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Influence of temperature variations on the cake resistance and EPS of MBR mixed liquor fractions

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ABSTRACT

Few studies have been carried out to correlate the influence of temperature on the filterability of different mixed liquor fractions, i.e. suspended solids, colloidal matter and soluble organic matter. It is known that temperature has a big impact on the flux in microfiltration processes. The aim of this research was to figure out the impact of temperatures in the range of 10ºC to 40ºC on fouling, especially in terms of the release of particles and soluble microbial products (SMP). Results based on the contribution of each fraction, namely mixed liquor (>1 μm), supernatant (1 μm to 1 nm) and soluble (<1 nm), to fouling, suggest that the colloids present in the supernatant are affected by temperature changes. Low and high temperatures result in deflocculation and formation/release processes respectively, resulting in a release of submicron particles and were observed due to retention changes. Similar values of the cake resistance for all the fractions and for the range of temperatures studied, indicate that all submicron/colloidal particles within the range 0.05–0.4 μm have a similar influence in terms of fouling. No correlation was found between the concentration of EPS and the cake resistance, which indicates that (an)other foulant(s) could contribute to the cake resistance. Finally, low retentions of both polysaccharides and proteins were observed at 40ºC.

Keywords: Cake resistance; Extracellular polymeric substances (EPS); Membrane fouling; Temperature

1. Introduction

Membrane bioreactors (MBR) have been widely used in wastewater treatment due to their important advantages over traditional technologies [1]. The advantages of the MBR process are its retention of solids, a high effluent quality, good retention of all microorganisms and viruses, maintenance of high biomass concentration and compactness [2].

However, a major obstacle in membrane filtration is the rapid decline of permeate flux caused by membrane fouling [2,3]. Such fouling leads to frequent membrane cleaning and even membrane replacement, both of which increase the maintenance and operating costs of MBRs [1].

Membrane fouling is caused by interactions between the membrane and the components of mixed liquor which reduce the permeate flux [4]. This decrease is usually described by the resistance-in-series model [5]. This model has been used due to its facility in quantifying

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the grade of fouling. Cake resistance (R_c) has been reported as the main contributor to total resistance (R_t) in MBR processes [5,6]. The cake layer could be formed by a variety of components such as microorganisms, inorganic and organic compounds [5].

The principal constituents of the wastewater considered to have the most significant impact on membrane fouling are mixed liquor-suspended solids concentration, sludge PSD, extracellular polymeric substances (EPS) and suspended solids in the supernatant (SS_s) [3].

Temperature is one of the most important environmental factors in biological wastewater treatment [7]. The variations in temperature in a treatment system usually follow the seasonal fluctuations of the local climate.

Temperature affects filtration through its impact on permeate fluid viscosity. Notwithstanding, correction of the permeate viscosity is not sufficient to explain the large variations in cake resistance during cold events. Moreau et al. [8] reported that activated sludge viscosity variations are not sufficient to explain membrane filtration performance loss under cold environmental conditions.

Components having a significant impact on process fouling have, for the most part, been characterized. However, not much has been studied about the influence of temperature. Since both fouling and filterability are determining factors in MBR efficiency and effectiveness, it is of interest to understand the effects that variations in temperature can generate. Such understanding would help to anticipate, and possibly control, the behaviour of MBRs due to temperature changes.

Extracellular polymeric substances (EPS) are a complex mixture of polysaccharides, proteins, lipids and humic substances, which are products that form a highly hydrated gel matrix and come from the cell lysis, secretion or are already present in the influent [9]. EPS are experimentally identified in two solution forms: bound (or extractable), and soluble EPS (called soluble microbial products, SMP) [10]. Bound EPS occur as a capsule surrounding the bacterial cell wall that enhances flocculation, and the soluble EPS are in solution in the supernatant [11]. The effect of EPS in terms of filterability is not yet clear. In many studies, EPS (soluble and extractable) are primarily thought to affect the filterability of active sludge, and to be the major cause of membrane fouling in MBRs [11,12]. On the other hand, many other authors could not find a correlation between the filterability of the activated sludge and the measured amount of EPS [13,14].

In this study, the first stage of experimentation consisted of determining the cake resistance offered by different fractions of the mixed liquor. This was carried out at four different temperatures (10, 20, 30 and 40ºC) in a dead-end filtration. Particle size distribution (PSD) and specific oxygen uptake rate (SOUR) were coupled with each experiment. In the second stage, EPS and SMP determinations were performed. The effects of temperature on the resistance of the different fractions of the mixed liquor were analyzed.

2. Materials and methods

2.1. Activated sludge samples and pilot MBR operation

In order to carry out the experiments, activated sludge from a Kubota flat sheet membrane pilot plant was used (Fig. 1). Some characteristics of these samples are shown in Table 1. The operating conditions and the average values of the influent and permeate characteristics are shown in Tables 2 and 3.

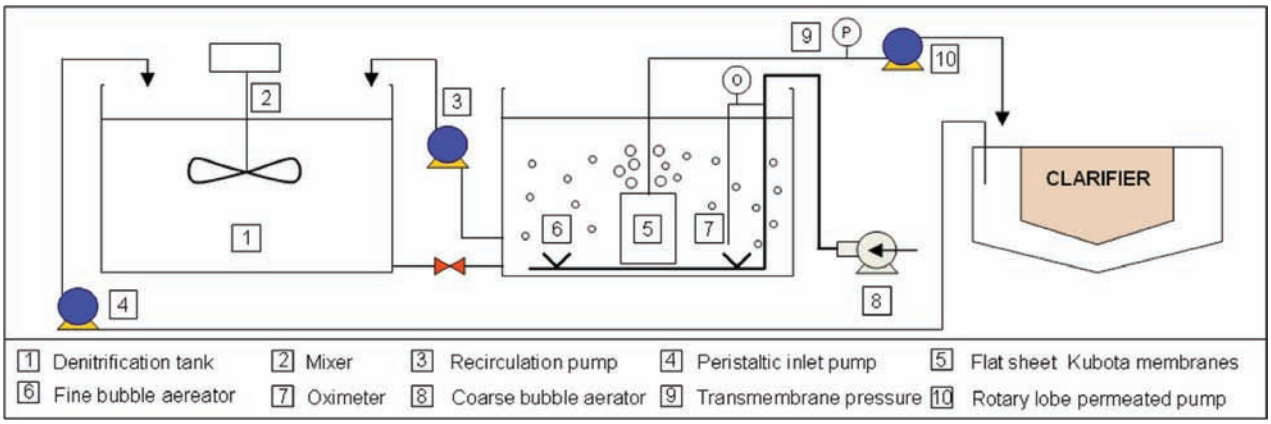

Fig. 1. Schematic view of the MBR.

