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The impact of deflocculation-reflocculation on fouling in membrane bioreactors

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Abstract

Membrane bioreactors (MBR) have proven to be a valuable alternative for conventional activated sludge plants. Membrane fouling remains, however, a significant drawback. Stable operation of a membrane bioreactor requires a good sludge condition. In this paper it is evidenced that bioflocculation is a crucial factor within that context. By changing the ratio of monovalent over polyvalent cations ([M/P]) in the influent, a deflocculation-reflocculation event was induced during which the impact of bioflocculation on fouling in membrane bioreactors could be studied. In a first phase, a high [M/P] influent was fed to the MBR which resulted in severe sludge deflocculation and worsened filtration characteristics. A low [M/P] influent was subsequently fed to the MBR. Within three weeks, the activated sludge reflocculated and filtration characteristics improved significantly. Monitoring of bioflocculation in MBRs is thus of extreme importance to start possible remediation as quickly as possible. Fragment surface proved to be a valuable parameter in that respect. In contrast, no clear relation between EPS and filtration characteristics could be found. This work substantiates the hypothesis that a *well functioning dynamic secondary membrane*, built up by robust activated sludge flocs, can prevent severe (irreversible) fouling in MBRs.

Keywords

membrane fouling; bioflocculation; cation bridging theory; monovalent / polyvalent cation ratio; extracellular polymeric substances (EPS)

List of abbreviations

μ	viscosity [Pa.s];
[M/P]	ratio of monovalent over polyvalent cations in equivalents;
ACTIAS	activated sludge image analysis system;
CER	cation exchange resin;
CFV_{mem}	cross-flow velocity near the membrane surface [m/s];
COD	chemical oxygen demand [mgO ₂ /L];
eEPS	extractable extracellular polymeric substances [mg/gMLVSS];
$eEPS_{PN/PS}$	ratio of protein eEPS over polysaccharide eEPS [-];
$eEPS_{tot}$	total amount of eEPS [mg/gMLVSS];
EPS	extracellular polymeric substances;
HRT	hydraulic retention time [d];
J	flux [L/m ² .h];
K	permeability [L/m ² .h.bar];
MBR	membrane bioreactor;
MLSS	mixed liquor suspended solids [g/L];
MLVSS	mixed liquor volatile suspended solids [g/L];
PBS	phosphate buffered saline;
SMP	soluble microbial products;
$SMP_{PN/PS}$	ratio of protein SMP over polysaccharide SMP [-];
SMP_{tot}	total amount of SMP [mg/gMLVSS];
SRT	solids retention time [d];
T	temperature [K];
T_{ref}	reference temperature [K];
TMP	transmembrane pressure [mbar];

1. Introduction

While the advantages of membrane bioreactors have by now been well established in the context of water reuse and footprint reduction, its major disadvantage, being the still unavoidable membrane fouling, has to be overcome (or reduced to a strict minimum) to genuinely obtain an economically and environmentally affordable alternative for the conventional activated sludge systems [1]. Therefore, MBR research still predominantly focuses on the investigation of the causes and effects of membrane fouling.

As clearly suggested by the roadmap of Chang *et al.* [2], the (*condition* of) the activated sludge takes a central position within this fouling context. While the majority of the research still concentrates on the activated sludge's *productivity*, i.e., the production and turn-over of the bound and soluble extracellular polymeric substances (e.g., [3,4]), the authors believe that these extra cellular polymeric substances (EPS) are only part of the picture and that probably the activated sludge's *biofloculation ability* is the higher level to take into account. In line with Le-Clech *et al.* [1], the quality of the formed cake layer is believed to be a decisive factor. If a *well functioning dynamic secondary membrane* can be formed, which can be removed by regular *physical* cleaning, severe membrane fouling, and, hence, more drastic *chemical* cleaning actions will be avoided. The qualifier *well functioning* means that the secondary membrane should be built up by robust activated sludge flocs that produce an appropriate amount of bound EPS (in terms of quantity and composition, further denoted by eEPS) but only a limited amount of soluble EPS (further denoted by SMP). Hence, EPS do play a role, but, if the dynamic secondary membrane hypothesis is correct, biofloculation in general is a key aspect to monitor and to stimulate in membrane bioreactors.

