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Fouling of membranes in MBRs for wastewater treatment: planktonic bacteria can have a significant contribution

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Abstract: Membrane bioreactors (MBRs) for wastewater treatment show great potentials in the sustainable development of urban environments. However, fouling of membranes remains the largest challenge of MBR technology. Dissolved extracellular polymeric substances (EPS) are often assumed be the main foulant in MBRs. However, single bacterial cells are often erroneously measured as EPS in traditional spectrophotometric analysis of EPS in activated sludge, so we hypothesized that single cells in many cases could be the true foulants in MBRs for wastewater treatment. To study this, raw MBR sludge and sludge supernatant with varying concentrations of planktonic cells were filtered on microfiltration (MF) membranes, and we found a direct correlation between the cell count and rate of flux decline. Addition of planktonic cells to fresh MBR sludge dramatically increased the flux decline. The identity of the most abundant planktonic

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cells in a full-scale MBR wastewater treatment plant was determined by DNA fingerprinting. Many of these genera are known to be abundant in influent wastewater suggesting that the influent bacterial cells may have a direct effect on the fouling propensity in MBR systems. This new knowledge may lead to new anti-fouling strategies targeting incoming planktonic bacteria from the wastewater feed.

Keywords: Modelling, fouling, membrane bioreactor, planktonic bacteria, influent wastewater

1. Introduction

Membrane bioreactors (MBR) are, due to multiple advantages, increasingly installed for wastewater treatment around the world. However, membrane fouling remains the Achilles heel as it reduces permeability and membrane lifetime, and elevates operating costs (Le-Clech et al., 2006).

The characterization of membrane fouling in terms of composition, characteristics, and identification of foulants has achieved lots of attention in the literature (Flemming, 2020, Hamedi et al., 2019, Meng et al., 2017). However, it is difficult to identify the foulants in activated sludge, as activated sludge is a complex mixture of multiple substances, including sludge flocs, single cells, filaments, EPS, and salts, all interacting within the MBR reactor and at the membrane surface (Christensen et al., 2018). Fouling mechanisms and the development of so-called biofouling layers on the membrane surfaces are still not fully explored in full-scale MBR systems for wastewater treatment (Vanysacker et al., 2014). A more detailed understanding should include interdisciplinary research to unify the knowledge from the fields of physical chemistry, process engineering, and microbial ecology to fully realize the potentials and to develop well-adapted control models for MBRs.

The liquid phase constituents of MBR sludge, either colloidal or soluble, have shown a clear relevance with regards to membrane fouling in full-scale surveys, case studies, and controlled laboratory-scale setups (Bugge et al., 2013, Faust et al., 2014, Hamedi et al., 2019, Jørgensen, Nierychlo, et al., 2017, Rosenberger et al., 2005, Van De Staey et al., 2015).

The liquid phase of activated sludge contains a variety of compounds. Lin et al. (2014) describe these compounds, here listed from the smallest to the largest; salts, dissolved organic matters, soluble microbial products (SMP) or soluble extracellular polymeric substances (EPS), colloids

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including organic macromolecules, and rigid inorganics such as silica, struvite, and others. However, also planktonic (single) bacterial cells are present in higher amounts in MBRs than in the conventional activated sludge (CAS) process due to the rejection of bacteria by the membrane in MBR and the selection of floc forming bacteria in CAS (Christensen et al., 2015). The differentiation between some of the compounds is not well defined. With overlapping particle sizes, colloids (ranging from 0.01 to 10 μm) includes SMPs and planktonic bacteria (Lin et al., 2014). The denomination of soluble EPSs and SMPs are often intertwined, however, soluble EPS will be used throughout this article. Soluble EPS have often been the usual suspects as membrane foulants (Ramesh et al., 2007, Shi et al., 2018) and they have been studied extensively and summarized in the review by Lin et al. (2014).

The main components of EPS are carbohydrates, proteins, humic substances, and nucleic acids (Flemming and Wingender, 2010, Lin et al., 2014). The determination of EPS concentration relies almost exclusively on polysaccharide and protein measurements with photometric DuBois (DuBois et al., 1956) or Anthrone (Gaudy, 1962, Raunkjær et al., 1994), and Lowry (Frølund et al., 1995) assays. The isolation of soluble EPS is unfortunately not straight forward as many strategies exits and no standard method have been agreed upon (Van den Broeck et al., 2011, Rosenberger et al., 2005). Centrifugation is commonly used to remove the sludge flocs and various particles (Fan et al., 2006, Gkotsis et al., 2020, Gkotsis and Zouboulis, 2019) whereas in other cases an additional filtration step (Van den Broeck et al., 2010) or heating step (Van De Staey et al., 2015) is included. Whether or not the planktonic bacterial cells are contained in the soluble EPS fraction can therefore vary. Therefore, the quantification of soluble EPS components might in some cases erroneously contain the cellular constituents of planktonic bacteria, while in studies where they are removed, their contribution to fouling is not included. When total protein is measured with the Lowry method, one average bacterial cell constitutes approximately $0.66 \cdot 10^{-13}$ g protein (Frølund et al., 1996, Wilen et al., 2000), meaning that planktonic cells may contribute to a surrogate concentration of EPS. Little attention has been drawn to this possible contribution from suspended planktonic cells to fouling propensity, although Wilén and co-workers have shown that the major constituent of released particles in controlled deflocculation experiments was actually bacteria and floc fragments with only little release of soluble EPS (Wilen et al., 2000). Other powerful analytical techniques have been used to identify the chemical fingerprints to unravel EPS's contributing to fouling (Iorhemen et al.,

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2019, Kimura et al., 2015), but they do not take into account the contribution of single cells. Hence, many of the previous foulant-characterization studies in MBRs may have overestimated EPS fouling and underestimated the importance of single planktonic bacteria.