Table 1
Mean values of the main characteristics of sludge samples used.

Parameter	Exp 1 - 05.03.2008	Exp 2 - 11.03.2008	Exp 3 - 13.03.2008
Sludge TSS (g L^{-1})	8.55	8.01	7.27
Sludge VSS (g L^{-1})	7.18	6.56	5.59
Sludge VSS (%)	84	82	77
SRT (d)	185	111	111
SOUR (mgO_2 gVSS^{-1} h^{-1})	0.90–0.050*	1.97–0.109*	3.18–0.290*

*Standard deviation (SD).

Table 2
Chemical parameters during a one-year test run.

Parameter	Influent			Permeate		
	Min–Max	Mean	SD	Min–Max	Mean	SD
BOD (mg L^{-1})	90–603	385	110	0–69	18	15.6
COD (mg L^{-1})	151–1129	500	141	16–83	32	10.3
NH_{4+} (mg L^{-1})	16–105	68	17.8	0–19.6	0.7	2.75
NO_3 (mg L^{-1})	0–6.8	3.1	0.98	2–102	42	13.4
T-N (mg L^{-1})	21–184	80	20.6	3–40	15	5.44

Table 3
Operating parameters during a one-year test run.

Parameter	Min–Max	Mean	SD
Bioreactor net volume (m^3)	–	6.4	–
Filtration flux (L m^{-2} h^{-1})	12–37	18	–
Cycle filtration/relaxation (min)	–	9/1	–
DO (mg O_2 L^{-1})	0.5–2	1	–
Organic loading (gBOD gMLSS^{-1} d^{-1})	0.02–0.32	0.07	0.040
HRT (h)	10.6–31.8	21	–
pH	6.8–7.7	7.2	0.14
Conductivity (mixed liquor) (mS cm^{-1} 20ºC)	1409–3110	2140	357
Membrane surface (m^2)	–	16	–

2.2. Experimental setup and operating conditions

In order to determine the flux in each experiment, a dead-end filtration system [15] with an AMICON model 8200 dead-end stirred cell was used (Fig. 2). A polyethylene shell was used as a water jacket around the cell body, allowing the water coming from the thermostatic bath to fill the space between the cell and the casing, keeping the temperature constant. Mass permeate and time data were automatically monitored using a computer connected to a balance COBOS, model M-620 CBC. Permeate flow calculations were performed using a linear regression of the last 50 points.

Two different types of membranes were used. In order to determine the resistance due to suspended solids and colloidal matter, the membrane (Kubota, 0.4 μm chloride polyethylene hydrophilic) was cut from a large flat sheet membrane module to fit the size and shape of the cell. To determine the resistance from soluble matter, a commercial Whatman membrane, 0.05 μm PC, was used. The sludge samples were acclimated for 24 hours, just after the SOUR was measured.

On the first day, the temperatures of the thermostatic baths 1 and 2 were set at 10ºC and 20ºC, respectively. At the end of the experiments, the temperature of these two thermostatic baths was changed to 30ºC and 40ºC, respectively. To do the experiments, two samples were taken at the same time. The temperature of the sludge in the MBR at the moment the samples were taken ranged from 20 to 25ºC. Sample 1 was used for experiments at 10ºC and 30ºC, and Sample 2 for experiments at 20ºC and 40ºC. The supernatant was obtained by centrifuging

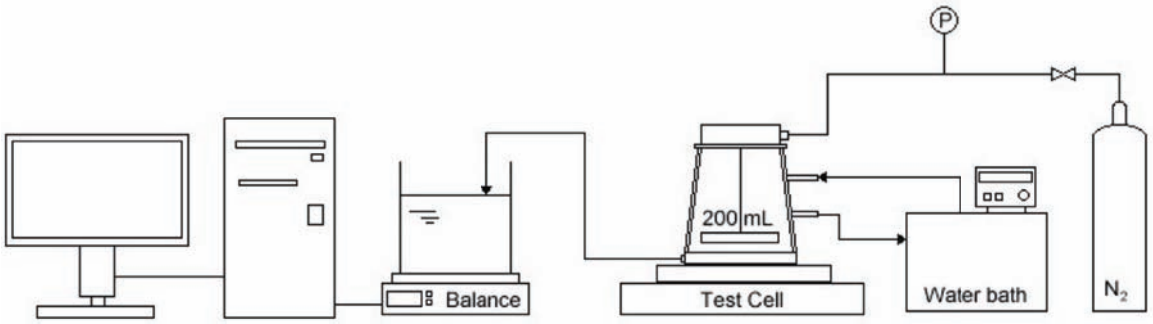

Fig. 2. Experimental setup and test cell.

Table 4
Steps involved in the different filtration experiments.

Step	Description	Filtration time (s)	Pressure (bar)	Membrane
1	Determination of J0 with distilled water	600	0.12	
2	Mixed liquor filtration	3600	0.12	Kubota 0.4 μm
3	Backwash cleaning with distilled water	600	0.12	
4	NaClO 0.05% cleaning	1800	0	
5	Distilled water cleaning	600	0.12	
6	Supernatant filtration	3600	0.12	
7	Backwash cleaning with distilled water	600	0.12	
8	NaClO 0.05% cleaning	1800	0	
9	Distilled water cleaning	600	0.12	
10	Permeate from mixed liquor filtration	1800	0.12	Whatman 0.05 μm
11	Distilled water cleaning	600	0.12	
12	NaClO 0.05% cleaning	1800	0.12	
13	Distilled water cleaning	600	0.12	

the mixed liquor samples for 15 min at 4000 g (4ºC). The driving pressure was set to 0.12 bar.

