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Impact of organic fractions identified by SEC and fluorescence EEM on the hydraulic reversibility of ultrafiltration membrane fouling by secondary effluents

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ABSTRACT

Loss of membrane filtration performance due to organic fouling is still a significant drawback for the application of low-pressure membranes in tertiary wastewater treatment. The present study investigates the relevance of different organic fractions present in secondary effluents in terms of hydraulically reversible and irreversible fouling of hollow-fibre ultrafiltration membranes. A good correlation between the hydraulically reversible filtration resistance and the total organic biopolymer concentration according to size exclusion chromatography (SEC) was observed. Qualitatively biopolymers consist mainly of polysaccharides as well as proteins with high molecular weight. Polysaccharides are retained by the membrane pores, but can be removed by simple UF backwashing. On the other hand, fluorescence excitation–emission matrix (EEM) analysis indicates that the extent of the hydraulically irreversible fouling correlates with the presence of protein-like substances. Removal of protein-like substances by biological slow sand filtration or chemical coagulation results in the significant reduction of the hydraulically irreversible fouling, which is presumably due to proteins in the molecular range of biopolymers. In contrast to the comparatively low sensitivity of colorimetric methods for the analysis of proteins and polysaccharides, the combined application of size exclusion chromatography and fluorescence EEM analysis is a promising tool for the determination of the organic fouling propensity of secondary effluents.

Keywords: Organic fouling; Ultrafiltration; Tertiary sewage treatment; Fluorescence analysis; Size exclusion chromatography; LC-OCD; EEM

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1. Introduction

Tertiary treatment of biologically purified municipal sewage is gaining increasing significance with regard to the enhanced protection of receiving water bodies and indirect or direct reuse of wastewater. Low-pressure membrane filtration provides an efficient tool for the removal of pathogens and particulate nutrients from secondary effluents. For example, the extension of Berlin's largest sewage treatment plant Ruhleben by tertiary ultrafiltration with coagulation is currently being planned for a hydraulic capacity of 247,000 $m^3 \cdot d^{-1}$. However, membrane fouling is still a considerable drawback, restricting the economic operation of membrane facilities and eventually limiting the lifetime of the installed membranes.

Hydraulically reversible fouling due to superficial deposition of particles and formation of concentration polarisation layers is controllable by the appropriate hydraulic operation of the membrane facility and, if applicable, by periodic backwashing of the membranes. However, organic substances adsorbed onto the membrane surface or inside the membrane pores are usually not easily removable by backwashing, thus resulting in hydraulically irreversible fouling and necessitating frequent chemical cleaning of the membranes [1]. The formation of hydraulically irreversible fouling is not yet fully understood. Recent investigations indicate the importance of dissolved organic carbon (DOC; i.e., dissolved organics < 0.45 μm) and/or colloidal organic matter in terms of long-term fouling of micro- and ultrafiltration membranes, which is less dependent on the hydraulic operational conditions [2,3]. Several studies suggest the influence of humic substances contained in municipal effluents due to their adsorption affinity towards polymeric membranes [4–7]. However, others point out the relevance of organic macromolecules of microbial origin usually referred to as extracellular polymeric substances (EPS), soluble microbial products (SMP), effluent organic matter (EfOM), or organic biopolymers, and primarily including proteins and polysaccharides [8–11].

Several analytical approaches for the identification of organic membrane foulants have been presented, including the application of colorimetric methods for the determination of proteins and polysaccharides (e.g., [12]), FTIR analysis (e.g., [13]), and fractionation methods based on size exclusion chromatography (SEC; e.g., [9]). In recent years, fluorescence analysis has been shown to be a sensitive tool for the qualitative differentiation between humic-like, fulvic-like, and protein-like DOC constituents in natural waters and treated sewage [14], as well as in activated sludge [15]. Therefore, fluorescence analysis may also be a potential method for the characterisation of membrane foulants, as has already been demonstrated regarding solutions of natural organic matter [16], surface water [17], and MBR sludge [18]. However, recent studies investigating the fouling of microfiltration membranes in membrane bioreactors using fluorescence spectroscopy resulted in different conclusions in terms of the principal foulants: Wang et al. [19] observed indications for a predominant role of protein-like substances, whereas Kimura et al. [20] found no correlation between the extent of membrane fouling and the fluorescence intensity of protein-like compounds within the foulants extracted from the applied membrane.

The present study focuses on the identification of those dissolved organic substances in secondary effluents which are relevant in terms of hydraulically reversible and irreversible ultrafiltration membrane fouling, using SEC, fluorescence analysis, and colorimetric methods in parallel. Moreover, the efficiency of effluent pre-treatment by biological slow sand filtration or coagulation for the reduction of the fouling potential of secondary effluents is examined.

2. Materials and methods

2.1. Analytical methods

2.1.1. Size exclusion chromatography

The molecular weight distribution of the effluent organic matter was characterised using size exclusion chromatography (SEC) with continuous $UV_{254\,nm}$ absorption, organic carbon (OC) and organic nitrogen (ON) detection (LC–OCD system by DOC-Labor Dr. Huber, Karlsruhe, Germany; SEC column supplied by AllTech-Grom GmbH Rottenburg–Hailfingen, Germany, with resin Toyopearl® HW-50S by Tosoh Bioscience, Tokyo, Japan). SEC pressure was 400 kPa, the mobile phase of the chromatographic system is disodium-hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2\ H_2O$, c = 8.4 $mmol \cdot L^{-1}$) and potassium-dihydrogen phosphate (KH_2PO_4, c = 18.4 $mmol \cdot L^{-1}$), ionic strength = 43.7 $mmol \cdot L^{-1}$, pH = 6.6, flow rate = 1 $mL \cdot min^{-1}$, acidification by phosphoric acid (c = 60 $mmol \cdot L^{-1}$), flow rate = 0.5 $mL \cdot min^{-1}$. The SEC column was tested using standard solutions of polyethylene glycols (PEG) between 106 $g \cdot mol^{-1}$ and 40,000 $g \cdot mol^{-1}$. The retention times observed during chromatography of standard solutions confirm that the void volume of carrier buffer is associated with compounds > 40,000 $g \cdot mol^{-1}$ (at t = 37 min), whereas PEG with M = 23,000 $g \cdot mol^{-1}$ is already retained by the SEC column to a certain extent (elution time t = 42 min), with the smallest PEG having an elution time of t = 72 [21]. However, a very strict correlation between the molecular weight of organic compounds and their chromatographic elution time cannot be expected, since functional groups and steric character of organic compounds may interact with the material of the SEC column.

Prior to LC–OCD analysis, every sample was filtered through 0.45 μm cellulose nitrate filters. Under the given conditions, four different DOC fractions can be distinguished in an LC–OCD chromatogram. In municipal

secondary effluent, the so-called biopolymer fraction eluting at $t \approx 42$ min includes macromolecular proteins and polysaccharides, as well as organic colloids. Characteristics of the secondary effluent's biopolymer fraction are low $UV_{254\,nm}$ absorption and low ON content. Further DOC fractions of lower molecular weight are the humic and fulvic substances (characterised by higher $UV_{254\,nm}$ absorption and increasing ON content, $t \approx 58$ min), low molecular weight acids (peak around $t \approx 65$ min), and low molecular weight neutral substances ($t > 65$ min). The organic carbon concentrations of the fractions are quantified using an infrared detector calibrated with potassium phthalate solution. The OC detection limit of the LC–OCD system is 10 μg·L^{-1}, the standard deviation is less than 1 % of the measured value (measurement range: 1–5 mg·L^{-1}; samples with higher DOC concentrations were diluted). The ON method (LC–OCD–ON) for the further characterisation of the fractions' organic nitrogen concentration can currently not convert 100% of ON and is therefore only used for qualitative interpretation.