To unravel the complexity of MBR fouling, the aim of this study was to investigate whether an increased level of planktonic bacteria can explain the elevated fouling propensity of activated sludge with higher apparent SMP concentrations. This was done by studying how MBR sludge with increasing levels of planktonic bacteria and colloids deteriorated membrane performance due to fouling by combining the use of model bacteria, sludge fractionation, sludge characterization, and filterability methods.

2. Material and Methods

2.1 Sludge samples and supernatants

Activated sludge samples were collected from an MBR pilot system installed at Aalborg West Wastewater Treatment Plant (WWTP). The pilot was fed with an inlet flow of 0.5 m^3/h and consisted of an anoxic tank (2 m^3) followed by an aerobic tank (2 m^3). In addition, an anaerobic tank (1.8 m^3) carried out sidestream hydrolysis for EBPR and denitrification as described in (Ziegler et al., 2016). The pilot MBR installed by Alfa Laval A/S contained 40 m^2 "hollow sheet" PVDF membranes (200 nm nominal pore size, MFP2) to treat pre-clarified raw wastewater with enhanced biological phosphorous removal and nitrogen removal. Samples of MBR sludge from the pilot system for analysis was collected the day of use and was left for approximately three hours to reach room temperature before characterization and filtration experiments. Supernatant was produced for filtration experiments by centrifugation (Sigma 6-16K, Buch&Holm, Osterode am Harz, Germany) at 880×*g* for 2 min to remove large flocs and leave small floc fragments, colloids, and soluble material in the supernatant (Wilén et al., 2008). In order to prepare activated sludge with different ratios of flocs and colloids, a colloidal fraction was produced after centrifugation at 3400×*g* for 8 min followed by a second clearing step of centrifugation at 5000×*g* for 5 min. The number of flocs in the activated sludge was then reduced to 50% (v/v) by dilution with the colloidal suspension thus obtaining a suspension with 100% (v/v) colloids and 50% (v/v) flocs (C100F50) compared to the original fresh sludge. The same procedure was followed to obtain a ratio of 100% (v/v) colloids and 75% (v/v) flocs, whereas a solution of 100% (v/v)

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colloids and 150% (v/v) flocs was prepared by resuspension of harvested flocs from the first centrifugation.

2.2 Preparation of bacterial cultures

The strain *Pseudomonas* sp. UK4 was chosen as model bacterium to represent the planktonic cells in activated sludge supernatant. *Pseudomonas* sp. UK4 is Gram-negative, rod-shaped, and was originally isolated from a biofilm formed in a drinking water reservoir (Larsen et al., 2007). Cultivation was done according to Dueholm et al., (2013) and the cells were harvested by centrifugation at 5000×*g* for 15 min and resuspended in Phosphate Buffered Saline 6.7 mM (PO_4) (PBS) (HyClone, Thermo Scientific) and stored at 4°C to prevent further growth. Before usage, the samples were homogenized in a glass tissue grinder (Thomas Scientific) and diluted in PBS buffer to the desired cell count for filtration tests. Washed and homogenized cells were added to the MBR sludge and sludge supernatant to increase the planktonic cell count by a magnitude of 4.

2.3 pH, turbidity, dry matter, protein, humus, and polysaccharide concentration

pH, turbidity and sludge total solids concentration (TS) was measured in duplicate according to Standard Methods (APHA et al., 2005). Sludge supernatant and bacterial suspension was analyzed for protein and humic substances according to a modified Lowry method with use of BSA (Fraktion V, AppliChem, Darmstadt) as standard for protein, and HA (Janssen Chimica, Geel, Belgium) as standard for humic substances (Frølund et al., 1995). The concentration of carbohydrates was determined by a modified Anthrone method (Raunkjær et al., 1994) with D(+)-glucose (BDH, Poole, England) as standard.

2.4 Cell counting

In order to count planktonic cells in sludge and bacterial suspensions, a specific fluorescent DNA stain, DAPI (4′,6′-diamino-2-phenylindoledihydrochloride-dilactate) was applied to a final concentration of 0.05 mg/ml for 5 min. Homogenized samples were filtered onto 0.22-µm-pore-size white polycarbonate membrane filters (Millipore, Bedford, Mass.) and fixed on microscopic slides. Total counts were determined by counting no fewer than 10 microscopic fields using an Axioskop epifluorescence microscope (Carl Zeiss, Oberkochen, Germany, 1000x magnification), and the mean number of cells L^{-1} was calculated for each sample.

