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Activated sludge characteristics affecting sludge filterability in municipal and industrial MBRs: unraveling correlations using multi-component regression analysis

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Keywords: membrane bioreactor (MBR); activated sludge filterability; membrane fouling; relative hydrophobicity; bioflocculation;

Abstract

In the field of membrane bioreactors (MBR) many membrane fouling related questions still remain unanswered. The goal of this research is to unveil some of the black-box features of activated sludge filterability by correlating the results from activated sludge filterability measurements following the Delft Filtration Characterization method (DFCm) with a large set of activated sludge characteristics. Ten different MBRs in Belgium and the Netherlands were sampled in both winter and summer. All samples were subjected to the DFCm, automated image analysis and an extensive set of standardized measurements. No clear correlation could be found between a single sludge parameter and activated sludge filterability. However, a combination of sludge morphology and relative hydrophobicity (RH) allows for a clear classification of activated sludge into two classes, i.e., *bad* and *poor to good*, implying that deflocculation and a low RH have a negative impact on activated sludge filterability. Furthermore, for sludge samples having *poor to good* filterability, accurate estimations of sludge filterability can be made when including more parameters. The main conclusion is that filterability can be predicted by analyzing the *bioflocculation state* of the activated sludge.

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List of abbreviations

ΔR_{20}	added resistance after filtration of 20L/m² in the DFCm [m^{-1}];
%1pixel	surface fraction of sludge particles equal to 1pixel [%];
#1pixel	average number of sludge particles equal to 1pixel [1/image];
A_i	area of activated sludge particle i [pixels];
A_{mean}	activated sludge mean particle size [pixels/particle];
ACTIAS	activated sludge image analysis system;
CFV	cross-flow velocity near the membrane surface [m/s];
DC	dissociation constant [Abs_{650nm}/washing step];
DFCm	Delft filtration characterization method;
eEPS	extractable extracellular polymeric substances [mg/gMLVSS];
eEPS PN	protein fraction of eEPS [mg/gMLVSS];
eEPS PS	polysaccharide fraction of eEPS [mg/gMLVSS];
EPS	extracellular polymeric substances;
HRT	hydraulic retention time;
MATH	microbial adhesion to hydrocarbons;
MBR	membrane bioreactor;
MLSS	mixed liquor suspended solids [g/L];
MLVSS	mixed liquor volatile suspended solids [g/L];
PB	polybrene;
PBS	phosphate buffered saline solution;
PLS	partial least squares;
PVSK	polyvinyl sulphate potassium salt;
R_{add}	added resistance to filtration [m^{-1}];
RH	relative hydrophobicity [%];
SC	surface charge [meq/gMLSS];
SMP	soluble microbial products;
SMP PN	protein fraction of SMP [mg/gMLVSS];
SMP PS	polysaccharide fraction of SMP [mg/gMLVSS];
SRT	solids retention time;
SSE	sum of squared errors;
TB	toluidine blue;
TMP	transmembrane pressure [mbar];
TOC	total organic carbon [mgC/L];

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1. Introduction

The market share of membrane bioreactors (MBR) in the field of biological wastewater treatment has increased significantly in the last decade [1,2]. The combination of an activated sludge process with a membrane filtration step for biomass separation offers a number of advantages amongst which an excellent effluent quality, a small footprint and a complete decoupling of the hydraulic and sludge retention time (HRT and SRT, respectively) [3]. However, despite extensive research efforts membrane fouling in MBRs is still far from being fully understood [4] mainly due to the inherent interactions among the activated sludge, the influent and applied process conditions. Activated sludge filtration in an MBR is indeed a physical process of which the efficiency is dictated by activated sludge filterability, which, on its turn, is dictated by the interactions between the biomass, the wastewater and the operational conditions. These interdependencies add complexity to the problem of fouling and often lead to conflicting conclusions in literature. As a result, controversial results are commonly encountered, e.g., for the influence of biomass concentration, relative hydrophobicity and EPS on membrane fouling.

Biomass concentration or mixed liquor suspended solids (MLSS). The impact of biomass concentration on membrane fouling has been examined extensively and in the early days of MBRs it was often considered as one of the main foulant parameters. However, with an increasing number of publications contradictory results are being presented. In the review paper of Le-Clech *et al.* [5], it is noticed that some authors report an increasing biomass concentration as a positive impact on membrane fouling, whereas others report a negative correlation while yet another group finds the influence of MLSS insignificant. More recently, also the existence of an optimal MLSS concentration range is suggested [6]. The lack of a clear correlation between MLSS concentration, which is basically the simplest parameter to measure, and any other foulant characteristics indicates that the MLSS concentration (alone) is a poor indicator for biomass fouling propensity [5].

Relative hydrophobicity (RH). Membranes used in MBRs are typically made hydrophilic to improve their water permeability. Low hydrophobicity of sludge flocs is thus expected to cause high fouling due to stronger interactions with the membrane surface. Moreover, a decreasing activated sludge hydrophobicity results in floc deterioration [7] which on its turn again leads to severe membrane fouling [8]. In contrast, Meng *et al.* [9] found that the RH of activated sludge is positively correlated with membrane fouling.

Extracellular polymeric substances (EPS). EPS have recurrently been labeled as the main cause for membrane fouling in membrane bioreactors. EPS are, by definition, of microbial origin and can be divided into two subclasses, i.e., extractable EPS (eEPS) and soluble microbial products (SMP) which are bound to the sludge flocs or free in the bulk solution, respectively. The composition and the amount of EPS, and thus their fouling propensity, are highly dependent on the condition of the activated sludge microbiology. An activated sludge under stress is known to produce more and/or other EPS [10]. Nonetheless, EPS is generally measured as such and no distinction is made between different fractions or compositions of EPS, e.g., sugars are usually measured as an equivalent of D-glucose according to the method of Dubois *et al.* [11]. Although some sugars cause more membrane fouling than others [12,13], most often no further classification is made between different types of polysaccharides. Since the actual fouling relevant fraction of EPS is still unknown, a standard method to measure this fraction does not yet exist [4] which leads to conflicting results for the reported impact of EPS on membrane fouling by different authors.