2.1.2. Fluorescence analysis

Fluorescence analyses were conducted using a FluoroMax-3 Spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ/USA) with a xenon lamp as excitation source. The samples are excited at excitation wavelengths between 240 nm and 450 nm at 10 nm increments. The corresponding emission intensity is measured at wavelengths between 290 nm and 500 nm at 2 nm increments. Slit widths were set to 10 nm, scan speed to 200 min^{-1}. Prior to analysis, the samples were diluted using potassium chloride solution (c = 0.01 mmol·L^{-1}) to a uniform DOC concentration (c = 1 mg·L^{-1}) and adjusted to pH = 2.8 using hydrochloric acid (c = 0.1 mmol·L^{-1}) according to recommendations by Westerhoff et al. [22]. This approach prevents absorbance interferences due to anions, reduces the interaction between metals and DOC, which might quench or enhance fluorescence, reduces absorbance interferences from DOC molecules, and eliminates the need for applying an inner-filter correction [23]. Sample volume was 2 mL at room temperature.

The fluorescence data are visualised in fluorescence excitation–emission matrices (F–EEMs). Interferences due to reflection at the cuvette (Rayleigh–Tyndall effect) were corrected using the software EEMScat and N-Way (Department of Food Science, University of Copenhagen, Denmark). Raman scattering of water molecules was considered by parallel fluorescence measurement of potassium chloride solution (c = 0.01 mmol·L^{-1}, pH = 2.8) and subtraction of its EEM from a sample's EEM.

2.1.3. Colorimetric determination of proteins and polysaccharides

Protein concentrations were determined using the colorimetric method according to Lowry et al. [24]. The resulting blue colouration of the sample was measured at λ = 750 nm with ultrapure water as reference and a blank based on ultrapure water. The method was calibrated with BSA standards up to 30 mg·L^{-1}. The limit of quantification was 3 mg·L^{-1} BSA equivalents.

Polysaccharide concentrations were determined according to Dubois et al. [25]. The resulting orange-brown colouration of the sample was measured at λ = 490 nm with ultrapure water as reference and a blank based on ultrapure water. The method was calibrated with glucose standards up to 100 mg·L^{-1}. The limit of quantification was 5 mg·L^{-1} glucose equivalents.

2.2. Experimental set-up

2.2.1. Effluent samples

Secondary effluent was obtained from the municipal sewage treatment plant Berlin–Ruhleben (accomplishing mechanical and biological treatment with biological nitrogen and phosphorus removal). For some tests, slow sand filter (SSF) filtrate of the secondary effluent was used, which was obtained from a pilot installation at the treatment plant (filter velocity: 0.5 m·h^{-1}; EBCT: 1.4 h). Furthermore, ultrafiltration (UF) permeate of the SSF filtrate was withdrawn from a UF pilot plant (MWCO = 100–150 kDa). One ultrafiltration test was conducted using the supernatant of sludge from a membrane bioreactor (MBR) being fed by municipal sewage.

Particles and larger colloids in municipal treated wastewaters contribute to the overall fouling of UF membrane installations; however, it has been shown that the contribution of the organic fraction being smaller than 0.45 μm is more than 50% of the overall fouling resistance [3]. Thus, the focus of the present study was the impact of dissolved effluent organic matter < 0.45 μm. Every sample was filtered through 0.45 μm cellulose nitrate filters after sampling from the sewage treatment plant in order to remove any particulate and large colloidal matter. LC–OCD chromatograms showed that secondary effluent, SSF filtrate and its UF permeate withdrawn at the same day were mainly different in terms of the biopolymer concentration, whereas the concentration of humic substances was quite similar. The supernatant of MBR sludge contained a fivefold higher biopolymer concentration compared to secondary effluent, but only 21% more humic substances.

2.2.2. Coagulation of secondary effluent

Pre-treatment of secondary effluent by coagulation was carried out using ferric chloride ($FeCl_3$) as coagulant. The coagulant solution was added to the secondary effluent at a stirring velocity of 400 min^{-1}. After 30 s, the stirring velocity was reduced and maintained at 60 min^{-1} for 10 min (procedure according to the German technical standard W 218 [26]). Following a 30 min sedimentation

time, the coagulated effluent was filtered through 0.45 μm cellulose nitrate filters in order to remove flocs.

2.2.3. Membrane module and ultrafiltration test procedure

A cross-flow membrane module for the execution of bench-scale ultrafiltration tests using outside-in PVDF hollow-fibre membranes by Memcor® (Memcor/Siemens Water Technologies, Windsor, NSW, Australia) was developed. The membrane fibres were potted into Plexiglas® mountings and fixed in the reusable Plexiglas® module. For each ultrafiltration test, a new bundle of hollow-fibre membranes was used. The principal characteristics of the membranes and test module are summarised in Table 1.

Prior to each test, the fresh membrane fibres were rinsed with 12 L of ultrapure water. The initial permeate flux J_0 of each membrane module was determined immediately before starting the test using ultrapure water. During the 25-h ultrafiltration tests, the filtration cycles consisted of 28.5 min of ultrafiltration, followed by 1.5 min of permeate backwashing at a flux of 3.5–4-fold of the permeate flux. Each ultrafiltration test was conducted using 10 L of the respective effluent at 23 ± 1°C. The permeate flux was continuously measured by an electronic balance; data were recorded by a computer. The experiments were carried out in recycle mode, returning the retentate continuously and the permeate periodically back to the feed tank. Consequently, the lab-scale UF module was tested at a low water conversion factor, since no concentration of the feed water occurred.

2.2.4. Resistance-in-series model

The hydraulically reversible and irreversible fouling due to dissolved effluent organic compounds was distinguished using the resistance-in-series model, which considers the total hydraulic filtration resistance to be the sum of separate resistances [27]:

Table 1
Characteristics of the membranes and test module

Outer diameter of the membrane fibres, mm	0.8
Inner diameter of the membrane fibres, mm	0.5
Nominal pore diameter, μm	0.04
Number of membrane fibres in the module	30
Length of each membrane fibre in the module, cm	30
Inner diameter of the module, cm	1.27 (= 1/2 inch)
Effective membrane area, cm^2	220
Constant trans-membrane pressure (TMP), bar	0.33
Permeability of the module at TMP = 0.33 bar, $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	280 ± 36
Average cross-flow velocity, $m \cdot s^{-1}$	0.25

$$R_{total} = \frac{\Delta p}{J \cdot \mu} = R_{membrane} + R_{rev.} + R_{irrev.} \quad (1)$$

with R_{total} — total filtration resistance, m^{-1}; Δp — trans-membrane pressure, $kg \cdot m^{-1} \cdot s^{-2}$, J — permeate flux, $m \cdot s^{-1}$; μ — dynamic viscosity, $kg \cdot m^{-1} \cdot s^{-1}$; $R_{membrane}$ — membrane resistance (to ultrapure water), m^{-1}; $R_{rev.}$ — hydraulically reversible resistance, m^{-1}; $R_{irrev.}$ — hydraulically irreversible resistance, m^{-1}.

Even during the 25-h ultrafiltration of ultrapure water, a reproducible loss of permeate flux of approximately 20% was observed due to the deposition of bacteria (confirmed by FE–SEM analyses, results not shown), which were inherent to the non-sterile test system and could not be removed despite frequent cleaning. Therefore, the filtration resistances of the ultrafiltration tests were corrected by the time-dependent resistance due to bacterial deposition in order to focus exclusively on fouling caused by dissolved effluent organic compounds. Substantial bacteria growth in deionised water during the stabilisation phase of the membrane module could be excluded by visual control.