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2.5 Submerged flat sheet membrane laboratory scale reactor set-up

The filtration system was a flat sheet system with aerated membranes, the so-called Aalborg Filtration Property Analyzer (AaFPA) which was previously described by Jørgensen, Bugge, et al. (2017). The total volume of the reactor was 5 L and the active membrane area was 84 cm^2 of an Alfa Laval MFP2 flat sheet membrane. The membrane consisted of a polyvinylidene fluoride active layer with 200 μm pore size and a polypropylene support layer. The airflow was kept constant at 7.5 L min^{-1} and the temperature was kept stable at around 22°C. The permeate flux was calculated by weighing the permeate online and the transmembrane pressure was controlled by the water level difference between sludge and the permeate beaker.

2.6 Sludge fouling propensity

Filtration experiments to determine fouling propensity were carried out on MBR sludge, MBR supernatant, model suspensions of bacterial cultures along with suspensions of MBR sludge, and supernatant with bacterial cultures added to increase cell count. Fouling propensity was determined by measuring flux decline during 1 h filtration experiments at fixed TMP of 5 kPa. The flux data obtained was fitted to a mathematical model using Equation 1

$$J = J_{SS} + k \cdot e^{-bt} \quad (1)$$

where J_{SS} is the steady state flux, k is the flux decline at steady state, and b is a rate constant for the flux decrease (Cheryan, 1998). A high b value represents a fast reach of J_{SS} whereas a high k value indicates a big loss in flux from J_0 to J_{SS}. Values from each filtration were obtained by minimizing the root mean square error (RMSE) between the experimental flux and the calculated flux, by adjusting the constants k, b, and J_{SS}, using the Microsoft Excel problem solver function.

Secondly, TMP-step filtration experiments were conducted to determine specific fouling layer resistance, fouling layer compression, and irreversible fouling. The filtration experiments were conducted at 1 h steps of TMPs varying from 1 to 13 kPa with 2 kPa increments. Between each step, a 1 h relaxation step was applied to remove reversible fouling. Another mathematical flux model was fitted to the experimental flux data to obtain limiting flux, J_{LIM}, specific resistance at no pressure, α_0, and a compressibility parameter, P_a, as described in (Jørgensen, Bugge, et al., 2017). The flux was modelled as function of TMP with the following equation, assuming that the main fouling mechanism is cake formation:

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$$J = \frac{TMP}{\mu(R_m + R_c)} \quad (2)$$

In which R_m and R_c are the hydraulic resistances of the membrane and cake (m^{-1}), of which the cake resistance and is the product of the specific cake mass, ω ($kg \times m^{-2}$), and the average specific cake resistance, α ($m \times kg^{-1}$). As cakes formed on membranes during filtration of MBR sludge are compressible, the specific cake resistance pressure dependency can be described by the following equation:

$$\alpha = \alpha_0 + \frac{\alpha_0}{P_a} TMP = \alpha_0 + k \cdot TMP \quad (3)$$

α_0 ($m \times kg^{-1}$) is the initial specific cake resistance (not compressed) and P_a (Pa) depends on the cakes compressive strength. The slope of a α-TMP plot, i.e. the ratio ratio α_0/P_a, denotes the cake compressibility, k. The amount of cake is simulated by solving the following equation numerically by a simple Euler approach, as described in (Jørgensen, Bugge, et al., 2017).

$$\frac{d\omega}{dt} = \left(J - J_{LIM}\left(1 - e^{-\omega/\omega_{crit}}\right)\right) C_b \quad (4)$$

In eq. (4) C_b is the bulk sludge concentration, J_{LIM} is the limiting flux ($m \times s^{-1}$) whereas ω_{crit} is the critical specific mass of cake ($m \times kg^{-1}$). By multiplying the specific cake resistances and specific amounts of cake (eq. (3) and (4)) the cake hydraulic resistance is simulated over time, and from this the permeate flux is simulated throughout the TMP step experiments using eq. (2). The modelled fluxes were fitted to measured fluxes in TMP step experiments by changing the values of the model parameters J_{LIM}, α_0 and k to reduce the error between measured and modelled flux values. This was done using the solver function in Microsoft Excel.

A high limiting flux represents low fouling propensity, as it is a measure of back transport of foulants away from the membrane due to air scouring. Hence, at high limiting flux, the rate of flux decline is lower than for higher limiting fluxes. The specific resistance of the fouling layers is described and compared between fouling layers by calculating the specific resistance at 5000 Pa, $\alpha_{(5000\ Pa)}$, is the specific resistance of a fouling layer at 5000 Pa, as this specific resistance is more comparable for operation than the extrapolated α_0 value. The ratio α_0/P_α will be used to describe the cake compressibility.

