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Correlation of EPS content in activated sludge at different sludge retention times with membrane fouling phenomena

Djamila Al-Halbouni[a], Jacqueline Traber[b], Sven Lyko[c], Thomas Wintgens[c], Thomas Melin[c], Daniela Tacke[d], Andreas Janot[e], Wolfgang Dott[a], Juliane Hollender[b,]*

[a]*Institute of Hygiene and Environmental Health, RWTH Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany*
[b]*Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstr. 133, CH 8600 Dübendorf, Switzerland*
[c]*Department of Chemical Engineering, RWTH Aachen University, Turmstr. 46, D-52056 Aachen, Germany*
[d]*Institute of Environmental Engineering, RWTH Aachen University, Mies-van-der-Rohe-Str. 1, D-52074 Aachen, Germany*
[e]*Erftverband, Paffendorfer Weg 42, D-50126 Bergheim, Germany*

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ABSTRACT

In this study, activated sludge characteristics were studied with regard to membrane fouling in membrane bioreactors (MBRs) for two pilot plants and one full-scale plant treating municipal wastewater. For the full-scale MBR, concentrations of extracellular polymeric substances (EPS) bound to sludge flocs were shown to have seasonal variations from as low as 17 mg g^{-1} dry matter (DM) in summer up to 51 mg (g DM)$^{-1}$ in winter, which correlated with an increased occurrence of filamentous bacteria in the colder season. Therefore, it was investigated at pilot-scale MBRs with different sludge retention times (SRTs) whether different EPS contents and corresponding sludge properties influence membrane fouling. Activated sludge from the pilot MBR with low SRT (23 d) was found to have worse filterability, settleability and dewaterability. Photometric analysis of EPS extracts as well as LC-OCD measurements showed that it contained significantly higher concentrations of floc-bound EPS than sludge at higher SRT (40 d) The formation of fouling layers on the membranes, characterised by SEM-EDX as well as photometric analysis of EPS extracts, was more distinct at lower SRT where concentrations of deposited EPS were 40-fold higher for proteins and 5-fold higher for carbohydrates compared with the membrane at higher SRT. Floc-bound EPS and metals were suggested to play a role in the fouling process at the full-scale MBR and this was confirmed by the pilot-scale study. However, despite the different sludge properties, the permeability of membranes was found to be similar.

1. Introduction

Membrane fouling is one of the main limitations in full-scale applications of membrane bioreactor (MBR) for wastewater treatment plants (WWTPs) and concerns scientists as well as operators (Le-Clech et al., 2005). Among the most observed operating parameters with an expected impact on fouling propensity is certainly the solid (or sludge) retention time (SRT), which in turn has effects on various sludge properties such as floc size, extracellular polymeric substance (EPS) content, settling characteristics, soluble microbial products (SMPs) and others (Le-Clech et al., 2006).

*Corresponding author. Tel.: +41 44 823 5493; fax: +41 44 823 5893.
E-mail addresses: djamila.al-halbouni@rwth-aachen.de (D. Al-Halbouni), jacqueline.traber@eawag.ch (J. Traber), lyko@ivt.rwth-aachen.de (S. Lyko), wintgens@ivt.rwth-aachen.de (T. Wintgens), melin@ivt.rwth-aachen.de (T. Melin), tacke@isa.rwth-aachen.de (D. Tacke), andreas.janot@erftverband.de (A. Janot), wdott@ukaachen.de (W. Dott), juliane.hollender@eawag.ch (J. Hollender).
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In several recent studies different aspects of SRT were examined; some researchers focused on lab-scale plants (Liao et al., 2006), some used synthetic wastewater (Liang et al., 2007), others analysed extremely low to extremely high SRT (Massé et al., 2006; Ng et al., 2006). Most of these studies present data on sludge supernatant and effluent as well as transmembrane pressure (TMP) development to assess the extent of fouling. However, what is lacking is a more detailed examination of the utilised membranes as well as insights into the fouling mechanisms and their relation to SRT-dependent parameters.

The observation of a negative impact on membrane performance by low SRT is generally explained by high concentrations of SMP or bound EPS (Ahmed et al., 2007; Chang and Lee, 1998; Liang et al., 2007, Massé et al., 2006; Ng et al., 2006). Special importance is ascribed to carbohydrates, particularly polysaccharides in the supernatant (Grelier et al., 2006; Jinsong et al., 2006). The commonly used TMP or flux measurements as indicators of advancing membrane fouling do not always reflect the observed differences in SMP concentrations at different SRTs. Interestingly, it was reported that SMP samples from bench-scale sequencing batch reactors at different SRTs did not affect the flux decline trends of reverse osmosis and nanofiltration membranes in dead-end filtration cell tests (Jarusutthirak and Amy, 2006) or that the overall fouling resistance in submerged MBRs even increased as SRT prolonged (Lee et al., 2003), although higher SRT is generally regarded as beneficial for membrane performance (Le-Clech et al., 2006).

Other factors that can vary with SRT and thus influence the actual fouling phenomenon are mixed liquor suspended solids (MLSS) and dissolved oxygen concentration. The rate of membrane fouling was found to be faster for low dissolved oxygen concentration due to preferential deposition of small particles on the membrane, thus leading to a less porous biofilm with worse filterability (Jin et al., 2006). Therefore control of aeration in submerged MBR studies is essential. In addition, sludge morphology changes significantly if the SRT is modified; this involves changes in sludge floc size and leads to the development of different predominant microbial communities, which can have different effects on membrane fouling (Ahmed et al., 2007; Meng et al., 2006). Massé et al. (2006) reported the development of non-flocculating organisms with increasing SRT and at the same time better settling properties of the sludge as shown by a decreasing sludge volume index (SVI). They explained their observations with less biopolymer production by non-flocculating bacteria as well as better degradation and hydrolysis of macromolecules at high SRT.

In a recent study (Lyko et al., 2007a, b), the long-term observation of activated sludge supernatant as well as membrane foulants of a full-scale MBR revealed a typical composition of three main components in the order metals > humic acids > carbohydrates > proteins. There it was also shown that temperature impacts on mixed liquor carbohydrate concentrations and that these variations can be related to conventional sludge parameters such as capillary suction time (CST) and SVI. Starting from those results, the aim of the present study was to investigate the influence of SRT on EPS and sludge characteristics more thoroughly in pilot-scale plants. For this purpose, two pilot plants with different SRTs, but at the same time with operational conditions that are close to full-scale mode, were run in parallel. The focus was to find correlations between sludge properties, especially EPS contents, and membrane fouling phenomena with regard to the different SRTs.

2. Materials and methods

2.1. *Full-scale MBR Nordkanal in Germany*

The operation of the full-scale MBR Nordkanal in Kaarst (North Rhine-Westphalia, Germany) started in the beginning of 2004. This municipal WWTP is operated by the Erftverband and has a capacity of 80,000 population equivalents, treating up to 45,000 m^3 wastewater per day. The composition of the raw wastewater is typical for German municipal wastewater, with a mean chemical oxygen demand (COD) of 489 $mg\,L^{-1}$ and biological oxygen demand (BOD) of 171 $mg\,L^{-1}$ during the sampling period. The plant consists of pre-treatment facilities including pre-screening followed by the MBR unit, which is divided into four parallel lines. Each line consists of a denitrification tank (pre-denitrification), a vario-zone and a nitrification tank (Engelhardt et al., 2005). The membrane modules for biomass separation are immersed directly in the nitrification tanks. The submerged hollow fibre modules (ZENON ZeeWeed® 500c PVDF, 0.04 μm nominal pore size) have a total membrane area of 84,480 m^2. The MLSS concentration varies between 9 and 12 $g\,L^{-1}$ depending on the season. The SRT is 28 d. For the present study, activated sludge samples were taken monthly from the nitrification tank (line 4).