3. Results and discussion

3.1. Ultrafiltration of different effluents

The ultrafiltration tests of the four effluent samples containing different amounts of biopolymers reveal significant differences in terms of the fouling behaviour. During the first hours of ultrafiltration, the pilot plant's UF permeate only causes a minor deterioration of the permeate flux (Fig. 1a). However, the flux decline within the separate filtration cycles increases with increasing biopolymer concentration of the effluent samples, with the most significant decline being caused by the supernatant of MBR sludge. In the case of slow sand filter (SSF) filtrate, the flux decline is completely reversible by permeate backwashing, whereas hydraulically irreversible flux losses are already observed in the initial phase of the ultrafiltration of secondary effluent and supernatant of MBR sludge.

After 20 h of ultrafiltration, two groups can be distinguished in terms of the filtration behaviour of the four effluents (Fig. 1b). In the case of SSF filtrate and its UF permeate, the permeate flux immediately after backwashing is approximately 80% of the initial flux J_0. This flux level corresponds to the filtration curve of ultrapure water after 20 h, so that the 20% net loss is not due to dissolved effluent organic matter. In contrast, regarding secondary effluent and supernatant of MBR sludge, only 60–70% of the initial flux is recoverable by permeate backwashing, thus indicating the occurrence of hydraulically irreversible fouling by dissolved organic substances.

The LC–OCD chromatograms of the feed and permeate samples of each ultrafiltration test illustrate that

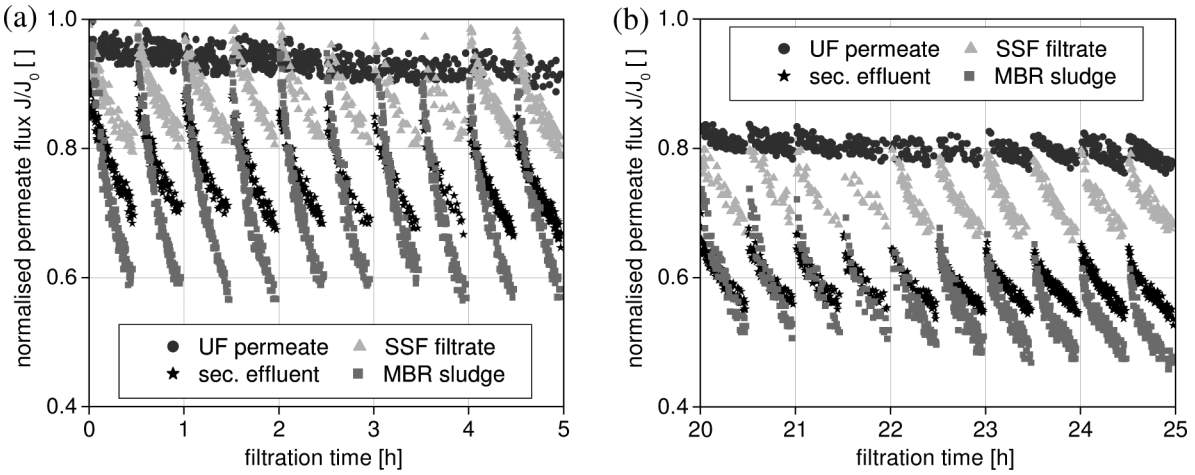

Fig. 1. Normalised permeate flux of ultrafiltration tests using supernatant of MBR sludge (DOC = 13.1 mg·L^{-1}), secondary effluent (DOC = 10.9 mg·L^{-1}), slow sand filter (SSF) filtrate (DOC = 9.4 mg·L^{-1}), and SSF filtrate's UF permeate (DOC = 8.7 mg·L^{-1}) (a) in the initial phase, (b) at the end of the tests (J_0: initial permeate flux).

the biopolymers are invariably largely retained by the hollow-fibre membrane (Fig. 2). However, the retention of humic substances is significantly lower and decreases even more during the ultrafiltration tests (Table 2). After an initial retention of 20–42%, which is probably caused by adsorption at the fresh membrane material, the transmission of humic substances increases due to the incremental saturation of the membrane surface. Apparently, despite the adsorption process, the membrane pores remain sufficiently open to allow for the passage of humic substances. The retention of humic substances remains relatively high only during ultrafiltration of the supernatant of MBR sludge. This may be explained by the formation of a gel layer of biopolymers at the membrane surface due to the much higher biopolymer concentration compared to other tests, thus changing the membrane's separation characteristics.

Regarding the composition of dissolved organic substances, the four effluents are different mainly in terms of the biopolymer content (except for minor differences in the overall content of humic substances; cf. peak at $t \approx 58$ min, Figs. 2 a–d, and Table 2). Since the biopolymers are the only fraction which is significantly retained by the membrane, the differences in the effluents' fouling behaviour are likely to be due to interactions between the biopolymers and the membrane pores. The humic substances fraction is considered to be of minor relevance in terms of fouling due to their high transmission through

Table 2
Feed water concentrations and retention percentages of ultrafiltration tests using different effluent samples

Ultrafiltration test	Feed concentration (mg·L^{-1} *C*)			Retention (%)		
	DOC	Biopolymers	Humic substances	Time (h)	Biopolymers	Humic substances
Supernatant of MBR sludge	13.1	2.40	5.07	0.5	93	42
				1	92	27
				24	92	24
Secondary effluent	10.9	0.50	4.20	0.5	86	27
				1	87	16
				24	88	8
Slow sand filter (SSF) effluent	9.4	0.34	3.95	0.5	81	27
				1	78	17
				24	83	13
UF permeate of SSF effluent	8.7	0.07	3.63	0.5	—	20
				1	—	8
				24	—	4

