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Table of contents

1. Objectives.....	1
2. Introduction	1
3. Development and validation of techniques for MBR monitoring.....	1
3.1. 2D-fluorescence for EPS monitoring - IBET	1
3.2. Organic matter characterisation through 2D-fluorescence combined with size exclusion chromatography - UNESCO-IHE	5
3.2.1. Analytical Characterization Methods.....	5
3.2.2. Preliminary Work.....	7
3.2.3. Future Work	9
3.3. Comparison of EPS extraction and characterisation methods - IBET	10
3.3.1. EPS extraction	10
3.3.2. Analysis of protein and polysaccharide content in each EPS fraction.....	11
3.3.3. Proteins and polysaccharides analysis: conclusions.....	13
3.3.4. Modified Method of Lowry for determination of humic substances	13
3.4. Monitoring of MBR performance with particle size distribution analysis - NTNU	17
3.5. Respirometry as a tool for monitoring feedwater characteristics and biological activity - UNITN	18
4. Implementation of characterisation and monitoring techniques on MBR installations.....	20
4.1. Delft University of Technology Filtration Characterisation Installation TM for determination of relationships between filterability characteristics and full-scale permeability data of MBR Varsseveld	20
4.1.1. Introduction	20
4.1.2. Material and Methods.....	21
4.1.3. Results	23
4.1.4. Conclusions	29
5. References	30
 ANNEX A	 31
Methods for EPS extraction compared in this study	31
Method 1 - Sperandio et al, 2005	32
Method 2 - Frolund et al., 1995 and 1996.....	32
 ANNEX B	 33
Methods for protein and polysaccharide measurement used in this study.....	33
Method of Lowry	34
Method of Dubois.....	35
Method of Anthrone	36
 ANNEX C	 37
Delft Filtration Characterisation Unit	37
C.1. INSTALLATION MAJOR PURPOSES	38
C.2. REQUIREMENTS ON SITE	38
C.3. MAJOR RESULTS	38
Dilution effect	38
pH effect.....	40
Aeration effect.....	41
Substrate addition effect.....	42
C.4. PERSPECTIVES FOR THE FUTURE.....	43

1. Objectives

This report summarises the activities developed within the Workpackage 5 towards: a) the development and validation of analytical techniques in order to monitor and obtain a pattern response from the MBRs; b) the implementation of characterisation and monitoring techniques on MBR installations.

2. Introduction

The previous deliverable of this WP (D7) comprised a compilation of the analytical procedures available within the EUROMBRA project for feedwater characterisation and MBR monitoring. Some of these techniques were developed and validated for MBR monitoring in the work presented in this deliverable. 2D-fluorescence was evaluated as a non-invasive technique to be used to obtain pattern responses from the MBR. Two EPS extraction techniques were compared, as well as methods for analysis of proteins and polysaccharides in the EPS fractions. Preliminary tests were carried out to evaluate the correlation of particle size distribution and fouling potential of a sample. Respirometry was evaluated as a tool to characterise (fractionate) the feedwater COD and the biological activity in the MBR.

Furthermore, the Delft University of Technology Filtration Characterisation Installation was implemented at a full-scale MBR wastewater treatment plant. Results of these tests are also described in this deliverable.

3. Development and validation of techniques for MBR monitoring

3.1. 2D-fluorescence for EPS monitoring - IBET

EPS detection is of high relevance in membrane bioreactors (MBRs). They are considered to be the main responsible for membrane fouling, which results in performance deterioration. Therefore, it is important to monitor and minimise EPS production in these systems. EPS contain large amounts of proteins and aromatic substances that possess natural fluorescence properties, making fluorescence a promising technique to monitor their production in MBRs. However, it is important to assess the feasibility in detecting these compounds in presence of humic- and fulvic-like substances, which can be present in wastewater in high concentrations, with a widely variable composition.

In this study, soluble EPS extracted from an activated sludge bioreactor were analysed by 2D-fluorescence. The results showed three excitation/-emission peaks, two of which could be attributed to proteins at around 350 nm of emission and the third peak at 375 nm of excitation and 450 nm of emission (figure 1c). To try to understand the nature of this last peak, it was compared to the peak obtained with commercial humic acids and with NADH, another compound with fluorescence properties likely to be present in the sample. Both compounds were found to have excitation-emission signals in the same region as the peak obtained in the EPS sample, therefore, both may be responsible for the unknown signal (figure 1).

