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Operational experience with a seasonally operated full-scale membrane bioreactor plant

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HIGHLIGHTS

- Seasonal full-scale MBR with flat sheet membrane was monitored for 2 years.
- MBR showed very good BOD_5, COD and NH_4-N removal efficiency.
- Lack of organic substrate led to increase of particularly carbohydrates and DNA.
- EPS and SMP content correlated with activated sludge morphological characteristics.
- Increasing number of pathogens was detected in permeate during the operation.

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ABSTRACT

A seasonally operated full-scale membrane bioreactor plant (flat sheet, 0.03 μm) treating municipal wastewater from a recreation facility was monitored for 2 years. In particular, membrane bioreactor operation characteristics and development and changes in extracellular polymeric substances and soluble microbial product concentrations were observed, which were both dependent on volume and quality of incoming wastewater. Microbiological effluent quality, nutrient removal efficiency and activated sludge characteristics were analysed on a regular basis. Correlations between activated sludge quality, extracellular polymeric substance and soluble microbial product concentrations were identified. Pathogen related changes in effluent quality during plant operation were also observed. Nutrient removal efficiency was very good, despite fluctuations in influent flow.

1. Introduction

Membrane bioreactor (MBR) technology usually results in high effluent quality with low concentrations of organic matter and suspended solids (SS), and a near complete absence of (pathogenic) bacteria (Monclús et al., 2010). Application of MBR technology, therefore, represents a great step forward in wastewater treatment. In comparison with the conventional activated sludge (AS) process, MBR offers several important advantages: (i) the membrane can be situated directly in the activation line (flow-scheme of AS process), thereby reducing the process footprint and, due to the high concentration of AS, reducing the volume required for the aeration tanks; (ii) SS concentrations in effluent are significantly better; (iii) the effluent is hygienically secure, i.e. all bacteria are retained and, therefore, the effluent is suitable for a wide range of re-use purposes (Judd, 2010). On the other hand, this technology

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Abbreviations: AS, activated sludge; C/N, carbon to nitrogen ratio; CFU, colony forming unit; COD, chemical oxygen demand; EPS, extracellular polymeric substances; eEPS, extracted extracellular polymeric substances; EPSc, extracellular polymeric substances with carbohydrates as a major component; EPSp, extracellular polymeric substances with proteins as a major component; F/M, food to microorganism ratio; HRT, hydraulic retention time; HS, humic substances; IR, internal recycle; MBR, membrane bioreactor; MLSS, mixed liquor suspended solids; MLVSS, mixed liquor volatile suspended solids; NH_4-N, ammonia nitrogen; NO_3-N, nitrates nitrogen; PES, polyethersulphon; PO_4-P, phosphate phosphorus; R_x, specific kinetic rate; SMP, soluble microbial products; SRT, sludge retention time; SS, suspended solids; SST, secondary settling tank; TMP, transmembrane pressure; WWTP, wastewater treatment plant.

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does have a few disadvantages, including higher operational costs due to higher aeration requirements and the necessity for chemical cleaning of the membrane due to fouling.

Mixed liquor characteristics influence membrane fouling significantly. Membrane filterability is affected by AS viscosity, AS concentration, amount of filamentous bacteria, floc size, extracellular polymeric substances (EPS), soluble microbial products (SMP), etc. (Judd, 2t010). The SMP fraction is considered the main contributor to membrane fouling via adsorption of macromolecules and pore clogging (Meng et al., 2009). Both EPS and SMP are substances produced by microorganisms that are released into the liquid phase as part of their metabolism or under biological or mechanical stress (Lesjean et al., 2005; Meng et al., 2008). Different processes and technological steps that allow minimization of fouling rate include pretreatment of raw wastewater, suitable dosing of chemical compounds and equalization of flow rate, optimization of aeration intensity, changes in basic operation parameters (e.g. increasing sludge retention time (SRT), decreasing the food to microorganism ratio (F/M), or increasing hydraulic retention time (HRT)), and optimization of reactor design (Dvořák et al., 2011; Judd, 2010; Meng et al., 2009).

Many papers have been published recently that deal with membrane fouling, though most of these relate to lab-scale or pilot-scale MBR plant studies. Consequently, there is a lack of articles related to the monitoring of operational problems of full-scale MBR plants, including microscopic analysis, microbiological and chemical effluent parameters, and content of foulant in AS, as well as their relationship to operational parameters.

2. Methods

2.1. Experimental set-up and operation

The MBR wastewater treatment plant (WWTP) was installed in a recreation area in Southern Bohemia (Czech Republic) in 2008 and its main operational period was from April to November. The recreation facility has a total capacity of 285 permanent beds and has an open camping area with shared bathrooms and toilets. This implies a large fluctuation in the F/M ratio and HRT over the holiday season (Fig. 1). The MBR WWTP, which was designed to remove chemical oxygen demand (COD) and total nitrogen (N_{tot}), was divided into two lines measuring 7000 mm × 2160 mm × 3080 mm (length × width × depth) with an overall volume of 84 m^3 (2 × 42 m^3) and able to handle an overall influent capacity of 60 $m^3\,d^{-1}$ (30 $m^3\,d^{-1}$ per line).