2.7 Bacterial community profile from full-scale MBR

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For the bacterial community analysis, a sample was collected from a Danish full-scale MBR at Lundtofte WWTP (150,000 PE). The sludge sample was handled and centrifuged as previously described. DNA extraction, 16S rRNA gene amplicon sequencing, and bioinformatics data processing were done according to Albertsen et al., (2015) using the MiDAS 2.0 bacterial reference database (McIlroy et al., 2017) and MiDAS fieldguide for functional assignments (www.midasfieldguide.org). The data supporting the findings of this study are available from the corresponding author upon request.

3. Results and discussion

3.1 Characteristics of sludge, supernatant, and bacterial suspension

Prior to filtration experiments for fouling propensity determination, the suspensions were characterized by TS, pH, cell count, turbidity, and EPS concentrations as listed in Table . After centrifugation of the MBR sludge, the amount of total solids in the supernatant was reduced to 7% of the original suspension. The number of planktonic cells in the supernatant was $2.49 \cdot 10^{10}$ cells L^{-1}, approx. 10 times higher than typically found in CAS supernatant of $0.2\text{-}0.7 \cdot 10^{10}$ cells L^{-1} (Morgan-sagastume et al., 2008, Wilen et al., 2000). From the measured protein concentration it can be estimated that the bacterial cells in supernatant constituted 20-60% of the total protein content assuming their protein content was $0.66 \cdot 10^{-13}$ g protein per cell (Frølund et al. (1996)) or with $2.3 \cdot 10^{-13}$ g protein per cell (calculated from our model bacteria suspension numbers from Table 1). This confirms the profound importance of direct cell counts when interpreting EPS data. As shown on the micrograph in Figure 1A, not only planktonic single cells remained in the sludge supernatant but also some bacteria with filamentous morphology, counting additionally $1.55 \cdot 10^{10}$ cells L^{-1}, so the cellular contribution was even higher. From the community profile (Figure 1C), it was confirmed that full-scale MBR sludge supernatant contained both bacteria with and without filamentous morphotypes, such as the filamentous *Ca.* Microthrix (McIlroy et al., 2017). Interestingly, *Arcobacter* and several other genera observed in the sludge supernatant are known to abundant in raw wastewater (Kristensen et al., 2020, Saunders et al., 2016), so their presence indicate that some of these influent bacteria were not removed by higher organisms such as ciliates or bound to the flocs (Ali et al., 2019, Eikelboom, 2000, Kristensen et al., 2020). Therefore, these free-living bacteria, which are continuously supplied with the influent wastewater, act as small colloids in bulk water with a size of 0.5-3 μm (Maddela et al., 2018, Snaidr et al., 1997). The bacterial strain chosen for the model suspension belongs to the family Pseudomonadaceae, which

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has been found in wastewater influent (Saunders et al., 2016). The strain *Pseudomonas* sp. UK4 forms rod-shaped cells with a size of 1 μm (Figure 1B), but, as the strain is a biofilm former (Larsen et al., 2007), some small aggregates were found after homogenization although much smaller than sludge flocs (65-125 μm) (Christensen et al., 2015). The bacterial model suspensions were prepared with fixed cell numbers by dilution in a nutrient free buffer. When comparing sludge supernatant to bacterial model suspension with similar numbers of planktonic cells, higher turbidity and TS were observed in the sludge supernatant (Table 1). This may be due to other colloids and humic substances present in the sludge supernatant, whereas the model bacteria suspension is washed bacteria suspended in phosphate buffer.

3.2 Fouling propensities of single cells

The effects of planktonic single cells on flux development were evaluated from 1 h filtration experiments at 5 kPa constant TMP. Flux development of bulk sludge, sludge supernatant, and model bacterial suspensions with increasing number of cells was monitored and resulted in a fast, initial decline in flux followed by an equalization of flux at the end (**Error! Reference source not found.**A). Equation 1 was fitted to the experimental flux data and the rate constant *b* were used for the comparing different experiments. The resulting parameters are listed in Table 2.

Most MBR plants are operated with cyclical relaxation or backwash procedures; hence, the rate of flux decline (*b*) is critical for the filtration performance. As observed from Figure 2B, the rate constant *b* increased with higher numbers of planktonic cells resulting in more rapid flux decline. The linear correlation between the fouling rate constant *b* and the number of planktonic cells indicates that there was a direct correlation between cell concentration and rate of fouling. This can be a result of higher convective drag of cells to the membrane surface, as classical fouling theory describes rate of foulant convection towards the membrane surface as the product of flux and foulant concentration (Christensen et al., 2018). The rate of flux decline and planktonic cells count for sludge supernatant and sludge supernatant with planktonic cells were in line and followed the trends of the model suspensions, indicating that the cell count in supernatant determined the rate of flux decline. The rate of cake formation affects the pore blocking and irreversible fouling of a membrane after physical cleaning (e.g., backwash), where the membrane surface is prone to fouling. Hence, suspensions showing high rate of flux decline have high fouling propensity.

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The addition of 4 times more planktonic cells from the model bacterial suspension clearly affected the filtration profiles of sludge and supernatant, as a faster flux decrease (higher rate constant, *b*) was observed for both sludge and supernatant when the number of planktonic cells were quadrupled (Figure 2B). This confirms that the planktonic cells were adsorbed or accumulated on the membrane surface due to their size and behaviour in the membrane concentration polarization layer (Christensen et al., 2018). Similar to our findings, free living cells have recently been identified as the most critical foulant in lab-scale anaerobic membrane bioreactors (Zhou et al., 2019).