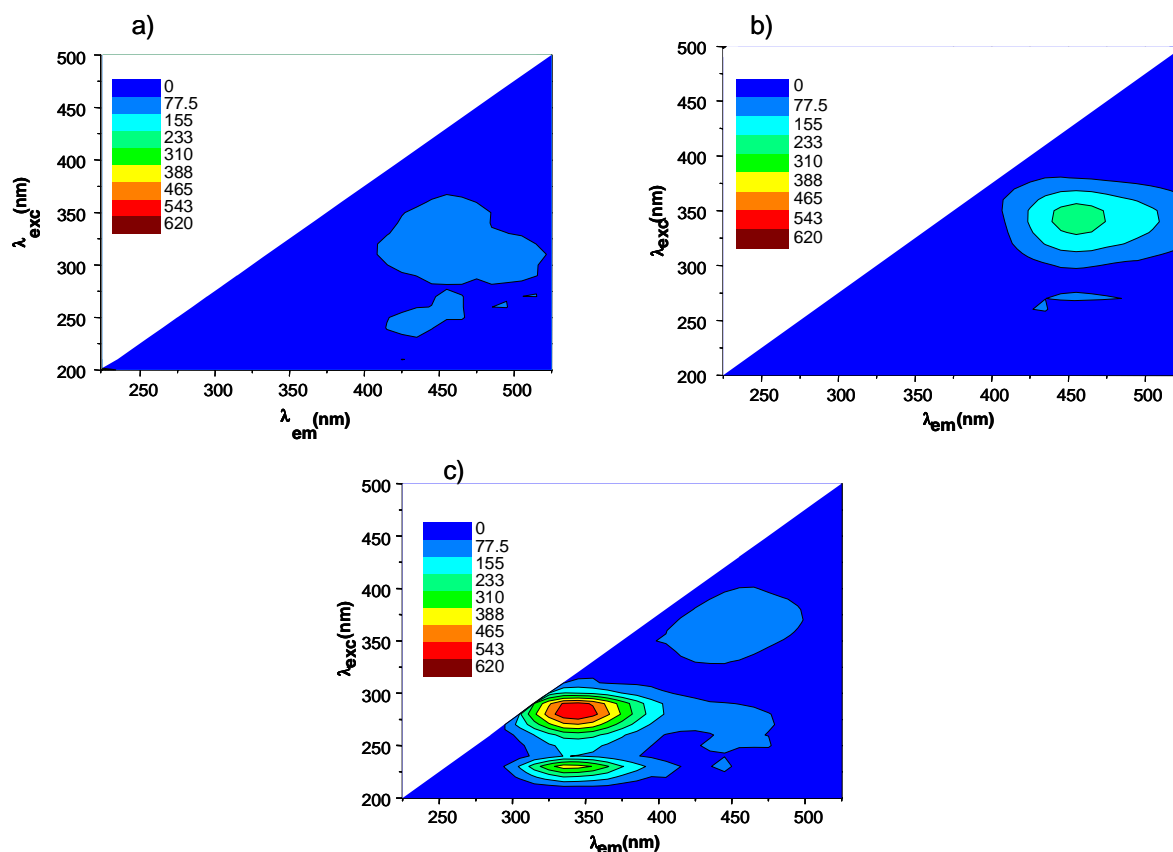


Figure 1 - 2D-Fluorescence spectra of (a) Commercial humic acids (10 mg/L), (b) NADH (9 mg/L) and (c) soluble EPS (diluted 1:10). Spectra were acquired in an excitation wavelength range from 200 to 600 nm and in an emission wavelength range from 225 to 625 nm.

Fluorescence spectra were also obtained with commercial humic acids and a model protein (bovine serum albumin BSA). The results (Figure 2) showed that the commercial humic acids had a strong quenching effect on the excitation-emission spectrum of BSA, reducing or even eliminating the protein peak in the 2D-fluorescence map (Figure 2c).

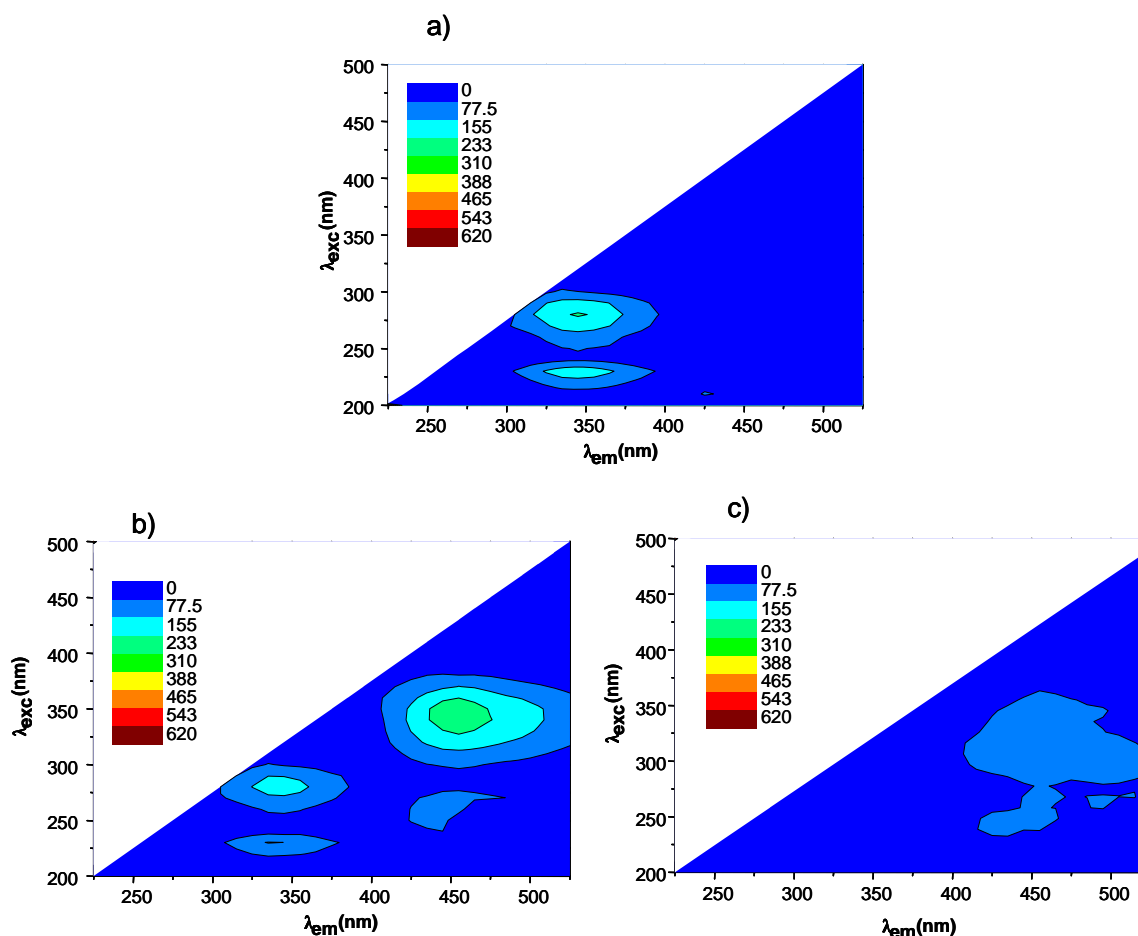


Figure 2 - 2D-Fluorescence spectra of (a) BSA (10 mg/L), (b) NADH with BSA (9 mg/L and 10 mg/L, respectively) and (c) commercial humic acids with BSA (both at 10 mg/L). Spectra were acquired in an excitation wavelength range from 200 to 600 nm and in an emission wavelength range from 225 to 625 nm.