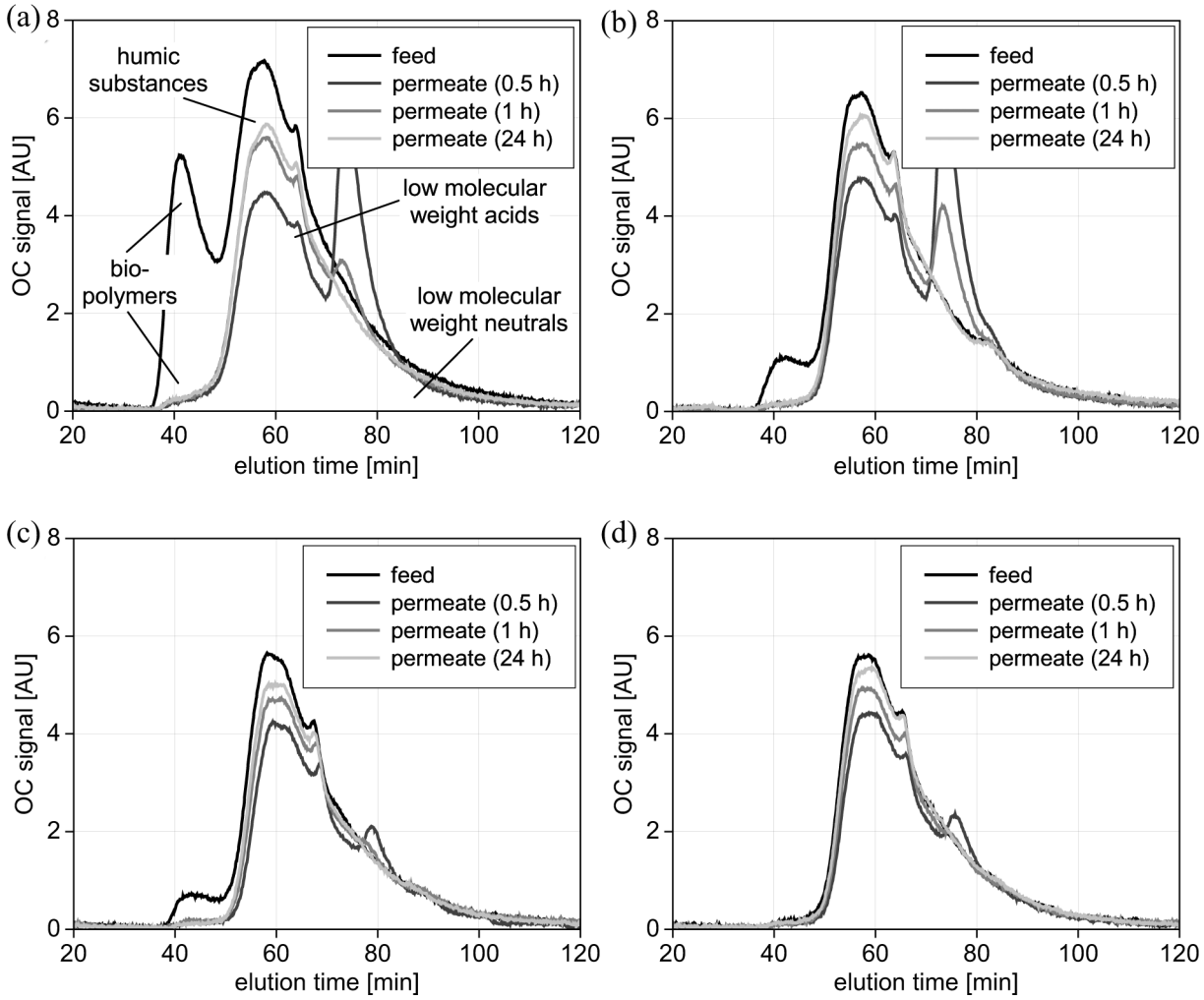

Fig. 2. LC–OCD chromatograms of feed and permeate samples of the ultrafiltration tests using (a) supernatant of MBR sludge, (b) secondary effluent, (c) SSF filtrate, (d) UF permeate of SSF filtrate (each sample threefold diluted; peaks at t ≈ 75 min are due to solvent residues originating from the membranes).

the applied mesoporous ultrafiltration membranes during the entire 25-h tests (Figs. 2 a–d, [28]).

3.2. Filtration resistances

The different extent of the flux decline within the filtration cycles and the hydraulic recoverability of the flux loss are reflected by the filtration resistances. The hydraulically reversible filtration resistance increases with increasing biopolymer concentration of the effluent (Fig. 3a). Within 24 h, the reversibility remains constant (except for the MBR supernatant with 2.4 $mg \cdot L^{-1}$ biopolymers, where the reversibility varies by less than 25% between start and end of the test).

In contrast, no correlation between the biopolymer concentration and the hydraulically irreversible filtration resistance in the initial filtration phase was observed (Fig. 3b). However, the different impact of the four effluents on the hydraulically irreversible organic fouling is confirmed by the irreversible resistances in the final stage of the tests. The ultrafiltration of secondary effluent and supernatant of MBR sludge results in the formation of relatively high irreversible filtration resistances at the end of the tests. On the other hand, no hydraulically irreversible fouling due to dissolved organic substances was observed during ultrafiltration of SSF filtrate and its UF permeate.

The biopolymer concentration of secondary effluents varies depending on the varying biological activity due to seasonal variations of the wastewater temperature and, especially in case of sewage treatment plants in combined sewer systems (as in Berlin–Ruhleben), the dilution by

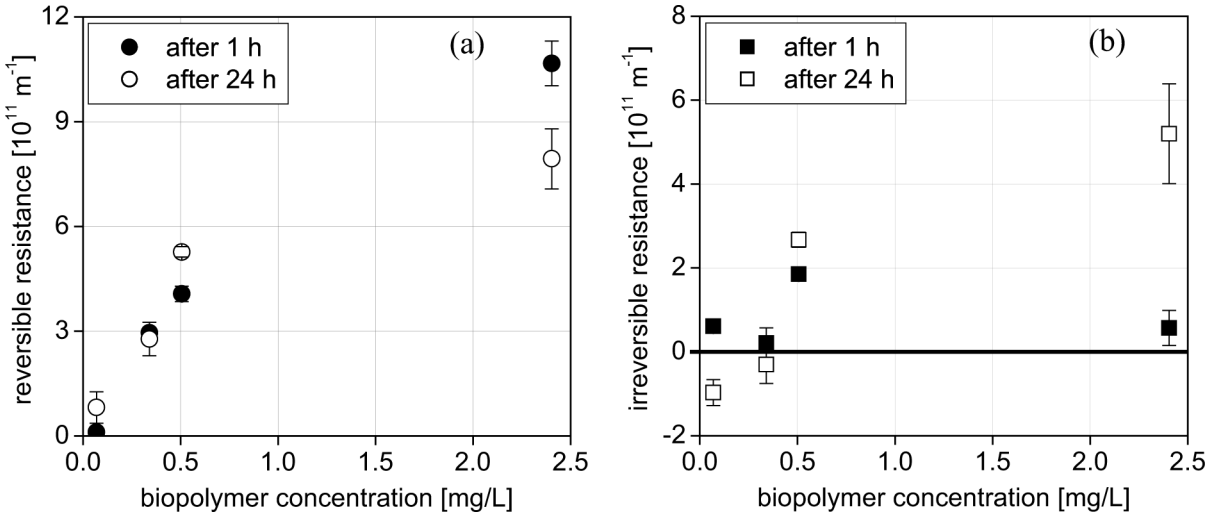

Fig. 3. Filtration resistances vs. biopolymer concentration (as carbon) of the ultrafiltration tests using supernatant of MBR sludge, secondary effluent, SSF filtrate, and UF permeate of SSF filtrate — (a) hydraulically reversible resistance, (b) hydraulically irreversible resistance (negative resistances are due to permeate fluxes after backwashing being slightly higher than in ultrafiltration of ultrapure water).

stormwater. The evaluation of further ultrafiltration tests using effluents with such varying biopolymer concentrations confirms the correlation between the carbon content of the biopolymer fraction and the hydraulically reversible membrane fouling, which can be well described by logarithmic functions (Figs. 4a and b).

The correlation between the biopolymer concentration and the hydraulically irreversible filtration resistance is again less distinct (Figs. 4c and d). However, while each sample of secondary effluent caused significant irreversible filtration resistances within 24 h, the ultrafiltration of SSF filtrate and its UF permeate resulted in no hydraulically irreversible fouling (see labelled data points in Fig. 4d). The differences between secondary effluents on the one hand, and SSF filtrate of secondary effluent and its UF permeate on the other hand indicate that dissolved effluent organic matter causing hydraulically irreversible fouling of ultrafiltration membranes is selectively removed by biological slow sand filtration. Therefore, biological sand filtration represents a space-consuming, but relatively simple pre-treatment method for the reduction of irreversible membrane fouling in tertiary filtration (cf. [29,30]), since the biopolymers remaining in secondary effluent after slow sand filtration apparently induce only hydraulically reversible membrane fouling.

3.3. Fluorescence analysis

In excitation–emission matrices (EEMs), the emission wavelength *Em* is plotted against the excitation wavelength *Ex* (Fig. 5). Usually, three main areas are distinguishable in EEMs of natural waters and wastewaters: The peaks A and B in Fig. 5a are based on the fluorescence of humic-like substances, and include both primary (B) and secondary (A) peaks, while the smaller peak C is referred to as protein-like compounds due to the fluorescence of the aromatic amino acids tryptophan, tyrosine and phenylalanine [31].