The analogy with and the importance of classic activated sludge (sedimentation failure) research cannot be disregarded. Long before the introduction of membrane bioreactor technology, biofloculation of activated sludge was already a very active research domain and it still is (e.g., [5,6,7,8,9,10,11]). As a result of this intensive research, it is commonly accepted that micro-organisms produce eEPS that form a gel-like matrix that encapsulates the micro-organisms and aids in biofloculation [12] by their negative charges (and, hence, electrostatic forces) as well as by their hydrophobic regions (and, hence, by hydrophobic interactions).

Within a broader research context on the impact of sludge retention and sludge loading on the activated sludge's fouling behavior, this paper presents a deflocculation – reflocculation event which substantiates the abovementioned secondary membrane theory. Deflocculation was triggered by a high mono- over polyvalent cation ratio in the influent and induced severe fouling. Given the negative charges of activated sludge flocs and its eEPS, the role of cations in biofloculation has, indeed, been highlighted several times, mainly in the classic activated sludge context (e.g., [9,12,13]) but also in MBR settings (see, e.g., [14,15]). By a better balancing of the cations, biofloculation as well as the membrane permeability was restored.

2. Materials and methods

2.1. Experimental setup

A submerged lab-scale membrane bioreactor with a total volume of 25 L was equipped with a pristine flat sheet membrane (Kubota, Type 203 cartridge). The main operational characteristics are summarized in Table 1. Reactor level, transmembrane pressure (TMP), permeate flow, pH and dissolved oxygen level are measured and controlled on-line with a data acquisition system (NI PCI-6229, National Instruments) and data acquisition software (LabVIEW 8.0, National Instruments).

The MBR was subsequently fed with two glucose based synthetic influents which mainly differ in their monovalent over polyvalent cation ratio ([M/P], in terms of equivalents), i.e., 17.4 for the high [M/P] influent and 2.02 for the low [M/P] influent (Table 2). Both influents were prepared in concentrated form and diluted in function of the flux to maintain a constant reactor loading rate of 1.5 gCOD/L.d. For a constant reactor loading rate, a higher flux corresponds with a more diluted influent. If possible, the reactor was operated at a flux of 20 L/m².h, however, during deflocculation permeability decreased and could not be sustained despite frequent chemical cleaning of the membrane (2 h soak in a sodium hypochlorite solution with 0.5 wt% free chlorine). The flux was

then lowered to 10 and even 5 L/m².h.

At start-up, the MBR was seeded with return activated sludge from a large-scale communal wastewater treatment plant. The solids retention time (SRT) was set to 50 days by controlling the daily amount of waste sludge.

Table 1 Summary of the main operational characteristics during the experiment.

Parameter	Value
Flux [L/m ² .h]	5 – 20
Filtration – relaxation [min]	8 – 2
HRT [d]	0.5 – 2.3
SRT [d]	50
Reactor loading rate [gCOD/L.d]	1.5
CFV _{mem} [m/s]	1.5
High [M/P] influent period [d]	1 – 35
Low [M/P] influent period [d]	36 – 70

Table 2 Composition of the original influent (high [M/P]) and the new influent (low [M/P]).

Original influent: high [M/P]		New influent: low [M/P]	
Component	Concentration [g/L]	Component	Concentration [g/L]
C ₆ H ₁₂ O ₆ (glucose)	2.550	C ₆ H ₁₂ O ₆ (glucose)	2.550
(NH ₄) ₂ HPO ₄	0.563	(NH ₄) ₂ HPO ₄	0.217
K ₂ HPO ₄	1.875	NH ₄ NO ₃	0.597
NaCl	0.075	KCl	0.086
CaCl ₂ .2H ₂ O	0.075	Na ₂ SO ₄	0.133
MgSO ₄ .7H ₂ O	0.075	MgCl ₂ .6H ₂ O	0.376
FeCl ₃ .6H ₂ O	0.015	CaCl ₂	0.083
		FeCl ₃	0.087
		Yeast extract	0.150
[M/P]	17.4	[M/P]	2.02