Incoming wastewater was pre-treated by manually-cleaned fine screens, following which the inlet divided into two streams (or lines). Each line was split into four zones connected with different operation conditions. These were, in the direction of flow, an anoxic section, an oxic section, an oxic section with a submerged flat sheet membrane, and a secondary settling tank (SST). Internal recycles (IR) were installed between the membrane and the anoxic section (IR1) and between the SST and the oxic section (IR2). The oxic section with membrane was equipped with two polyethersulphon (PES) submerged flat sheet membrane modules (2 × 50 m^2; siClaro FM 642) with a nominal pore size of 0.03 μm. The MBR WWTP was run under a constant transmembrane pressure (TMP) without backwashing and the operating vacuum/TMP was set at 50–80 mbar (max. 250 mbar). Subsequent operation showed a flux decrease over time due to membrane fouling. Permeate was obtained by suction pump controlled by a level switch. This resulted in variable suction frequency depending on influent volume. Aeration of oxic section with membrane separation was provided by blower working in different work cycles during the day (diurnal operation 1.5 h switched on and 0.5 h switched off; nocturnal operation 1.5 h on and 1.5 h off). Hence, the MBR relaxation period was variable throughout the operation seasons. The MBR WWTP was also equipped with a coagulant pump (addition of $Fe_2(SO_4)_3$ solution) for simultaneous phosphorus precipitation in the oxic section without membranes. Precipitation was only initiated, however, in the 1st season. The SST was constructed as a precaution against membrane separation or other MBR WWTP operational problem. Between the operational seasons the membranes were chemical cleaned by 0.5% natrium hypochlorite solution and 0.5% citric acid solution.

Due to low flow of incoming wastewater for most of the operation season, only one line of the MBR WWTP operated over both seasons (see Fig. 1).

2.2. Analytical methods

The AS was regularly subjected to microscopic analysis according to Jenkins et al. (1993). Activated sludge flocs were classified into three size groups: small (50–150 μm), medium (150–500 μm) and large (>500 μm). Influent and effluent waters were tested for COD, NH_4-N, NO_3-N, NO_2-N and PO_4-P. All parameters were measured using standard WTW sets or according to standardised methodologies (APHA, 2005). Mixed liquor suspended solids (MLSS) measurement was performed gravimetrically and kinetic tests of

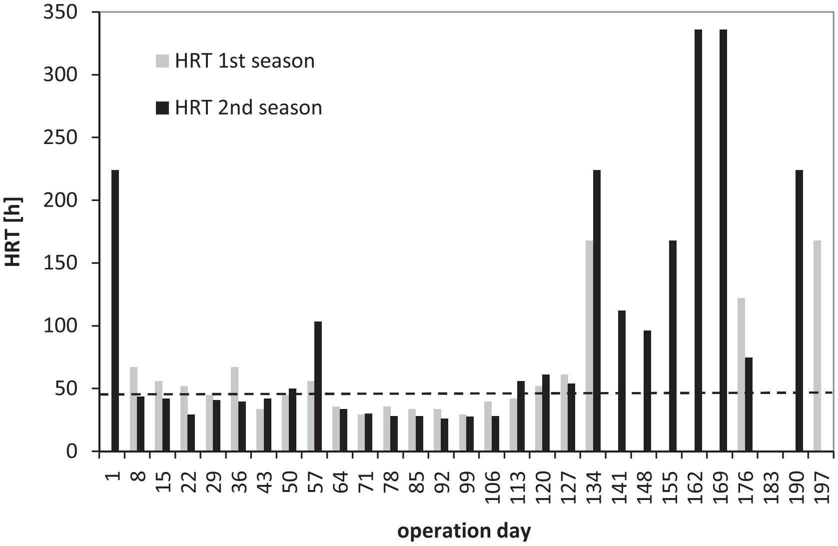

Fig. 1. Evolution of HRT over both seasons (dashed line shows design HRT).

nitrification and de-nitrification were carried out according to methods described by Kristensen et al. (1992).

Enumeration of culturable microorganisms in the influent and effluent was performed by counting the colonies formed in a nutrient agar culture medium following aerobic incubation at 37 °C and 22 °C according to EN ISO 6222. Intestinal enterococci were quantified by the membrane filtration method described in EN ISO 7899-2. Detection and enumeration of *Escherichia coli* and thermotolerant coliform bacteria was performed according to EN ISO 9308-1 and *Clostridium perfringens* levels were determined according to EN ISO 6461-2.