In our opinion, there are two main reasons why results in MBR research are often contradictory, i.e., (*i*) there is a lack of standardized methods for crucial analyses, like EPS and all its sub-fractions and (*ii*) most of the studied sludge characteristics are interconnected in one way or another to other sludge characteristics. For example,

bound EPS serve as a matrix for microbial aggregates and are as such linked with bioflocculation, and thus with sludge morphology. Apart from that, some components present in the eEPS (e.g., proteins) strongly contribute to sludge floc hydrophobicity. On top of that according to Drews [4], depending on the applied method, the group of eEPS overlaps with other polymer fractions. This further stresses the need for (*i*) standard methods to be used for all MBR-related research and (*ii*) a broader approach for MBR-data interpretation trying to unravel some of the interdependencies of sludge characteristics in relation with membrane fouling. With this paper, we want to focus on the latter aspect.

The goal of this research is to unveil some of the black box features of activated sludge filterability. Ten different MBRs in Belgium and the Netherlands, treating both municipal (4) and industrial (6) wastewaters, were sampled in both winter and summer. The Delft Filtration Characterization method (DFCm) was chosen to determine activated sludge filterability [14], instead of calculating sludge properties from process permeability, i.e. flux and TMP, in each installation. The process permeability itself is a weak indicator for sludge filterability since it cannot be completely decoupled from, e.g., the operational history of the membrane. The DFCm on the other hand, is an off-line method in which the filtration behavior can be related exclusively to the fouling propensity of the activated sludge. Each sample was subjected to a set of activated sludge analyses, such as relative hydrophobicity, image analysis and EPS. The selection of parameters was based on state-of-the-art literature on membrane fouling in MBR.
The obtained relationships can help in pinpointing the main causes of fouling and can assist in implementing the correct remedial actions to improve process efficiency.

2. Methods and materials

2.1. Delft characterization method (DFCm)

Delft University of Technology has developed a small-scale filtration characterization installation combined with a measuring protocol to investigate the activated sludge filterability [14]. The DFCm comprises several steps, from the determination of membrane resistance to the membrane cleaning. The main step of the DFCm is the activated sludge filtration step, a short-term experiment, performed in an installation (Figure 1a) with the following characteristics. A single sidestream tubular ultrafiltration membrane tube (Norit X-flow, diameter=8mm, length=1m, membrane area=0.025m^2 and nominal pore size=0.03µm) forms the basic filtration system of the unit. A peristaltic pump is used for the activated sludge recirculation with a cross-flow velocity (CFV) of 1m/s (single-phase flow). The permeate is extracted at a constant flux of 80L/m^2.h. Activated sludge samples collected from different MBRs are filtered under identical operational circumstances. In this way, differences in filterability can be related, exclusively, to the properties of the MBR activated sludge sample.

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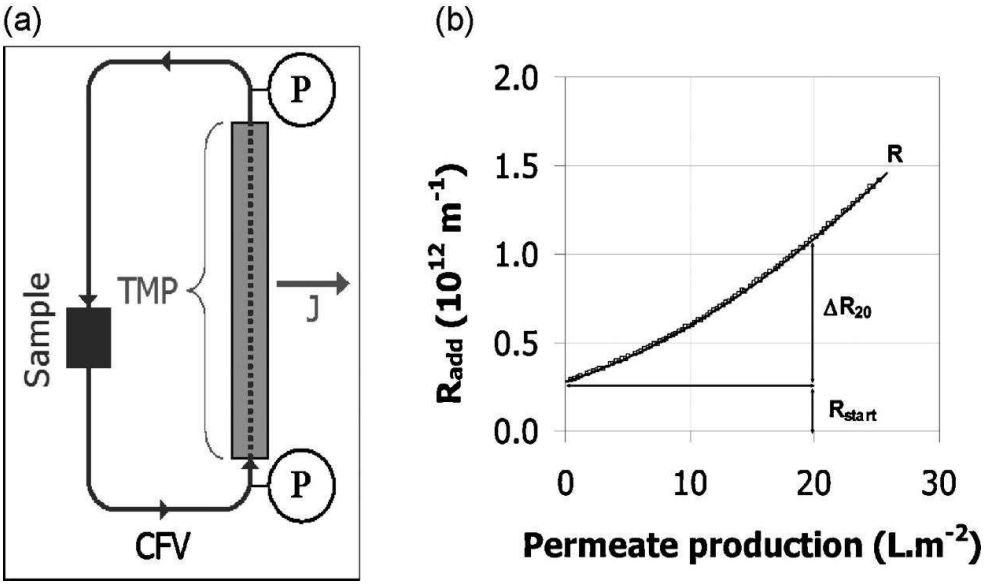

Figure 1: Schematic representation of the DFCm installation (a) and a typical output example (b).

During the filtration step, several parameters, such as, transmembrane pressure, flux, temperature and pH are monitored and stored in a computer data file using the software application Testpoint (National Instruments). The main output of an experiment is the evolution of the resistance during filtration. This resistance is calculated using Darcy's law. The filtration resistance is plotted as a function of the permeate production per unit of membrane surface. As a result of the fouling of the membrane during filtration, filtration resistance will increase. The slope of the curve gives an indication of the activated sludge filterability, e.g., a steep curve corresponds to poor filterability. For easy comparison between different tests, the value ΔR_{20} is used. This value is defined as the increase in resistance after a specific permeate production of 20L/m^2 (Figure 1b). Using MBR sludge with bad filterability, i.e., > 3.0, too high cake resistance resulted in permeate production less than 20L/m². In those cases ΔR_{20} values were computed by mathematically extrapolating resistance build-up until a predicted total volume of 20L/m² permeate was reached. For the interpretation of ΔR_{20} values, an adaptation of the classification proposed by Geilvoet [15] is used in this paper. The original classification comprised only 3 classes and was only based on experiments with municipal activated sludge samples, which usually have better filterability compared to industrial activated sludge samples. In this paper, both municipal and industrial samples were analysed. Hence, the scale was extended with an additional low class with 3.0 as cut-off level.