3.3 Effect of planktonic cells on filtration parameters

3.3.1 Limiting flux

To link fouling propensities to the planktonic single cells, TMP step test filtration series were performed on MBR bulk sludge, sludge supernatant, sludge suspensions with different rations of sludge flocs and colloids, and a bacterial model suspension with similar single cell count as the sludge supernatant. The measured flux profiles are shown in Figure 3. After fitting the flux model described previously by Jørgensen, Bugge, et al. (2017), filtration parameters were determined, see Table 3. It was clear from the results that the model works for "sludge-like" suspensions that form compressible cakes on the membrane, and that the bacterial suspension did not show the same behaviour (Figure 3). Furthermore, the model takes the dry matter concentration into account, which was very low in the model bacterial suspension. The values obtained for the bacterial suspension should therefore be interpreted with care, but it shows the specific cake resistance was high for single cells, i.e., low concentrations of single cells can have a high impact on fouling resistance and rate constant. However, it was very clear that J_{LIM} decreased with higher ratio of colloids to flocs, thus indicating the beneficial roles of the sludge flocs.

3.3.2 Specific resistance

A recent survey of 29 different activated sludge samples from various types of pilot and full-scale wastewater treatment plants in Denmark, using the same step test model to estimate the specific resistance at 5000 Pa of the fouling layer, presented values ranging from $0.45 \cdot 10^{12}$ to $8.4 \cdot 10^{12}$ m kg^{-1} (Jørgensen, Nierychlo, et al., 2017). The sludge used in this study from a pilot-scale MBR

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plant had similar filtration properties with a specific resistance of $1.88 \cdot 10^{11}$ m kg^{-1} (Table 3). Previous studies have demonstrated that cake resistance is highly dependent on SMP in sludge supernatant (F. Wang et al., 2014) and specific resistances of SMPs have been reported to be a magnitude of 700 larger than of sludge (Teng et al., 2019). For extracted EPS, specific resistances have previously been reported to be of the order of 10^{14} up to 10^{17} m kg^{-1} (Nagaoka et al., 1996, Z. Wang et al., 2009). Assuming that the bacteria in the model suspension are rigid spheres of equivalent diameters of 0.5 µm, a specific resistance of $6.75 \cdot 10^{12}$ m kg^{-1} can be predicted from the Carman-Kozeny equation. However, the modelled specific resistance at 5000 Pa from the filtration data was $2.53 \cdot 10^{14}$ m kg^{-1} for the bacterial model suspension, indicating that the bacterial cells form highly compact fouling layers, where the cells are deformed or soft material on the cell surface occupy the void between the cells like previously observed for yeast cells (Meireles et al., 2002). The data from Table 3 clearly shows that specific resistance at 5000 Pa decreases with higher ratio of flocs to colloids, hence that flocs form less compact layers than colloids/bacteria. In previous studies from water treatment, it was found that the hydraulic resistance is higher for EPS filter cakes than planktonic cell filter cakes (Dreszer et al., 2013, Vrouwenvelder et al., 2016). However, this may be a consequence of EPS forming larger deposits with lower specific resistance than planktonic cells.

3.3.3 Compressibility

The most compressible fouling layer was formed during the step filtration of model bacteria and supernatant (Figure 3). This is seen as the k increased by a magnitude of 20 when filtering supernatant compared to bulk sludge, resulting in a less compressible fouling layer formed from sludge. This is unexpected as the more compact layer would be expected to be the less compressible fouling layer. It is also in contradiction to other studies, e.g., dead-end filtrations by Poorasgari et al. (2015), showing higher compressibility of fouling layers formed by sludge than supernatant. The compressibility of the bacterial suspension is even higher, as would be expected due to the homogeneity of the suspension lacking filaments and macromolecular structures. Hence, the compressibility of the fouling layers formed by model bacteria was higher than expected.

3.3.4 Irreversible fouling

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The percentage decline in flux (ΔJ) from first to last filtration at 1 kPa in the TMP step filtrations, listed in Table 3, revealed significant formation of irreversible fouling ranging from 15-19% for supernatant and bulk sludge, to 61% for planktonic cells. This demonstrates the severe effects of the planktonic cells on membrane performance. Similar effects were seen by Hassan and co-workers who added smaller (1-2.5 μm) *Escherichia coli* bacterial cells to a filtration of yeast, which are larger cells, and observed severe irreversible fouling (Hassan et al., 2013). The bacterial cells might cause pore blocking or adsorb to the membrane, but also the formation of a biofilm where cells adsorbs to the surface, which are not easily removed by air scouring or during relaxation, could be an explanation to the irreversible fouling. This study, along with many others, demonstrates the severe fouling effects of colloidal and particular matter in the sludge supernatant (Lin et al., 2014, Rosenberger et al., 2005). However, where many studies rely on particle detection or extractable EPS measured by colorimetric assays (that may or may not include single cells), our study directs the attention to the planktonic cells. Therefore, focusing on a well-defined bacterial suspension this study was able to eliminate the other particular matter thus highlight the effects of the planktonic single cells.