Total EPS content in AS was calculated as the sum of concentrations of carbohydrates, humic substances (HS), proteins, and DNA. SMP were analysed in the supernatant, obtained using gravitational sedimentation. Thermal extraction of extracellular polymeric substances (EPS) from AS was carried out according to Morgan et al. (1990). Retention of SMP was calculated as the difference in SMP concentration between the supernatant and the effluent. HS were analysed according to Sharma and Krishnan (1966) and protein concentration was measured spectrophotometrically using the Lowry method (Lowry et al. (1951). Revised values for protein and HS concentrations were obtained using the Frølund method (Frølund et al., 1955). DNA was analysed according to Burton (1956) and carbohydrate content analysed using the Dubois method (Dubois et al., 1956), using 5% phenol instead of 80% (Raunkjaer et al., 1993).

3. Results and discussion

3.1. Influence of operation conditions on flux decrease

A decrease in flux occurred during both the 1st and 2nd operation seasons (Fig. 2). As indicated in the figure, effluent was filtered using two membranes and a total filtration surface of 100 m^2 until the 100th operational day of the 1st season. Subsequently, one membrane was taken out of operation and filtration undertaken with one membrane only (50 m^2) until the end of the operation season. This decision was made by the WWTP operator due to (i) a decrease in the amount of incoming wastewater, and (ii) as an attempt to minimise operational costs related to chemical cleaning of the 2nd membrane. For the same reasons, the 2nd membrane was turned off throughout the 2nd operation season. This step led to a notably faster (×2.5) flux decrease compared to the 1st operational season (Fig. 2). A flux value of 5 $L\,m^{-2}\,h^{-1}$ was reached during the 2nd season after only 32 operation days (membrane A), compared with 80 operation days (membrane A) and 88 operation days (membrane B) during the 1st season. For the first 32 operation days in both seasons, the mixed liquor suspended solids (MLSS) concentration was very similar (~4 $g\,L^{-1}$). The flux increase for membrane A (1st season) between the 40th and 50th operation day can be explained by a temporary break in filtration resulting in an interruption to the cake layer on the membrane's surface (long-term relaxation). Relaxation is often used as a method of prolonging MBRs operation cycle (Judd, 2010).

The flux increase from the 130th operation day of the 2nd season was caused by an extreme decrease in AS concentration and its influence on membrane fouling with SS (minimization of reversible fouling). MLSS concentration (Figs. 3 and 4) is one of the main operational parameters influencing flux decrease (Trussell et al., 2006). The decrease in MLSS highlighted in Fig. 4 was due to WWTP operator error, whereby the main volume of AS was separated from the rest of the MBR in the SST until the end of the season.

Two phases of flux decrease were observed over both seasons (Fig. 2). In the 1st season, the decrease lasted from the 1st to the 80th day and from the 80th day to the end of operation. During the 2nd season, the character of flux decrease differed between the first 32 operation days and the rest of the season. During the first phase, flux decrease was faster and membrane fouling proceeded along the inner side. The second phase was slower and characterised by the creation of a cake layer on the membrane's surface (Lim and Bai, 2003). Subsequently, the filtrate was run through the cake layer and flux decrease was slower. Accumulation on the inner side of the membrane, therefore, is likely to be the predominant contributor to flux decrease (Dvořák et al., 2011).

Flux decrease reached 90% (membrane A) and 85% (membrane B) during the 1st season (149 filtration days), and 71% (membrane A) during the 2nd operation season (182 filtration days). All values exceeded 66%, which represents the limit for chemical cleaning as recommended by the membrane supplier. On the other hand, based on the flow of incoming wastewater at the end of both seasons, the flux was sufficient for WWTP operation in both cases.

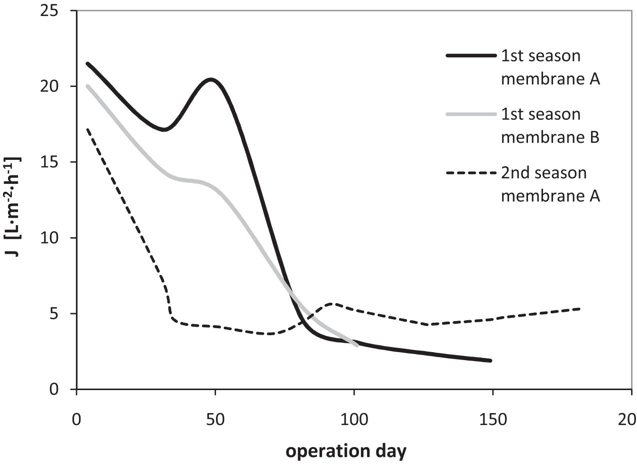

Fig. 2. Flux decrease during the 1st (membrane A and B) and 2nd (membrane A) operational seasons.