Bad: $\Delta R_{20}>3.0$
Poor: $1<\Delta R_{20}<3.0$
Moderate: $0.1<\Delta R_{20}<1$
Good: $\Delta R_{20}<0.1$

2.2. Image analysis

A fully automated image analysis procedure, ACTIAS (ACTivated sludge Image Analysis System), is used for the characterization of the activated sludge composition [16].
All sludge samples are diluted to 1.0gMLSS/L prior to microscopic analysis. The dilution is made with permeate to maintain the same environmental matrix, since a

dilution with demineralized water could cause sludge deflocculation. The reason for diluting the sludge sample to 1.0gMLSS/L is twofold: (*i*) at high sludge concentrations sludge flocs touch each other and no distinction can be made between neighboring flocs, which would lead to an overestimation of object sizes; (*ii*) working at a normalized sludge concentration enables comparison of different sludge samples, i.e., with different biomass concentrations.
Activated sludge images are captured manually from two 10µL drops on a carrier slide using a light microscope (Olympus BX 51) with phase contrast illumination (Ph1) and a total magnification of 100 times. The microscope is equipped with a 3CCD color video camera (Sony DXC-950P) which is connected to a computer. Microscopic images (90/sample) are digitized and stored as JPG (768x576 pixels) using Zeiss KS100.3 acquisition software. These images are subsequently processed by the developed image analysis procedure which is embedded in the MATLAB Image Processing Toolbox 4.2 (The Mathworks Inc., Natick, MA).
Two parameters are selected to represent the sludge flocculation state, i.e., activated sludge mean particle size and the surface fraction of activated sludge particles equal to 1pixel (with 1pixel=1.675µm x 1.675µm (square) for this microscope-camera configuration). These parameters can be described as follows:

A_{mean} = the average size of a sludge particle, calculated from all particles present in all processed images [pixels/particle]:

$$A_{mean} = \frac{\sum_{i=1}^{all} A_i}{\#\,particles}$$

with A_i the surface of particle i [pixels].

%1pixel = total surface of particles with a size equal to 1 pixel divided by the total surface of all particles, calculated from all particles present in all processed images [%]:

$$\%1pixel = \frac{\sum_{i=1}^{all} A_i \mid A_i = 1pixel}{\sum_{i=1}^{all} A_i} \cdot 100\%$$

with A_i the surface of particle i [pixels].

2.3. EPS – extraction and analysis
The term *EPS* is used as a general term for different classes of macromolecules such as polysaccharides, proteins, nucleic acids, (phospho-)lipids and other polymeric compounds which can be found at or outside the cell surface and in the intercellular space of microbial aggregates [5]. EPS can be divided in two different classes, soluble EPS or SMP and bound or extractable EPS (eEPS).
SMP are extracted from a 100mL activated sludge sample by means of centrifugation (5000G for 10min, at 4°C) followed by a filtration step on a pre-rinsed filter (Whatman Grade 1, rinsed with 100mL of Milli-Q water). The filtrate is analyzed for SMPs.
The eEPS is extracted by a heat treatment step (80°C, 10min) [5] followed by a centrifugation (5000G for 10min, at 4°C) and a filtration step on a pre-rinsed filter (Whatman Grade 1, rinsed with 100mL of Milli-Q water). The SMP and eEPS fractions are then analyzed for proteins (PN) and polysaccharides (PS) according to a corrected Lowry method [17] and the method of Dubois *et al.* [11], respectively.

2.4. Hydrophobicity

A procedure based on the MATH-test [18] was used to determine sludge RH. Sludge samples were diluted and washed twice with a PBS-buffer to 2.5gMLSS/L. This dilution sample is measured as the initial value at 650nm (Abs_i) in a spectrophotometer (DR5000, Hach Lange) with the filtrate from this sample as a blank. Next, 3mL of this dilution sample is shaken vigorously with an equal amount of n-hexadecane for 2min. After that, the sample is allowed to separate again for 5min. The absorbance in the aqueous phase is then measured at 650nm (Abs_f) and compared to the absorbance of the dilution sample (blank). The RH can then be calculated as follows:

$$RH = 1 - \left(\frac{Abs_f}{Abs_i} \right) \cdot 100\%$$

2.5. *Surface charge (SC)*

Activated sludge SC was measured with a colloid titration according to the work of Mikkelsen [19] and Wilén *et al.* [20]. For colloid titration, polybrene (PB) and polyvinyl sulphate potassium salt (PVSK) were used as cationic and anionic reactants, respectively. The endpoint was determined from the colour change (blue to pink) of toluidine blue (TB). 1mL of a 1.0gMLSS/L activated sludge sample was diluted to 100mL with permeate. To this solution, 5mL of 0.001N PB and 5mL of 0.05g/L TB was added and stirred with a magnetic stirrer. Backtitration with PVSK was performed in triplicate. Backtitration of 100mL permeate with PB and TB served as blank. The SC was expressed as milli-equivalents per gram MLSS of negative colloidal charge [meq/gMLSS].

2.6. *Floc stability*

The activated sludge dissociation constant (DC), being a measure for floc stability, was determined with a procedure similar to the method described in Zita and Hermansson [21]. First, activated sludge samples were diluted with permeate to 1.0gMLSS/L. Next, 1L of the dilution sample was gently stirred (Heidolph RZR2051, 200rpm) for 15min in a glass beaker. After 15min of sedimentation, 200mL of the supernatant was taken and the turbidity was measured on a spectrophotometer (DR5000, Hach Lange) at 650nm with the filtrate from this sample as a blank. Then, 200mL of Milli-Q was added to the beaker and the sample was gently stirred again for 15min. This procedure was repeated five times. The DC is defined as the slope of the accumulated turbidity versus the number of washing steps [21].