3.4 Planktonic cells in MBRs

The planktonic cells found in MBR sludge might have different origins. As shown from the bacterial community analysis many bacterial genera including *Arcobacter*, *Acidovorax*, *Romboutsia*, *Trichococcus*, and *Blautia* are abundant in influent wastewater (Figure 1C). Especially *Arcobacter* seems to integrate poorly into the sludge flocs and could be a key foulant. A recent study found that *Arcobacter* was abundant in the influent, the bulk water phase of the sludge, and the effluent of 14 Danish CAS wastewater treatment plants (Kristensen et al., 2020). Other planktonic bacteria in MBR sludge likely originated from disrupted sludge flocs, such as *Rhodoferax* and *Tetrasphaera,* which also are known to be process-critical and abundant in the sludge flocs (Saunders et al., 2016). Bacterial community analyses of fouling layers indicate the presence of pioneer species, however, the fouling layers becomes with time more similar to the bulk sludge in terms of bacterial composition (Ziegler et al., 2016). This would indicate that the immigrating bacteria, which are not absorbed by the sludge flocs, might play a key role in initial fouling. After initial adhesion, the fouling potential of bacteria may be species/strain-dependent and some features shared by key fouling-causing bacteria (Ishizaki et al., 2016, Maddela et al., 2018) Hence, future studies should further investigate how specific influent planktonic cells

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contribute to initial membrane fouling and how this can be mitigated. In addition, as it is shown that a significant part of measured soluble EPS may be planktonic cells, it is suggested for further studies to include bulk water cell count in the analysis of MBR sludge fouling potential.

3.5 Control strategies

As we have demonstrated, the planktonic cells in MBRs play a crucial role in fouling. However, mitigation of planktonic cells in MBR sludge is not a straightforward task due to the nature of membrane technologies – all particular components are held back. Operation at lower TMP and higher hydraulic retention time (HRT) are generally recommended to avoid severe fouling caused by soluble EPS (Hamedi et al., 2019), whereas constant and high TMP accumulates the colloid-like free cells and shows severe fouling (Hong et al., 2019) and should therefore be avoided. Data indicates that SRT is important for the concentration of single cells as these comes from the feed and are only removed with the sludge. On the other hand, shear stress may not be that important for fouling as single cells comes from feed. Hence, a central control strategy to mitigate fouling is to ensure high degree and good conditions for flocculation, e.g., by coagulation or electrocoagulation, etc.

4. Conclusions

This study aimed to describe the influence of planktonic single cells on sludge fouling propensity in MBRs. The planktonic cells may often be hidden under a surrogate concentration of EPS or encountered as colloidal particles from turbidity measurements and particle size distributions. Based on direct cell counts, it was found that the planktonic bacterial cells constituted up to 60% of the total protein content of the "soluble EPS". Based on this, it is recommended to carry out bulk water bacterial counts along with EPS measurements in future studies of sludge fouling potential.

Filtration tests showed that fouling was governed by the amount of free cells in the bulk liquid, as the cell count was directly proportional to rate of fouling by cell deposition. Accordingly, experiments with varying fractions of flocs and colloids in sludge showed higher rates of fouling formation and higher specific fouling layer resistance for sludge with higher colloidal/bacterial cell fractions. Bacterial community analysis of the suspended cells showed several genera to be enriched in the water phase, many of them known to be abundant in influent wastewater. In order

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to mitigate fouling, it is important to ensure high degree and good conditions for flocculation and to avoid high numbers of single cells in the supernatant.

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Tables

Table 1 - Characteristics of sludge, supernatant, and bacterial suspensions.

Suspension	TS ($g\ L^{-1}$)	pH	Cell count ($cells\ L^{-1}$)	Turbidity (OD_{650nm})	Protein ($mg\ L^{-1}$)	Humics ($mg\ L^{-1}$)	Carbo-hydrates ($mg\ L^{-1}$)
Bulk sludge	9.6	7.2	-	0.034	-	-	-
Supernatant	0.68	7.3	$2.49 \cdot 10^{10}$*	0.026	14.58	14.28	5.4
Model bacteria	0.01	7.4	$2.74 \cdot 10^{10}$	0.006	6.18	0.13	-

*the cell count corresponds to the planktonic single cells, however, additional $1.55 \cdot 10^{10}$ cells L^{-1} were present as filaments.

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Table 2 – Filtration model parameters from 1 h filtration experiments at TMP of 5 kPa.