The comparison of the EEMs of feed and permeate samples of the ultrafiltration test using supernatant of MBR sludge indicates the reduction of the peak intensities of humic-like and protein-like substances (Figs. 5a and b). Subtraction of the permeate EEM (b) from the feed EEM (a) results in a differential EEM (Fig. 5c), which gives information about the character of organic substances being retained by the membrane. In the case of the supernatant of MBR sludge, the differential matrix indicates a high retention of protein-like substances (labelled area in Fig. 5c), while humic-like substances are retained to a lesser extent. This observation is in accordance with the LC–OCD chromatograms of the same samples, which show retentions of biopolymers (including proteins) and humic substances of 92% and 27%, respectively (cf. Fig. 2a and Table 2).

Further differential EEMs have been prepared from the EEMs of feed and permeate samples of the ultrafiltration tests using secondary effluent, SSF filtrate of secondary effluent, and UF permeate of SSF filtrate, respectively (Fig. 6). The differential EEM of secondary effluent (Fig. 6a) also reflects the increased retention of protein-like compounds and a higher transmission of humic-like substances in ultrafiltration. This coincides with the corresponding LC–OCD chromatograms, reveal-

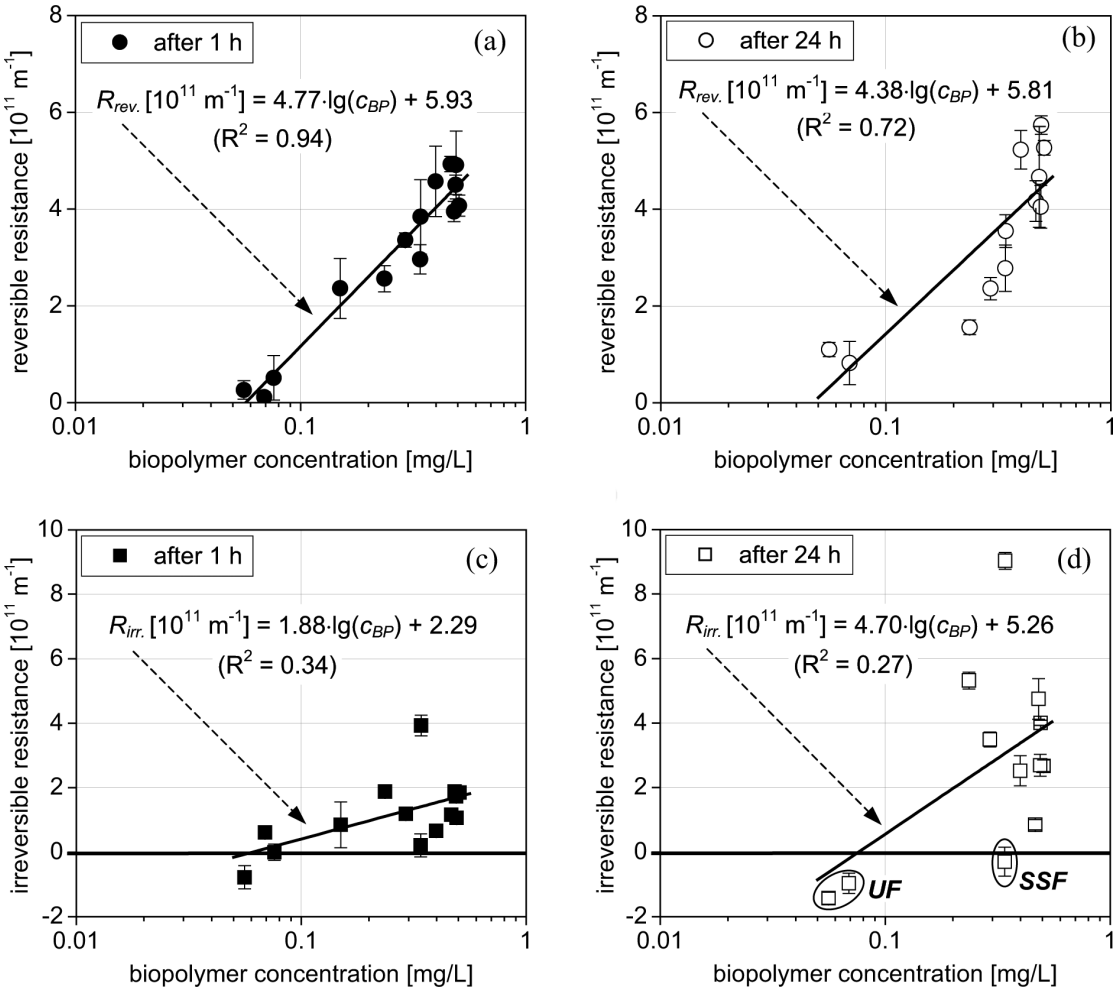

Fig. 4. Correlation between the biopolymer concentration c_{BP} (as carbon concentration) of different effluents and (a) hydraulically reversible resistance after 1 h, (b) hydraulically reversible resistance after 24 h, (c) hydraulically irreversible resistance after 1 h, (d) hydraulically irreversible resistance after 24 h.

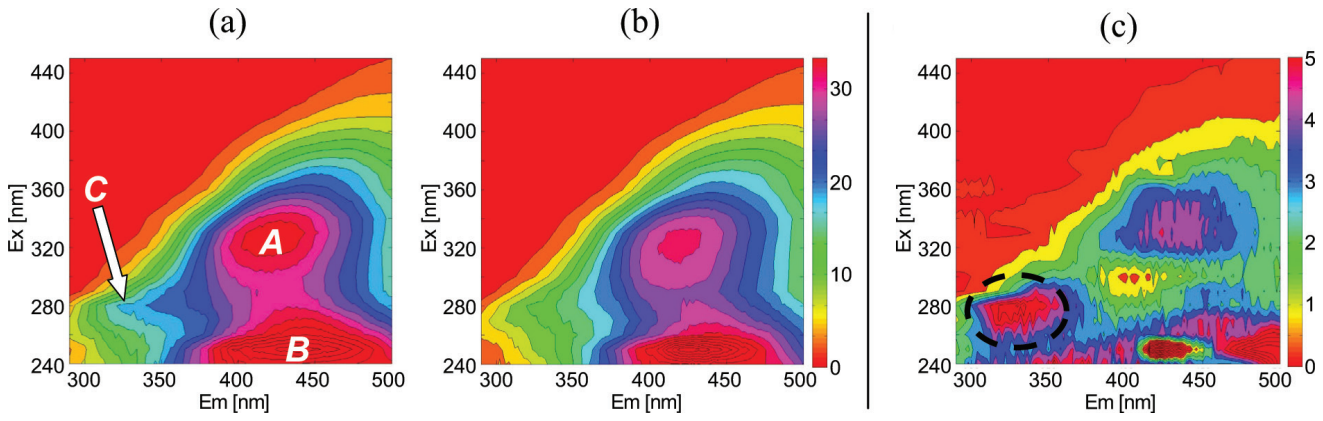

Fig. 5. EEMs of the ultrafiltration test using supernatant of MBR sludge after 1 h of filtration — (a) feed, (b) permeate, (c) differential EEM: (a)–(b).