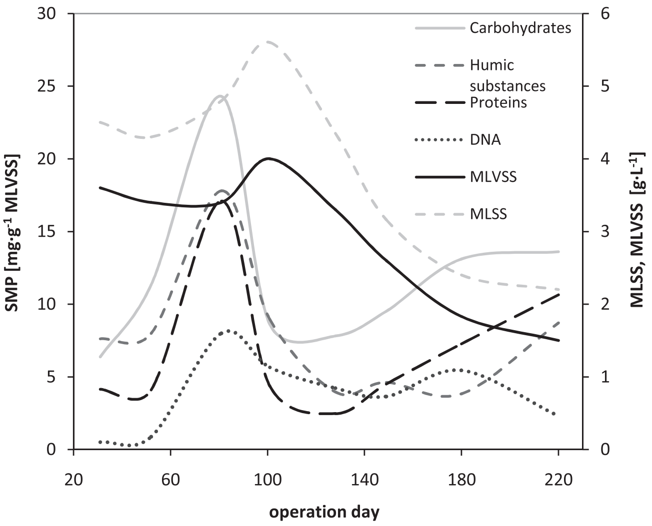

Fig. 3. Evolution of SMP components, MLSS and MLVSS concentrations during the 1st operational season.

3.2. Soluble microbial products (SMP)

3.2.1. First season

SMP are comprised of soluble cellular components released during cell lysis, which then diffuse through the cell membrane and are lost during synthesis or are excreted for some other purpose (Li et al., 2005). SMP concentration is an important contributor to

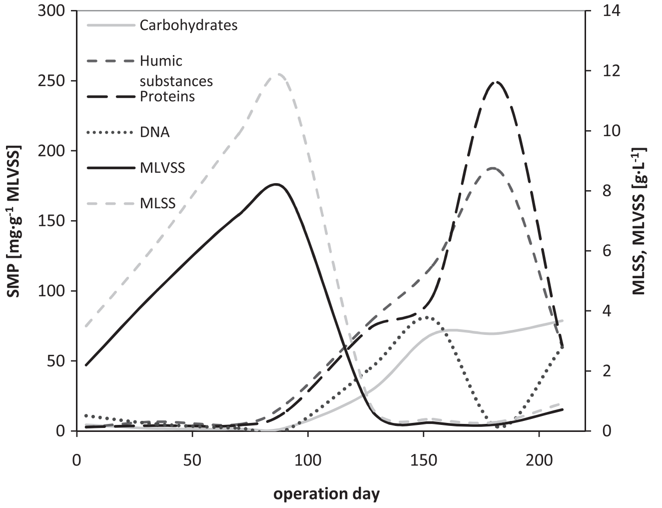

Fig. 4. Evolution of SMP components, MLSS and MLVSS concentrations during the 2nd operational season.

membrane fouling and has a higher impact than MLSS (Zhang et al., 2006). SMP, therefore, can help to create a significant barrier to permeate flow in membrane processes. Their effects on MBR fouling have previously been discussed by, for example, Ishiguro et al. (1994) and, over recent years, the influence of SMPs on membrane fouling has grown in importance (Meng et al., 2009; Holba et al., 2012).

Four main groups of SMP (HS, protein, carbohydrate, and DNA) were analysed over the whole period of operation in order to assess any relationships between the development of mixed liquor volatile suspended solid (MLVSS) concentration and evolution of individual groups of SMP (e.g. SMP sum), as well as to confirm previous results of SMP studies. Relationships between MLVSS, EPS and SMP concentrations are generally well-known and have been observed by many research groups (e.g. Dvořák et al., 2011). These relationships were confirmed by monitoring of SMP, extracted extracellular polymeric substances (eEPS) and MLVSS concentrations during both operation seasons in this study.

Between the 30th and 80th operation day of the 1st season, MLVSS concentration decreased by about 12% (Fig. 3). This resulted in a significant increase in all four SMP groups analysed (24.3 mg g^{-1} → 67.2 mg g^{-1}, ∼360%), indicating a release of internal cell content into the surrounding supernatant and a stress reaction by the microorganisms. Many microorganisms produce carbohydrates as a protective barrier (Wingender et al., 1999). With increasing attendance at the recreation facility (from the 64th day), the F/M ratio increased markedly (200% over previous rate) and HRT decreased to designed values (Fig. 1). This acted as a catalyst for an increase in MLVSS concentration, a decrease in SMP to concentrations found before the decline in MLVSS concentration (67.2 mg g^{-1} → 27.6 mg g^{-1}; Fig. 3), and an improvement in AS morphological characteristics such as floc size and distribution (increase in floc of >500 μm diameter, from 15% to 60%; Table 1). Enlargement of AS floc led to a decrease in SMP in the supernatant and an increase in bonded EPS concentration (Fig. 5).