2.2. Image analysis

A fully automated image analysis procedure, ACTIAS (ACTivated sludge Image Analysis System), was used for the characterization of the activated sludge composition [16,17]. To this end, activated sludge images were captured (from two 10 µL drops on a carrier slide) manually using a light microscope (Olympus BX 51) with phase contrast illumination (Ph1) and a total magnification of 100 times. The microscope was equipped with a 3CCD color video camera (Sony DXC-950P) which was connected to a computer. Microscopic images (90 images per day) were digitized and stored as JPG (768x576 pixels) using Zeiss KS100.3 acquisition software. These images were

subsequently processed by the developed image analysis procedure which is embedded in the MATLAB Image Processing Toolbox 4.2 (The Mathworks Inc., Natick, MA).

Through a consecutive segmentation and recognition algorithm, ACTIAS is able to make a distinction between three object classes, i.e., flocs, filaments and fragments. For each object class, a specific set of morphological variables is calculated, e.g., floc size, floc elongation, filament length and fragment surface. An extensive description on how these variables are computed can be found in Jenné *et al.* [17]. From this set of variables, the mean fragment surface [pixels] per image is selected as a measure for deflocculation. These fragments can be regarded as the *primary particles* which constitute, according to Mikkelsen and co-workers, the building blocks of an activated sludge floc which is built up by the concept of multilayer primary particle adsorption under the influence of an upper level of incorporation. Deflocculation is said to resemble a desorption process of these primary particles (see, e.g., [18]).

2.3. EPS – extraction and analysis

Soluble microbial products (SMP) are extracted from a 100 mL activated sludge sample by means of centrifugation (5000 G for 10 min, at 4°C) [1]. The supernatant is analyzed for SMPs while the sludge pellet is resuspended in a PBS buffer solution. A cation exchange resin (CER, Dowex Marathon C Na⁺ form, Sigma–Aldrich, USA) is added to this suspension at a ratio of 70 gCER/gMLVSS. This solution is then stirred at 600 rpm in a baffled erlenmeyer for 1 h [19]. A last centrifugation step (12000 G for 15 min, at 4°C) separates the CER from the supernatant which contains the extractable EPS fraction (eEPS). The SMP and eEPS fractions are analyzed for proteins and polysaccharides according to a corrected Lowry method [19] and the method of Dubois *et al.* [20], respectively.

2.4. Analytical methods

In addition to the on-line measured permeability (flux/TMP), the time to filter has been selected as a filtration characteristic. A 10 mL activated sludge sample is filtered on a 0.60 µm filter (GF-3, ø 47 mm, Macherey Nagel) and the time to filter the 10 mL sample is measured. The measurement is done in duplicate, hence, each presented value is the mean of both measurements.

The chemical oxygen demand (COD) was measured with Hach Lange COD Cell Tests (LCK 414 and LCK 514) on a spectrophotometer (Hach Lange DR5000). Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) measurements were conducted in accordance with the procedures described in APHA Standard Methods [21].

3. Results and discussion

The MBR was seeded with return activated sludge from a large communal wastewater treatment plant. From day 1 until day 36 the high [M/P] influent was fed to the MBR, with then a switch to the low [M/P] influent until day 70.

Figure 1 represents the on-line measured permeability and the imposed fluxes. Since flux is influenced by water viscosity and water viscosity varies with temperature, flux (and thus permeability) is normalized to a reference temperature, i.e., 15°C, according to the temperature – viscosity relation given by Touloukian *et al.* [22]:

$$K_{T_{ref}} = \frac{J_{T_{ref}}}{TMP}$$

with $J_{T_{ref}} = J_T \cdot factor$

$$\text{where } factor = \frac{\mu_T}{\mu_{T_{ref}}} = 10^{\left[\frac{B}{(T-C)} - \frac{B}{(T_{ref}-C)} \right]}$$