The potential quenching effect of NADH over the protein fluorescence signal was also studied through mixing BSA and NADH (Figure 2). It seems that NADH can produce a slight decrease in the BSA peak intensity when compared with BSA alone (Figure 2a), and consequently has a small quenching effect. However, this effect might have been amplified by the fact that the NADH concentration used in this experiment was much higher than it is likely to be present in real samples. Nevertheless, quenching effect in protein measurements, and peak overlapping in the humic-like compounds region has to be considered since it causes interferences in EPS analyses by 2D-fluorescence.

In order to assess the extension of the quenching effect in the application of fluorescence for wastewater treatment monitoring, a sample of surface water was tested. It was expected that this sample contained a different composition of humic- and fulvic-like substances than the commercial humic acids employed before, since these compounds are known to have a high geographical variation. The results (Figure 3) showed that there was some quenching effect of the natural organic substances over BSA, although to a lower extent than with the commercial humic acids, suggesting that only some of the compounds included in this broad class interfere with protein detection by fluorescence.

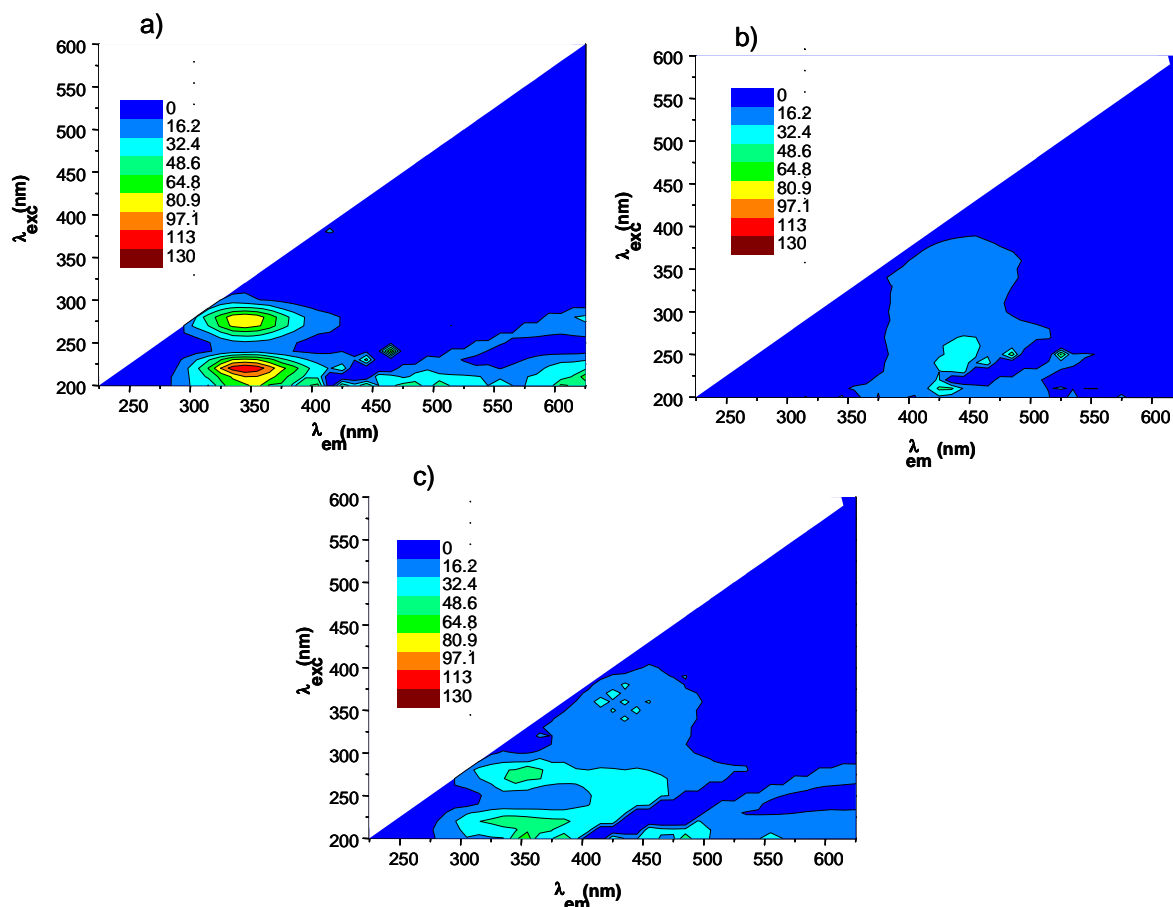


Figure 3 - 2D-Fluorescence spectra of (a) BSA (10 mg/L), (b) surface water and (c) surface water with BSA (10 mg/L). Spectra were acquired in an excitation wavelength range from 200 to 600 nm and in an emission wavelength range from 225 to 625 nm.