2.2. *Description of the pilot plants*

Two pilot MBRs with a bioreactor volume of 260 L each were operated as nitrification zones in parallel with the primary effluent of the WWTP Soers (Aachen, Germany). This WWTP treats typical German municipal wastewater with a COD and BOD in the range of 600–800 and 300–400 $mg\,L^{-1}$, respectively. After an initial phase of approximately 4 months, the continuous operation mode was established in the pilot plants. The plants contained sensors for online measurements of temperature (mean $19 \pm 3\,^{\circ}C$), pressure, level and flux. The pH value (mean 7 ± 0.1) and oxygen concentration (in the range of 2.2–2.6 $mg\,L^{-1}$) were determined manually. The two different solid retention times were achieved by adjusted daily excess sludge removal. In order to minimise the impact of biomass concentration on fouling, the MLSS concentration was set to 12 $g\,L^{-1}$ in both pilot plants (Table 1). This required a higher inflow rate of wastewater to the plant with a lower SRT, otherwise the sludge production would be insufficient to keep the MLSS concentration. At the same time it was aimed at keeping the permeate flux at 15 $L\,m^{-2}\,h^{-1}$ for both plants, which in turn explains the bigger membrane area in the plant with a lower SRT. The hydrodynamic conditions for the filtration were comparable in both plants as the reduced membrane area in pilot plant 1 (SRT 40 d) was achieved by sealing the fibres of one bundle in the otherwise

Table 1 – Summary of design of pilot plants and measured parameters used for the study in 2006

Parameter (unit)	Pilot plant 1	Pilot plant 2
SRT (design) (d)	30	15
SRT (calculated) (d)	40±17.9	23±11.2
MLSS (design) ($g L^{-1}$)	12	12
MLSS (measured) ($g L^{-1}$)	12±1.5	13±2.5
Active membrane area (m^2)	1.43	2.23
Permeate flow (design) ($L m^{-2} h^{-1}$)	15	15
Permeate flow (calculated) ($L m^{-2} h^{-1}$)	13±3.1	14±0.3
F/M ratio (calculated) (kg COD (kg MLSS d)$^{-1}$)	0.09±0.025	0.14±0.050
HRT (calculated) (h)	12±2.3	9±5.7

identical module. The applied membranes were immersed hollow fibre modules (PURON, KMS Germany) of three polyethersulphone (PES) fibre bundles (nominal pore size 0.05 μm, Judd, 2006). The filtration cycle with cross-flow aeration was 4 min filtration and 30 s relaxation without backwashing. SRT, hydraulic retention time (HRT) and food to microorganisms (F/M) ratios were calculated according to Metcalf and Eddy (2004) (Table 1).

2.3. Sampling

The design SRTs of 15 and 30 d were chosen in a range that is relevant for full-scale operation and close to the SRT of the full-scale plant Nordkanal with 28 d. After continuous operation had been established in the pilot plants, activated sludge and MBR effluent were sampled every 2 weeks for 3.5 months in 2006. The collected data allowed an analysis of sludge characteristics with regard to low and high SRT and related EPS concentrations. Grab-activated sludge samples as well as effluent (MBR permeate) samples were taken from the pilot MBRs and the full-scale plant and cooled during transport, storage and preparation. Membrane autopsies were conducted once in October 2006. Three single fibres were cut from each of the two membrane modules and sealed in a plastic bag to be transported on ice to the laboratory.

For the full-scale MBR Nordkanal, activated sludge samples were taken monthly for a period of 2.5 years.

2.4. Determination of sludge properties

The filtration index (FI) of activated sludge supernatant is a measure of filterability and was determined in a stirred dead-end filtration cell according to Rosenberger (2003). Flat PES sheet membranes with a molecular cutoff (MWCO) of 150 kDa were used with a constant TMP of 1 bar. Membrane permeability (L_P) was determined after the filtration and recovery of 15 mL permeate. The FI_{15} was calculated as the ratio of activated sludge permeability and clean water permeability after the filtration of 15 mL.

The SVI is a measure for settleability of activated sludge and was determined in a 1 L glass cylinder after a settling time of 30 min according to German standard norm DIN 38414 part 10. Sludge had to be diluted if the settled sludge volume exceeded 250 mL L^{-1}. MLSS were analysed according to German DIN 38414 part 2. Dewaterability of the mixed liquor was measured as the CST using a capillary suction timer (HWT Wassertechnik, Germany).

2.5. EPS extraction

A method using Dowex (Sigma-Aldrich 91973) as a cationic exchange resin was used to extract EPS from activated sludge (Froelund et al., 1996). Briefly, activated sludge samples were centrifuged twice for 15 min at 30,000*g* (4 °C) to separate the supernatant including soluble substances from the biomass with bound EPS. Extraction of bound EPS was performed with 75 g Dowex per gram dry matter for 2 h stirring at 900 rpm in a beaker at 4 °C. The same principle was adapted to extract EPS from membranes. For these experiments, the membrane surface was first rinsed with distilled water to remove any macroscopically visible sludge remainder; fibres were then cut into pieces and extracted as described above for the sludge. The ratio of Dowex to membrane surface area was 15 g to 60–80 cm^2. In a second step, membrane pieces were eluted in acidic (citric acid, 2.0 g L^{-1}) or basic (sodium hypochlorite, 1.2 g L^{-1}) solution for 24 h at ambient temperature with vigorous shaking in order to leach the remaining, more tightly bound substances including metals from the membrane pores. The ratio of cleaning solution to membrane material was approximately 10–15 mL for a membrane length of 140 cm (corresponding to a surface of 75 cm^2).

2.6. Photometric analysis of EPS

Carbohydrates were determined according to Dubois et al. (1956). Samples were measured at 490 nm in triplicate. Glucose was used as a standard for calibration from 0 to 80 mg L^{-1}. Samples were diluted prior to the experiment, if necessary. The limit of detection (LOD) was 1.3 mg L^{-1}.

Proteins and humic substances were determined according to the method described by Froelund et al. (1995), which is a modification of the Lowry et al. method (1951). For protein measurements, sodium dodecyl sulphate (SDS) was added to the samples to a final concentration of 1% (w/v) in order to improve the solubility of lipoproteins (Flemming et al., 2000). Calibration was done with BSA (with 1% w/v SDS) from 0 to 80 mg L^{-1}. For humic acids measurements, a humic acid standard (Fluka 53680) was used for calibration from 0 to 300 mg L^{-1}. Samples were measured at 750 nm in duplicate. The LOD was 0.1 mg L^{-1} for proteins and 1.2 mg L^{-1} for humic acids. Absorption was detected with a UV/VIS-Spektrometer, Perkin-Elmer, Lambda 14. If necessary, samples were concentrated by freeze-drying prior to quantification of EPS.

2.7. Analysis of metal ions

Metal ions (Mg^{2+}, Mn^{2+}, Ca^{2+}, Fe^{3+}, Al^{3+}) were quantified by ICP-MS using a Perkin-Elmer Elan DRC II. Liquid samples were acidified with 65% HNO_3 (suprapur) and 100 μL of a 1 μg m L^{-1} rhodium stock solution was added as an internal standard to each sample. A standard mix of 30 elements (Merck 110580) was used for calibration.

2.8. Size exclusion chromatography

Size exclusion chromatography (SEC) was performed on two PSS Suprema columns in series (first 1000 Å, 10 μ, second 30 Å, 10 μ, both 8 × 300 mm) coupled to an Agilent HPLC system with a diode array detector (HP 1040 M Series II). Injection volume was between 50 and 100 μL and mobile phase was a 66 mM phosphate buffer at pH 6.6 with a flow of 1 mL min^{-1} at room temperature. Dextran standards (PSS Suprema) with molecular weights (MWs) from 180 to 277 kDa were used for calibration. This SEC method with UV detection was employed directly after sampling and EPS extraction, respectively. Furthermore, samples were stored at −20 °C and used for liquid chromatography-organic carbon detection (LC-OCD) a few months later. In this application, a size exclusion column (Toyopearl TSK HW-50S; 250 × 20 mm) was used and the organic carbon was detected as CO_2 by an IR detector. A Graentzel Thin-Film Reactor (DOC-Labor Dr. Huber, Germany) with UV was used for total oxidation (Huber and Frimmel, 1992). Mobile phase was a 24 mM phosphate buffer (pH 6.6) at 1 mL min^{-1}, the detection limit was 10 μg L^{-1} and the fractionation ranged from 100 to 20,000 Da. The classification of peaks into certain fractions was done according to Huber and Frimmel (1996) and was confirmed by measurements of several proteins (BSA, globulin, albumin) and humic acid (Fluka 53680). This established method is based on the characterisation of peak maxima location and the spectral absorption coefficient/organic carbon ratio as well as calibration with reference substances, i.e. humic substances from the International Humic Substances Society and dextrans. According to this, six possible fractions can be distinguished in the LC-OCD chromatograms, in order of elution: mineral colloids (probably silicate, hydroxides), polysaccharides (hydrophilic, high molecular), humic substances (mix of humic and fulvic acids), building blocks (humic substance base units), low MW acids and amphiphilic/neutral substances (e.g. proteins, peptides, aminosugars).

