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Monitoring of Transparent Exopolymer Particles (TEP) in a Membrane Bioreactor (MBR) and Correlation with other Fouling Indicators

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Abstract The occurrence of Transparent Exopolymer Particles (TEP), an acidic fraction of polysaccharides, was monitored for more than six months in the activated sludge of three MBR units and the relationship between TEP and other fouling indicators was studied. These compounds consist mainly of exopolysaccharides of a sticky nature, a characteristic which makes them a group of interesting substances in processes like sedimentation, flocculation and membrane fouling. The relationship between capillary suction time (CST) and polysaccharides (PS) was linear for the three tested sludges, although the correlation with TEP concentrations was stronger. A slight linear correlation of both TEP and PS was found with the critical flux (CF) measured with a small filtration test cell, which was submerged in the membrane tank to assess the filterability performance of the sludge *in situ*. However, the correlation CF-PS was clearer. The relationship between TEP, polysaccharides and sludge filterability highlights the potential of this parameter for the monitoring of membrane systems.

Keywords Membrane bioreactor (MBR); Transparent exopolymer particles (TEP); fouling; capillary suction time (CST); Extracellular polymeric substances (EPS); critical flux

INTRODUCTION

In the last decade, MBR technology has become a competitive technology for advanced treatment and recycling of industrial and municipal wastewater in many regions of the world (Lesjean and Huisjes, 2008). In order to find a solution to one of the biggest hindrances of this technology, membrane fouling studies have multiplied in the recent years. Among these studies, foulant characterization is a major research issue in MBR technology. Some of these studies concluded that extracellular polymeric substances (EPS) in the sludge are involved in the fouling process, nevertheless the relationship between EPS and fouling is not clear yet. The term EPS encompasses a large quantity of compounds of different nature produced by the micro-organisms in the biomass, but in the practise they are monitored as a sum of the polysaccharides (PS) and proteins contained in the sludge. A linear correlation between PS concentration and fouling rate was found (Lesjean *et al.*, 2005), while in other studies this could not be observed (Drews *et al.*, 2007a). All agree that PS are one of the major contributors to the membrane fouling process. However, fouling investigations have mainly focused on their concentration, and rarely on their nature. Nevertheless, in order to clarify the importance of EPS in membrane fouling it is necessary to get a better understanding of their properties and composition. In other fields of membrane filtration like wine filtration, Vernhet *et al.* (1999) found that the flux decline related to polysaccharides appeared to depend more on the respective amounts of the different polysaccharides than on the total polysaccharide content. Applying this to MBR technology would convert the EPS paradox in a question of quality and not of quantity.

EPS are also relevant in other fields like oceanography or seawater desalination. In these fields, the transparent exopolymer particles (TEP), one fraction of EPS, have received increasing attention in the last decade. They are found abundantly in the ocean and play an

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important role in many fields of marine ecology (Alldredge, 1993). TEP are very sticky particles that exhibit the characteristics of gels, and consist predominantly of acidic polysaccharides (Passow, 2002). Although TEP analysis was not yet applied to wastewater treatment technology, the relationship between TEP and fouling has been already mentioned. Some authors proposed that TEP in source waters is a prime factor leading to biofilm growth on membrane surfaces and suggested measuring TEP concentrations to determine the efficiency of pre-filtration arrays upstream from high pressure membranes (Berman and Holenberg, 2005).

TEP is measured spectrophotometrically using alcian blue, a cationic dye which binds to acidic mucopolysaccharides. For the measurement of exopolysaccharides concentration in wastewater, the phenol method of Dubois *et al.* (Dubois *et al.*, 1956) requires concentrated acid to break all the carbohydrates into monosaccharides, which are subsequently measured spectrophotometrically using glucose as standard for the calibration. The whole analysis takes about one hour and involves sulphuric acid, which makes the method rather tedious. Besides, it has been found that high concentrations of nitrite and nitrate disturb the analytical method so that the obtained polysaccharide concentrations must be readjusted using a correction equation (Drews *et al.*, 2007b). Therefore, the method used for TEP analysis offers various advantages over the conventional method for polysaccharide analysis in MBR fouling research: it is simpler and quicker, the dye is non toxic and no strong acids are used (de la Torre *et al*., 2008).