This study evaluates the potential of 2D-fluorescence techniques for monitoring the behaviour of wastewater treatment systems. The results suggest that **quantitative determination of proteins in such complex media is not feasible with 2D-fluorescence** in view of the interference of other compounds likely to be present in the system. However, a correlation between the **2D-fluorescence maps and the system performance may be established through non-mechanistic methods which can be used as a tool to optimise these biological systems**. This approach will be employed to monitor a bench-scale MBR implemented in IBET and in UNITN. 2D-fluorescence maps will be taken regularly or on-line from the feedwater, the effluent and, eventually, at the membrane surface, in order to assess the evolution of the microbial activity and growth in the MBRs.

Alternatively, it is possible to use size exclusion chromatography to separate different fractions as a function of molecular weight and then apply 2D-fluorescence to characterise each fraction (Her et al. 2003). The application of this technique enables monitoring the proteins in a MBR in a deterministic way using 2D-fluorescence, as described below.

3.2. Organic matter characterisation through 2D-fluorescence combined with size exclusion chromatography - UNESCO-IHE

Activities at UNESCO-IHE have focused on development and implementation of analytical characterization methods for effluent organic matter (EfOM) present in the sludge-water phase of membrane bioreactor (MBR) activated sludge. These methods include: (i) size exclusion chromatography with dissolved organic carbon detection (SEC-DOC) to show the polysaccharide (PS), humic substances (HS), and low molecular weight acids (LMA) composition of organic matter; (ii) fluorescence excitation-emission matrix (EEM) to differentiate humic-like from protein-like organic matter; and (iii) dissolved organic nitrogen (DON) to compliment dissolved organic carbon (DOC) measurements.

Our first hypothesis is that EfOM consists of natural organic matter (NOM) derived from the drinking water source and carried through the water cycle, to which soluble microbial products (SMPs) are then added during biological wastewater (i.e., MBR) treatment. Our second hypothesis is that organic-related fouling of MBRs correlates with PS-DOC, protein-like organic matter, and DON, all features of SMP. Here, the term SMP relates to water-phase extracellular polymeric substances (EPS) that are present in the sludge water phase of a MBR.

3.2.1. Analytical Characterization Methods

EfOM is first characterized by dissolved organic carbon (DOC), a measure of the amount of organic matter, and UV absorbance at 254 nm, a measure of aromatic character. With these results, specific UV absorbance ($SUVA = UVA_{254} \times 100/DOC$) is calculated with a classification scheme of $SUVA \geq 4$ L/mg-m for sources dominated by humic substances and $SUVA \leq 2$ L/mg-m for sources dominated by non-humic material. Humic substances are more dominant in NOM while non-humic material is more dominant in EfOM.

Size exclusion chromatography with on-line dissolved organic carbon (DOC) detection (SEC-DOC) involves a high-pressure liquid chromatograph (LC600 Shimadzu Corp., Japan) coupled with a TSK HW-50S column (26 mm x 300 mm). There are two detectors in series, a variable-wavelength UV detector and an on-line DOC detector (modified Sievers 800 *Turbo*, Ionics Instruments, USA), used to separate organic matter by molecular size distribution according to polysaccharides (PS), humic substances (HS), and low-molecular-weight (organic) acids (LMA). These individual peaks can be integrated and expressed as mg/L as DOC.

SEC-DOC separates EfOM components by size (molecular weight). The sample is passed through a size exclusion column which elutes large molecular weight organic components first, while smaller organic components are retained in the column and elute later. On-line UV and DOC analyzers measure the particular intensity as a function of retention time. The results are graphically represented showing the UV absorbance and DOC concentration relative to retention time or, if the column is calibrated with known compounds, as a function of molecular weight (size). Results are reported as arbitrary DOC units (i.e., detector signal).

When applied to EfOM, the technique yields a graph (chromatogram) typically with three peaks (Figure 1). The initial peak corresponds to high molecular weight substances designated as the polysaccharide (PS) peak, which is comprised of polysaccharides and proteins in both macromolecular and colloidal forms. The second, often larger, peak is comprised of humic substances (HS). Finally, the third peak encompasses low molecular weight acids (LMA). Other materials such as low molecular weight neutrals are poorly resolved.

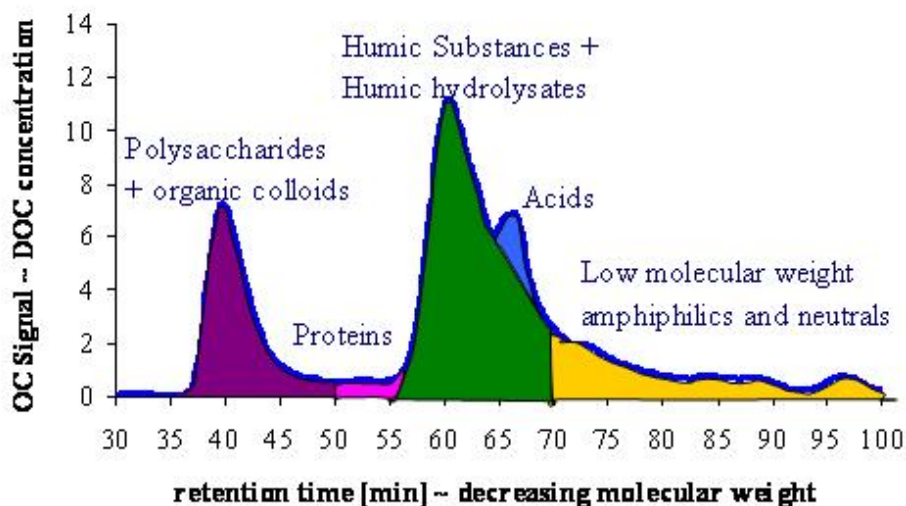


Figure 4. Ideal SEC-DOC chromatogram

Some EfOM components contain fluorophores that can be used to fingerprint the NOM of a water sample. Fluorophores are bonding structures which, when exposed to UV light (i.e., excitation or ex), will absorb then re-emit (i.e., emission or em) that light at a variety of wavelengths. By exposing a sample to a particular wavelength while monitoring other wavelengths, it is possible to develop a three-dimensional spectrum where excitation (ex) and emission (em) wavelengths are the y- and x-axes with fluorescence intensity (FI) as the z-axis.