2.7. Analytical methods

The total organic carbon of permeate (TOC_{perm}) and supernatant (TOC_{sup}: supernatant of activated sludge centrifugated at 5000G for 10min at 4°C) was measured with a Hach Lange IL-550 TOC/TN analyzer. MLSS and mixed liquor volatile suspended solids (MLVSS) measurements were conducted in accordance with the procedures described in APHA Standard Methods [22].

2.8. Activated sludge samples

Ten different MBRs in Belgium and the Netherlands were selected for the sampling campaign. The selected set of MBRs includes plants treating municipal (4) and a variety of industrial (6) wastewaters, of different scales and equipped with different membrane types (Table 1). All MBRs were sampled twice, both in winter and summer, except MBR 10, which was only sampled in winter due to technical problems during the summer sampling campaign. A sample of 30L was taken directly from the membrane tank, whenever possible. If this was not feasible, the sample was

taken from the recirculation flow from the membrane tank back to the bioreactor, i.e. the carbon and/or nutrient removal tank.

Table 1: type of wastewater, scale and membranes of the sampled MBRs.

Location	Feed water	Scale	Type of membrane module (pore size, manufacturer)
1	Municipal	Full-scale	Flat sheets (0.08µm, Toray)
2	Municipal	Full-scale	Flat sheets (0.4µm, Kubota)
3	Municipal	Full-scale	Hollow fibers (0.04µm, Zenon)
4	Municipal	Pilot-scale (15m³)	Flat sheets (0.08µm, Toray)
5	Industrial – food	Full-scale	Hollow fibers (0.04µm, Koch – Puron)
6	Industrial – waste	Full-scale	Hollow fibers (0.04µm, Zenon)
7	Industrial – food	Full-scale	Hollow fibers (0.04µm, Koch – Puron)
8	Industrial – chemistry	Pilot-scale (9m³)	Flat sheets (0.04µm, Microdyn-Nadir)
9	Industrial – food	Full-scale	Hollow fiber (0.4µm, Mitsubishi – Sterapore)
10	Industrial – food	Full-scale	Micro-tubular (0.03µm, Norit – X-flow)

All 30L sludge samples were placed in the sludge reservoir of the DFCm installation within two hours after sampling. According to the DFCm, the sludge in the reservoir is continuously aerated to ensure aerobic conditions (as in the membrane tanks) and to keep the sample well-mixed during the course of the experiment. Prior to the start of each experiment, a 1L sample was taken from the DFCm reservoir for all other sludge analyses, to ensure the exact same sample composition and condition.

2.9. Mathematical techniques

To correlate the activated sludge characteristics (input) with the activated sludge filterability (output) a Partial Least Squares (PLS) approach is exploited. PLS is a multiple linear regression technique that seeks the best correlation between a reduced set of input variables and a reduced set of output variables. The *reduction* of the input and output data aims at removing the noise and co-linearity between the data by creating new input and output variables that are weighted combinations of existing ones. These so-called *score* vectors capture the majority of the variance in the input and output data. To infer the score vectors, and, hence, the weights of the measurement data, a leave-one-out cross-validation is employed. For more detailed information about PLS, the reader is referred to the work of Geladi and Kowalski [23].

3. Results and discussion

The influence of biomass concentration, relative hydrophobicity, sludge morphology, EPS, sludge dissociation constant, TOC and surface charge on sludge filterability (ΔR_{20}) was studied. The results are discussed below.

3.1. DFCm results

The DFCm results on activated sludge filterability are presented in Figure 2. There is a large variation between the sludge filterability in the different plants. If we compare the results plant-wise, in general, summer sludge samples exhibit better sludge filterability compared to winter samples, with the exception of MBRs 7 and 8. Given that results from the DFCm are corrected for temperature, this seasonal variation exceeds the mere temperature effect on viscosity. In this analysis, filtration data are corrected for viscosity changes due to temperature.

Our present results, and especially those for the municipal installations 1 to 4, confirm the observation of several authors that a seasonal decrease in reactor temperature negatively affects membrane fouling rates [24-26].

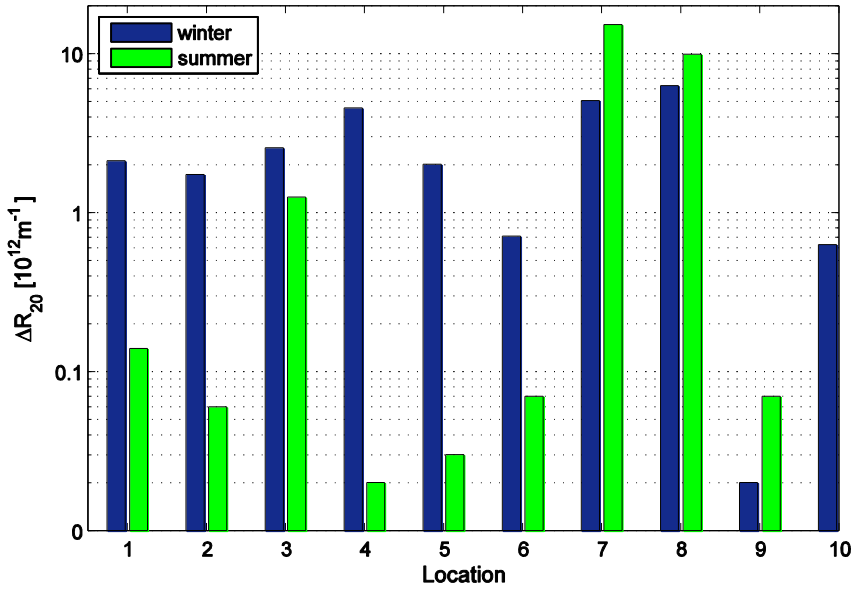

Figure 2: ΔR_{20} results from the DFCm with blue = winter sample, green = summer sample.

No correlation could be found between the membrane configuration and activated sludge filterability; the dissimilarities are evidently due to different operational conditions applied in the different plants, e.g., type of wastewater, HRT, SRT, seasonal period, etc.