In this study, the concentration of TEP in permeate, influent filtrate and mixed liquor supernatant was monitored during more than six months in three MBR units. The occurrence of TEP, retention rate and the relationship between TEP and other fouling indicators were studied for the first time in a membrane bioreactor.

METHOD

MBR units

Activated sludge, influent and permeate analysed were taken from an MBR pilot plant and a demonstration plant operated in Berlin and equipped with the same submerged PVDF flat sheet microfiltration (MF) membrane. In the pilot plant, two identical units of approx. 1.5 m^3 equipped with a membrane module each (22 m^2) are assessed side-by-side (MBR1 and MBR2). Both are fed with municipal wastewater and in one of them (MBR2) a flocculant for permeability enhancement is added periodically. The demonstration plant of approx. 10 m^3 treats domestic water for 250 p.e. with a membrane surface of 31 m^2 (MBR3). All units are operated under constant aeration and with a filtration / relaxation regime (no backwash). Further operating characteristics are detailed in Table 1.

Table 1. Operation conditions of the three investigated MBR units

	SRT	MLSS*	COD supernatant*	COD influent	Temperature
	(d)	(g/L)	(mg/L)	(mg/L)	(°C)
MBR1	12	7-8	30-110	500-1500	10-25
MBR2	12	5-11	30-110	500-1500	10-25
MBR3	25	8-21	150-300	750-2500	17-20

*of mixed liquor taken from the membrane tank

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Activated sludge samples were taken from the membrane chamber. TEP, PS, CST, time to filter (TTF), sludge volume index (SVI), proteins, and critical flux were monitored in these three plants for more than six months.

Analytical methods

The analysis method used for the determination of the TEP concentrations (de la Torre *et al.*, 2008) is based on the protocol developed for TEP quantification in sea water (Arruda *et al.*, 2004). The former consists of mixing 5 mL of prefiltered sample with 0.5 mL of 0.055% (m/v) alcian blue solution and 4.5 mL of 0.2 mol/L acetate buffer solution (pH 4) in a flask. The flask is then stirred for 1 min and then centrifuged (Centrifuge MR23i Jouan GmbH, Germany) at 15,300 rpm (23,292 x g) for 10 min. TEP react with the alcian blue solution yielding a low solubility dye–TEP complex. The concentration of the alcian blue in excess is determined by reading the absorbance at 602 nm (UV-vis spectrophotometer, Analytic Jena, Germany). Xanthan gum is used for the calibration, and the results expressed in mg/L xanthan gum equivalent. PS concentrations were analysed using the phenol-sulphuric acid method (Dubois *et al*., 1956). The bound EPS were extracted using the cation ion exchange method described by Frølund *et al*. (1996). Proteins were analysed according to Frølund *et al.* (1995). Rapid tests were used for the determination of the COD (Hach Lange, Germany).

Physical methods

A small portable filtration test cell (Fig. 1) was submerged directly in the sludge tank in order to measure the sludge filterability under defined conditions, independent of the membrane age and operating conditions of the plant. This test cell used a UF flat sheet membrane of PES (Microdyn-Nadir, Germany) of approx. 0.024 m², is aerated and the evolution of the pressure on the permeate side is recorded via a pressure sensor. The protocol for the measurement of the critical flux consisted of a flux-stepping method with relaxation based on Le-Clech *et al.* (2003). Additionally to the critical flux, CST (Triton CST Filterability Tester Model 200 Allied Colloids GmbH, Germany) and SVI were measured twice a week following Standard Methods (Clesceri *et al*., 1998). Time to filter (TTF) was registered as the time to collect 25 mL of sludge filtrate from a paper filter.