2.9. Microscopic observation of membranes and activated sludge

Membranes were fixed in a 2.5% glutaraldehyde solution for 10 h and dehydrated in a series of ethanol/water according to Diao et al. (2004). Scanning electron microscopy (SEM) was employed using an Environmental Scanning Electron Microscope (ESEM) XL 30 Field Emission Gun (Philips, Eindhoven, NL). The ESEM contained an EDAX Falcon Genesis so that energy-dispersive X-ray spectroscopy (EDX) was used to determine elements on the membrane surface.

Activated sludge samples were observed under 100–400 × magnification of a light optical microscope with and without staining (Gram, Neisser) in triplicate. For the identification of filamentous bacteria, the identification key according to Eikelboom and van Buijsen (1992) and Jenkins et al. (1993) was used. Based on experiences with conventional activated sludge and by comparison with references, samples were classified into five categories of filamentous characteristics: category 0 (rare occurrence), 1 (marginal), 2 (modest), 3 (strong) and 4 (very strong).

2.10. Statistical evaluation

Statistical evaluation of data was done using the free software PAST ver. 1.73 (Hammer et al., 2001). All data rows were tested for normal distribution in normal probability plots (QQ plots). The probability plot correlation coefficients for the presented data were between 0.92 and 0.99; it is therefore justified to assume normal or almost normal distribution, which is a prerequisite for *t*-tests. The paired *t*-test was used for determining probabilities of different mean values.

3. Results

3.1. Variation of EPS concentration in a full-scale MBR

In a long-term monitoring of the full-scale MBR Nordkanal (Germany), the observation of bound EPS contents in activated sludge flocs showed a significant seasonal variation (Fig. 1). EPS concentrations were as low as 17 mg (g DM)$^{-1}$ in the summer and as high as 51 mg (g DM)$^{-1}$ in the winter. In the presented data set in Fig. 1, for the first half of the observation period (until September 2005) the variation of EPS concentrations is reflected by the filtration properties of the sludge as the FI_{15} was higher and thus indicated better filterability for low EPS concentrations. For the second half of the observation time this relation between EPS concentration and FI_{15} was not that distinct. The SVI was determined almost daily (mean for 2 years 72 ± 11 mL g^{-1}) and showed higher values (up to 124 mL g^{-1}) in cold seasons. The following parameters were measured at the WWTP over the sampling period of 2.5 years: amount of inflowing wastewater, COD of inflow and permeate, ammonia and nitrate of inflow and permeate, phosphorous of inflow and permeate, temperature and turbidity of permeate, pH and acid capacity of inflow and permeate, acid consumption (for membrane cleaning) and precipitant consumption. No clear seasonal variations or correlations were detected.

In addition, microscopic techniques were applied to determine microbial populations in each sludge sample (see Supplementary Information 1). For this full-scale MBR, nocardioform actinomycetes, known as foam-causing bacteria in WWTP (Müller et al., 2005), were detected at higher frequency in two periods when also EPS concentrations and SVI were higher: November 2004–April 2005 (filamentous category 3) and February–May 2006 (filamentous category 2). Another filamentous bacterium, *Microthrix parvicella*, was detected at higher frequency in the period from November 2004 to April 2005 but was then found only occasionally and with no clear relation to other parameters.

3.2. Activated sludge properties in pilot MBRs

In order to investigate the influence of different EPS concentrations on sludge characteristics more thoroughly, a set-up of two pilot MBRs was used and studied also with respect to the membrane performance. The SVI was lower for the higher SRT of 40 d (mean 182 ± 24 mL g^{-1}), indicating better settling properties than for the sludge with 23 d SRT (SVI mean 215 ± 37 mL g^{-1}) (Fig. 2). The FI_{15}, which is higher for better

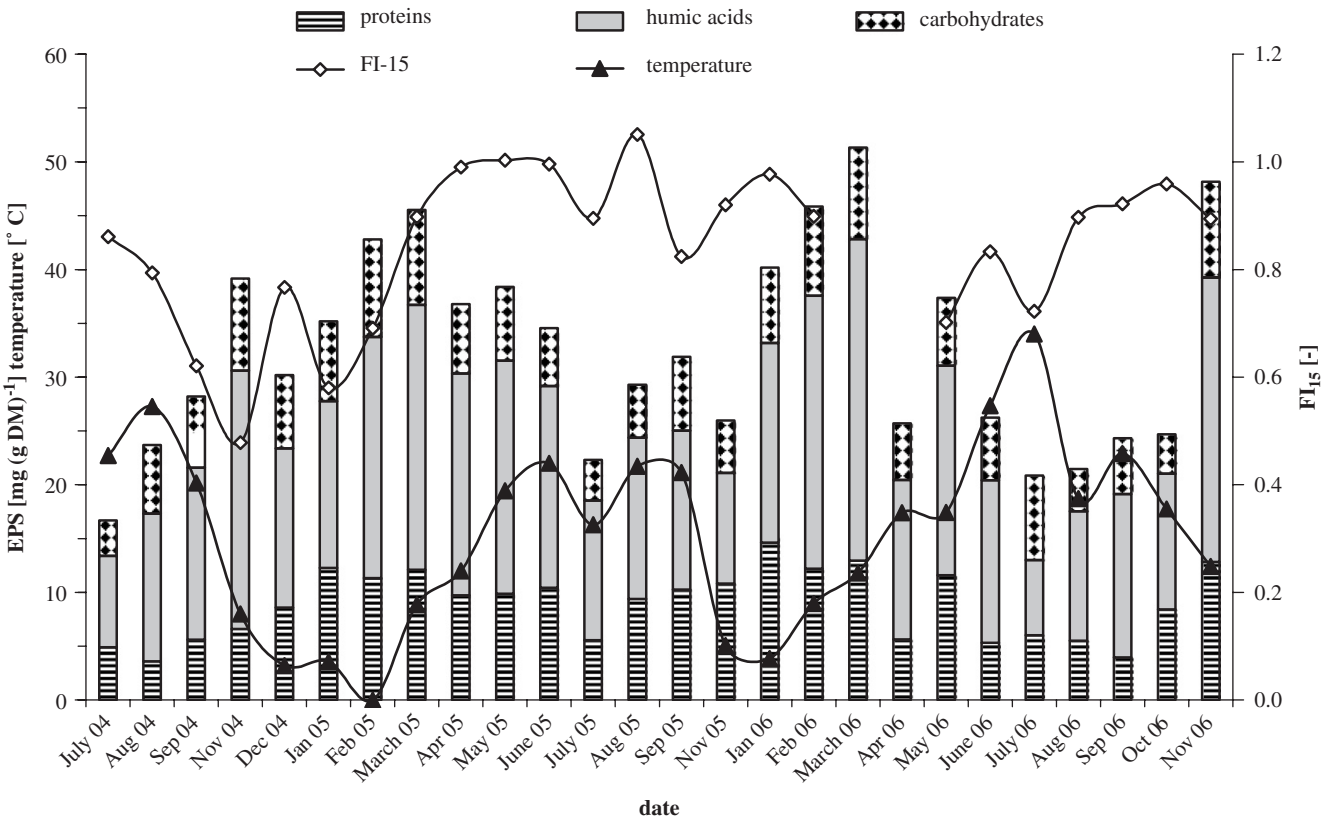

Fig. 1 – EPS bound to activated sludge flocs in a full-scale MBR compared to the filtration index FI_{15} and the maximum air temperature (averaged over 8 d before the sampling day). Data are based on monthly samples over 2.5 years. For March and April 2006 the FI_{15} values were not determined.

filterable sludge (maximum value 1.0) was significantly lower at 23 d SRT (mean 0.59±0.09) compared with 40 d (mean 0.79±0.07), thus indicating a better filterability of activated sludge at higher SRT (Fig. 2). In addition, dewatering of the sludge was better at higher SRT (CST mean 36.5±6.4 s) compared with lower SRT (CST mean 41.6±9.3 s). The determined differences of these parameters with regard to different SRTs were statistically significant as shown by high probabilities (p) for different means in the paired t-test: p was 95.8% for SVI, 99.9% for FI_{15} and 95.9% for CST. The relatively high SVI in both pilot plants is supported by the detection of filamentous bacteria, e.g. nocardioform actinomycetes, *Thiothrix* sp. and *Haliscomenobacter*. The relative frequency of these bacteria was similar at both SRTs (data not shown). With respect to the type of treated wastewater and the detection of the mentioned filamentous bacteria, sludge of both pilot plants can be regarded comparable to sludge of the full-scale MBR.