Attendance at the recreational facility decreased after the 110th operational day and wastewater flow decreased accordingly (∼34.5 m^3 d^{-1} → ∼6 m^3 d^{-1}, reduced to 0 m^3 d^{-1} several weeks later; see Fig. 1). This produced a decline in the F/M ratio and a decrease in the content of large floc (from 60% to 5% by the 128th operational day) compared to the 81st operational day, which was connected with a slight but constant increase in all four SMP groups up until the end of the 1st operational season (except for DNA concentration; Fig. 3). DNA concentration improved up until the 180th operational day, whereupon it decreased. This was due to poor AS condition and elimination of most of the active bacteria, a situation confirmed by increased presence of AS fragments with a diameter <50 μm and the concentration of small floc (on average 85%; Table 1), as well as by comparison of specific kinetic rates (R_x) of nitrification and denitrification measured on the 101st and 180th operational days (R_x (COD) −5.49 → −3.27 mg g^{-1} h^{-1} and R_x (NO_3-N) −0.53 → −0.08 mg g^{-1} h^{-1} for denitrification; R_x (NH_4-N) −1.84 → −0.24 mg g^{-1} h^{-1} and R_x (NO_3-N) 2.21 → 0.35 mg g^{-1} h^{-1} for nitrification). Compared to the previous MLVSS decline, the SMP concentration change was not as significant (Fig. 3). This was due to a significantly lower quantity of incoming organic substrate between the 30th and 80th operational day than in the former case (18 m^3 d^{-1} × 0 m^3 d^{-1}, ↓F/M ratio → ↑HRT; Fig. 1). This led to consumption by other microorganisms of SMP dissolved in the supernatant. Degradation of EPS can have a number of consequences, e.g. the formation of products serving as potential carbon and energy sources for bacteria (Xun et al., 1990).

Table 1
Size and structure of activated sludge flocs and AS characteristics during the 1st operational season (fragments < 50 μm: –, absent; –+, few; +, common; ++, abundant; +++, excessive).

Characteristics of flocs and AS	Operation day					
	31	52	81	101	128	220
Small [%]	20	30	35	60	60	85
Medium [%]	50	55	5	30	35	15
Large [%]	30	15	60	10	5	0
Compact [%]	0	0	5	5	5	0
Compact core [%]	75	80	75	75	80	100
Diffused [%]	25	20	20	20	15	0
Fragments < 50 μm	+	+++	++	++	++	+++
Filament abundance	3	2	2	2	3	4

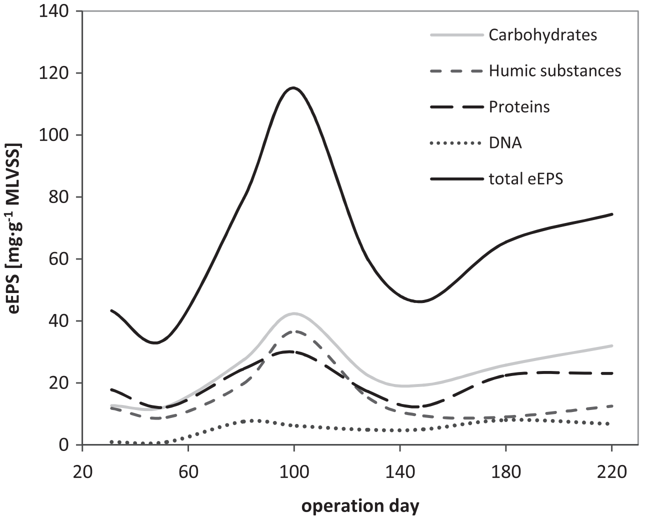

Fig. 5. Evolution of components and total eEPS concentration during the 1st operational season.

3.2.2. Second season

The WWTP operator did not inoculate the MBR with new AS at the start of the 2nd season, but began operation with AS that was aerated for several months with almost no substrate. During these months the MBR was fed with a minimum of incoming wastewater (∼0–1 m^3 d^{-1}), which resulted in a low concentration of MLVSS at the beginning of WWTP operation and a 10x higher DNA concentration in SMP compared to the previous season (10 mg g^{-1} in the 2nd season compared with 1 mg g^{-1} in the 1st; Figs. 3 and 4). With an increase in incoming wastewater (~24.5 m^3 d^{-1}), DNA concentration in the supernatant decreased and MLVSS concentration increased (Fig. 4). This resulted in an improvement in floc size and number of diffused fragments <50 μm in AS morphological characteristics (Table 2).