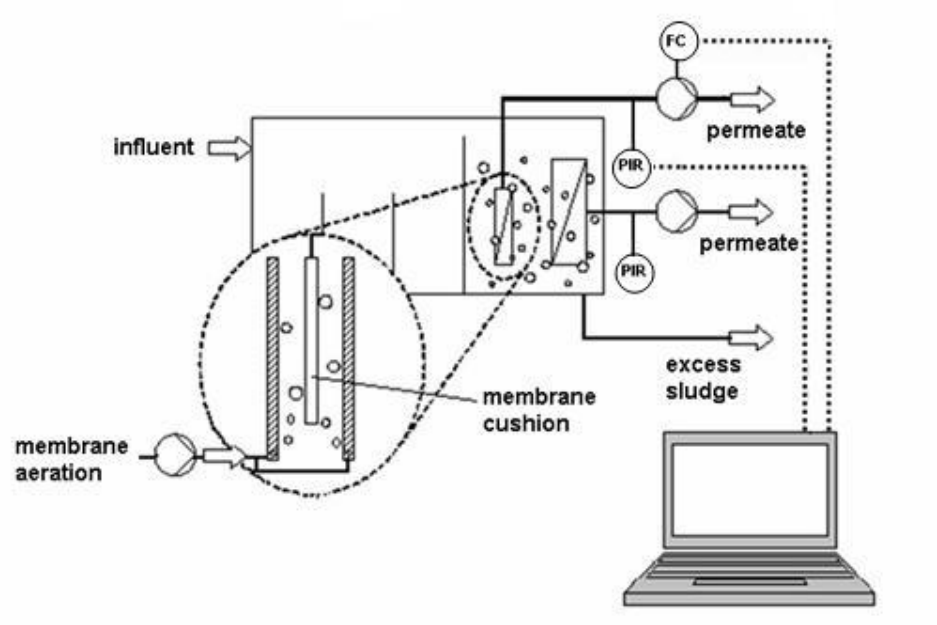

Figure 1. Scheme of the *in situ* filtration test cell.

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Preparation of the samples
Filter papers (Schleicher and Schuell / Whatman, black ribbon Ø 90 mm, Germany) were rinsed with 200 mL deionized water. After that, 50 mL of sample was filtered to obtain the filtered mixed liquor and filtered influent.

RESULTS

TEP evolution

In Fig. 2, 3 and 4 the evolution of TEP concentrations in filtered influent, filtered mixed liquor and permeate are represented for the three investigated units. Compounds belonging to the TEP group can be found, i.e., in the human urine; therefore it is not surprising to find a high concentration of these compounds in the paper filtered wastewater influent. As can be seen, the concentration in the sludge filtrate of MBR1 and MBR2 varies between 10-20 mg/L xanthan gum equivalent, whereas the concentration in MBR3 is up to 5 times higher (20-100 mg/L). The reason may be that the influent of the demonstration plant is more concentrated and also that the mixed liquor of the demonstration plant is highly concentrated in comparison with the pilot units. The concentration in the permeate is higher in the demonstration plant than in the pilot units, with about 11 mg/L, and its time evolution is quite stable for all units.

The concentration of TEP in the influent is quite variable, and it keeps in the range of the concentration in the mixed liquor, which indicates that there is no evidence of accumulation in the reactors. The retention of these compounds is very variable, between 0 and 40% in the pilot units and about 60% in the demonstration plant. Due to the implementation of the same membrane material, the variable rejection rate can be attributed to a difference in the salt concentration of the different mixed liquors or to a difference in the properties of the TEP, like a larger size of the molecules of TEP in the demonstration plant, or to the differences in the operational conditions like flux or cross-flow velocity, or finally to a difference of floc size distribution resulting in a different dynamic filtration layer.

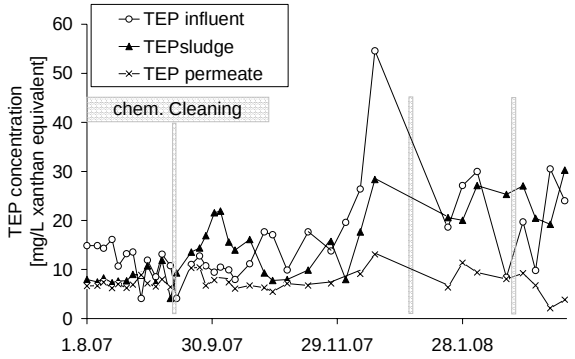

Figure 2. TEP evolution in MBR1 in influent filtrate, mixed liquor filtrate and permeate.