3.3. *EPS and metals in activated sludge and effluent of pilot MBRs*

In order to evaluate the observed differences of sludge filterability and settleability at low and high SRT, data of EPS and metal concentration were compared for activated sludge and MBR effluent (i.e. membrane permeate). The results presented here are from the pilot study in 2006; however, an earlier examination with a similar set-up of two MBRs in 2005 had revealed similar tendencies for the sludge.

The term bound EPS is used in the following, referring to the fraction that was extracted from the flocs using the cationic exchange resin Dowex. The tendency of higher bound EPS concentrations in the sludge flocs with lower SRT was evident for humic acids but less pronounced for proteins and carbohydrates (Fig. 2). The EPS data are less significant ($p<95\%$) than the parameters of sludge properties; the probability p for different means of humic acids is 93.4%, for proteins 70.9% and for carbohydrates 80.6%. For the predominant metals in the sludge flocs (iron, calcium, aluminium, magnesium), the situation was opposite to that of the EPS: sludge with a lower SRT contained lower metal concentrations (see Supplementary Information 2), with the highest significance ($p>99\%$) for calcium, iron and aluminium. This is explained by an accumulation of metals with extending SRT.

The results for supernatant and MBR effluent are summarised in Table 2. The concentration of soluble substances in the activated sludge supernatant showed the same tendency as bound EPS for humic acids; they were present in higher amounts at lower SRT ($p=92.5\%$) whereas differences in carbohydrate concentrations were of less significance ($p=72.7\%$). Protein was below the detection limit in supernatant and effluent of the pilot MBRs. Calcium and magnesium were the predominant metals in both the

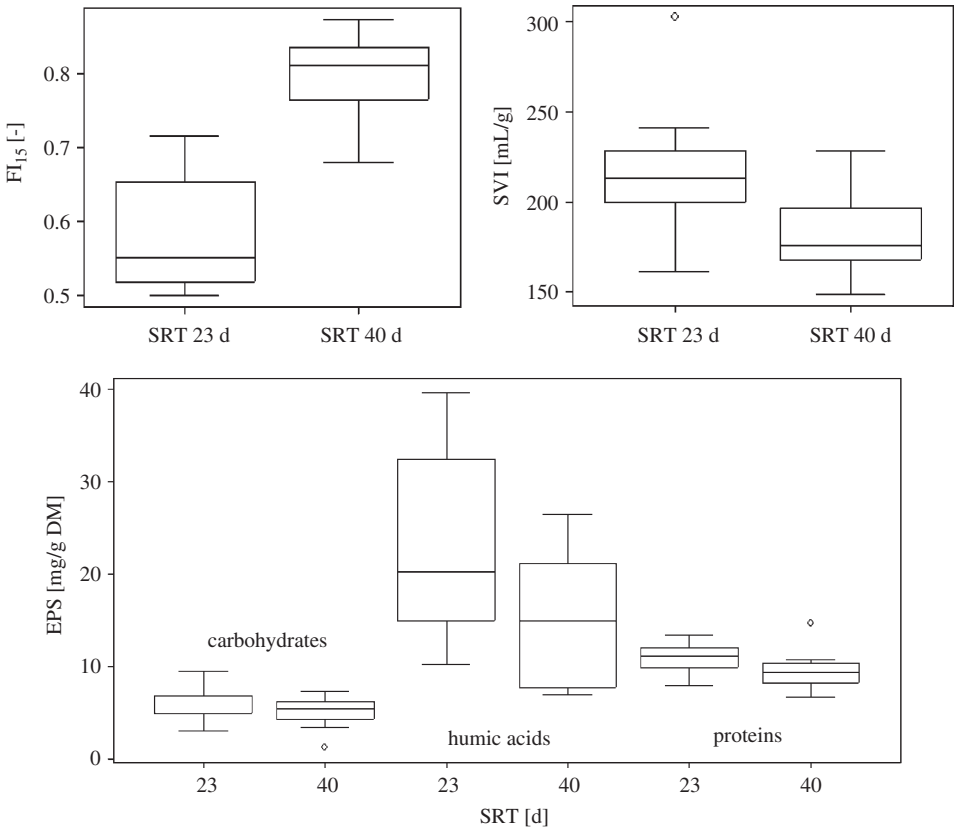

Fig. 2 – (Top left) Filtration indices FI_{15} for activated sludge. (Top right) Sludge volume index for activated sludge. (Below) Bound EPS in activated sludge flocs. Data for all graphs are based on seven separate samples for each of the two pilot MBRs. The presented box plots show the median with a horizontal line inside the box, the 25–75% quartiles as a box and minimal/maximal values as whiskers.

Table 2 – Soluble metals and substances in supernatant of activated sludge and MBR effluent of both pilot plants

Concentrations	Activated sludge supernatant		MBR effluent	
	SRT = 23 d	SRT = 40 d	SRT = 23 d	SRT = 40 d
Ca^{2+} ($mg\,L^{-1}$)	47.0 (±3.9)	47.3 (±4.3)	47.2 (±5.3)	47.2 (±5.0)
Mg^{2+} ($mg\,L^{-1}$)	9.9 (±1.6)	10.1 (±1.9)	9.9 (±1.7)	10.0 (±1.7)
Humic acids ($mg\,L^{-1}$)	11.8 (±5.5)	8.9 (±5.3)	9.9 (±5.7)	7.6 (±5.1)
Carbohydrates ($mg\,L^{-1}$)	5.6 (±3.7)	4.5 (±1.8)	2.6 (±1.4)	3.0 (±1.7)

No metals other than Ca and Mg were detected.
Protein was below LOD. Data are mean values based on seven separate samples for each of the two MBRs.

supernatant and effluent; however, no significant difference was observed in their concentrations at 23 and 40 d SRT.

3.4. *Microscopic characterisation of fouled membranes*

Membrane performance in the pilot plants was monitored by flow and TMP measurements and it declined in both MBRs from an initial permeability of approximately 1000 to 200 $L\,m^{-2}\,h^{-1}\,bar^{-1}$ after 3 months of operation (Al-Halbouni et al., 2007). The relatively high fouling rates are explained by the absence of any permeate backflush cycles being common for the applied hollow fibre system. At that time (October 2006) when membrane performances were comparably low in both MBRs, modules were removed from the plants and fibres were cut out for further analysis. Visual inspection of membranes showed almost white fibres with no adhesive sludge from the MBR with 40 d SRT, whereas membranes from the 23 d module appeared covered by a sludge cake layer.

Further microscopic investigation of the membrane surface revealed dense deposits covering almost all the surface of the fibre with 23 d SRT. Various elements were detected on the surface layer and implied organic (carbon, oxygen) as well as inorganic (metals, phosphorous, silicium) origins of foulants (Fig. 3). In contrast, only thin layers of deposits were found on