The intensity signifies the amount of a fluorophore, or material, in a particular peak. The large peak located at em-ex 450-270 is associated with humic-like material, whereas the slightly smaller peak located at em-ex 450-330 is associated with fulvic-like material (Figure 2); there is a final peak of interest located at much lower intensity at em-ex 350-280, corresponding to protein-like substances.

A fluorescence excitation-emission matrix (EEM) is developed by scanning over an excitation range of 240 to 450 nm by 10-nm increments and an emission range of 290 to 530 nm by 2-nm increments using a FluoroMax-3 spectrofluorometer (HORIBA Jobin Yvon, Inc., USA). The fluorescence samples are first adjusted to 1 mg/L of DOC and a pH of ~3.0. The result is a three-dimension spectrum in which fluorescence intensity (arbitrary units) is represented as a function of excitation and emission wavelengths. There are two dominant peak areas observed in EEM: one at excitation/emission wavelengths of ~340 nm/~420 nm, corresponding to humic-like organic matter; and a second at excitation/emission wavelengths of ~270 nm/~350

nm, corresponding to protein-like organic matter. EfOM, compared to NOM, typically shows more protein-like and less humic-like organic matter. Based on an EEM, a fluorescence index (FI) can be calculated by the ratio of fluorescence intensity at emission 450 and 500 nm at excitation 370 nm. A higher FI (~ 1.7 - ~ 2.0) reflects organic matter of an autochthonous (microbial) origin while a lower FI (~ 1.3 - ~ 1.4) reflects organic matter of an allochthonous (terrestrial) origin. EfOM typically shows an autochthonous signature (higher FI) while NOM typically shows an allochthonous signature (lower FI).

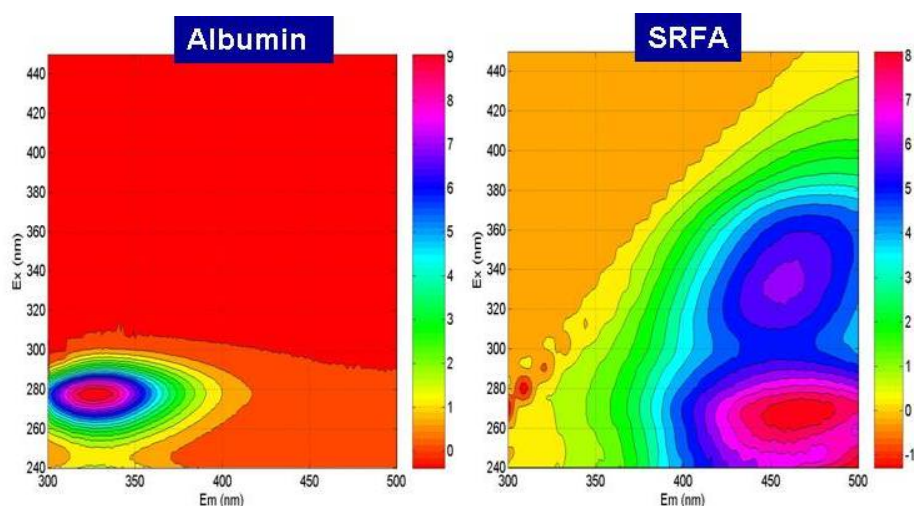


Figure 5. Fluorescence EEM of a protein (albumin) and Suwannee River fulvic acid

Dissolved organic nitrogen (DON) is accomplished by a two-step process, first involving elimination of dissolved inorganic nitrogen (DIN) by continuous dialysis through dialysis bags with a molecular weight cutoff (MWCO) of 100 Daltons, followed by direct measurement of DON as total nitrogen (TN) with a TN analyzer.

3.2.2. Preliminary Work

We have completed some preliminary work as part of our methods development and preliminary hypotheses testing. This work has focused on two operating MBRs in North America:

- Arapahoe MBR
 - SRT = 19 days; HRT = 6 hours; MLSS = 11,100 mg/L
 - Immersed Zenon 0.02 μm ; Anoxic/Oxic
- Georgia MBR
 - SRT = Unknown; HRT = 10 hours; MLSS = 3,360 mg/L
 - Sidestream Memcor 0.1 μm MF; Aerobic

Samples were taken from the sludge water (~ 1.0 μm filtrate) and the MBR permeate (Table 1) and analyzed according to SEC-DOC, EEM, and DON. Stirred cell simulations were also performed using a 0.1 μm PDVF flat-sheet membrane.

SEC-DOC results are presented in Figure 3. The PS peak for the Arapahoe sludge water was 4.5 mg DOC/L while that of the Georgia sludge water was 0.8 mg DOC/L. Corresponding EEM results are shown in Figure 4. Some retention of protein-like NOM is observed in both cases.

The stirred cell tests (Figure 5) clearly show greater fouling trends for the Arapahoe versus the Georgia sludge water. Based on these stirred cell simulations, fouling correlates with high PS-DOC, high DON, and high protein-Like NOM.

Table 1. MBR Samples Summary.