Chemical cleaning of the membrane was carried out with sodium hypochlorite to ensure the same resistance of the membrane (R_m) at the beginning of each experiment. Membranes were replaced when R_m increased due to irremovable fouling. In the case of the 0.05 μm Whatman membrane, 0.12 bar driving pressure was used. No driving pressure was used with the 0.4 μm Kubota membrane. Table 4 shows the procedure followed within the three different filtration experiments.

2.3. Analytical methods

2.3.1. SOUR determination

To determine SOUR, a portable oxymeter, WTW model 340i, together with a stirrer, was used. The oxygen uptake rate was determined by monitoring the dissolved oxygen concentration in the mixed liquor. SOUR was then calculated by dividing the oxygen uptake rate by the mixed liquor volatile suspended solids (MLVSS) concentration.

2.3.2. PSD determination

Particle size distribution was obtained using a COULTER LS 230 particle counter. After acclimating, the samples were introduced into the device to check the influence of temperature on the size of the flocs.

2.3.3. EPS extraction

EPS were extracted from the mixed liquor using a cation exchange resin (CER) Dowex 50x8, 20–50 mesh and in the sodium form (Fluka 44445) [16]. Before extraction, sludge was centrifuged at 4000 g for 15 minutes at 4ºC using a SIGMA 4K10 centrifuge. For the centrifuge, a 150 ml of sample was used and the sludge pellets were re-suspended to their original volume using a buffer consisting of 2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl and 1 mM KCl at pH 7 [16]. The procedure consisted of stirring the sludge with the CER for 16 hours at 900 rpm [16]. The amount of CER was calculated by using 70 g CER/g VSS. The extracted EPS were first separated from the CER/sludge by centrifugation (4000 g, 4ºC, 1 min.), and then

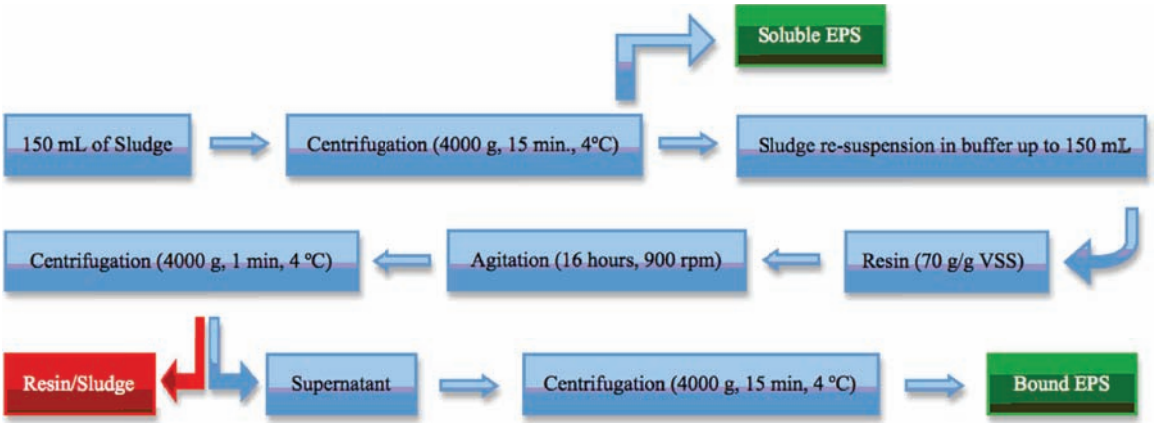

Fig. 3. Procedure for EPS extraction.

the supernatant was centrifuged (4000 g, 4ºC, 15 min) in order to remove the remaining floc components. Figure 3 illustrates, in detail, the procedures of the extraction.

Colorimetric methods were used to perform EPS determination. To determine the carbohydrate content, the phenol-sulphuric acid method of Dubois et al. [17] was used. Glucose was used as a standard. Therefore, the carbohydrate concentration is given as mgL^{-1} glucose equivalent. A Total Protein Kit (Sigma-Aldrich, TP 0330), based on Lowry's description [18] and modified by Peterson [19], was used to determine the protein content. The results shown in the below sections concerning EPS were calculated as the sum of carbohydrate and protein content.

2.3.4. EPS retention

The concentration of EPS involved in the cake layer, and thus in the cake resistance, was calculated as the difference in the concentration of EPS in each fraction before and after the filtration step. For example, to quantify the concentration of EPS involved in the cake layer during the mixed liquor filtration, the EPS concentration in the permeate of the mixed liquor was subtracted from the concentration of both the bound EPS and SMP in the mixed liquor. The difference is the amount of EPS that was retained on the membrane and thus related to the cake resistance. These results are discussed in Section 3.4.

2.4. Filtration resistance determination

The filtration flux through a uniform membrane surface in an MBR can be described by the general form of Darcy's law [20]

$$J = \frac{\Delta P}{\mu R_i}$$

where J is the permeation flux, ΔP is the transmembrane pressure (TMP) that is applied, μ is the viscosity of the permeate, and R_i is the resistance, offered by the particles/substances retained by the membrane. R_i can adopt different names depending on the fraction of the mixed liquor subjected to filtration. The resistance due to suspended solids is R_{ss}; R_{sup} is the resistance due to the supernatant, and R_m the intrinsic membrane resistance. R_{sup} is the sum of R_{col}, the resistance that takes into account the colloidal matter, and R_{sol}, which takes into account the soluble matter. In order to calculate the cake resistance, the R_m of both membranes were calculated by passing demineralized water through both membranes before each experiment.