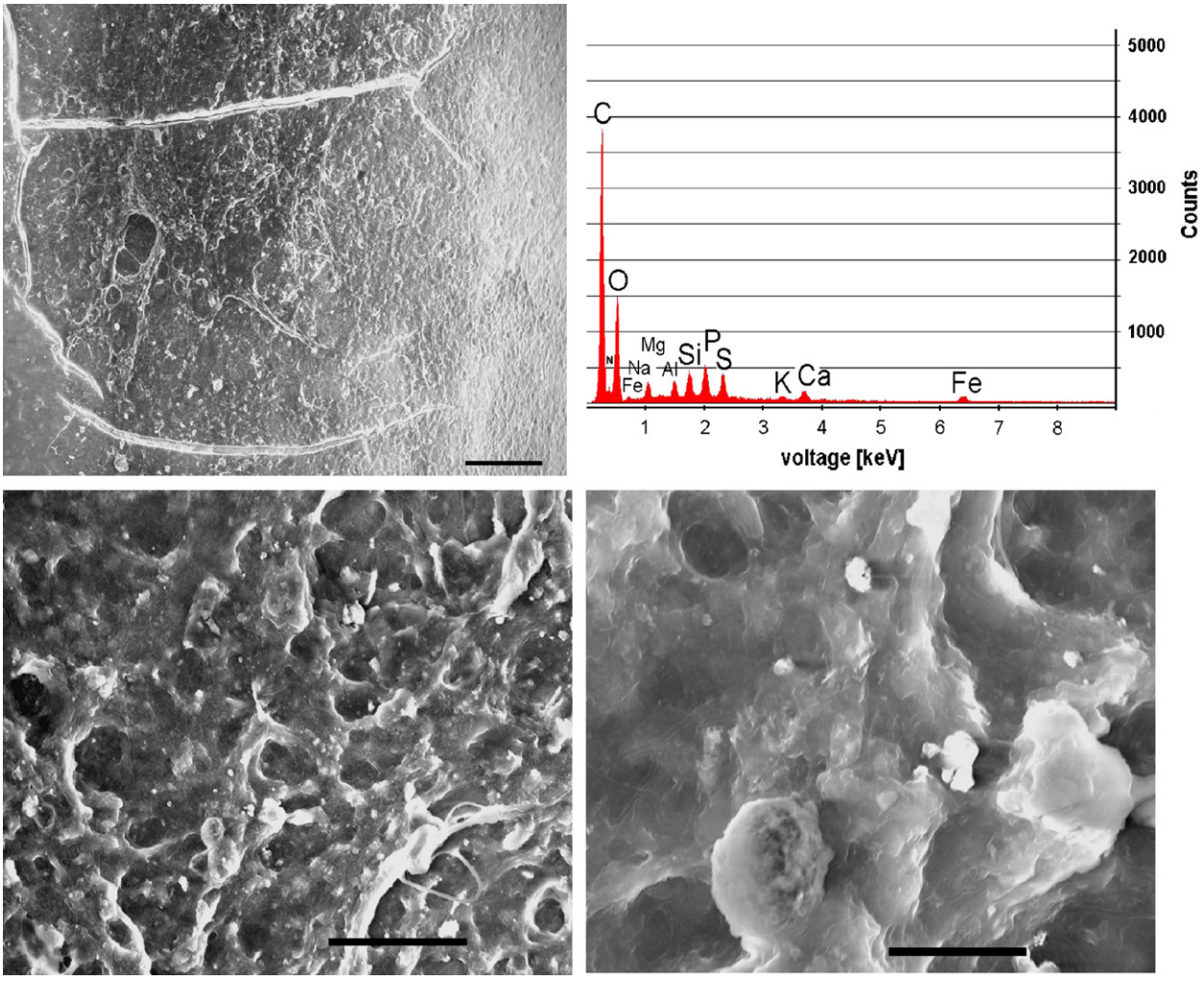

Fig. 3 – SEM of fouled membrane surface (SRT 23), scale bar 200 μm (top, left), 50 μm (below, left) and 10 μm (below, right). Elements detected by EDX on this fouled membrane surface (top, right).

the fibre with 40 d SRT while the original membrane structure was visible. Elements detected on this surface were mainly those of the PES membrane material (C, O, S); only traces of Al and P were present (Fig. 4).

In the full-scale MBR Nordkanal, membranes exhibited very inhomogeneous fouling, depending on the position of fibres within the module, on the age of the membrane and on the position of the module in the filtration line. Fig. 5 shows an example of a membrane surface with obvious deposits that contain organic and inorganic foulants. When parts of the membrane were examined that appeared free of deposits, the corresponding peaks of membrane material PVDF, especially fluorine, were the only elements detected by EDX.

3.5. *Extraction of foulants from membranes of pilot MBR*

In addition to the microscopic observation of fouled membranes, EPS and metals were extracted from membranes of both pilot MBRs in order to quantify the amount of deposited substances. Results from the first step in this experiment (CER extraction) are presented in Table 3 (left part). The CER method offers the advantage of obtaining EPS in their native structure without further chemical hydrolysis. The EPS released through this were mainly Ca^{2+}- and Mg^{2+}-bound polymers because the CER is targeting these metal ions. It was found that CER extractable membrane-bound EPS at 23 d SRT were more than 40-fold (protein) and 4.8-fold (carbohydrates) higher than at 40 d; humic acids were not detected at all in the extracted EPS at 40 d (Table 3).

Results from the subsequent second step (chemical extraction) are shown in Table 3 (right part). Aluminium and iron were released from the membranes, with iron being eluted more effectively by citric acid than by NaOCl and this with a 1.5-fold higher concentration at 23 d SRT compared with 40 d. The fact that carbohydrates were also present in the acidic membrane eluates suggests the existence of EPS bound to Fe^{3+} and Al^{3+}. Other EPS (humics and proteins) might have been part of this group, too; however, only carbohydrate analysis of the eluates was performed due to limited available eluate volume. It has been shown by Park and Novak (2007) that Fe-bound EPS differ from Ca/Mg-bound polymers and that they can be selectively extracted by using sodium

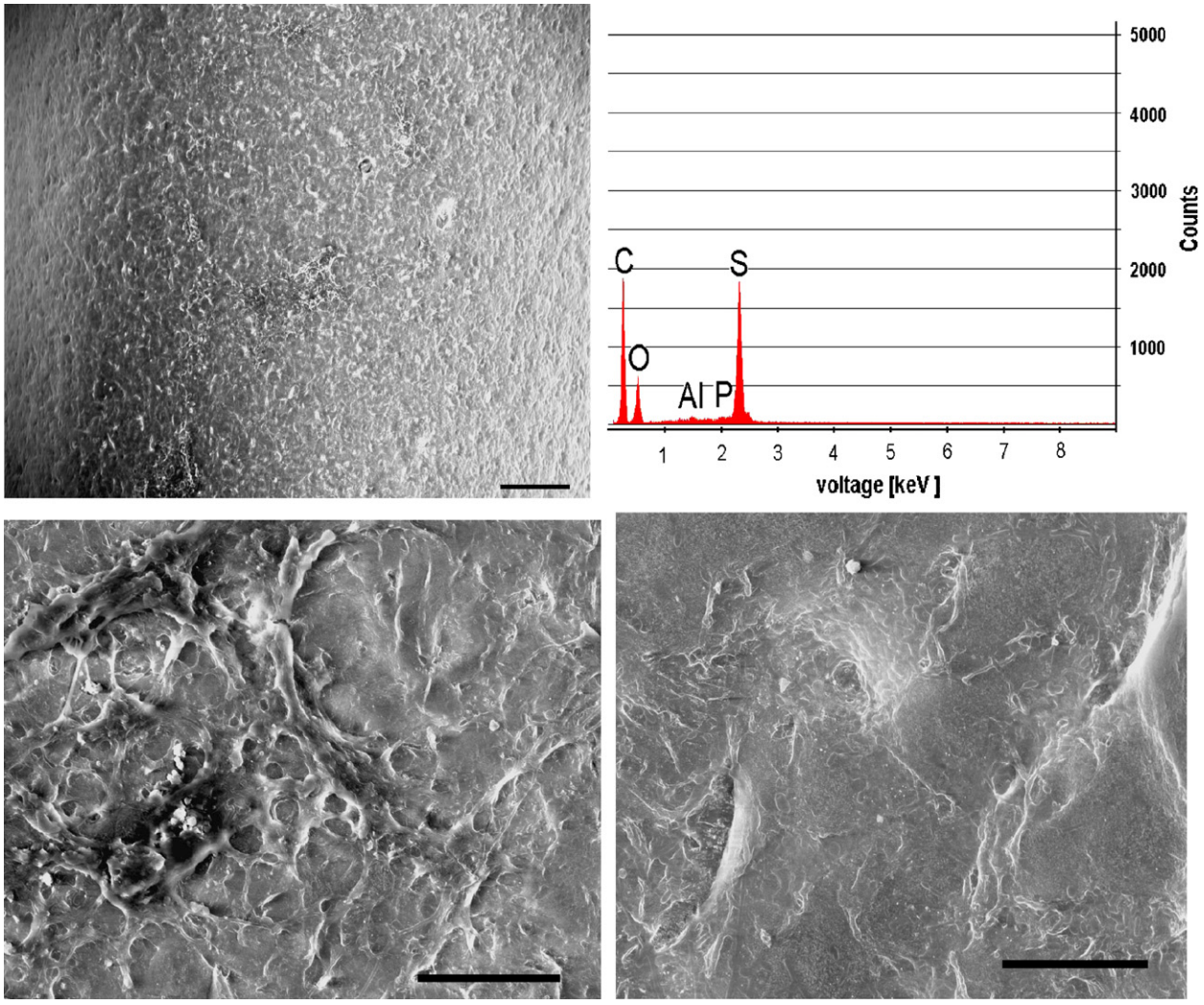

Fig. 4 – SEM of fouled membrane surface (SRT 40), scale bar 200 μm (top, left), 50 μm (below, left) and 10 μm (below, right). Elements detected by EDX on this fouled membrane surface (top, right).