A mistake by the WWTP operator caused the main volume of AS to be diverted to the SST on the 120th operational day. This led to an exceptional drop in MLSS concentration and complications with the wastewater treatment process. As can be seen in Fig. 4, concentrations of all four SMP groups increased as mg g^{-1} of MLVSS following the faulty procedure, while concentrations in mg L^{-1} remained almost the same. The increase in MLSS concentration was limited between the 134th and 169th operational day by low influent volume (~6 m^3 d^{-1}), which resulted in high HRT (Fig. 1). A slight improvement was noted after the 176th operational day following an increase in the volume of wastewater influent. The AS diversion and inlet decline (HRT ↑) after the 134th operational day (Fig. 1) caused significant changes in AS morphology, i.e. the total elimination of large and medium floc (>500 and 150–500 μm) and 100% occurrence of small floc (<150 μm).

Experience gained through full-scale plant monitoring indicated that operational staff discipline is extremely important for correct functioning of the WWTP. The key factors contributing to correct MBR functioning are clear operator instructions and, especially, motivation toward operational discipline. One way of solving this problem could be increasing the level of automation and introducing centralised remote data transfer.

3.3. Influence of EPS on MBR fouling

Typically, extracellular polymeric substance (EPS) solutions are characterised according to their relative content of proteins (EPSp) and carbohydrates (EPSc). While monitoring the MBR WWTP, it was observed that extracted EPS (eEPS) composition differed over the two seasons. During the 1st season, the major eEPS component was carbohydrate (eEPSc). This situation changed during the 2nd season, when the major eEPS component was protein (eEPSp). This was probably caused by a lower pH during the 1st season (pH 1st season ~6.0; pH 2nd season ~7.0). Many microorganisms produce carbohydrates that are either excreted into the medium or form a capsule around the cell, both as a protective barrier and as a gel formation for adsorbing inorganic ions (Wingender et al., 1999). A further explanation for the higher content of carbohydrates, therefore, could be their excretion by microorganisms. While carbohydrates can adsorb ferric ions due to simultaneous phosphorus precipitation, ferric sulphate solution was only added during the 1st season.

While EPSp has hydrophobic tendencies, EPSc are more hydrophilic (Liu and Fang, 2003). These hydrophilic characteristics could explain the apparent higher propensity for EPSc to interact with the membrane's surface, rather than EPSp. The strong hydrophilic interaction between carbohydrates and the membrane surface, therefore, may be the cause of the initial fouling observed in the MBR system (Drews et al., 2006).

PES is basically hydrophobic but membranes made of PES can be modified to some extent through the use of additives such as co-polymers or by post-treatment (Judd, 2010). We analysed content of protein and carbohydrate to determine which of these two important groups had highest impact on MBR fouling. Correlations were observed among all four groups of SMP and eEPS during both operating seasons (Figs. 3–6), clearly indicating continuous conversion of eEPS to SMP.

The EPS fraction retained by the membrane differed between seasons (Table 3). During the 1st season, carbohydrates were the main component influencing membrane fouling, whereas proteins were identified as the main component during the 2nd season, probably due to a difference in F/M ratio. Kimura et al. (2008) observed a relationship between an increase in F/M ratio and increasing quantity of protein in foulant; however, the results of this study indicated the opposite relationship. A change in membrane surface character (PES originally hydrophobic → hydrophilic) over long-term operation could probably affect interaction with compounds present in suspension, i.e. different compounds interact at the membrane's surface at the start of operation than at the end. As carbohydrates are more hydrophilic (Liu and Fang, 2003), they could have interacted more strongly with the membrane during the 1st season. During the 2nd season, proteins interacted significantly more as the membrane became less hydrophilic and more hydrophobic. Alternatively, a change in the carbon to nitrogen (C/N) ratio may also explain the process. During the 1st season, the C/N ratio was approximately 17:1, but had dropped to 11:1 by the 2nd season. The influence of C/N ratio on protein and carbohydrate production was monitored according to Fenxia et al. (2011). These authors observed a decline in carbohydrates and an increase in proteins within the SMP when the C/N ratio decreased.

The 2nd season was characterised by an increase in the influence of HS on membrane fouling (Table 3), caused by higher HS than carbohydrate in the SMP. In general, HS would not contribute significantly to fouling due to its lower molecular weight (Drews et al., 2006). In this case, however, HS content was much higher than carbohydrate content and, therefore, HS leverage was more

Table 2
Size and structure of activated sludge flocs and AS characteristics during the 2nd operational season (fragments < 50 μm: –, absent; – +, few; +, common; ++, abundant; +++, excessive).