Three different filtration tests were carried out, as laid out in Figure 4. In the first step, the mixed liquor filtration was performed in order to obtain R_{ss}. In the second step, the supernatant from the centrifuge was filtered resulting in R_{sup}. In the final step, the permeate from the mixed liquor filtration was filtered, obtaining R_{sol}. The first two filtrations used the 0.4 μm Kubota membrane, while the last filtration used the Whatman 0.05 μm membrane.

3. Results and discussion

3.1. Particle size distribution

Floc size ranged from 2.1 μm to 223.4 μm, with a maximum percentage value of 5.6% and a particle diameter of 21.7 μm. Wilen et al. [21] reported that deflocculation occurred under anaerobic conditions and low temperatures. Therefore, a change in the particle size distribution due to a decrease in temperature was expected. However, this behaviour was not observed. This could be due either to the conditions in our reactor—low dissolved oxygen concentration and excess filamentous bacteria leading to

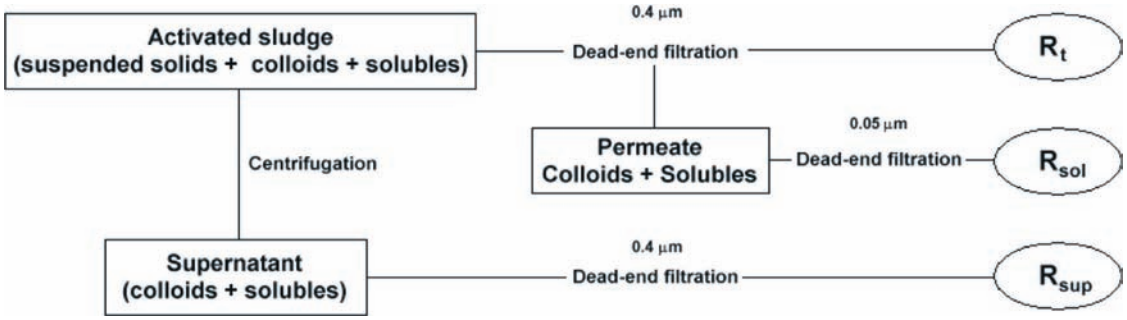

Fig. 4. Filtration procedures to determine the three different resistances.

Table 5
Intrinsic resistance of the membranes used in this study.

T (ºC)	0.4 μm membrane (R_m x 10^{11} m^{-1})			0.05 μm membrane (R_m x 10^{12} m^{-1})		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
10	0.531	0.527	0.654	0.901	1.150	0.736
20	0.511	0.501	0.980	0.969	0.870	0.930
30	0.847	0.818	0.946	1.021	0.763	1.082
40	0.860	0.871	0.967	0.881	1.351	1.412

small size flocs—or to a short acclimation time. A longer acclimation time was not applied since the properties of the sludge can be affected by the absence of oxygen and substrate.

3.2. Resistance of the different fractions to the range of temperatures analyzed

A set of three experiments for each temperature was conducted. The values of R_m are shown in Table 5.

The cake resistance of the different fractions to the range of temperatures studied is shown in Figure 5. As a general trend, the values of the cake resistance are quite similar for all fractions. For both the mixed liquor and the soluble fraction, no tendency was observed for the range of temperatures studied. The cake resistance of the mixed liquor ranged from 1.59×10^{12} m^{-1} to 1.89×10^{12} m^{-1}. In the case of the soluble fraction, no influence of the temperature is observed in the range of values from 1.19×10^{12} m^{-1} to 1.25×10^{12} m^{-1}. Only for the supernatant fraction did the cake resistance increase with the temperature from 1.04×10^{12} m^{-1} to 2.44×10^{12} m^{-1}. These similar values indicate that all submicron/colloidal particles within the range 0.05-0.4 μm have a similar influence in terms of fouling. This is in accordance with Roorda et al. [15] who reported an increase in the cake resistance with the temperature. Campbell et al. [22] suggested that this increase in cake resistance could be due to the stretching of foulants as the temperature rises.

The dynamic viscosity of water depends on the water temperature and thereby influences the cake resistance. Based on this fact, many authors found an increase in the flux when increasing the temperature [20,23,24]. However, the release of SMP at high temperatures was also reported by Drews et al. [25]. It seems that at high temperatures, likely above 30ºC, temperature could induce modifications of the microbial kinetics and of the sludge characteristics/composition. Small fractions of SMP could be metabolized/released. This could explain the data shown in Figure 5. There is no increase in the cake resistance for the mixed liquor fraction, since the small particles are likely to be retained in the floc. As the temperature increases, these particles remain within the flocs since deflocculation takes place mainly at low temperatures. These flocs lead to the formation of a cake layer which seems not to be influenced by temperature. In the case of the super-

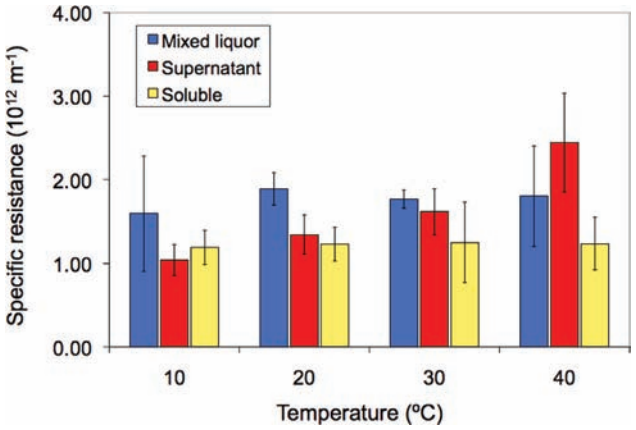

Fig. 5. Average values of the cake resistance at filtration time = 1000 s for the range of temperatures studied.

natant, the flocs have been removed, producing an increase in free particles as the temperature increases and, thus, an increase in cake resistance. The soluble fraction was not influenced by temperature, showing the lowest cake resistance since a higher percentage of soluble matter is able to pass through the membrane.