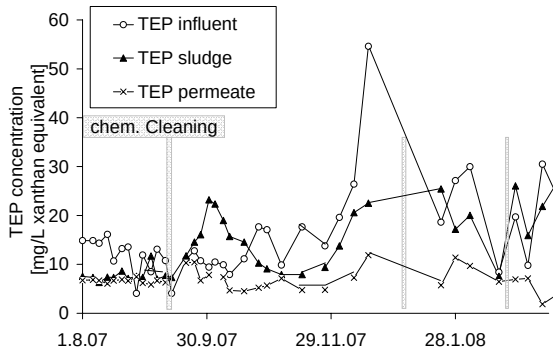

Figure 3. TEP evolution in MBR2 in influent filtrate, mixed liquor filtrate and permeat

After chemical cleaning of the pilot plant in September (first grey line in the graphs) a big increment in the TEP concentration of MBR1 an MBR2 was observed. This increment in the TEP did not follow a parallel increase neither in the temperature nor in the concentration of TEP in the influent, what indicates that TEP formed as a result of a disturbance in the

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environmental conditions. This was already observed for EPS (total polysaccharides and proteins) in the literature (Drews *et al.*, 2007b). This pattern could not be seen after other chemical cleanings. A smaller peak could also be observed for the concentration of polysaccharides in MBR1 after the chemical cleaning, whereas in MBR2 the concentration remained stable (Fig. 5). The second peak for the TEP concentration in the pilot unit and the generally higher lever in January and February is probably attributable to the great increase in the influent concentration. Another reason for the higher level in the last months can be a change in the protocol of the TEP analysis, which may lead to differences in the concentration, like already mentioned in the method description (de la Torre *et al.*, 2008).

Fig. 6 shows that the bound TEP concentrations are extremely high in MBR3 reaching up to 1000 mg/L. That is connected to the high COD concentration in the influent of the plant and to the high mixed liquor concentration (Table 1). A reason for the variations in the concentration of bound TEP in MBR2 is difficult to explain because of the irregular addition of flux enhancer. However, the measured concentrations of bound TEP may be subjected to a significant error because of the matrix influence and the dilution effect. These can play an important role in the case of the bound TEP, because the samples extracted with the ion exchange resin contain a lot of potentially interfering substances which can cause a significant matrix effect, and the error derived from that will be multiplied by diluting (25:1 dilution is necessary due to the high concentrations of bound TEP in the samples).

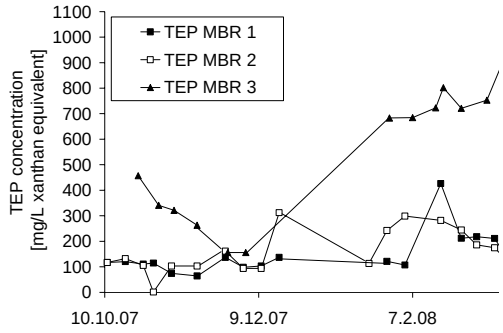

Figure 4. TEP evolution in MBR3 in influent filtrate, mixed liquor filtrate and permeate.

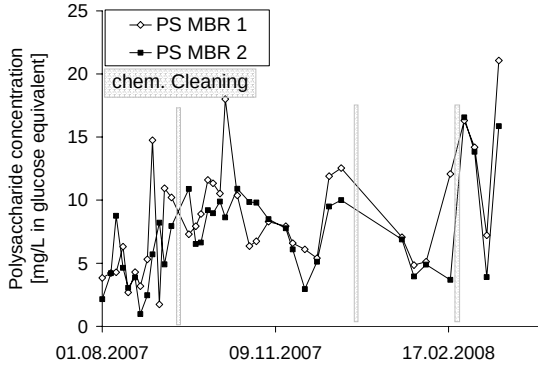

Figure 5. Polysaccharide evolution in MBR1 and MBR2 in influent filtrate, mixed liquor filtrate and permeate.

Cross-correlations between TEP and other fouling indicators

As can be seen in Fig. 7, there is a general relationship between the values measured with the Dubois method and the TEP values obtained with the method developed in this study but no direct proportionality can be appreciated. That demonstrates that TEP represents a different fraction of polysaccharides not studied in wastewater treatment technology before. A good linear correlation between TEP and CST was found for the three MBR units investigated (Fig. 8), whereas the correlation between CST and the polysaccharide concentration of the sludge filtrate is weak in the same period (Fig. 9). No clear relationship was found between the concentration of TEP in the mixed liquors and the other parameters monitored in the plants: proteins, COD, TTF or SVI.