Characteristics of flocs and AS	Operation day					
	4	35	91	127	182	210
Small [%]	35	25	10	95	100	85
Medium [%]	20	25	45	5	–	15
Large [%]	45	50	45	–	–	–
Compact [%]	–	–	20	90	100	60
Compact core [%]	65	100	70	10	–	20
Diffused [%]	35	–	10	–	–	20
Fragments < 50 μm	> +++	– +	+	+	++	+++
Filament abundance	2	1	2	1	3	4

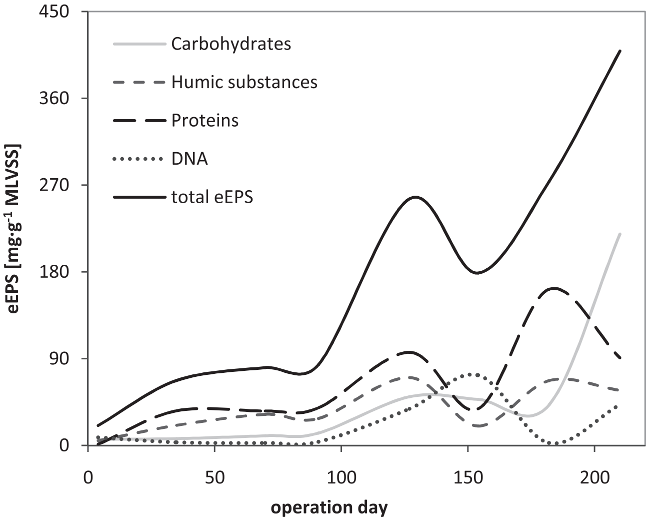

Fig. 6. Evolution of components and total eEPS concentration during the 2nd operational season.

Table 3
Average amount of eEPS and SMP in the supernatant and their retention by the membrane over both operational seasons.

Season	eEPS component in activated sludge [%]				SMP component in supernatant [%]				Retention of SMP by membrane [%]			
	C	HS	P	DNA	C	HS	P	DNA	C	HS	P	DNA
1st	37.5	23.8	30.8	7.8	38.9	26.1	22.4	12.6	40.2	19.3	28.7	11.9
2nd	28.9	22.0	36.4	12.7	17.7	33.0	35.0	14.3	20.8	25.9	35.9	16.5

significant. The high levels of HS SMP (Fig. 4) could be explained through biodegradation of carbohydrates, a mechanism previously described by Hedges (1988), i.e. HS are examples of polymers whose chemical structure is the result of partial enzymatic degradation of various biopolymers and condensation of refractive small organic breakdown products by spontaneous abiotic and enzymatic processes (Hedges, 1988).

One of the goals of this study was to assess the relationship between concentration of SMP and eEPS and the morphological characteristics of AS. The relationship between SMP concentration and AS characteristics, however, was primarily assessed during the 1st season as the results of SMP analysis were affected by the retention of AS in the SST during the 2nd season, i.e. a decrease in SMP concentration (Fig. 3) with increasing contribution of medium and large floc in the AS (Table 1). The smooth increase in carbohydrate content in eEPS (180 → 410 mg g^{-1} of MLVSS) up until the 150th operational day in the 2nd season (Fig. 6) could be explained by a reaction to a change in aeration character and may be linked to shear intensity (due to technical complications, fine aeration was changed for rough aeration during this period). The same microorganism reaction was also described by Lesjean et al. (2005), who found that an increase in EPS floc production was associated with an increase in shear intensity, the same trend that was observed in this study. Ramasamy and Zhang (2005) have previously shown that an increase in shear intensity causes overproduction of carbohydrates in the EPS of biofilm systems.

3.4. Influent and effluent chemical parameters

In this study, the influent could be characterised as high strength wastewater (except BOD_5; Table 4) The BOD_5, COD and NH_4-N removal efficiencies were all at very good levels (Table 4), despite WWTP flow volume (0–39 $m^3\ d^{-1}$) and HRT (Fig. 1) fluctuating significantly over both seasons. While the average C:N:P ratio was 180:11:1 in the 1st season, content of organic substances in the influent decreased and the C:N:P ratio changed to 115:10:1 during the 2nd season.

The efficiency of phosphorus removal was clearly influenced by operational staff discipline, especially during the 2nd season when the WWTP operator decided to terminate simultaneous phosphorus precipitation (Table 4), which led to a decrease in average phosphorus removal efficiency (45.4% → 36.5%; Table 4). The decrease in efficiency was not so apparent, however, due to a peak in MLVSS concentration during the 2nd season caused by higher phosphorus biomass assimilation.

Operation of the MBR WWTP resulted in unnecessarily high oxygen concentrations in the oxic section over both seasons. Values fluctuated between 3.9 and 6.3 mg L^{-1} (mean 5.3 mg L^{-1}) and, in general, higher average oxygen concentrations (∼8.0 mg L^{-1}) were observed in the membrane separation section over the 1st season. During the 2nd season, oxygen concentrations were between 0.35 and 7.8 mg L^{-1} (mean 3.5 mg L^{-1}) in the oxic section and ∼5.0 mg L^{-1} in the separation section. These high values, especially as regards the membrane separation section, provided an opportunity to decrease blower performance and thus the operational costs of the WWTP.