3.3. Influence of temperature in EPS and SMP concentrations for the different fractions analyzed

The variation in the concentration of EPS in the different fractions and temperatures was analyzed, are shown in Figures 6 and 7. A decreasing tendency in the concentration of bound EPS as the temperature increased can be observed in Figure 6. Values decreased from 1618.3 mgL^{-1} at 10ºC, to 1390.6 mgL^{-1} at 40ºC. This suggests that high temperatures could be related to deflocculation processes, releasing bound EPS attached to the floc, to the supernatant fraction. The difference between 10 and 40ºC gives a concentration of 227.7 mgL^{-1} of bound EPS released to the supernatant fraction. As shown in Figure 7, an increase in SMP can be observed in the range of temperatures studied. Drews et al. [25] also observed this formation/release of smaller SMP fractions at high temperatures. The concentration of SMP ranges from 42.7 at 10ºC to 76.0 at 40ºC. The difference in SMP content between the two temperatures is only 33.3 mgL^{-1}. Only 33.3 mgL^{-1} out of 227.7 mgL^{-1} bound EPS have been identified as part of the SMP fraction. The 194.4 mgL^{-1} of bound EPS not identified, which represents 85.3% of the total bound EPS released, suggests that high temperatures not only form/release SMP to the media, but also modifies the microbial kinetics of the microorganism that use SMP as a substrate. As a final result of this deflocculation-formation-release-degradation SMP process, the final balance increases the concentration of SMP and thus increases the cake resistance, as can be seen in Figure 5.

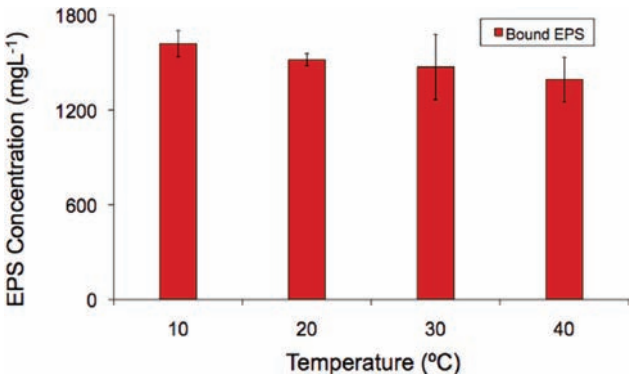

Fig. 6. Concentration of bound EPS at different temperatures.

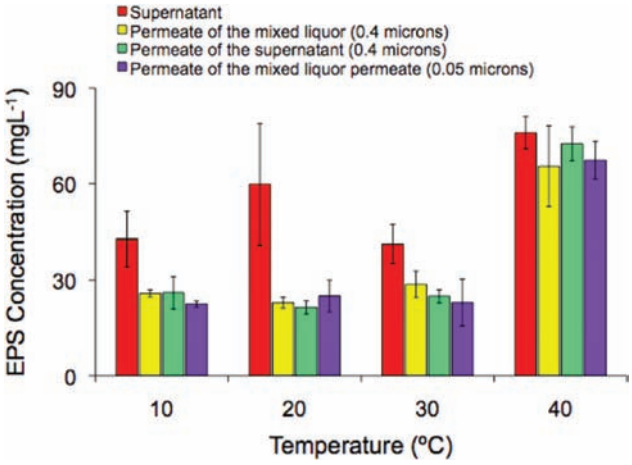

Fig. 7. Concentration of EPS at different temperatures in different fractions.

For the rest of the fractions, as shown in Figure 7, similar concentrations of SMP were identified in the range from 10ºC to 30ºC. The concentration of SMP in the permeates of the mixed liquor, the supernatant and the mixed liquor permeate varied in a very narrow range from 28.6 mgL^{-1} to 21.4 mgL^{-1}. These similar values suggest that for this range the concentration of SMP is not influenced by temperature. Another finding is related to the particle size. The average concentration of SMP in the supernatant is 47.9 mgL^{-1}. After the first filtration of the supernatant with the 0.4 μm membrane, the average concentration for all the fractions was 24.5 mgL^{-1}. This indicates that 48.9% of the SMP has a particle size above 0.4 μm. The remaining 51.1% is mostly below the size of 0.05 μm. According to Drews et al. [25], an increase in the SMP concentration was detected when the temperature was increased from 30ºC to 40ºC. The average value increased from 29.4 mgL^{-1} to 70.3 mgL^{-1} showing a strong impact of temperature on the increase of the SMP concentration. At 40ºC temperature, the values of all the fractions were more similar than for the other temperatures. This suggests that temperature reduces the size of SMP. Further discussion, including processes like denaturation of proteins, will be undertaken in Section 3.5.

3.4. Correlation between EPS and the cake resistance

Attempts made to correlate EPS concentration and sludge characteristics are often contradictory due to the complex nature of the biological system and differences in experimental methods [26]. Figure 8 shows the correlation between the concentration of EPS and the cake resistance of the different fractions for the range of temperatures

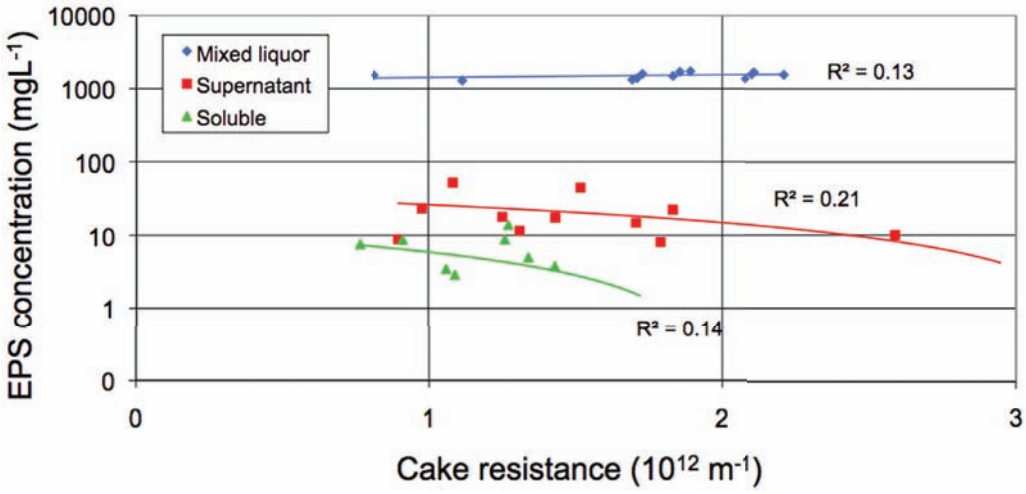

Fig. 8. Correlation between EPS concentration and the cake resistance of different fractions for the range of temperatures studied.