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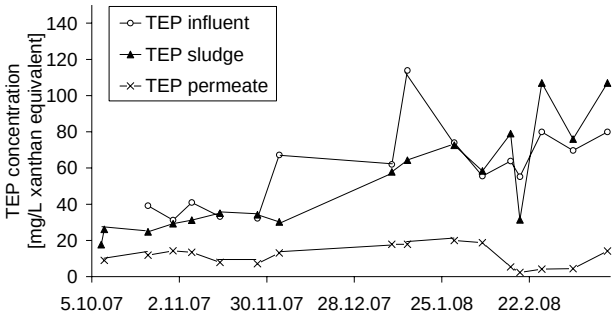

Figure 6. Evolution of bound TEP in MBR1, MBR2 and MBR3

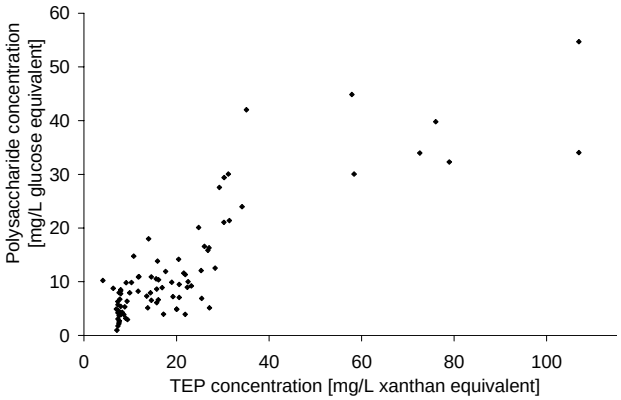

Figure 7. TEP concentrations against polysaccharide concentrations in mixed liquor filtrate from MBR1, MBR2 and MBR3.

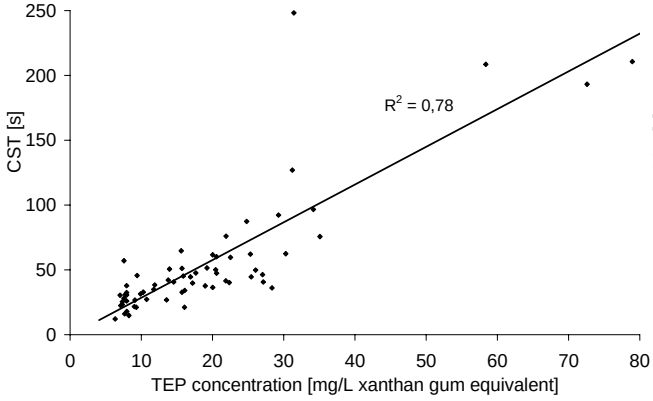

Figure 8. TEP concentrations in mixed liquor filtrate against CST in MBR1, MBR2 and MBR3.

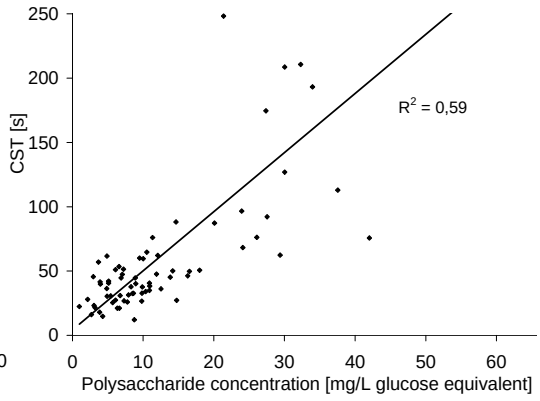

Figure 9. PS concentrations in mixed liquor filtrate against CST in MBR1, MBR2 and MBR3.

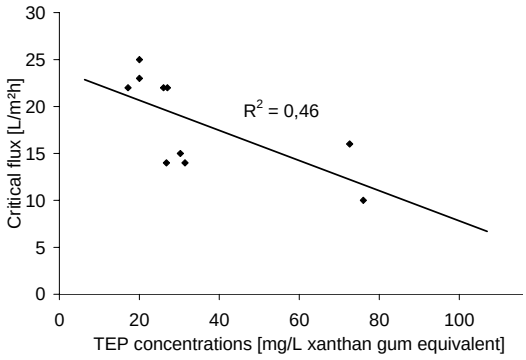

Figure 10. TEP concentration in mixed liquor filtrate against critical flux for the three investigated units.