Suspension	TS ($g\ L^{-1}$)	Cell count* (cells mL^{-1})	k	b	J_{SS}	RMSE	$\Delta J_{(after\ 55min)}$**
Model bacteria	0.001	$0.26 \cdot 10^7$	196.3	0.00058	196.7	16.58	224
Model bacteria	0.005	$1.73 \cdot 10^7$	289.2	0.00135	86.7	19.37	83.9
Model bacteria	0.01	$2.74 \cdot 10^7$	293.3	0.00172	64.2	18.10	58.8
Model bacteria	0.02	$5.01 \cdot 10^7$	326.7	0.0029	52.0	19.65	42.3
Model bacteria	0.05	$8.16 \cdot 10^7$	265.2	0.00464	32.1	17.44	24.6
Model bacteria	0.1	$16.3 \cdot 10^7$	285.7	0.00823	26.8	16.03	20.8
Supernatant	2.0	$2.49 \cdot 10^7$	199.4	0.00346	35.0	16.00	29.2
Supernatant + bact.	2.1	$10.6 \cdot 10^7$	201.0	0.00556	20.5	15.35	15.9
Bulk sludge	9.6	$2.49 \cdot 10^7$	243.4	0.00947	27.1	16.30	25.9
Bulk sludge + bact.	9.7	$10.6 \cdot 10^7$	378.8	0.02769	36.3	15.02	33.3

*Planktonic cells only

**$\Delta J_{(after\ 55min)}$ denotes the average flux during the last 5 min of filtration approximating the observed J_{SS}

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Table 3 *- Filtration parameters from TMP step test modelling*

Suspension	J_{LIM} (LMH)	α_0 *(*m kg^{-1})	$\alpha_{(5000\ Pa)}$ *(*m kg^{-1})	$\alpha_0/P_\alpha = k$ *(*m kg^{-1} Pa^{-1})	RMSE	ΔJ*
Model bacteria	101	$2.51 \cdot 10^{14}$	$2.53 \cdot 10^{14}$	$4.18 \cdot 10^{9}$	23	61 %
Supernatant ($C_{100}F_0$)	37	$6.71 \cdot 10^{12}$	$8.04 \cdot 10^{12}$	$2.66 \cdot 10^{9}$	17	15 %
$C_{100}F_{50}$	52	$3.17 \cdot 10^{11}$	$3.77 \cdot 10^{11}$	$1.13 \cdot 10^{8}$	10	
$C_{100}F_{75}$	75	$1.70 \cdot 10^{11}$	$1.93 \cdot 10^{11}$	$4.51 \cdot 10^{7}$	23	
Bulk sludge ($C_{100}F_{100}$)	71	$8.63 \cdot 10^{10}$	$1.88 \cdot 10^{11}$	$2.04 \cdot 10^{8}$	29	19 %
$C_{100}F_{150}$	80	$1.45 \cdot 10^{11}$	$1.82 \cdot 10^{12}$	$6.48 \cdot 10^{7}$	26	

* *ΔJ is the decrease in mean flux in percentage from the first filtration at 1 kPa and the last filtration at 1 kPa after the TMP step series.*

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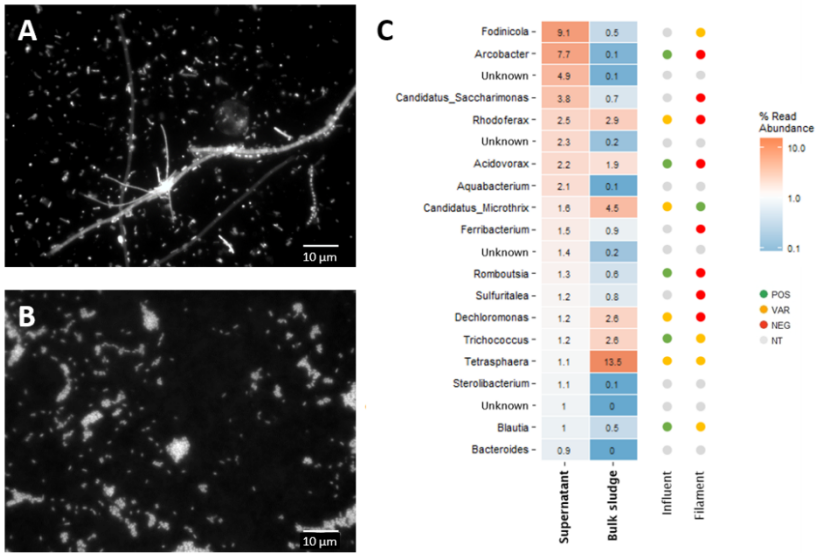