3.2. Biomass concentration

MLSS and MLVSS results are listed in Table 2. The ten plants cover a wide variety of biomass concentrations, ranging from 4.6gMLSS/L to 19.4gMLSS/L. Plant-wise comparison indicates that most of the sampled MBRs operate at a constant biomass concentration. The MLSS increase in MBR 5 is due to an urgent process water requirement at this location; a surplus of permeate was extracted from the sludge to be used in the production process. Nonetheless, the sludge filterability was good at this location. These results confirm the controversy about the impact of biomass concentration on sludge filterability; also in this sampling campaign, the results of MLSS and MLVSS could not directly be correlated to sludge filterability.

3.3. Relative hydrophobicity

The results for RH are presented in Figure 3. Generally, RH is higher in summer samples, especially for MBRs that are subjected to varying influent temperatures, i.e., MBRs treating municipal wastewater: 1, 2, 3 and 4.

For the majority of the sampled MBRs, sludge filterability and RH appear to be positively correlated, i.e., hydrophobic particles interact less with the hydrophilic membrane, as was expected. However, the RH-value alone renders inconclusive information on the filterability for some samples, e.g., the RH for MBR 6 decreases from 83% in winter to 60% in summer but sludge filterability improves as is reflected by a ΔR_{20} decrease from $0.71 \cdot 10^{12} m^{-1}$ to $0.07 \cdot 10^{12} m^{-1}$. In the case of MBR 8, the summer sludge sample is more hydrophobic with a RH of 62% versus 53% in winter, although sludge filterability deteriorates with a ΔR_{20} increase from $6.28 \cdot 10^{12} m^{-1}$ to $9.90 \cdot 10^{12} m^{-1}$. It is obvious that with RH and biomass concentration as sole input parameters, we are unable to estimate sludge filterability.

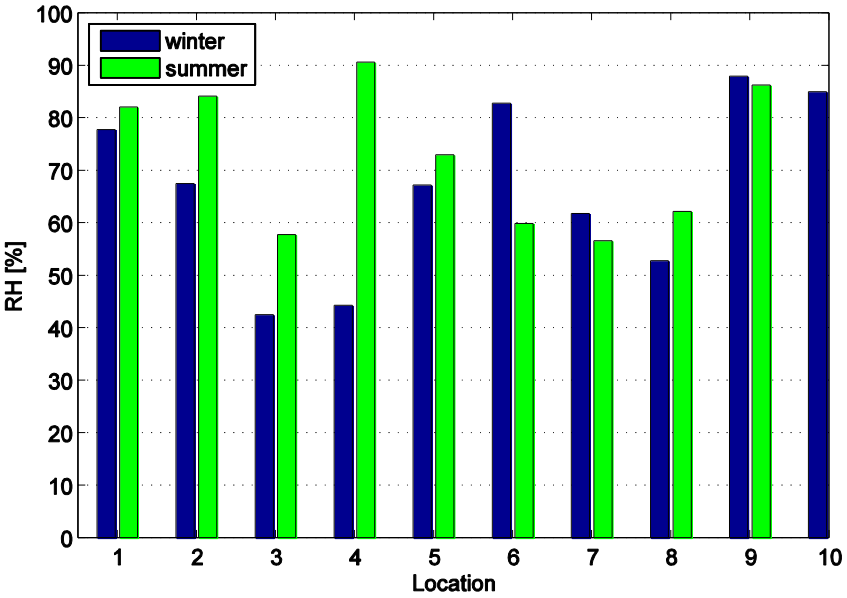

Figure 3: Relative hydrophobicity (RH) of activated sludge samples. Blue = winter sample, green = summer sample.

3.4. Sludge morphology

Sludge morphology was monitored by means of an automated microscopic image analysis procedure, the results are summarized in Figure 4 and Figure 5. Since it has been demonstrated that deflocculation deteriorates sludge filterability [8,27], two parameters are selected to represent the sludge flocculation state, i.e., activated sludge mean particle size A_{mean} and the 1%pixel value being the surface fraction of sludge particles equal to 1pixel.

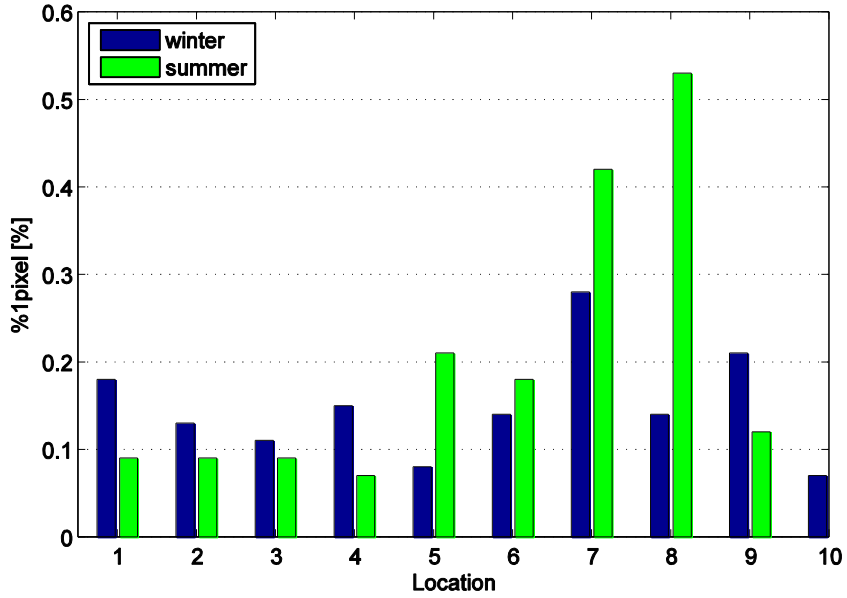

Figure 4: Surface fraction of activated sludge particles equal to 1pixel. Blue = winter sample, green = summer sample.

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Table 2: summary of the results from activated sludge characteristics.