Sample/ Parameter	Arapahoe MBR Sludge Water	Arapahoe MBR Permeate	Georgia MBR Sludge Water	Georgia MBR Permeate
MLSS (mg/L)	11,140	---	3,660	---
TOC (mg/L)	14.3	6.3	8.3	5.2
DOC (mg/L)	13.1	6.2	8.2	5.2
UVA ₂₅₄ (cm-1)	0.165	0.118	0.178	0.092
SUVA (L/mg-m)	1.26	1.90	2.17	1.77
DON (mg/L)	1.33	0.66	0.59	0.41

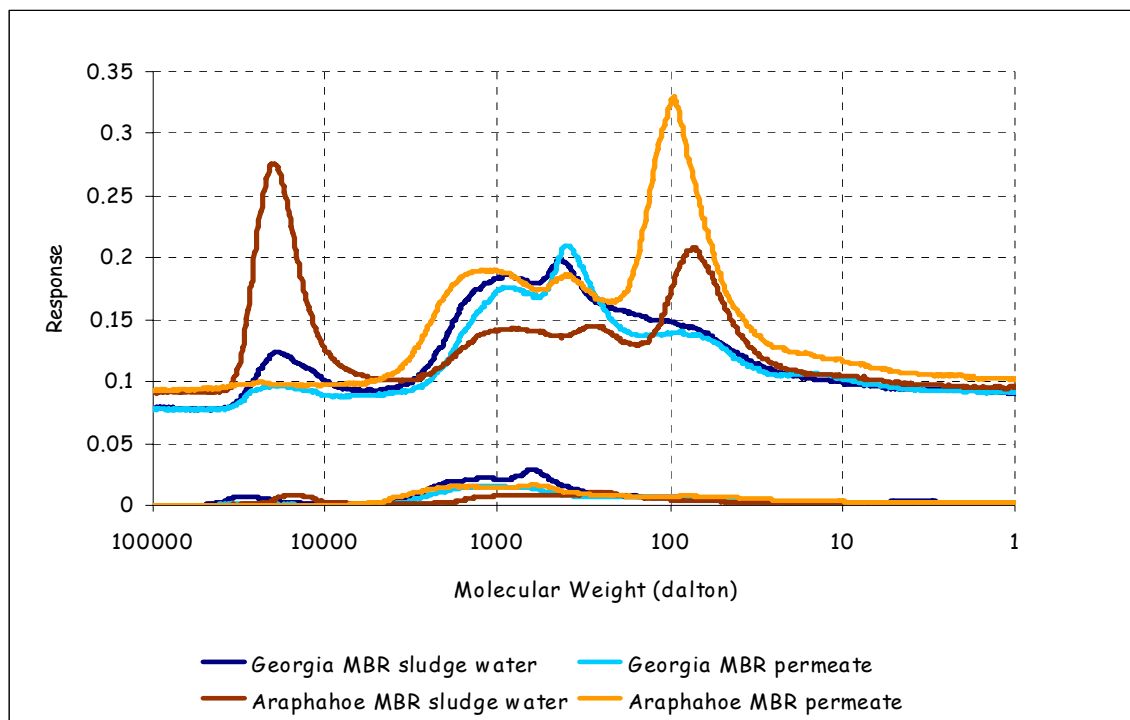


Figure 6. SEC-DOC Results.

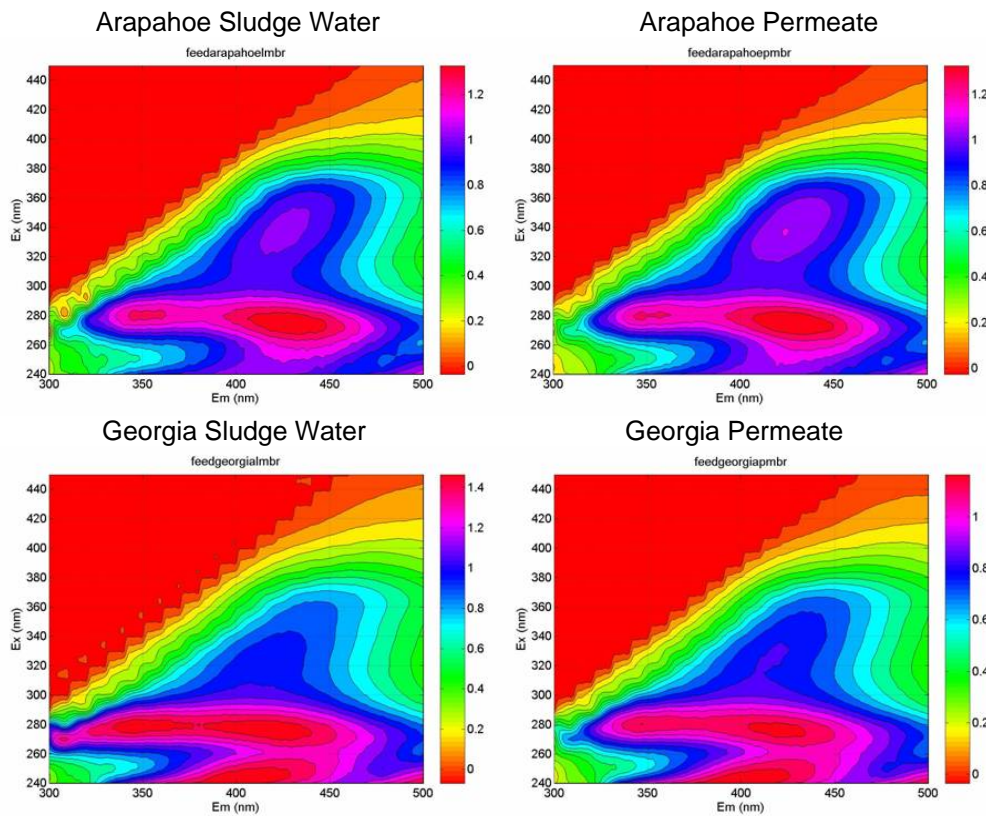


Figure 7. EEM Results.