studied. The values of R^2 were 0.13, 0.21 and 0.14 for the mixed liquor, supernatant, and soluble fraction, respectively. The low value for the mixed liquor fraction can be attributed to the bound EPS, which represents more than 96.3% of the total EPS, and does not contribute to the fouling process as much as SMP. Besides, the presence of flocs, mitigate the effect of free particles able to participate in the fouling process. For the three fractions, the correlations are similar, suggesting that for the range of temperatures studied, SMP particles ranging from 0.4 μm to 0.05 μm have the same influence on the fouling process. Nevertheless, all the fractions showed a poor correlation, indicating that (an)other foulant(s) not identified in this study could be responsible for the values of the cake resistance. This will be discussed in the next section.

3.5. Polysaccharides and proteins retention

Throughout the study, polysaccharide (PS) concentrations ranged from 342.6 mg L^{-1} to 189.3 mgL^{-1} in the centrifuged sludge, from 11.1 mgL^{-1} to 4.6 mgL^{-1} in the supernatant, and from 5.8 mgL^{-1} to 0.8 mgL^{-1} in the permeate. The concentration of proteins ranged from 1396.5 mgL^{-1} to 1113.9 mgL^{-1}, from 70.6 mgL^{-1} to 26.5 mgL^{-1} and from 67.3 mgL^{-1} to 19.7 mgL^{-1}, respectively. Figures 9 and 10 show the retention of PS and proteins for all the fractions within the range of temperatures studied. Retentions higher than 95% were reached for both PS and proteins when filtrating the mixed liquor for all the range of temperatures. In the case of PS, filtration of the supernatant and the soluble matter for the range of 10–30°C gave retentions between 67.1–75.3% and 33.1–20.6%, respectively. Since elevated temperatures lead to stronger movement of the molecules (whereby solubility is increased and adhesive forces are decreased), a higher

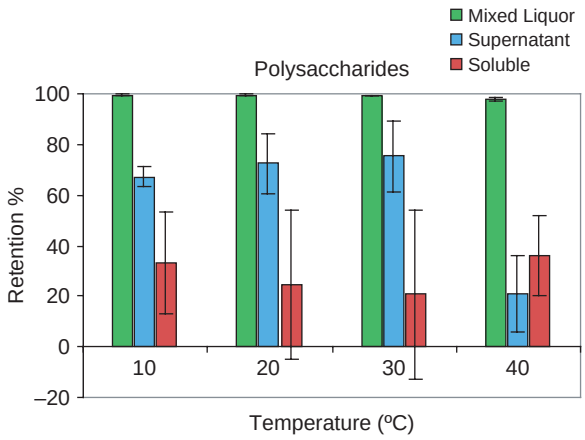

Fig. 9. Percentage retention of PS for the different fractions within the range of temperatures studied.

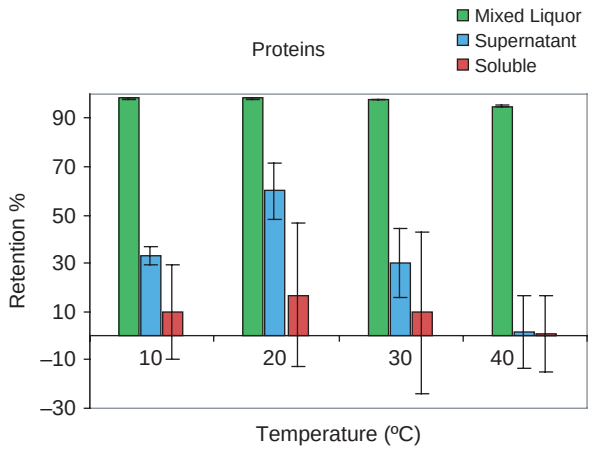

Fig. 10. Percentage retention of proteins for the different fractions within the range of temperatures studied.

mass flux of soluble microbial products (SMP) through the membrane was expected at higher temperatures.

As shown in Figure 9, this behavior can be observed at 40ºC, but with a higher retention of PS in the filtration of the soluble matter than in the supernatant. Campbell et al. [22] found that foulants could stretch due to increased temperature . This could explain the difference in retention between the supernatant and the soluble fraction at 40ºC, even knowing that colloidal PS exhibit a high fouling potential [27–29]. Previous studies have shown important relationship between MBR fouling and the sludge carbohydrate fraction [30–33]. Moreover, high molecular weight carbohydrates can promote the formation of "sticky" hydrogel membrane surfaces [32,33]. Because a high PS concentration in the SMP has been related to high fouling rates [34], the values of retention of PS at 40ºC could be correlated with the high cake resistance encountered for the supernatant fraction at 40ºC. Nevertheless, in comparing the increasing tendency of the cake resistance of the supernatant with the retention values between 30ºC and 40ºC, their difference suggests that just proteins and PS alone are not involved in the fouling process [12,13]. This agrees with the poor correlations that all the fractions described in Section 3.4 exhibited and indicate the importance of other possible foulant(s) not identified in this study in the fouling process.

In terms of retention, the 0.4 μm membrane has an MW cut-off (MWCO) higher than 170 kDa [35]; thus the dissolved low-to-medium size organics are able to pass the membrane. Jang et al. [36] found that most of the protein and the PS at steady-state existed above a molecular weight (MW) of 10 kDa, In general, a broad spectrum of molecular weights from 0.5 to 50 kDa has been reported for the SMP components [37]. Thus, adsorption effects are considered to be the dominant retention mechanism displayed by the membrane, and not a steric exclusion [38–40].