Fluctuations in incoming wastewater flow could be minimised by using part of the second unused WWTP line as a buffer tank. This would lead to improved chemical parameters in the effluent and more constant conditions between the various process steps.

3.5. Pathogen concentration in the permeate

Five groups of pathogens (six in the 2nd year) were monitored over the 2 years of WWTP operation (Table 5). In general, the effluent contained a minimum of pathogenic microorganisms at the start of operation and numbers grew with increasing number of operation days. This was observed for all microorganisms analysed over both seasons.

Decarolis and Adham (2007) studied content of total coliforms in effluent over several months of MBR WWTP operation. They operated four MBR pilot plants with different types of membrane: the MenJet B10 R (US Filter) with a nominal pore size of 0.08 μm (microfiltration) for 212 days; the Type 510 (Kubota) 0.4 μm (microfiltration) for 96 days; the ZW 500 D (Zenon) 0.04 μm (ultrafiltration) for 112 days, and the Sterapore HF (Mitsubishi) 0.4 μm (microfiltration) for 105 days. The authors observed total effluent coliform concentrations (MPN/100 mL) of 386 ± 674 (US Filter), 13 ± 69 (Kubota), 807 ± 1314 (Zenon) and 7 ± 7 (Mitsubishi); all concentrations being lower than those observed in the present study (30–13.3 × 10^3 CFU/1 mL; siClaro® FM 642 Martin system 0.03 μm). The difference in results could be caused either by different raw wastewater pre-treatment or by different membrane operation time, i.e.:

Table 4
Average values (min; max) for basic influent and effluent chemical parameters, removal efficiency, MLSS and MLVSS concentrations at the MBR WWTP.

Parameter	1st season			2nd season		
	Influent [mg L^{-1}]	Effluent [mg L^{-1}]	Removal efficiency [%]	Influent [mg L^{-1}]	Effluent [mg L^{-1}]	Removal efficiency [%]
BOD_5	470 (340; 570)	2 (1; 5)	99 (99.0; 99.8)	380 (110; 810)	3 (1; 7)	99 (99.3; 99.9)
COD	1840 (520; 4600)	35 (10;100)	96 (85.8; 99.3)	710 (430; 980)	75 (10; 160)	91 (77.6; 98.7)
NH_4-N	110 (36; 137)	13 (28.2; 2.5)	81 (49.4; 97.6)	55 (36.7; 81.1)	15 (1.2; 35.0)	76 (55.1; 97.5)
PO_4-P	10 (5.4; 13.8)	4.8 (1.4; 8.2)	45 (5.1; 73.0)	5.8 (3.9; 7.7)	3.8 (2.75; 5.77)	36 (15.8; 56.9)
MLSS [g L^{-1}]		3.9 (2.2; 5.6)			4.2 (0.3; 11.6)	
MLVSS [g L^{-1}]		3.0 (1.5; 4)			3.0 (0.2; 8.0)	

Table 5
Microbiological analysis of permeate (* – CFU in 1 mL; ** – CFU in 10 mL; *** – CFU in 500 mL; MO – microorganisms).

Characteristics	1st season	2nd season
Thermotolerant coliforms	0–20**	0–300**
Escherichia coli	0–3**	0–400**
Clostridium perfringens	3–25 ***	10–200***
Intestinal enterococci	6–20**	0–55**
Culturable MO at 22 °C	650–13 × 10^{3*}	10–7.3 × 10^{3*}
Culturable MO at 37 °C		30–13.3 × 10^{3*}

(i) During this study, highly concentrated wastewater (undiluted by rainwater) was pre-treated by screens only. In the study of Decarolis and Adham (2007), wastewater was subjected to coagulation (27 mg L^{-1} ferric chloride and long-chain, high molecular weight anionic polymer 0.15 mg L^{-1}; in both cases average concentrations) upstream of the WWTP and then replaced after primary sedimentation. These pre-treatment steps could have resulted in lower pathogen concentrations in incoming wastewater.

(ii) As previously mentioned, the lower results in this study could have been caused by the longer operation time of the membrane compared with the four pilot plants. In present study, the MBR was operated continuously for 3 years, whereas new membranes were regularly installed in the study of Decarolis and Adham (2007). Long-term membrane operation often leads to the formation of micro-cracks, which allow pathogen microorganisms to pass through and contaminate the permeate.

4. Conclusions

A considerable increase in the amounts of all SMP groups, especially of DNA and carbohydrates in the case of an insufficient amount of organic substrate was identified.

There was a clear relationship between eEPS and SMP concentrations and AS morphological characteristics.

Very good BOD_5, COD and NH_4-N removal efficiencies were observed, despite fluctuation in influent flow (99%, 96% and 81%, respectively in the 1st season and 99%, 91% and 76%, respectively in the 2nd season).

Increasing pathogen numbers were detected in the permeate as a consequence of changes in membrane integrity during long-term operation.

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