Location		MLSS	MLVSS	SC	DC[a]	TOC_{sup}[b]	TOC_{perm}[b]	SMP PS	SMP PN	eEPS PS	eEPS PN
1	Winter	9.89±0.11	7.45±0.12	-0.453±0.023	0.0113	39.96	7.88	1.15±0.07	2.74±0.23	26.52±1.56	44.26±0.59
	Summer	8.35±0.01	6.07±0.01	-0.373±0.162	0.0041	30.86	8.80	0.30±0.20	0.68±0.25	22.93±1.55	40.76±0.25
2	Winter	16.11±0.12	9.00±0.05	-0.347±0.201	0.0191	35.97	17.27	0.83±0.002	2.69±0.07	19.41±1.29	33.97±0.44
	Summer	14.89±0.10	6.72±0.04	-0.400±0.069	0.0074	15.52	5.80	0.19±0.01	0.21±0.02	23.50±0.01	36.00±0.11
3	Winter	9.01±0.09	6.18±0.08	-0.293±0.122	0.0158	33.65	11.11	1.68±0.01	2.09±0.27	34.89±0.86	71.68±0.74
	Summer	8.23±0.10	5.21±0.06	-0.720±0.106	0.0141	40.33	18.26	1.28±0.11	1.14±0.34	25.93±0.02	48.04±0.37
4	Winter	4.97±0.01	3.21±0.01	-0.507±0.115	0.0550	40.74	21.12	10.21±1.05	6.00±0.09	23.21±0.78	68.01±2.61
	Summer	5.81±0.04	3.41±0.01	-0.307±0129	0.0044	16.29	8.21	1.18±0.24	0.74±0.29	18.23±0.31	43.86±0.27
5	Winter	7.77±0.14	6.85±0.14	-0.707±0.266	0.0757	134.9	71.02	8.87±0.44	27.66±0.80	36.88±0.22	40.92±0.60
	Summer	19.35±0.45	16.80±0.40	-0.773±0.122	0.0599	219.2	93.46	5.81±0.36	12.36±0.08	14.72±0.04	25.54±0.46
6	Winter	8.40±0.04	7.22±0.02	-0.587±0.180	0.0608	55.28	27.15	2.17±0.17	6.89±0.25	21.76±4.31	36.23±0.21
	Summer	10.54±0.21	8.62±0.16	-0.573±0.295	0.0694	55.54	29.91	1.98±0.13	4.86±0.12	24.20±0.18	28.42±0.20
7	Winter	17.26±0.06	7.96±0.02	-0.453±0.061	0.0610	55.20	15.30	2.90±0.07	3.85±0.84	15.70±0.47	35.68±0.25
	Summer	16.80±0.36	6.05±0.11	-0.813±0.092	0.1051	102.5	13.17	15.28±0.27	6.59±0.10	17.94±0.33	23.18±0.77
8	Winter	13.97±0.12	10.00±0.07	-0.200±0.106	0.0625	142.9	48.60	2.77±0.02	7.58±0.06	22.09±0.43	47.82±0.49
	Summer	14.77±0.25	10.60±0.18	-0.653±0.083	0.2642	284.7	51.14	16.19±0.18	17.29±0.21	21.71±0.37	36.13±0.25
9	Winter	15.32±0.09	14.30±0.07	-0.347±0.046	0.0505	119.0	36.20	6.71±0.44	2.98±0.03	17.71±0.07	20.24±0.11
	Summer	13.71±0.14	12.60±0.13	-0.653±0.046	0.0197	40.33	18.26	0.65±0.01	0.83±0.07	19.37±0.02	19.65±0.17
10	Winter	4.61±0.04	4.53±0.02	-0.427±0.101	0.0501	131.2	24.29	30.34±0.27	5.85±0.23	22.97±019	30.43±0.24
Unit		g/L	g/L	meq/gMLSS	Abs/washing step	mgC/L	mgC/L	mg/gMLVSS	mg/gMLVSS	mg/gMLVSS	mg/gMLVSS

[a]R^2 of slope > 0.97, [b]maximum allowed standard deviation 2%

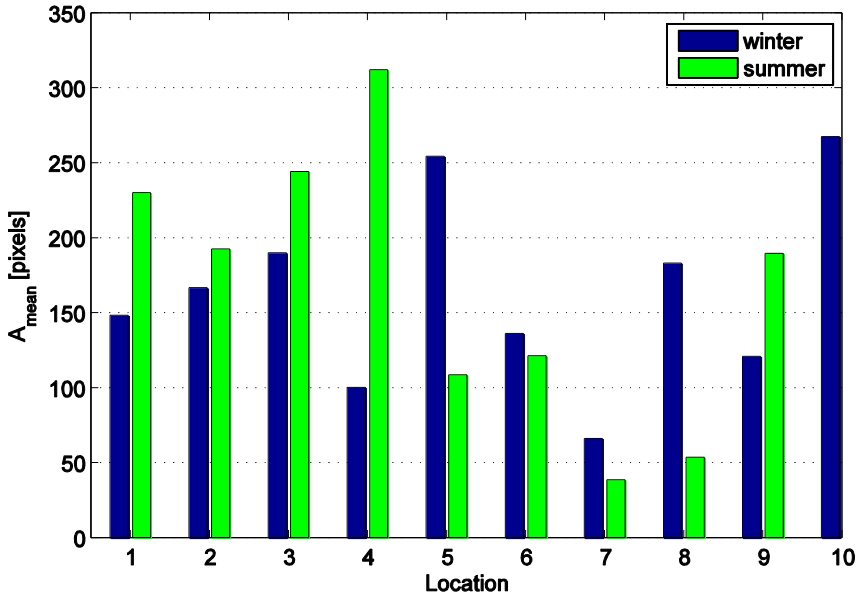

Figure 5: Activated sludge mean particle size. Blue = winter sample, green = summer sample.