In the case of proteins, a decrease in retention when increasing the temperature was observed for all the fractions, except at 10ºC. Carbohydrate constituents are present at generally higher molecular weights, and also wider molecular weight distributions, than the proteinaceous materials. As the cake layer consists of an organic matrix, it could be that at low temperatures the matrix does not swell, keeping the porosity constant, allowing low molecular weight components like proteins to pass through the membrane. Nevertheless, proteins showed a low retention percentage from 30ºC onwards. At 40ºC the retention was negligible. Campbell et al. [22] studied the effect of temperature on the conformation of the a-amylase protein and its activity during ultrafiltration. They worked in the range from 12ºC to 60ºC and their data showed that increasing the temperature led to a decrease in retention. Proteins do not have a fixed conformation, but are in a dynamic state where conformation and activity are a compromise between flexibility of the structure and stability of the molecule. This has been reviewed [41] and it has been demonstrated that protein structure and folding are controlled by the free energy of stabilization, which is a result of the difference between stabilizing and destabilizing forces, and is approximately 50 $kJmol^{-1}$. Further, protein stability depends on the balance between enthalpic and entropic changes (i.e. for globular proteins, the G of unfolding is maximum in the range of 10–30°C). The energy required to destabilize such a structure can be supplied in many different forms such as thermal, pressure or shear forces, and by concentration or chemical interactions. Once a denaturing agent has been removed, proteins will often rapidly reconfigure to obtain their native structure. Therefore, proteins are more sensitive to changes in temperature than PS are. Heat can be used to disrupt hydrogen bonds and non-polar hydrophobic interactions. This occurs because heat increases the kinetic energy and causes the molecules to vibrate so rapidly and violently that the bonds are disrupted.

Therefore, when increasing the temperature, proteins are quicker to suffer changes in their structure like denaturation, changing their structure from complex and larger structures, like a quaternary, into a simpler and smaller primary structure. The denatured species that are found in the permeate arise from their passage through the membrane. This may be due to either the tortuosity of the pores inducing shear forces or to a surface interaction between the protein molecule and the membrane pore. Thus, both denaturation and lower molecular weights can explain the very low retentions below 5% observed at 40°C.

4. Conclusions

- Since deflocculation occurs at low temperatures, a change in the particle size distribution due to a decrease in temperature was expected. However, this behaviour was not observed. This could be either due to the conditions in the MBR, low dissolved oxygen concentration and excess filamentous bacteria leading to small size flocs, or to a short acclimation time.
- As a general trend, the values of the cake resistance are quite similar for all the fractions. There is no increase in cake resistance for the mixed liquor fraction, since the small particles could be retained in the floc. In the case of the supernatant, the flocs have been removed, producing an increase in free particles as the temperature increases and, thus, an increase in the cake resis-

tance. The soluble fraction was not influenced by temperature at all, showing the lowest values of cake resistance since a higher percentage of soluble matter is able to pass through the membrane. These results suggest that the colloidal fraction is the one which could be more susceptible to changes when varying the temperature.

- A decrease in the concentration of bound EPS was noticed when the temperature increased. This suggests that deflocculation processes could be related to high temperatures, releasing bound EPS into the SMP fraction. A significant percentage of the released, bound EPS was not identified in the supernatant fraction for the step from 10°C to 40°C. This suggests that when the temperature is increased, the kinetics of the microorganism can be modified and thus their necessity for substrate. The final balance is the increase of SMP in the media leading to high cake resistance of the supernatant fraction.
- The concentration of SMP was not influenced by temperature within the range 10°C to 30°C. Similar values of EPS were detected at 40°C for all the fractions, while different values were encountered for the range from 10°C to 30°C. This suggests that high temperatures reduce the size of SMP.
- Low correlations between the different fractions and the concentration of EPS indicate that (an) other foulant(s) not identified in this research could contribute to the cake resistance variations for the range of temperatures studied. This is in concordance with the different values obtained for the retention of PS and the increasing value of the cake resistance for the supernatant fraction.
- More proteins than polysaccharides were detected in all the fractions. Almost no retention of protein was measured at 40°C. Denaturation can be present at high temperatures, leading to a high concentration of proteins in the permeate.