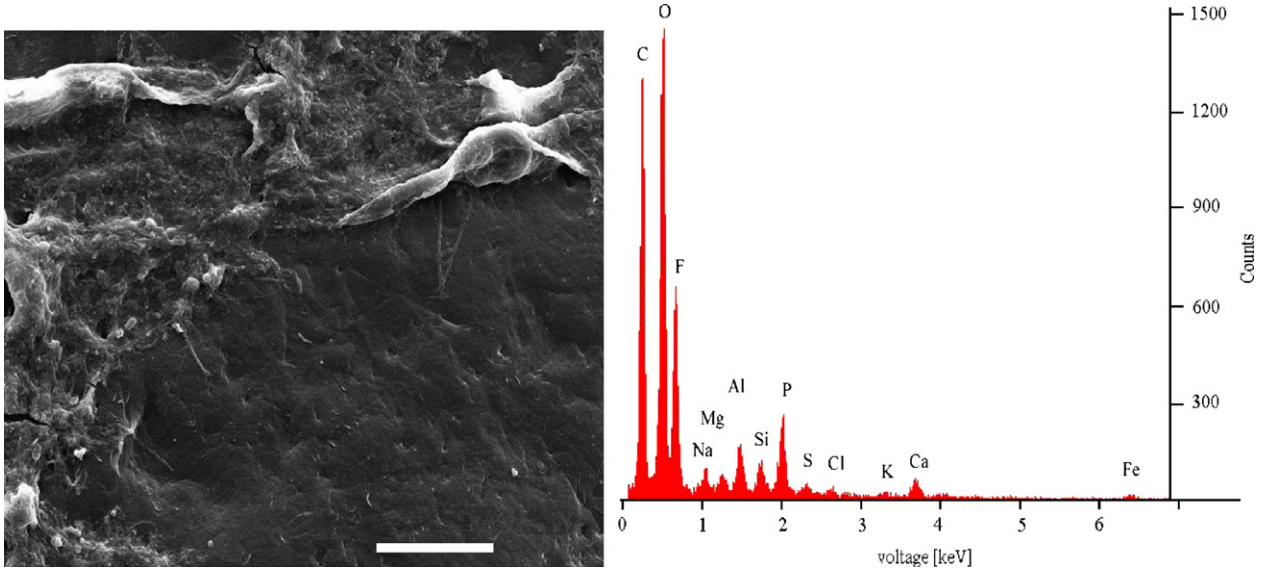

Fig. 5 – (Left) SEM of fouled membrane surface, sample taken from the full-scale MBR in 2004, scale bar 20 μm. (Right) Elements detected by EDX on this membrane surface (fluorine derives from PVDF membrane material).

Table 3 – Results of EPS extraction and cleaning of autopsied membranes that were removed from the modules of the pilot MBRs after 3 months of operation

SRT (d)	1st step: extraction with CER (Dowex)			2nd step: extraction in acidic or basic solution				
	Protein ($mg\,m^{-2}$)	Humic acids ($mg\,m^{-2}$)	Carbohydrates ($mg\,m^{-2}$)	Fe ($mg\,m^{-2}$) after acidic elution[a]	Fe ($mg\,m^{-2}$) after basic elution	Ca ($mg\,m^{-2}$) after basic elution	Al ($mg\,m^{-2}$) after basic elution	Carbohydrates ($mg\,m^{-2}$) after acidic elution[b]
23	92.7 (±1.2)	116.3 (±5.5)	84.9 (±2.3)	34.7[c]	8.8[c]	0.4[c]	4.6[c]	15.1 (±0.8)
40	1.9 (±1.2)	<LOD	17.5 (±0.9)	20.8[c]	7.0[c]	1.0[c]	5.9[c]	12.8 (±1.8)

[a] No metals other than Fe were detected in the acidic wash eluate.
[b] Carbohydrates could not be quantified in the basic eluates due to black colouring after the addition of test reagents.
[c] The standard deviation for metals determined by ICP-MS is in the range of 1–5%.

sulphide for iron removal and Dowex for calcium and magnesium removal. In the present study, the citric acid elution can be regarded as an Fe-selective extraction method using the strong iron-chelating properties of this acid (Welch et al., 2002).

3.6. *Molecular weight characterisation of EPS*

Size exclusion chromatography with UV detection (SEC-UV) and SEC with organic carbon detection (SEC-OCD) were applied in order to examine the MW distribution of the samples.

Results for activated sludge (bound and soluble EPS) and effluent of pilot MBRs at low and high SRT are shown in Fig. 6. For bound EPS from activated sludge the presence of macromolecular substances was evident (Fig. 6C and F). The macromolecular peak appeared in bound EPS of both MBRs and seemed to consist of polysaccharides associated with proteins or colloids which exhibit UV absorption as described previously for MBR sludge (Rosenberger et al., 2006). The main differences between bound EPS of activated sludge with regard to different SRTs are the humics and building blocks, which were almost undetectable for 40 d SRT sludge (Fig. 6F) compared with 23 d sludge (Fig. 6C).

In the activated sludge supernatant of pilot MBRs, a small amount of polysaccharides was detectable by SEC-OCD (Fig. 6B and E); however, this macromolecular peak was not UV-detectable (data not shown) and did therefore not contain proteins—which is in accordance with the photometric EPS quantification. The polysaccharide peak was much more prominent for the supernatant at 23 d SRT (Fig. 6B) than at 40 d SRT (Fig. 6E). No macromolecular substances were present in the pilot MBR effluent, which consisted of humics, building blocks and low molecular weight (LMW) acids and showed no differences with respect to SRT (Fig. 6A and D).

For the CER-extracted EPS from the pilot MBR membranes samples had to be concentrated by freeze-drying in order to obtain signals with SEC-UV. EPS extracted from the 23 d SRT membranes exhibited a macromolecular peak with a MW >277 kDa (Fig. 7A) that was not present in the 40 d SRT membrane extract (Fig. 7B). The macromolecular peak in EPS of the 23 d membrane probably contained proteins as the quantification of CER-extracted EPS revealed a high protein concentration in this membrane extract, whereas proteins in the EPS of the 40 d membrane were present in 40-fold lower concentrations and would therefore not produce a detectable UV signal in the applied SEC-UV.

As a comparison, SEC-OCD of samples from the full-scale MBR Nordkanal is shown in Fig. 8. The principal occurrence of SEC fractions is similar to the pilot MBR samples. The high MW peak was present in a high concentration in the sludge-bound EPS (Fig. 8A) and in a lower concentration in the soluble fraction of the supernatant (Fig. 8B) but not in the permeate (Fig. 8C). Furthermore, humics, building blocks and LMW acids were detected in all these samples. It has to be noted that the particular peak height can differ for samples taken at different seasons; however, the retention of macromolecular substances by the membrane was observed throughout the long-term investigation of the full-scale MBR Nordkanal. No macromolecular peaks were detected in EPS extracted from membranes of the full-scale MBR (Fig. 7C).

4. Discussion

4.1. *Full-scale MBR*

Long-term observation of the full-scale municipal MBR Nordkanal showed a seasonal dependence of floc-bound EPS in activated sludge. Higher amounts of EPS were produced at low temperatures in the winter and this correlated with worse sludge filterability (lower FI_{15}) and worse settling behaviour (higher SVI). A probable reason for enhanced microbial EPS production could be a change in the microbial population towards more EPS-producing bacteria like nocardioform species, which occurred in the sludge of this MBR preferably at colder temperatures. Another reason could be environmental stress through changing temperature as shown elsewhere (Barker and Stuckey, 1999). Similar observations of temperature-dependent polysaccharide concentrations were made by Rosenberger et al. (2006).