$$K = \text{permeability} \left[\frac{L}{m^2 \cdot h \cdot bar} \right]$$

$$J = \text{flux} \left[\frac{L}{m^2 \cdot h} \right]$$

$$\mu = \text{viscosity} [Pa.s]$$

$$B = 247.8K \text{ (constant)}$$

$$C = 140K \text{ (constant)}$$

$$T = \text{actual temperature [K]}$$

$$T_{ref} = \text{reference temperature} = 15^\circ C = 288.14K$$

At the conception of the experiment, the reactor was meant to be operated at a flux of 20 L/m².h. However, due to the dramatic decrease in permeability, this flux could not be sustained during the deflocculation period, despite frequent chemical cleaning of the membrane (e.g., day 20 and 28). The flux was then lowered to 10 and even 5 L/m².h. Due to these changes, it is difficult to conclude anything from the permeability evolution with time (apart from fast decreases, especially between day 20 and 40). One of the main reasons is that this measurement does not provide conclusive information about the instantaneous sludge filterability. In a constant flux process, a rise in TMP indicates an increasing fouling resistance. This is best explained by the resistance-in-series-model mentioned by several authors (e.g., Chang *et al.* [2]; Field *et al.* [23]).

$$J = \frac{TMP}{\mu R_t} = \frac{TMP}{\mu(R_m + R_f)} = \frac{TMP}{\mu(R_m + R_r + R_{ir})}$$

J is the filtration flux, TMP is the transmembrane pressure, μ is the viscosity and R_t is the total filtration resistance. This total resistance comprises the membrane resistance R_m which is a constant characteristic of the membrane and R_f , the overall fouling resistance. R_f can further be subdivided in the (physically) reversible fouling resistance (R_r , being fouling which can be removed by physical cleaning, e.g., backwashing or relaxation) and the irreversible fouling resistance (R_{ir} , being fouling which can be (partly) removed by chemical cleaning, e.g., soaking or chemical backwash). Monitoring the TMP in a constant flux process thus provides information about the sum of R_r and R_{ir} . However, while the process is running it is impossible to define the immediate contribution of the activated sludge to the R_{ir} -value since irreversible fouling is only reset after a chemical cleaning. To decouple the actual fouling propensity of the sludge from the already built-up (more irreversible) fouling, an off-line characterization to quantify the sludge filterability is preferred over the classic on-line permeability index. In this work, the time-to-filter has been selected as a filtration characteristic. This measurement is only influenced by the instantaneous sludge filterability (TMP, μ and R_m are constant and R_f depends on the actual sludge condition). A similar off-line quantification of the sludge filterability is the Delft filtration characterization method [24], which, unfortunately, requires too much sample volume (i.e., 25 L) to be applied for this MBR setup.

Figure 1 The blue line represents the normalized online permeability ($T_{ref}=15^{\circ}\text{C}$), the dashed red line indicates the imposed flux.

Figure 2 illustrates the total fragment surface per image (i.e., a measure for deflocculation) and the filtration time for the MBR sludge (i.e., a measure for the fouling degree) during the experiment.

Figure 3 provides images of the activated sludge in the MBR at day 1, at day 36 and at day 70. It is evidenced by these figures, that the sludge in the MBR starts to deflocculate after 3 weeks, i.e., the fragment surface per image increases significantly. This deflocculation strongly correlates with the fouling propensity of the mixed liquor, demonstrated by the increase of filtration time. After the switch to the low [M/P] influent, deflocculation continues for about 10 days but is then reversed. After 3 weeks of low [M/P] influent, robust sludge flocs are restored together with a good filterability of the activated sludge. It must be noted that, during the experiment, no loss of COD removal efficiency could be observed (Figure 4, top) and because of the complete retention of fragments, biomass levels could be maintained (Figure 4, bottom).