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References

[1] Y. Shui-li and Z. Fang-bo, Effects of components in activated sludge liquor on membrane fouling in a submerged membrane bioreactor, J. Environ. Sci., 18 (2006) 897–902.
[2] M. Hernández and R. Van Kamm, Role and variations of supernatant compounds in submerged membrane bioreactor fouling, Desalination, 179 (2005) 95–107.
[3] F. Meng and H. Zhang, Identification of activated sludge properties affecting membrane fouling in submerged membrane bioreactors, Separation and Purification Technologies, 51 (2006) 95–103.
[4] E. Bouhabila and R. Ben Aïm, Microfiltration of activated sludge using submerged membrane with air bubbling (application to wastewater treatment), Desalination, 118 (1998) 315–322.
[5] J. Lee and W. Ahn, Comparison of the filtration characteristics between attached and suspended growth microorganisms in submerged membrane bioreactor, Water Res., 35 (2001) 2435–2445.
[6] I. Chang and C. Lee, Membrane filtration characteristics in membrane-coupled activated sludge system — the effect of physiological states of activated sludge on membrane fouling, Desalination, 120 (1998) 221–233.
[7] A.A. Moreau, N. Ratkovich, I. Nopens and J.H.J.M. van der Graaf, The (in)significance of apparent viscosity in full-scale municipal membrane bioreactors, J. Memb. Sci., 340 (2009) 249–256.
[8] C. Chiemchaisri and K. Yamamoto, Performance of membrane separation bioreactor at various temperatures for domestic wastewater treatment, J. Memb. Sci., 87 (1998) 221–233.
[9] E. Reid and L. Xingrong, Sludge characteristics and membrane fouling in full-scale submerged membrane bioreactors, Desalination, 219 (2008) 240–249.
[10] Y. Ye and P. Le Clech, Evolution of fouling during crossflow filtration of model EPS solutions, J. Memb. Sci., 264 (2005) 190–199.
[11] S. Rosenberg and M. Kraume, Filterability of activated sludge in membrane bioreactors, Desalination, 146 (2002) 373–379.
[12] S. Liang and C. Liu, Soluble microbial products in membrane bioreactor operation: Behaviors, characteristics, and fouling potential, Water Res., 41 (2007) 95–101.
[13] H. Evenblij and J.H.J.M. van del Graaf, Ocurrence of EPS in activated sludge from a membrane bioreactor treating municipal wastewater, Water Sci. and Technol., 50 No 12 (2004) 293–300.
[14] H. Evenblij, S. Geilvoet, J.H.J.M. van del Graaf and H.F. van der Roest, Filtration characterisation for assesing MBR performance: three cases compared, Desalination, 178 (2005) 115–124.
[15] J.H. Roorda and J.H.J.M. Van der Graaf, SUR test used for optimisation of membrane filtration plants treating wastewater effluents, Desalination, 179 (2005) 131–150.
[16] B. Frolund and R. Palmgrem, Extraction of extracellular polymers from activated sludge using a cation exchange resin, Water Res., 30(8) (1996) 1749–1758 1996.
[17] M. Dubois and K. Gilles, Colorimetric method for determination of sugars and related substances, Anal. Chem., 28 (1955) 350–356.
[18] O. Lowry and N. Rosebrough, Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193 (1951) 265–275.
[19] G. Peterson, A simplification of the protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem., 83(2) (1977) 346–356.
[20] P. Le-Clech and V. Chen, Fouling in membrane bioreactors used in wastewater treatment, J. Memb. Sci., 284 (2006) 17–53.
[21] B. Wilén and K. Keiding, Anaerobic deflocculation and aerobic reflocculation of activated sludge, Water Res., 34 (2000) 3933–3942.
[22] J.M. Campbell, P.R. Walter, R. McLoughlin and C.J. Knowles, Effect of temperature on protein conformation and activity during ultrafiltration, J. Memb. Sci., 78 (1993) 35–43.
[23] X. Huang, P. Gui and Y. Quian, Effect of sludge retention time on microbial behaviour in a submerged membrane bioreactor, Process Biochem., 36 (2001) 1001–1006.
[24] T.W. Tan, H.Y. Ng and S.L. Ong, Effect of mean cell residence time on the performance and microbial diversity of predenitrification submerged membrane bioreactors, Chemosphere, 70(3) (2008) 387–396.
[25] A. Drews and J. Mante, Impact of ambient conditions on SMP elimination and rejection in MBRs, Water Res., 41 (2007) 3850–3858.

[26] A. Drews, C.H. Lee and M. Kraume, Membrane fouling - a review on the role of EPS, Desalination, 200 (2006) 186–188
[27] S. Rosenberger, Ph.D. Thesis, TU Berlin, Fortschr.-Ber. VDI Reihe 3 Nr. 769, VDI-Verlag (2003) Düsseldorf.
[28] S. Rosenberger, H. Evenblij, S. tePoele, T. Wintgens and C. Laabs, J. Memb. Sci., 263 (2005) 113–126.
[29] C. Jarusutthirak, G. Amy and J.P. Croué, Desalination, 145 (2002) 247–255.
[30] B.M. Wilen, B. Jin and P. Lant, The influence of key chemicals constituents in activated sludge on surface and flocculating properties, Water Res., 37 (2003) 2127–2139.
[31] P. Le-Clech, S. Jefferson and J. Judd, Impact of aeration, solids concentration and membrane characteristics on the hydraulic performance of a membrane bioreactor, J. Memb. Sci., 218 (2003) 117–129.
[32] B.P. Frank and G. Belfort, Polysaccharides and sticky membrane surfaces: critical ionic effects, J. Memb. Sci., 212 (2003) 205–212.
[33] C. Harscoat, M.Y. Jaffrin, R. Bouzerar and J. Courtois, Influence of fermentation conditions and microfiltration processes on membrane fouling during recovery of glucoronane polysaccharides from fermentation broths, Biotechnol. Bioeng., 65 (1999) 500–511.
[34] S. Rosenberg, C. Laabs, B. Lesjean, R. Gnirss, G. Amy, M. Jekel and J.C. Schrotter, Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment, Water Res., 40 (2006) 710–720.
[35] S. Lyko, D. Al-Halbouni, T. Wintgens, A. Janot, J. Hollender, W. Dott and T. Melin, Polymeric compounds in activated sludge supernatant. Characterisation and retention mechanisms at a full-scale municipal membrane bioreactor, Water Res., 41 (2007) 3894–3902.
[36] N. Jang, X. Ren, G. Kim, C. Ahn, J. Cho and I. Kim, Characteristics of soluble microbial products and extracellular polymeric substances in the membrane bioreactor for water reuse, Desalination, 202 (2007) 90–98.
[37] D.J. Barker and D.C. Stuckey, A review of soluble microbial products (SMP) in wastewater treatment systems, Water Res., 33(14) (1999) 3063–3082.
[38] S. Rosenberger, C. Laabs, B. Lesjean, R. Gnirss, G. Amy, M. Jekel and J.C. Schrotter, Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment, Water Res., 40 (2006) 710–720.
[39] S. Liang, C. Liu and L. Liu, Soluble microbial products in membrane bioreactor operation: behaviours, characteristics, and fouling potential, Water Res., 41 (2007) 95–101.
[40] F. Fan and H. Zhou, Interrelated effects of aeration and mixed liquor fractions on membrane fouling for submerged membrane bioreactor processes in wastewater treatment, Environ. Sci. Technol. (2007).
[41] R. Jaenicke, Protein folding: Local structures, domains, subunits, and assemblies, Biochemistry, 30(13) (1991) 3147–3161.