The results presented in Figure 4 and Figure 5 confirm the link between (de)flocculation and filtration performance, e.g., for MBRs 7 and 8 a sludge deflocculation event, reflected by a decrease in A_{mean} and an increase in %1pixel, results in a dramatic deterioration of sludge filterability whilst maintaining roughly constant levels of MLSS. In the case of MBR 8 specifically, sludge morphology parameters explain the deteriorated filterability which, in this case, completely overrule the effect of increased sludge hydrophobicity.

3.5. Filterability prediction: combining relative hydrophobicity and sludge morphology for primary classification

On the basis of the above presented results we propose to combine relative hydrophobicity and sludge morphology into a parameter *t*, as such yielding reliable information for a rough first estimation of the sludge filterability into two classes, i.e., (*i*) *bad* sludge filterability and (*ii*) *poor* to *good* filterability (Figure 6). *t* is defined as:

$$t = \frac{\%1\,\text{pixel}}{RH}$$

$t > 0.00325 \rightarrow$ *bad* sludge filterability ($\Delta R_{20} > 3.0$)
$t < 0.00325 \rightarrow$ *poor* to *good* filterability ($\Delta R_{20} < 3.0$)

The theory behind this observation is relatively simple, e.g., small sludge flocs are dramatic for sludge filterability [8], however, if these small flocs are very hydrophobic they tend to foul the hydrophilic surface of the membrane less. Similarly, robust sludge flocs improve sludge filterability, however, if these sludge flocs are very hydrophilic, they interact more with the membrane surface. The latter effect is hardly ever observed, since hydrophilic sludge particles do not flocculate well. Evidently, the proposed threshold value can be refined when additional data is gathered.

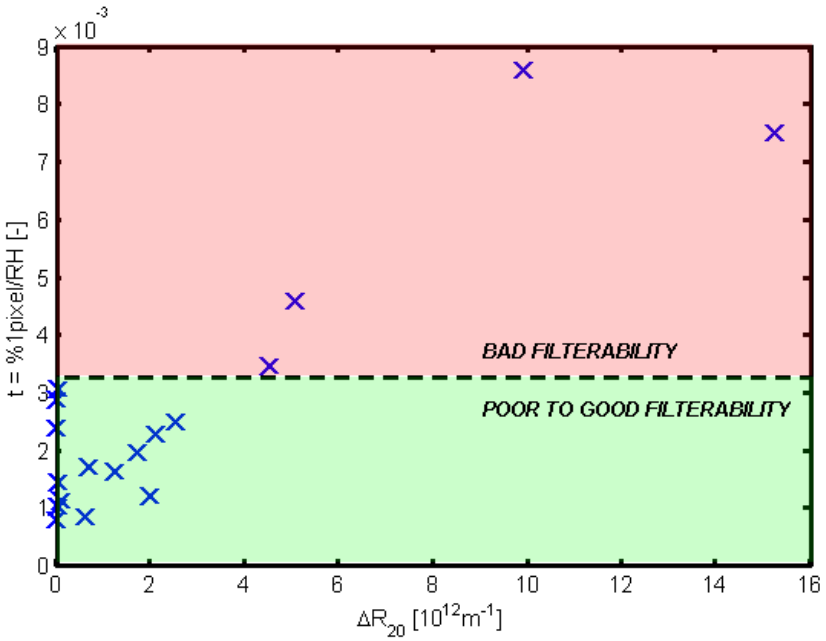

Figure 6: *t* versus ΔR_{20}. The combination of sludge morphology (%1pixel) and relative hydrophobicity marks a clear threshold for two filterability classes, i.e., *bad* (t>0.0325, ΔR_{20}>3.0) and *poor* to *good* (t<0.0325, ΔR_{20}<3.0).

3.6. Filterability prediction: including extra measurements for fine-tuning

Indicator *t* permits a classification of *poor to good* and *bad* sludge filterability, however, it does not allow a more accurate estimation of the actual sludge filterability value, i.e., ΔR_{20}. The difference between *poor, moderate* and *good* cannot be explained by hydrophobicity and sludge morphology alone, more detailed measurements are needed.

Apart from RH and image analysis, all samples were analyzed for eEPS (PN and PS), SMP (PN and PS), TOC in permeate and supernatant, floc surface charge and floc stability (Table 2).

For sludge samples with a ΔR_{20}<3.0, partial least squares was used to infer a correlation between ΔR_{20} and other sludge characteristics in order to accurately estimate activated sludge filterability.

The PLS results were obtained with a leave-one-out (LOO) crossvalidation. Best results were obtained with ten input variables (Table 3) and ten latent variables. Because the number of latent variables is high compared to the number of model inputs and the number of available samples, the effective rank of the model is investigated.

Using the criteria formulated by Seipel and Kalivas [28], the model's effective rank is less than one. The criterion developed by Van der Voet [29] is also used to estimate the model's degrees of freedom, and yields a rank of six. The real model rank is located between these two values, but closer to the lower bound [28]. Therefore, the developed PLS model is estimated to have a rank of approximately two or three, well below the number of available samples.

To further assess the accuracy of the model, the estimated ΔR_{20}-values from the LOO crossvalidation are plotted against the measured ΔR_{20}-values in Figure 7. Estimated negative values were set to zero prior to SSE calculation. The R^2 (0.9936) and SSE (0.07554) observed in the crossvalidation confirm the accurate estimation of the sludge filterability with the proposed model. Note that, due to the leave-one-out cross validation procedure, the R^2 value is not the traditional goodness-of-fit

regression coefficient but reflects the *validation* performance of the model on other data.

Table 3: PLS parameters used to estimate sludge filterability.