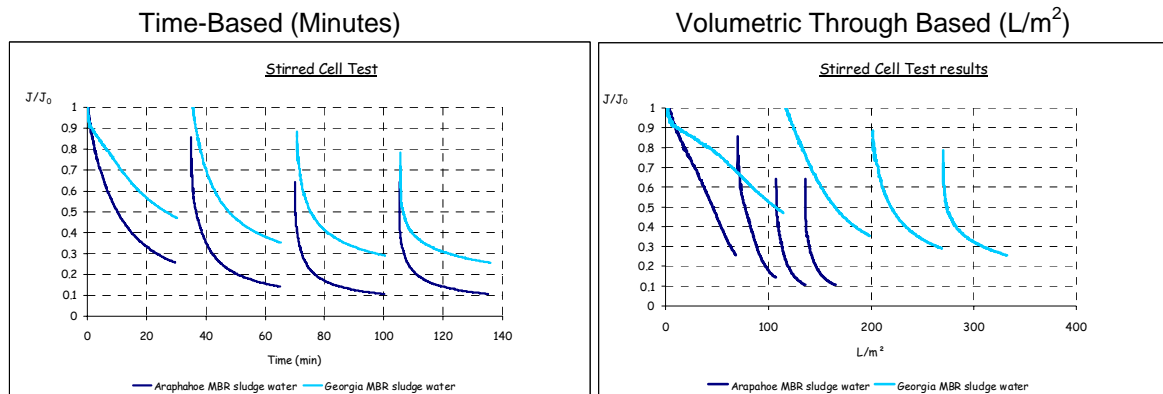


Figure 8. Stirred Cell Tests.

3.2.3. Future Work

We propose applying these techniques to two operating MBRs located in the Netherlands, with the work coordinated by our TU-Delft partner, and to the pilot being operated by EAWAG. This work will commence in August, 2007 upon appointment of a MSc student for a period of 8 months.

3.3. Comparison of EPS extraction and characterisation methods - IBET

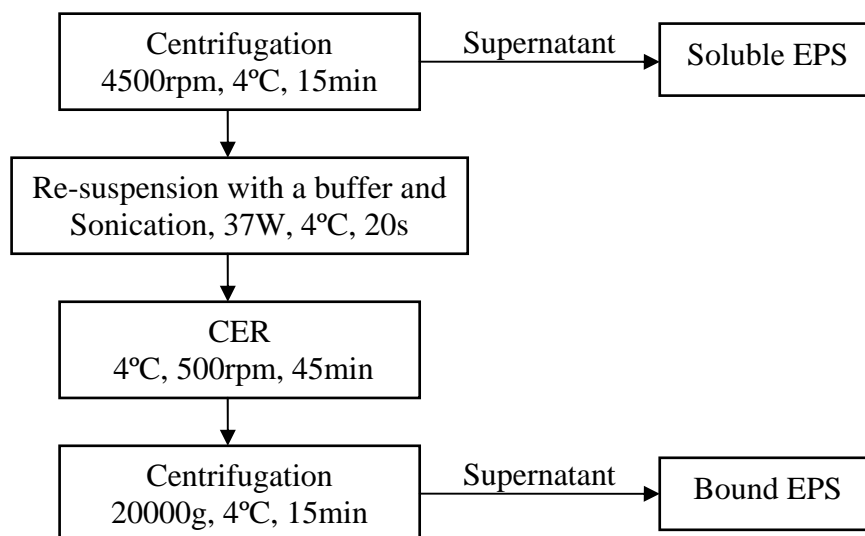
Several methods for EPS extraction and characterisation were collected in deliverables D2 and D7. This study aimed at comparing two of these EPS extraction methods and to further evaluate different analytical techniques for EPS characterisation. The selection of the methods was based on the utilisation of a cation exchange resin, since this resin has been described as the best reagent for EPS extraction with less cell lysis (Frolund *et al.*, 1996).

3.3.1. EPS extraction

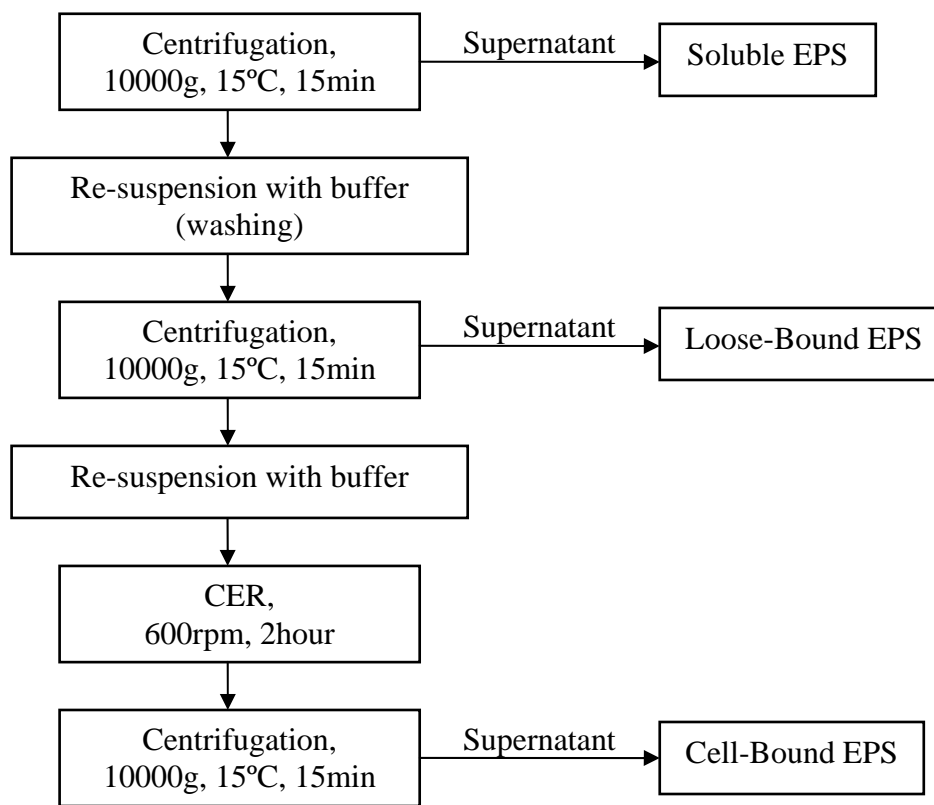
The two selected methods for this benchmarking are based on the use of a cation exchange resin (CER) for separation of the cells from the EPS. Method 1 (Sperandio *et al.*, 2005) has an intermediate sonication step, which is not applied in method 2 (Frolund *et al.* 1995, 1996). Method 2 has an additional centrifugation step as compared to method 1, resulting in three EPS fractions (soluble, loose-bound and cell-bound) whereas method 1 only distinguishes between soluble and bound EPS.