From Figures 2 and 3 it is clear that the addition of the low [M/P] did not invoke an instantaneous reflocculation, or at least it was not immediately visible. This can probably be attributed to the following reasons. First of all, a high SRT can promote deflocculation, which results in a self-enhancing process due to (the faster) growth of non-flocculating organisms, certainly at low F/M ratios [25]. Secondly, the exchange of ions can take quite some time due to the interaction with new biopolymers that first have to be formed during the growth process. The latter results are in full agreement with the *cation bridging theory* of which Sobeck and Higgins [12] claim that it best describes the role of cations in bioflocculation in conventional activated sludge systems. A similar positive effect of a low [M/P] ratio on bioflocculation (and on the subsequent sedimentation and dewaterability) has been reported in full-scale industrial (conventional) activated sludge installations [26,27,28]. It is, therefore, realistic to assume that the observed phenomena are not restricted to laboratory setups fed with synthetic influents.

Figure 2 Fragment surface (blue crosses) and filtration time (green triangles) versus time. The black, vertical line represents the day when the high [M/P] influent was switched to a low [M/P] influent (day 36) in the MBR.

Figure 3 Microscopic images (x100, Ph1) of a. start-up sludge (day 1), b. deflocculated sludge (day 36) and c. restored sludge (day 70) in the MBR.

Figure 4 Top: COD removal efficiency [%]. Bottom: MLSS (solid line) and MLVSS (dashed line) during the experiment.

In line with the current research trend, SMP and eEPS analyses were carried out in order to monitor the effect of deflocculation on the production of extracellular polymeric substances in the MBR (Figure 5). It is clear that these results cannot be directly correlated with the observed deflocculation, and thus with membrane fouling. It is arguable that the highest total concentrations (of especially SMP_{tot}) are noted during the deflocculation period; the maximum measured SMP_{tot} value corresponds with the peak of deflocculation. However, no explanation could be found for the lower SMP_{tot} and EPS_{tot} between day 35 and 41. Values measured on those days are within the range of values found for reflocculated sludge where good filtration characteristics were observed. In recent literature there is a lot of controversy about the relation between EPS (SMPs as well as eEPS) and membrane fouling [29,30]. The fact that there is no uniform method available for the extraction and measurement of EPS and the different EPS fractions hampers comparison of results obtained by different authors. Moreover, it is suggested in the work of Liao *et al.* [31], that it is not the total amount of EPS that dictates bioflocculation but rather the composition of the EPS. A more hydrophobic composition ($\approx$ high protein/polysaccharide ratio) would result in better flocculation. The results obtained in this experiment contradict this, since elevated protein/polysaccharide ratios were observed during deflocculation but it can be seen that during the deflocculation period (i.e., between day 24 and 55) the eEPS protein/polysaccharide ratio covers a much wider range than during the reflocculated period (i.e., after day 55).

Figure 5 Top: Evolution of SMP_{tot} and $eEPS_{tot}$ concentrations in time. Bottom: protein/polysaccharide ratios (PN/PS) in SMP and eEPS versus time. The red dashed vertical lines indicate the onset and the end of the deflocculation event in the MBR. The black vertical line indicates the day when the high [M/P] influent was switched to the low [M/P] influent in the MBR.

Conclusion

Based on the results presented the following conclusions can be drawn.

- Biofloculation is a key aspect to monitor and to stimulate in membrane bioreactors. Filtration characteristics deteriorate dramatically when activated sludge deflocculates while a reflocculated sludge improves the filtration performance significantly. These observations corroborate the hypothesis that the activated sludge acts as a *well functioning dynamic secondary membrane* that protects the actual membrane from fouling when large, robust flocs are formed.
- The ratio of monovalent over polyvalent cations in the influent plays a crucial role in biofloculation. A high [M/P] ratio in the influent can cause severe deflocculation. If an influent with a low [M/P] is subsequently fed to the reactor biofloculation is promoted again. However, it takes time before permeability can be restored. In this experiment it took approximately 3 weeks to regain a stable process. The restoration period will most probably also be influenced by the imposed sludge age.
- During this experiment, no clear relation between EPS (eEPS and SMP) and filtration characteristics could be found. These results enhance the controversy about the impact of EPS on membrane fouling. A standard method for the extraction and measurement of EPS and the different EPS fractions is needed to compare results obtained by different authors so that the true relation between EPS and membrane fouling in MBRs can be unraveled.

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