Variable (x)	Mean ($\bar{x}$)	Standard deviation (σ_x)	Model weight (ρ)
1. SMP PS [mg/L]	35.57	46.23	-4.86
2. eEPS PN [mg/gMLVSS]	34.48	9.17	2.05
3. RH [%]	77.01	10.92	2.57
4. DC [-]	0.0344	0.0269	1.02
5. %1pixel [%]	0.129	0.051	-7.30
6. #1pixel [#objects/image]	39.90	20.91	-3.78
7. %1pixel/RH [-]	0.00173	0.00075	-3.49
8. 1/ln(%1pixel/RH) [-]	-0.156	0.011	-14.07
9. (SMP PS)/RH [mg/L.%]	0.457	0.568	7.40
10. (eEPS PN)/RH [mg/gMLVSS.%]	0.462	0.160	-1.75
Standard deviation on output (σ_y)	0.817		
Output mean ($\bar{y}$)	0.680		

Using the variables from Table 3 and their respective parameters, the following equation allows calculating the estimated ΔR_{20} for each sludge sample.

$$\Delta R_{20,estimated} = \left(\sum_{i=1}^{10} \frac{x_i - \bar{x}_i}{\sigma_i} \cdot \rho_i \right) \cdot \sigma_y + \bar{y}$$

with x_i the measured value of variable i, $\bar{x}_i$ the mean value of all measured values of variable i, σ_i the standard deviation of variable i, ρ_i the model weight of variable i, σ_y the standard deviation of output y and $\bar{y}$ the mean value of all measured output values, i.e., ΔR_{20}.
It is important to notice that some of the variables are calculated through a combination of other measurements. The set of essentially needed measurements is, therefore, not that extensive, i.e., SMP PS, eEPS PN, RH, DC and image analysis.

For a given MBR activated sludge sample, the obtained relationships can assist in pinpointing the main causes of fouling and can help in implementing the correct remedial actions to improve activated sludge filterability.

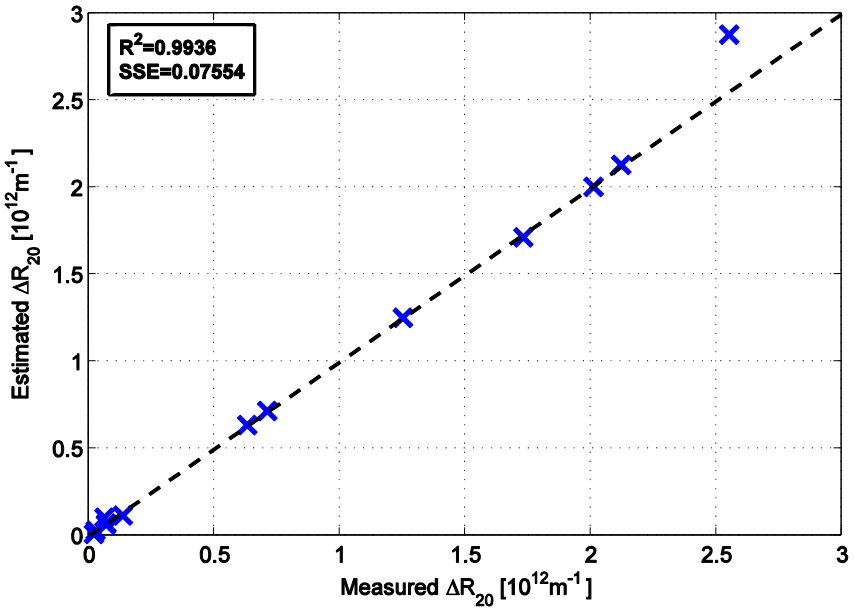

Figure 7: Estimated ΔR_{20} in leave-one-out crossvalidation with PLS versus measured ΔR_{20}.

3.7. Practical implications for MBR operation

As was mentioned in paragraph 3.1., the filterability of municipal summer samples is better compared to samples taken in winter. According to Rosenberger *et al.* [24] and Drews *et al.* [25] this is probably due to an increased SMP concentration in the mixed liquor supernatant of winter samples. Our results confirm that SMP PS negatively impact activated sludge filterability, i.e., all municipal MBRs have lower SMP PS concentrations in summer compared to winter. However, these summer samples also indicate an improved sludge flocculation, i.e., sludge morphology and hydrophobicity. Also EPS PN, which contribute to the structural integrity of the flocs and to hydrophobicity, as well as the dissociation constant, i.e., a measure for the stability of activated sludge flocs, influence the filterability of activated sludge. It can thus be concluded that the *bioflocculation state of activated* sludge is a major indicator for activated sludge filterability, not only for municipal MBRs, but for industrial MBRs as well.

To improve the filterability of activated sludge samples with ΔR_{20}>3.0 (bad) it is important to enhance flocculation and increase hydrophobicity. Commercial flocculants can be used as a fast and easy remedy, whilst adjusting process and environmental conditions to favor good bioflocculation and increase sludge hydrophobicity to avoid the excessive cost of constant flocculant dosing.

4. Conclusions

Ten different MBRs in Belgium and the Netherlands, treating both municipal (4) and industrial (6) wastewaters, were sampled in both winter and summer. Based on the results discussed in this paper, the following conclusions can be drawn.

- The lack of a clear correlation between a single sludge parameter and any other foulant characteristic indicates that every single parameter alone is a poor indicator of biomass fouling propensity, a combination of activated sludge parameters is more reliable to predict sludge filterability.
- A combination of sludge morphology, characterized by the parameter %1pixel, and relative hydrophobicity allows for a first classification of sludge filterability.

Deflocculation and low RH have a negative impact on activated sludge filterability whereas good flocculation and high RH improve sludge filterability.

- For sludge samples that have *poor to good* activated sludge filterability, accurate estimations of sludge filterability can be calculated on the basis of eEPS PN, SMP PS, sludge morphology, the dissociation constant and RH. Partial least squares proved to be a valuable tool for unveiling these correlations.
- No correlation could be found between activated sludge filterability and membrane configuration or wastewater to be treated. Sludge filterability is independent of membrane configuration.
- In general, summer samples had better activated sludge filterability compared to samples collected in winter. This effect exceeds the mere temperature effect as filtration data are corrected for viscosity changes due to temperature.

Overall, it can be concluded that the *bioflocculation state* of the activated sludge is a major indicator for activated sludge filterability.

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