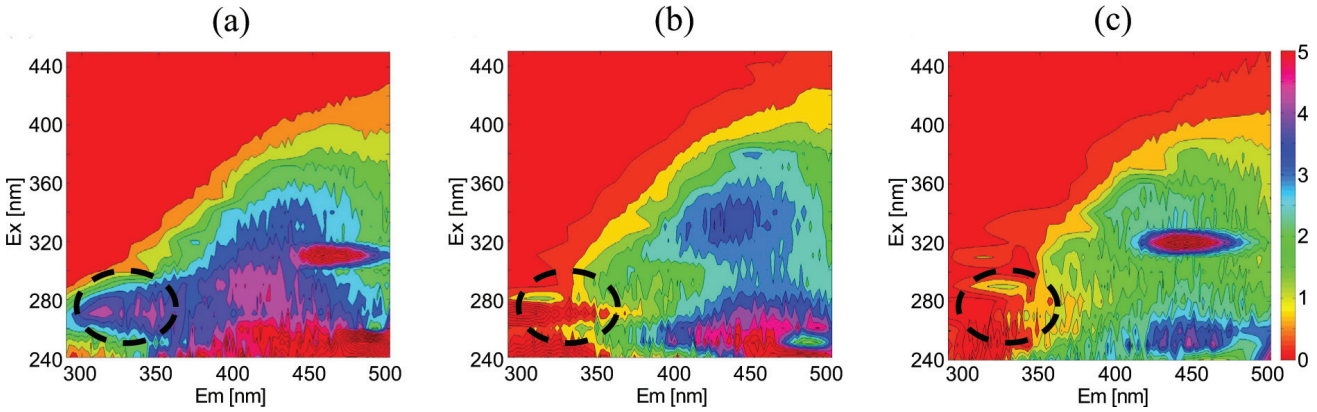

Fig. 6. Differential EEMs of the ultrafiltration tests using (a) secondary effluent, (b) SSF filtrate, (c) UF permeate of SSF filtrate (each based on samples taken after 1 h of filtration).

ing retentions of biopolymers and humic substances of 87% and 16%, respectively (cf. Fig. 2b and Table 2).

According to the LC–OCD analyses, the SSF filtrate still contains a significant amount of biopolymers, i.e., only approximately 30% less than the original secondary effluent (Figs. 7a and b, Table 2). When SSF is ultrafiltered, the remaining biopolymers are retained by approximately 80% (related to OC) by the UF membrane (Figs. 7b and c, Table 2). What is the character of these retained organics? The differential EEM of SSF and its UF permeate reveals practically no retention of protein-like compounds, but only a minor retention of humic-like substances (Fig. 6b). The reason might be that during slow sand filtration, proteins in the biopolymer range (t = 35–47 min) are predominantly removed from the secondary effluent compared to polysaccharides.

This hypothesis is supported by LC–OCD–ON data (Figs. 7a and b), showing that the ON content of secondary effluent in the biopolymer range decreases by 45%, compared to an OC decrease of approximately 30%. The $UV_{254\ nm}$ absorption behaviour is also in accordance since the integral of the $UV_{254\ nm}$ curve in the biopolymer range of secondary effluent is overproportionally reduced compared to the decrease of the OC content of its biopolymer fraction. Even if the LC–OCD–ON curve currently does not reflect 100% of the total ON content, we assume comparable results in terms of the ON signal when secondary effluent, its SSF and its UF permeate are compared directly to one another in the range of the biopolymer peak.

The approximately 80% retention of organic compounds in UF permeate of SSF is likely to be based on the retention of polysaccharide-like substances, which are only moderately removed by slow sand filtration and are not fluorescence-active either.

The ultrafiltration of both secondary effluent and its SSF filtrate results in the formation of hydraulically reversible filtration resistances, whose intensities correlate with the organic carbon concentration of the biopolymer fraction (cf. Fig. 3a). However, ultrafiltration of secondary effluent results in the increase of the hydraulically irreversible filtration resistance, whereas no irreversible fouling is observable in ultrafiltration of SSF filtrate (cf. Fig. 3b). The absence of irreversible fouling is likely to be due to the SSF filtrate's significantly lower concentration of protein-like substances interacting with the membrane. The biopolymers remaining in the secondary effluent after slow sand filtration include predominantly polysaccharide-like substances, which may be explained by their decreased biodegradability as compared to extracellular proteins [32]. Thus, the correlation between the biopolymer concentration and the hydraulically reversible fouling is predominantly due to the retention of long-chain polysaccharide-like substances, which are removable from the membrane surface by permeate backwashing. In contrast, the more compact protein molecules [33] are likely to enter the membrane pores to a certain extent, therefore eventually constricting and/or blocking the pores.

The UF permeate of the SSF effluent from the pilot plant contains almost no protein-like substances, thus no protein-like peak appears in Fig. 6c (labelled area). Accordingly, there is no irreversible fouling during the ultrafiltration test (cf. Fig. 3b).

3.4. Colorimetric determination of proteins and polysaccarides

In addition to LC–OCD analyses, protein and polysaccharide concentrations of samples taken after 0.5 h, 1 h, and 24 h of ultrafiltration were determined by applying the colorimetric methods of Lowry et al. [24] and Dubois et al. [25], respectively. In contrast to the biopolymer concentrations being reproducibly measured by LC–OCD, the colorimetrically determined concentrations were

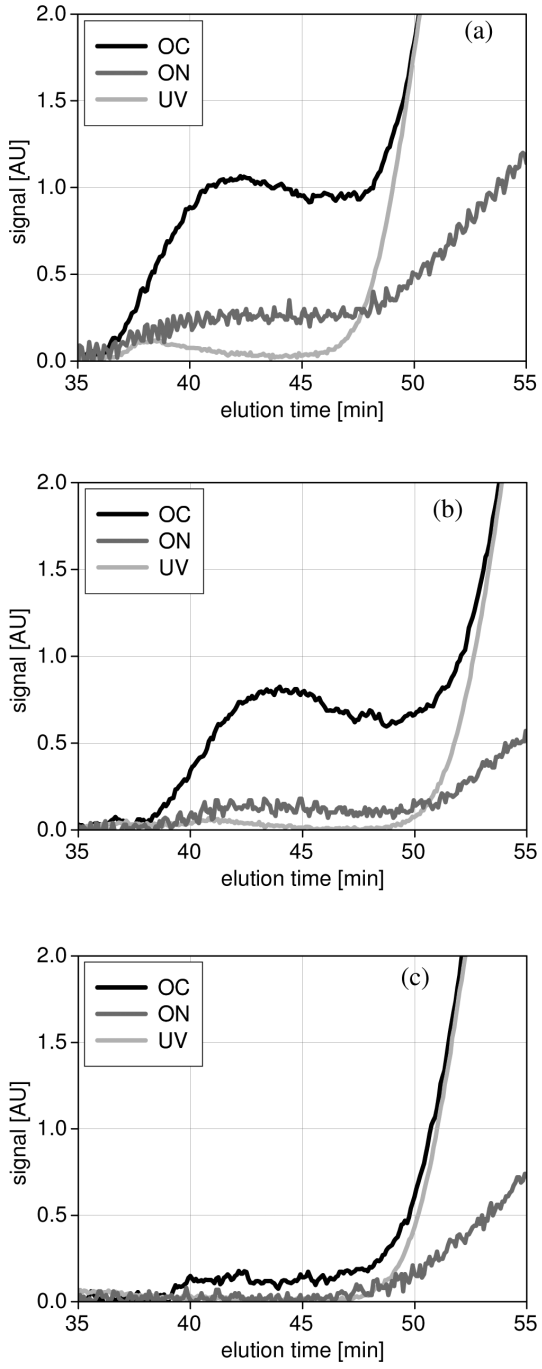

Fig. 7. Organic carbon (OC), organic nitrogen (ON) and $UV_{254\,nm}$ (UV) signals of the LC–OCD biopolymer fraction — (a) secondary effluent, (b) SSF filtrate, (c) UF permeate of SSF filtrate (each sample threefold diluted).

varying without showing any clear trend during the ultrafiltration tests. Thy calculated filtration resistances in the UF tests did not correlate at all with measured mean concentrations of the protein and polysaccharide concentrations (data not shown).