A significant retention of macromolecular compounds by the membrane has been shown previously for the full-scale

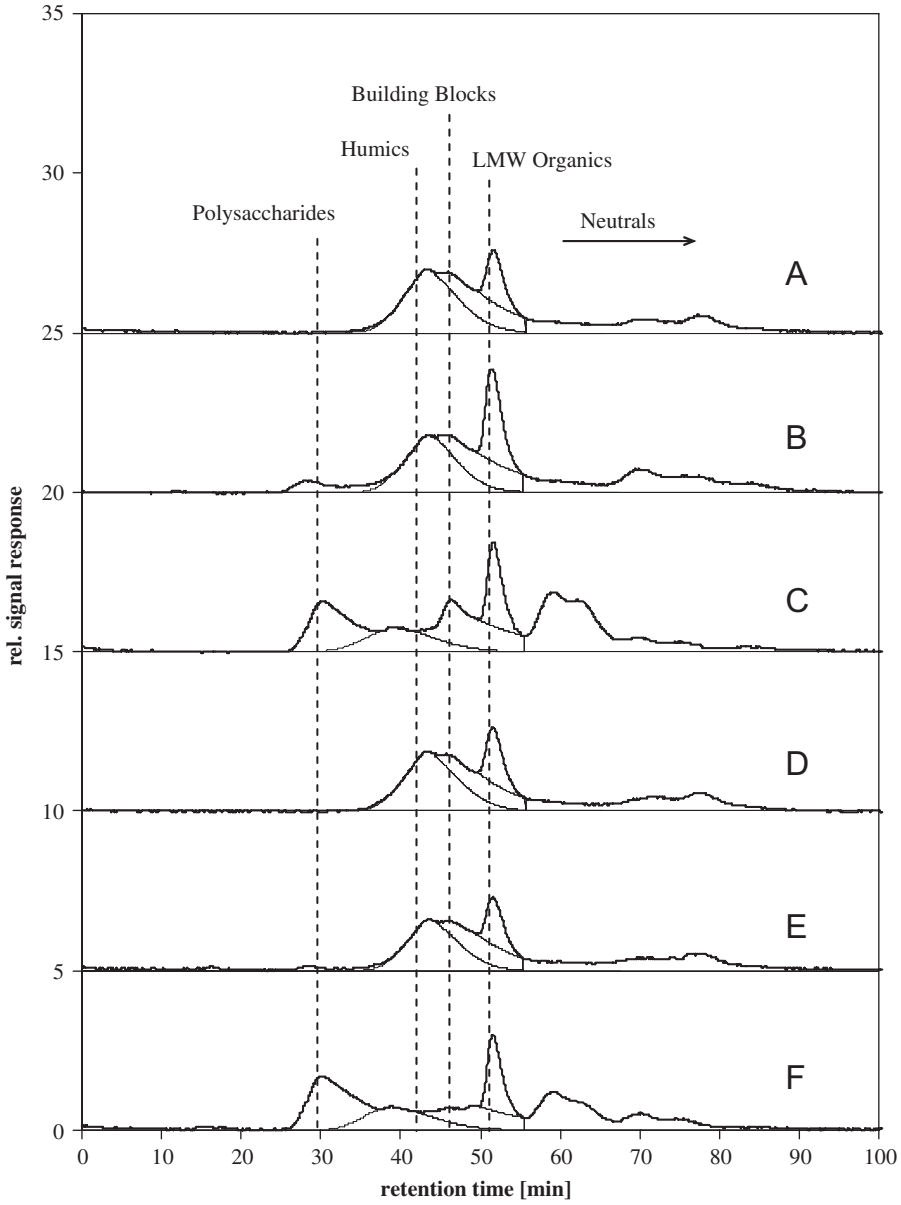

Fig. 6 – SEC with organic carbon detection of EPS in samples of pilot MBR, October 2006. (A–C) Samples from MBR with 23 d SRT. (A) Effluent, (B) activated sludge supernatant, (C) bound EPS from activated sludge flocs. (D–F) Samples from MBR with 40 d SRT. (D) Effluent, (E) activated sludge supernatant, (F) bound EPS from activated sludge flocs.

MBR (Lyko et al., 2007a); however, the application of frequent and optimised membrane cleaning strategies prevented the detection of pronounced fouling rates in this full-scale MBR (Lyko et al., 2007b). The fouling phenomenon in the full-scale plant, as determined by investigations of autopsied membranes, was found to be mainly caused by the deposition of a foulant layer that was partly porous but did not embed large quantities of microorganisms. Similar findings were described by Geng (2006) for PVDF hollow fibres in the aerated zone of a MBR. It is therefore justified to assume that vigorous aeration and frequent chemically enhanced backwashing of the membranes—as it was performed in Nordkanal—prevents or mitigates microbial attachment to a degree that biofilm development and surface deposits are not dominant.

4.2. *Pilot-scale MBRs*

Investigations of two pilot MBRs with 23 and 40 d SRT showed that higher SRT leads to sludge with significantly better settling and filtering properties as well as better dewaterability compared with lower SRT. In addition, floc-bound EPS were found to have higher concentrations in sludge with lower SRT; however, this was not correlated with the frequency of filamentous bacteria, which were present at both SRTs in comparable numbers. Although the significance of the different EPS concentrations is lower than of the sludge properties, the tendencies are clear.

It is therefore justified to assume that high floc-bound EPS concentrations at lower SRT have a negative influence on sludge properties in terms of settling behaviour, filterability

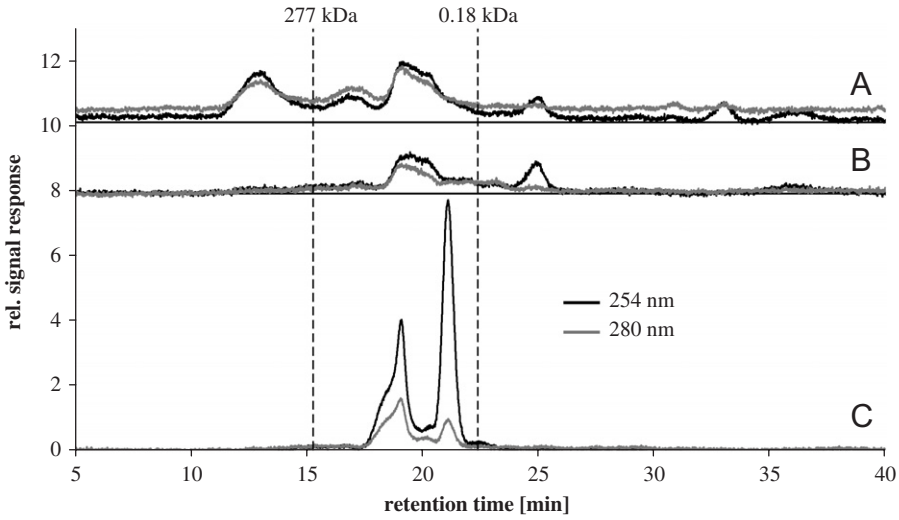

Fig. 7 – SEC with UV detection of membrane-bound EPS. (A) CER-extracted EPS from membranes of 23 d SRT. (B) CER-extracted EPS from membranes of 40 d SRT. (C) CER-extracted EPS from membranes of the full-scale MBR Nordkanal in April 2006. Samples (A) and (B) were concentrated four-fold by freeze-drying, injection volume was 100 μL for all samples.

and dewaterability. In the present study, flocs were not analysed; there is, however, evidence in the literature that high amounts of EPS can prevent the formation of larger flocs and thus lead to bad flocculation (Liao et al., 2006) and possibly to higher membrane fouling due to floc size (Liao et al., 2004). Other studies have also shown that the excessive production of EPS leads to higher hydrophobicity of sludge flocs and more irregularly shaped flocs (Liss et al., 2002; Meng et al., 2006). Such a relation between high EPS concentration at low SRT and irregular floc structure and size might have an impact on the results of the present study, assuming that some beneficial effects on the sludge flocs at higher SRT (Lee et al., 2003) will be reflected by improved sludge properties. The proposed positive effect of larger flocs is supported by findings of other researchers who argued that flocs with a larger size can act as a barrier for further foulants after the formation of a porous cake layer (Le-Clech et al., 2006) or might have a steric hindrance effect (Geng, 2006).

