, MBBR: moving bed biofilm reactor, MBR: membrane bioreactor)

3.3.4. Membrane cleaning

In fouling studies related to the wastewater filtration, often, model foulants such as humic acids, bovine serum albumin (BSA), sodium alginate, and colloidal silica particles are used to simulate polysaccharide, refractory organic matter, protein and colloidal particles that are ubiquitous in secondary-treated wastewater [98]. The simplicity of those fouling systems probably leads to an unrealistic estimation of the MPs rejection by the clean and fouled membranes. Here, we used artificial wastewater, probably leading to a better judgement about the rejection behavior of membranes. But it also allows us to study another aspect of these multilayers, that they can be used as sacrificial layers to allow easy membrane cleaning [61,99].

In Fig. 10, the wastewater permeability of pristine and fouled salt-annealed $(PAH/PAA)_{15}$ membranes is shown at different cleaning steps. First of all, we evaluated whether the PEMs were completely removable by rinsing a pristine coated membrane with rinsing trigger solution (pH 3, 3M NaCl) at 2 bar for 180 min. Indeed, the membrane permeability increased up to the level of a pristine uncoated membrane (~ 625 $L.m^{-2}.h^{-1}.bar^{-1}$) (bar C). After filtration of MP-bearing wastewater, the membrane permeability of fouled membrane was about 4.6 $L.m^{-2}.h^{-1}.bar^{-1}$ (bar D). Membrane cleaning with the rinsing solution at 2 bar for 180 min increased the permeability to ~170 $L.m^{-2}.h^{-1}.bar^{-1}$, confirming the incomplete removal of the PEMs and foulants (bar E). Hence, an additional rinsing step was incorporated as described in experimental section i.e. immersion of pre-rinsed membrane in the same cleaning solution for overnight. This step resulted in an outstanding increase in the membrane permeability up to one that is nearly equal with the permeability of the pristine uncoated membrane (bar F). This demonstrates the full elimination of both sacrificial PEMs and foulants. Shan et al. [99] and Ilyas et al. [61] have successfully used a PEMs as both a sacrificial layer and as the separating layer of a NF membrane. They both could only completely remove all foulants by backwashing. Here, we could remove the PEMs along with attached foulants without employing any shear forces. Manual re-coating of the rinsed membrane with $(PAH/PAA)_{15}$ multilayers and its subsequent salt annealing in 100 mM $NaNO_3$ caused an evident reduction of the permeability that was roughly identical to the permeability of the pristine coated membrane (Part G). Our results demonstrate that the sacrificial layer approach is also promising in real wastewater applications.

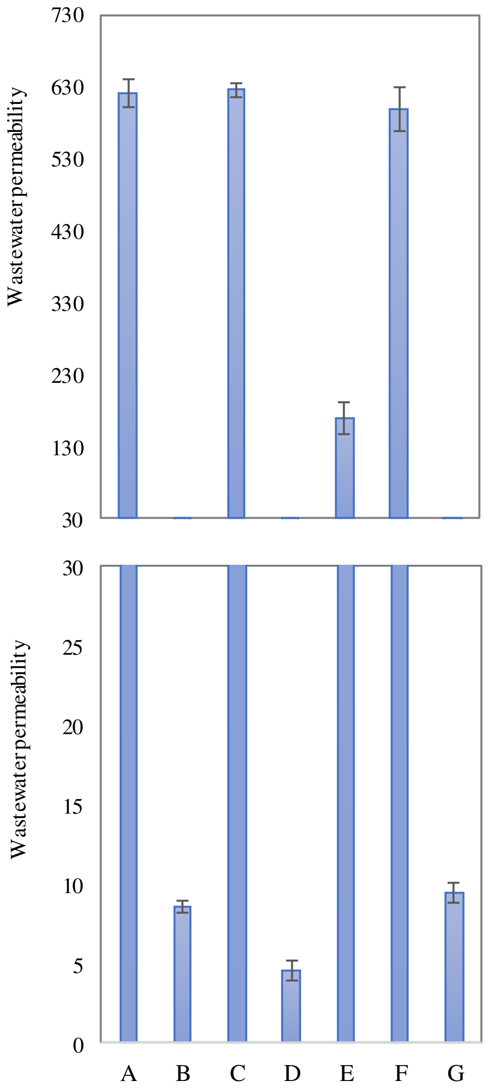

Fig. 10. MPs-bearing wastewater permeability (L.m^{-2}.h^{-1}.bar^{-1}) of the salt-annealed (PAH/PAA)$_{15}$ membrane after the following steps: (A) uncoated pristine membrane; (B) pristine salt-annealed coated membrane; (C) rinsing of pristine coated membrane with cleaning solution for 180 min; (D) fouled salt-annealed coated membrane; (E) rinsing of fouled membrane with cleaning solution for 180 min; (F) rinsing of fouled membrane with cleaning solution for overnight; (G) regeneration of PEMs on the cleaned UF membrane.

4. Conclusion

Current municipal WWTPs were never designed for MP removal, and persistent MPs are still seen in the secondary-treated wastewater. The effect of thermal and salt-annealing was evaluated on the performance of polyacrylonitrile-supported NF membranes made from weak PEMs for MPs polishing. In contrast to thermal annealing, salt annealing of PEMs enhanced salts rejection. The membrane also achieved a significantly improved rejection for some selected MPs. At initial steps of filtration, apparent rejections for both hydrophobic and hydrophilic MPs were governed by adsorption phenomena, whose role fade-away over time. The membrane then became more hydrophilic when steady-state rejection of MPs was achieved. Contribution of the molecular weight was higher than other dimensional parameters in steady-state rejection of all MPs by salt-annealed PEMs membranes, while MPA was a better surrogate parameter for the non-annealed membranes. A quite high removal of MPs next to the easy cleaning of both PEMs and foulants without employing any physical force are achievable in salt-annealed PEMs membranes, making them a promising technology for advanced wastewater treatment. These results were also accompanied with a relatively low salts rejection, allowing the production of low-saline concentrate streams that would make biological treatment much more straightforward.

Acknowledgments

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IMAGINE PEACE

Mehran Abtahi

Spring 2018, France.