The protein concentrations of the secondary effluents vary over the range of 8–10.5 mg·L^{-1} BSA equivalents, with the lowest concentration) measured in the UF permeate. The comparatively high colorimetrically determined protein concentration is probably due to false-positive values caused by reactions of humic substances with the Folin–Ciocalteau reagent. In the method according to Lowry et al. [2,] the Folin–Ciocalteau reagent reacts with proteins but also unspecifically with humic substances [33]. Application of the modified method with correction for humic substances as suggested by Frølund et al. [34] resulted in negative values, so that the correction for humic substances was not further carried out. The colorimetrically determined polysaccharide concentrations vary between 2.5 and 5 mg·L^{-1} as glucose equivalents, which were invariably below the method's limit of quantification (5 mg·L^{-1}).

Although the application of the colorimetric methods is faster, easier, and less expensive in comparison to LC–OCD analysis, their applicability for the determination of the organic fouling potential of secondary effluents is restricted by ineffective data resolution of proteins and polysaccharides in the considered concentration ranges. Interferences with further constituents of secondary effluents are another constraint, especially in the case of the protein analysis. the presented results indicate that the colorimetric methods at their current development stage are inappropriate for the determination of the organic fouling potential of secondary effluents. Conversely, the LC–OCD and F–EEM techniques offer much greater analytical sensitivity.

3.5. Ultrafiltration of coagulated secondary effluent

The impact of previous coagulation on the hydraulically reversible and irreversible fouling was investigated using 0.05–0.2 mmol·L^{-1} $FeCl_3$ as coagulant. LC–OCD analyses indicate a disproportionately high removal of biopolymers in comparison to humic substances (Table 3), confirming the results of a former study using higher coagulant dosages [28].

In the subsequent ultrafiltration tests, the removal of biopolymers resulted in a proportional decrease of the hydraulically reversible filtration resistance (Fig. 9a), thus confirming the correlation between the biopolymer concentration and the hydraulically reversible resistance (cf. Fig. 4). However, after 24 h of ultrafiltration, two pairs of hydraulically irreversible filtration resistances can be distinguished (Fig. 9b). During ultrafiltration of both untreated secondary effluent and effluent after coagulation at 0.05 mmol·L^{-1} $FeCl_3$, the hydraulically irreversible resistance increases, whereas no hydraulically irreversible fouling by dissolved organic substances can be observed after coagulation at higher $FeCl_3$ dosages. Thus, the filterability of the secondary effluent is significantly increased by coagulation at dosages > 0.1 mmol·L^{-1} $FeCl_3$.

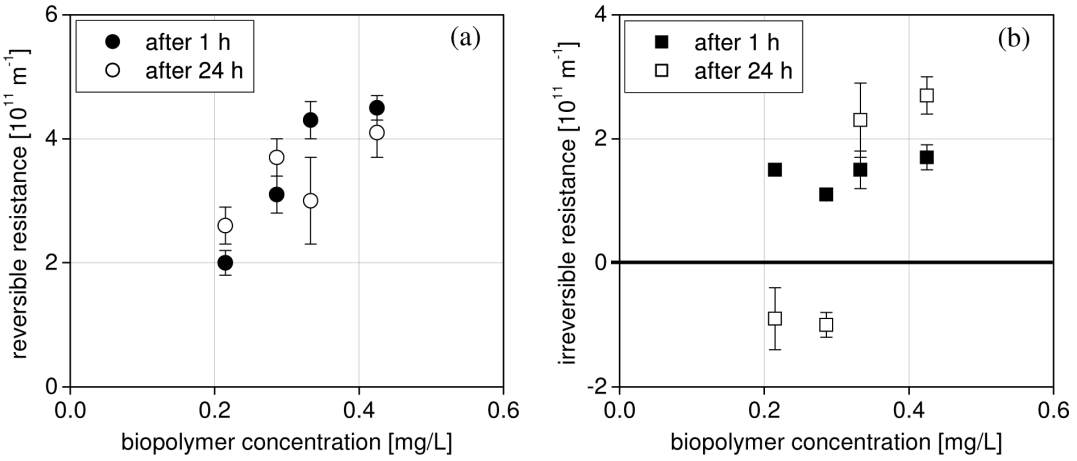

Fig. 8. Filtration resistances vs. biopolymer concentration (as carbon) of the ultrafiltration tests using untreated secondary effluent with and without previous coagulation — (a) hydraulically reversible resistance, (b) hydraulically irreversible resistance.

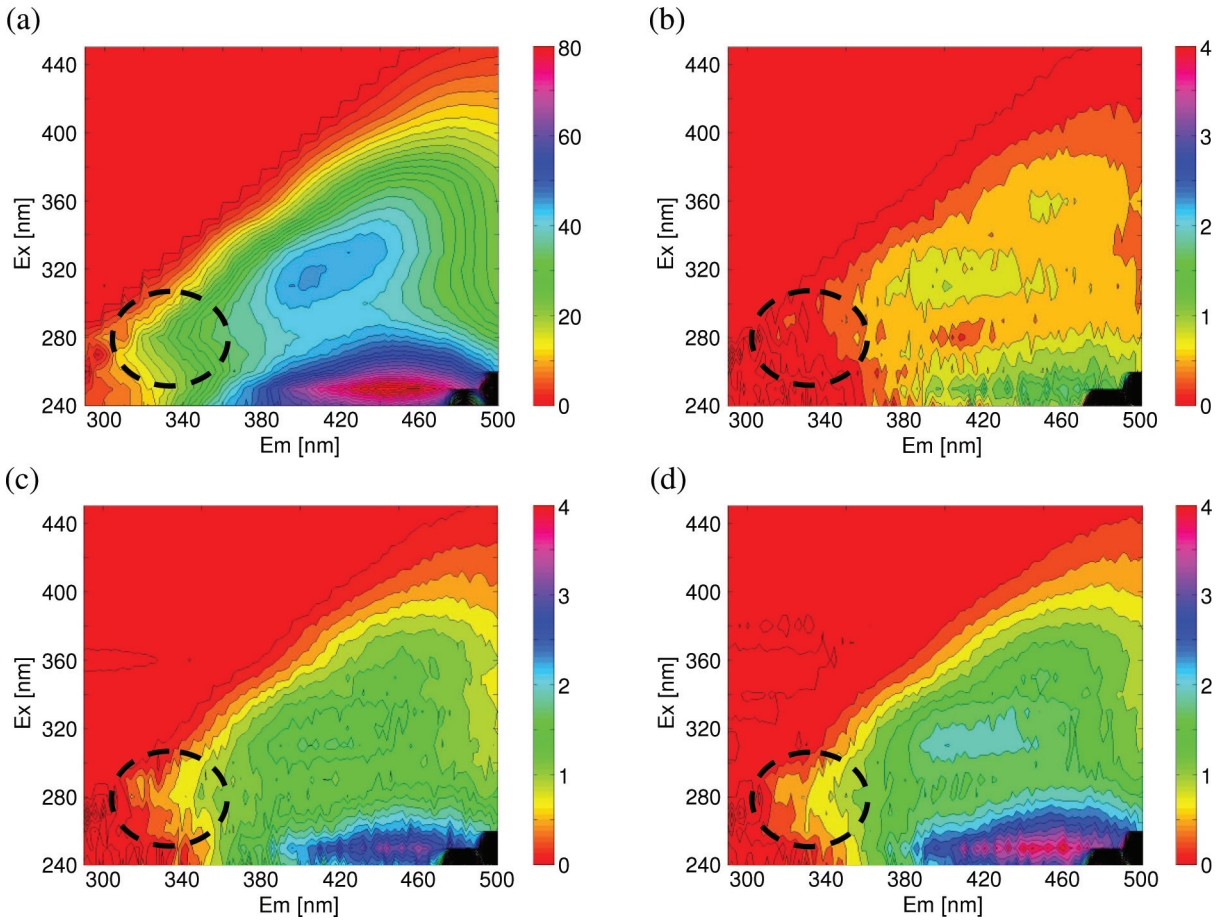

Fig. 9. EEMs of ultrafiltration tests using secondary effluent after coagulation (a) EEM of untreated secondary effluent (protein-like substances are labelled); (b) Differential EEM: secondary effluent – secondary effluent after coagulation at 0.05 mmol·L^{-1} Fe^{3+}; (c) Differential EEM: secondary effluent – secondary effluent after coagulation at 0.1 mmol·L^{-1} Fe^{3+}; (d) Differential EEM: secondary effluent – secondary effluent after coagulation at 0.2 mmol·L^{-1} Fe^{3+}.