On the other hand, under constant flux operation, the deposited macromolecules themselves might even be beneficial as they could act as a prefilter and prevent in-depth deposition of other species into the membrane (Le-Clech et al., 2006). Therefore, the detected deposition of high MW substances on membranes at low SRT does not necessarily mean that the filtration performance will be diminished. In fact, findings of different amounts of deposited EPS and metals on the membranes in the present study were not reflected by membrane permeabilities, which were comparably low at both SRTs in the stationary phase.

In other studies, soluble and colloidal substances in the sludge supernatant such as polysaccharides and SMP were regarded as solely responsible for high fouling rates (Rosenberger et al., 2006) and EPS extracted from sludge floc were constant, regardless of SRT (Jinsong et al., 2006). Contrary to that, the present study emphasises the role of floc-bound EPS as well as metals in the formation of fouling layers. Membrane examination revealed higher fouling propensity with the lower SRT (23 d) compared with higher SRT (40 d) in terms of surface deposits (shown by SEM/EDX) as well as pore clogging (determined by chemical extraction) by EPS and inorganic material. Proteins were obviously involved in membrane fouling; however, they were always below LOD within the soluble sludge supernatant fraction. Complexing metals that were present only in sludge flocs, especially iron, were obviously deposited on the membrane and in the pores together with humic substances.

4.3. *Transferability of results from pilot- to full-scale plants*

The studied full-scale MBR (mean SRT 28 d) was comparable to the pilot plant with higher SRT (40 d) in terms of treated wastewater, floc-bound EPS and metals in the sludge as well as the principal occurrence of typical fractions in the SEC and retention of macromolecules by the ultrafiltration. The main difference between sludge samples of the full-scale MBR and samples of pilot-scale MBRs with varying SRT was the amount of protein in the soluble EPS of activated sludge supernatant: protein was sometimes detected in the supernatant from the full-scale plant but below LOD in the pilot plants. High MW substances were neither involved in the formation of membrane fouling layers in the full-scale plant nor in the pilot MBR with higher SRT.

The impact of iron on membrane fouling has recently been shown in the full-scale MBR study (Lyko et al., 2007a) and it was concluded that Fe-complexed organic molecules as well as iron hydroxides are able to precipitate on the membrane surface. This phenomenon was confirmed by investigations of membranes from the pilot plants. However, higher amounts of iron in the activated sludge—as shown for the higher SRT in the present study—do not necessarily lead to higher membrane fouling.

The most significant results concern the relation between EPS concentration and sludge properties. It is evident that in

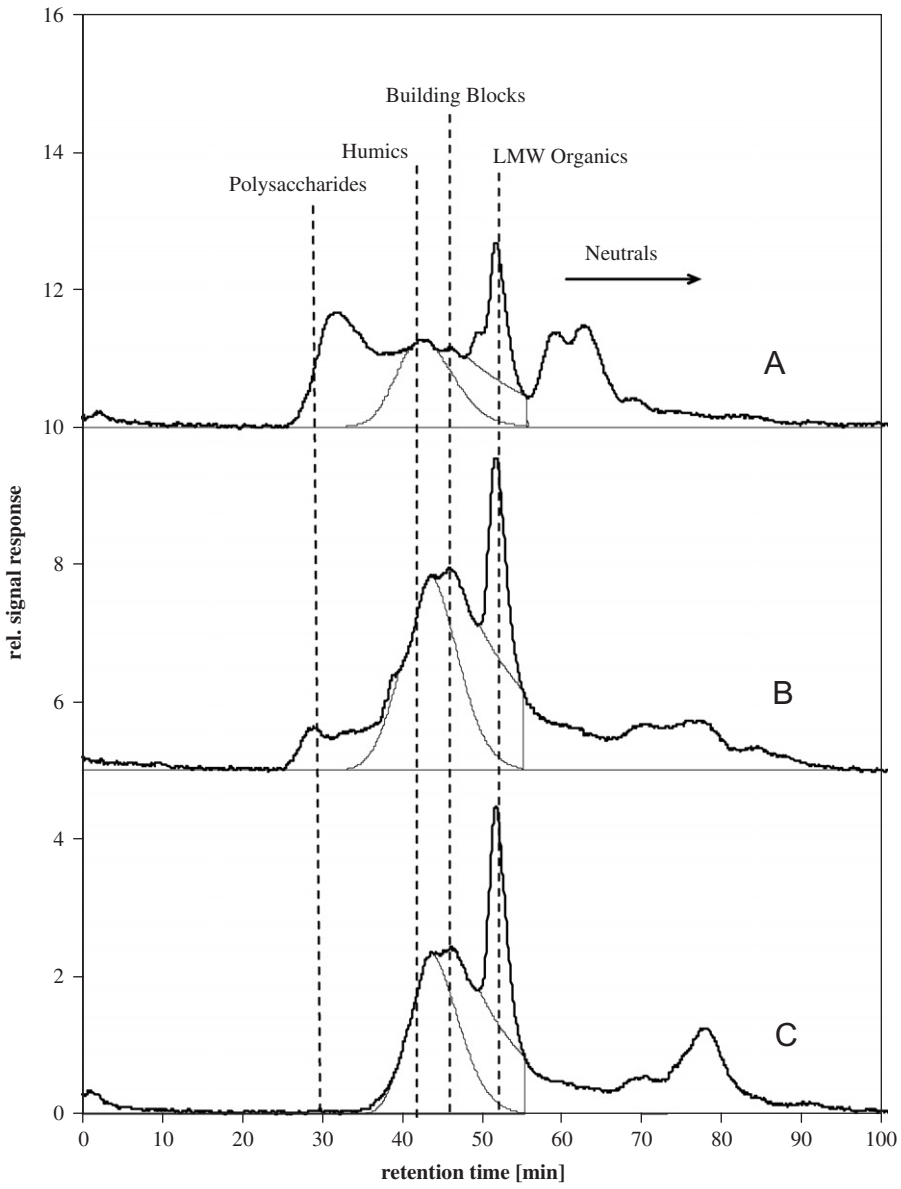

Fig. 8 – SEC with organic carbon detection of EPS in samples of the full-scale MBR Nordkanal, April 2006. (A) Bound EPS from activated sludge flocs, (B) activated sludge supernatant, (C) effluent after membrane filtration.

the studied pilot- as well as full-scale MBRs higher amounts of EPS have a negative impact on sludge properties (FI, settling behaviour, dewaterability). Obviously, the excess production of EPS can be related to low SRT (as shown for the pilot plant) or to colder seasons and probably a shift in microbial populations (as shown for the full-scale MBR). However, there is no evidence so far that all those described sludge parameters would have any dramatic impact on membrane permeability in real operation. Even though the influence of EPS on membrane performance was stressed as being more important than the actual microbial population (Ivnitsky et al., 2007), it might not be the distinct parameter for the prediction of full-scale operational performance. For a reliable description of fouling phenomena, probably a combination of several factors has to be considered, i.e. not only EPS amounts but also metals, precipitation agents, physico-chemical parameters in the system, membrane characteristics and applied cleaning chemicals. For further research, it can be useful to look at EPS not only as a sum parameter but also to obtain more detailed insight into specific components such as fatty acids and their origin as well as natural organic matter and its interaction with microbial products.

5. Conclusions

The present study was focused on sludge characteristics with regard to membrane fouling in pilot-scale MBRs at low and high SRT. The obtained data were used for a comparison with

results from a full-scale MBR. The results lead to the following conclusions:

- Higher amounts of floc-bound and soluble EPS have a negative impact on sludge properties (FI, settling behaviour, dewaterability). The excess production of EPS can be related to low SRT in the pilot plants and to seasonal variations in the full-scale MBR.
- The formation of fouling layers was more distinct in the pilot MBR with lower SRT where concentrations of EPS and metals deposited into the membrane were significantly higher compared with high SRT; however, there was no difference in permeability at both SRTs.
- A high MW fraction of EPS, containing polysaccharides and proteins, was involved in membrane fouling at lower SRT but was not detected in fouling layers at higher SRT or in the full-scale MBR.

Future research should involve studies on fouling mechanisms under defined conditions in order to gain a deeper understanding especially of the role of different EPS. Long-term monitoring of membrane permeability in pilot-scale studies is necessary in order to identify distinct parameters for the prediction of fouling. This knowledge would be beneficial for operators of full-scale MBRs in terms of adjusting the amount of precipitating agents, choosing the appropriate cleaning methods and working cost-effectively.

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Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.watres.2007.10.026.

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