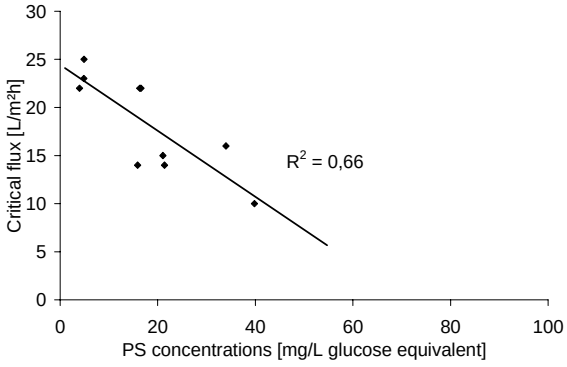

Figure 11. PS concentration in mixed liquor filtrate against critical flux for the three investigated units.

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Fig. 10 and 11 represent the values for the critical flux obtained visually from the graphics TMP-Flux with the flux-stepping protocol already mentioned against TEP and PS concentrations, respectively. Although the TEP measurement was not done at the same time (and in some cases not exactly on the same day) as the critical flux measurements, the diagram gives an idea about the sensitivity of the filterability (measured as critical flux) with the TEP concentrations. The higher the concentration of these compounds, the sooner the critical flux is reached (and the lower the mixed liquor filterability). In this case, the relationship of the critical flux with PS is vaguely stronger than the one found with TEP. That could mean that the TEP fraction is not the only one responsible for fouling, and that an important fraction for fouling investigation would be forgotten when measuring only the acid-PS fraction of the total EPS. Due to the low significance of the difference, this should be confirmed by further investigations.

DISCUSSION

The results obtained from the first monitoring study of TEP in MBRs show the significance of this parameter for the MBR fouling research. The relationship of this parameter with the dewaterability of the sludge has been elucidated after finding a linear correlation between CST values and TEP concentrations measured in three MBR units for more than six months. When monitoring the polysaccharide concentration in the units, only a slight trend could be found between CST and polysaccharide concentration using the conventional method (Dubois *et al.*, 1956). However, a slight linear correlation was found for both parameters (TEP and PS) against critical flux *in situ* measured with a filtration test cell. Against this parameter the relationship was clearer for PS concentrations than for TEP concentrations. That could mean that the TEP fraction of the PS is not the only one responsible for fouling. Nevertheless, the correlation found with CST shows the importance of this parameter in processes like sludge dewatering or flocculation. After chemical cleaning of the two units working in parallel (MBR1 and MBR2), an increment of the concentration of TEP was observed, which indicates a disturbed elimination or even a production of these compounds by the biomass after experiencing a disturbance in the environment, an effect already observed in the literature (Drews *et al.*, 2007b). This phenomenon could only be slightly observed when monitoring the polysaccharide concentration. This fact and the only weak correlation between PS and TEP concentrations demonstrate that the alcian blue method measures a different fraction of polysaccharides than the conventionally measured one according to Dubois *et al.* (1956). The occurrence of TEP in permeate and wastewater filtrate was also studied in the MBR units investigated. Additionally, bound TEP were extracted from the flocs and monitored in the three plants. These represent the main fraction of the total TEP, showing values up to 1 g/L in the demonstration plant. The influents showed high concentrations of TEP and the retention of this compound rounded 40% in MBR1 and MBR2, whereas in MBR3 it varied between 60 and 96%.

The alcian blue method for TEP analysis offers various advantages over the conventional method for PS analysis: it is simple and quicker, the dye is non toxic and no strong acids are used. Besides, using the method of Dubois et al. (Dubois et al., 1956), the interference of nitrate and nitrite need to be corrected, which provides a further time and cost disadvantage.

CONCLUSION

The first attempt to monitor the TEP concentration in sludge filtrate highlights the potential of this parameter as a fouling indicator for MBR systems. Linear correlations of this parameter were found with CST and critical flux measured *in-situ* with a portable filtration test cell

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which was submerged directly into the sludge tank. Only weak correlations were found for the CST with total carbohydrates concentration measured with the phenol method. However, the correlation between critical flux values and PS concentrations was stronger than with TEP concentrations. It was demonstrated that the phenol method measures a different fraction than the alcian blue method, which is simpler, quicker and does not use toxics. TEP showed the typical behaviour of microbial by-products after experiencing a process disturbance, after which the concentration of TEP increased, whereas the PS concentrations in the units stayed stable. The significance of this new parameter is not clear from the results of this study, but TEP monitoring seems to be an additional useful tool for MBR investigation that may help understanding the complex phenomenon of membrane fouling.

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