Table 3
Feed concentrations of ultrafiltration tests using secondary effluent without further pre-treatment and after coagulation using $FeCl_3$

	DOC		Biopolymers		Humic substances	
	($mg \cdot L^{-1}$)	(%)	($mg \cdot L^{-1}$)	(%)	($mg \cdot L^{-1}$)	(%)
Untreated secondary effluent	10.2	100	0.42	100	4.26	100
Secondary effluent after 0.05 $mmol \cdot L^{-1}$ Fe^{3+}	9.8	96	0.33	79	3.96	93
Secondary effluent after 0.1 $mmol \cdot L^{-1}$ Fe^{3+}	9.0	88	0.29	69	3.49	82
Secondary effluent after 0.2 $mmol \cdot L^{-1}$ Fe^{3+}	8.5	83	0.22	52	3.07	72

A similarly significant increase of the filterability of surface water was observed by Bagga et al. [35] in microfiltration with previous coagulation using $FeCl_3$. While the filterability was not influenced by coagulation at 0.1 $mmol \cdot L^{-1}$ $FeCl_3$, the fouling decreased after coagulation using 0.2 $mmol \cdot L^{-1}$ $FeCl_3$. According to Bagga et al. [35], the reduction of the fouling is due to the increased embedding of colloids and macromolecules with aromatic structures into the ferric hydroxide flocs.

Further information regarding the influence of coagulation on the organic composition of secondary effluent was obtained by fluorescence analyses of the ultrafiltration feed waters. The EEM of the untreated secondary effluent is dominated by humic substances, but shows also the presence of protein-like compounds (Fig. 9a). Despite the relatively high reduction of the biopolymer concentration by coagulation at 0.05 $mmol \cdot L^{-1}$ $FeCl_3$ (cf. Table 3), the differential EEM of the original and coagulated effluent indicates no removal of protein-like substances (Fig. 9b). However, coagulation using 0.1 $mmol \cdot L^{-1}$ and 0.2 $mmol \cdot L^{-1}$ $FeCl_3$ results in an increased removal not only of humic substances, but also of protein-like compounds (Figs. 9c and d).

The fluorescence analyses indicate that the reduction of the hydraulically irreversible organic fouling at coagulation dosages > 0.1 $mmol \cdot L^{-1}$ $FeCl_3$ is due to the increased removal of protein-like substances. This test series confirms the correlation between the total biopolymer concentration and the extent of the hydraulically reversible organic fouling, as well as the dependency of the hydraulically irreversible organic fouling on the qualitative composition of the biopolymer fraction, again indicating the high relevance of protein-like substances. Furthermore, it is shown that relatively low coagulant dosages may be sufficient for the considerable reduction of the irreversible fouling potential of secondary effluent.

In the present study, the flocs produced by coagulation were separated from the effluent prior to ultrafiltration due to the focus on fouling by dissolved organic compounds. With regard to the practical application of coagulation as pre-treatment method, the impact of the floc properties on the membrane permeability should be examined in further investigations.

4. Conclusions

The influence of different organic fractions present in secondary effluents on the hydraulically reversible and irreversible organic fouling of hollow-fibre ultrafiltration membranes has been investigated by different analytical approaches including (i) SEC followed by organic carbon detection (LC–OCD), (ii) SEC followed by $UV_{254\,nm}$ absorption (LC–OCD–UV), (iii) SEC followed by organic nitrogen detection (LC–OCD–ON) as well as (iv) fluorescence analysis excitation–emission matrices (EEM). The results obtained suggest the following conclusions:

- Due to the high retention of the macromolecular biopolymers (including mainly proteins and polysaccharides) by the ultrafiltration membrane, this fraction has a high fouling potential despite its comparatively low concentration in secondary effluents (generally < 0.5 $mg \cdot L^{-1}$ C as measured by LC–OCD).
- In contrast, the more abundant humic substances are mostly transmitted through the membrane and consequently have a minor impact on organic membrane fouling.
- The hydraulically reversible filtration resistance correlates well with the effluents' total biopolymer concentration according to LC–OCD analysis. However, no correlation was observed between the biopolymer concentration and the hydraulically irreversible organic fouling.
- Regarding the fouling mechanisms of biopolymers, it is suggested that the hydraulically reversible filtration resistance depends on (long-chain) polysaccharide-like substances, which are removable by permeate backwashing.
- Fluorescence excitation–emission matrices indicate that the extent of the hydraulically irreversible organic fouling in ultrafiltration of secondary effluents depends mainly on the concentration of protein-like substances.
- The hydraulically irreversible organic foulins is presumably caused by the deposition of more compact protein molecules at or inside the pores, thus resulting in the constriction and/or blockage of the membrane pores. Beside the results of fluorescence

analysis, LC–OCD–ON and LC–OCD–UV confirm the predominant role of protein-like compounds within the biopolymer fraction in terms of the hydraulically irreversible fouling.

- The hydraulically irreversible organic fouling of ultrafiltration membranes can be reduced by pre-treatment with biological slow sand filtration (SSF) or chemical coagulation with comparatively low coagulant dosages (in this case > 0.1 mmol·L^{-1} Fe^{3+}). EEM analysis indicates that for both pre-treatment methods, the removal of protein-like substances is disproportionately high in comparison to the reduction of the total biopolymer concentration.
- Regarding the determination of the organic fouling propensity of secondary effluents, the commonly applied colorimetric methods according to Lowry et al. [24] and Dubois et al. [25] are considered to be inappropriate due to low effluent concentrations of proteins and polysaccharides, as well as interferences with other effluent constituents.

At the current state of the art, the technologically more elaborate size exclusion chromatography with continuous organic carbon detection (LC–OCD) allows only for the evaluation of the less relevant hydraulically reversible organic fouling potential by measuring the biopolymer concentration. Fluorescence EEM analysis provides the potential for the selective determination of proteins. However, the fluorescence peak of the so-called protein-like substances includes not only macromolecules, but also smaller molecules containing aromatic amino acids (e.g., polypeptides), which are not necessarily membrane foulants.

A combination of size exclusion chromatography with $UV_{254\ nm}$, OC, ON detection and subsequent fluorescence analysis may provide an alternative for the quantitative determination of the irreversible fouling potential of effluents. Focussing on the characterisation of organic compounds, Her et al. [37] and Park [38] investigated a sequential experimental set-up in three different waters. With fixed fluorescence wavelengths for protein-like compounds (*Ex*: 278 nm, *Em*: 353 nm) and humic/fulvic-like compounds (*Ex*: 337 nm, *Em*: 423 nm), both authors conclude that the identified fractions can give additional information in terms of the optimisation of the applied water treatment. Considering the sensitive detection limit on dissolved organic matter and the detailed identification of especially protein-like substances, we expect the combination of size exclusion chromatography and fluorescence analysis to be promising with regard to the optimisation of the pre-treatment for fouling reduction and the operation of future membranes applications.

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