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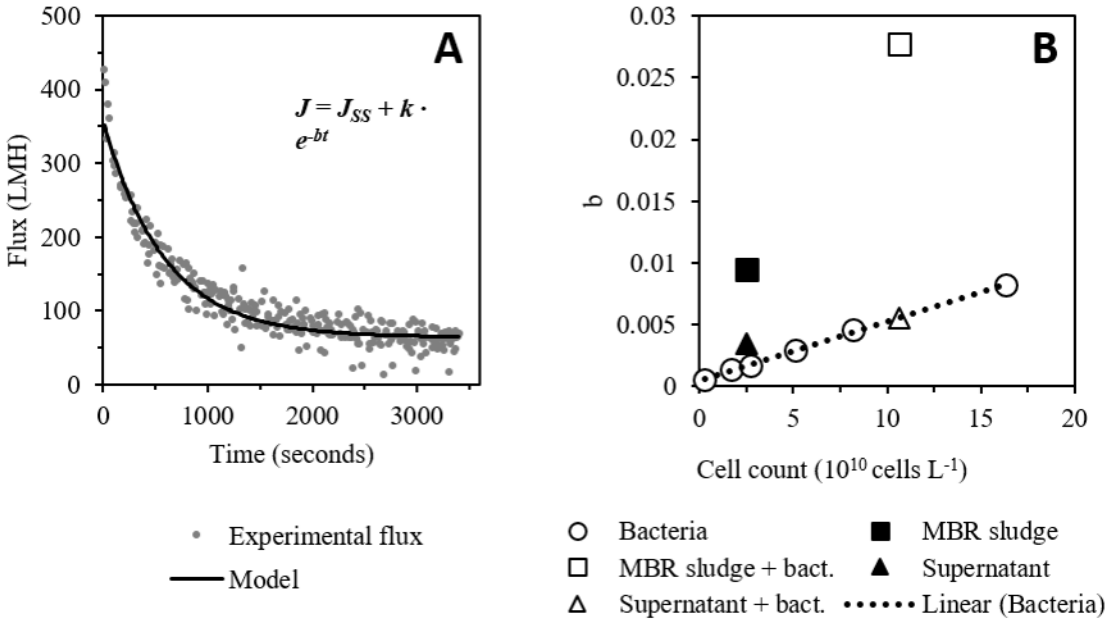

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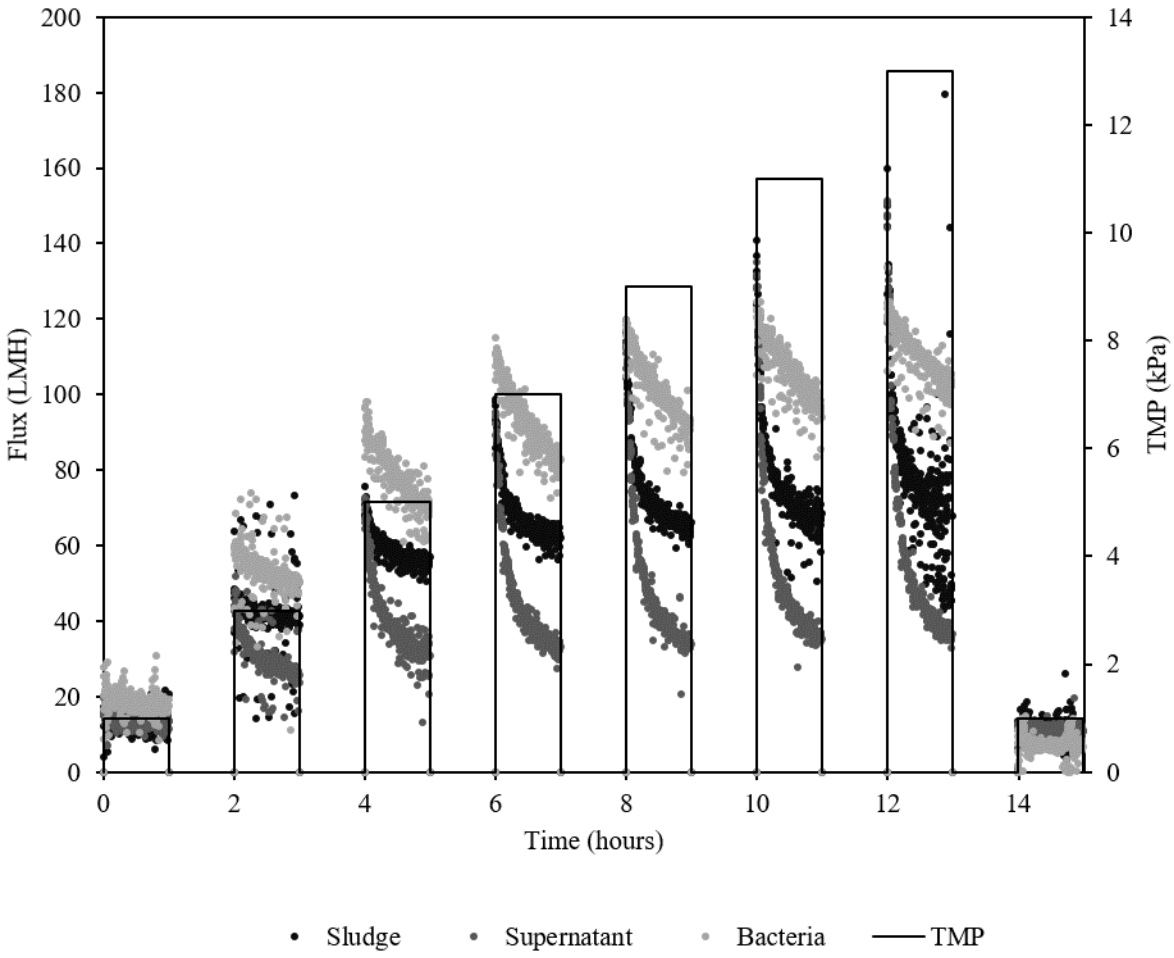

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