Method 1 - Sperandio et al, 2005

See detailed protocol in Annex A.



Method 2 - Frolund et al., 1995 and 1996
See detailed protocol in Annex A.



EPS characterisation of all five fractions was done by analysis of proteins and polysaccharides. The protein content was determined by the method of Lowry. The method of Dubois and the method of Anthrone were compared for determination of the polysaccharide content. The comparison of the two EPS extraction methods was based on the results obtained for the protein and polysaccharide characterisation of the different EPS fractions.

3.3.2. Analysis of protein and polysaccharide content in each EPS fraction

The method of Lowry is a widely used method, employed by all partners that do protein determination (see deliverable D7). Therefore, this was the method selected in this study to measure the protein content of the extracted EPS fractions. The protocol is presented in Annex B. The methods Dubois and Anthrone for polysaccharide analysis were compared in this study. Detailed description of these protocols is given in Annex B.

The results for protein and polysaccharide analysis of the different EPS fractions obtained in this study are shown in tables 2, 3 and 4. All samples were analysed in duplicate. The presented value is the average between the two results obtained for each sample and Stdev is the standard deviation.

Table 2 - Protein analysis (Method of Lowry).

Method 1			
		Stdev	
Soluble	225	7	mg/L
Bound	80	3	mg/L
total	306	10	mg/L

Method 2			
		Stdev	
Soluble	229	2	mg/L
Loose Bound	56	0.3	mg/L
Cell Bound	31	1	mg/L
Bound total	87	1	mg/L
total	316	3	mg/L

Table 3 - Polysaccharide analysis with the Method of Anthrone.

Method 1			
		Stdev	
Soluble	189	4	mg/L
Bound	77	8	mg/L
total	266	12	mg/L

Method 2			
		Stdev	
Soluble	167	3	mg/L
Loose bound	51	2	mg/L
Cell bound	17	3	mg/L
<i>Bound total</i>	68	5	mg/L
total	235	8	mg/L

Table 4 - Polysaccharide analysis with the Method of Dubois.

Method 1			
		Stdev	
Soluble	189	4	mg/L
Bound	78	2	mg/L
total	267	6	mg/L

Method 2			
		Stdev	
Soluble	169	3	mg/L
Loose bound	54	2	mg/L
Cell bound	23	1	mg/L
<i>Bound total</i>	77	3	mg/L
total	246	6	mg/L

3.3.3. Proteins and polysaccharides analysis: conclusions

The results obtained with the two methods of EPS extraction studied in this work were very similar (Tables 2 to 4), suggesting that both methods probably retrieve the totality of the EPS existent in the samples. Method 2 could present some advantages as compared to method 1: a) it does not require sonication; b) it distinguishes two fractions of bound EPS (loose- and cell-bound) which could have different impact on membrane fouling. **Therefore, for practical reasons, method 2 is recommended for EPS extraction in this project.**

The comparison between the Methods of Dubois and Anthrone for polysaccharide analysis showed that they give very similar results. The Method of Dubois is technically easier to perform than the Method of Anthrone. The colour of the reaction mixture is stable for a longer period allowing more time to measure the absorbance, thus resulting in higher accuracy. Moreover, the Anthrone solution has to be freshly prepared a few hours before the analysis, requiring a new calibration curve to be done with more frequency. **Therefore, this study recommends the Method of Dubois for polysaccharide analysis.**

Note: The Method of Lowry was carried out without the correction for humic substances suggested in Frolund *et al.*, 1995. The value of the correction seemed to depend on humic acids concentration and it is known to vary according with the kind of sample (feedwater, treated water, etc), probably because of the different humic substance composition. This interference is further analysed below.

3.3.4. Modified Method of Lowry for determination of humic substances

In the modified Method of Lowry (Frolund *et al.*, 1995), colour interference due to humic compounds is corrected by measuring samples with and without CuSO₄ addition. Without CuSO₄ addition, the colour development is attributed mainly to humic compounds. Frolund *et al.* (1995) describe a decrease of colour development of 20% for BSA standards when measured without CuSO₄, and no decrease for humic acid standards. The mutual interference of proteins and humic compounds was thus addressed by Frolund *et al.*, 1995 through the following equations:

$$A_{withCu} = A_{protein} + A_{humic} \quad (1)$$

$$A_{withoutCu} = 0.2A_{protein} + A_{humic} \quad (2)$$

So,

$$A_{protein} = 1.25(A_{withCu} - A_{withoutCu}) \quad (3)$$

$$A_{humic} = 1.25A_{withoutCu} - 0.25A_{withCu} \quad (4)$$

These equations were applied in this work to two BSA standard sets of solutions ranging from 0 to 150 mg/L, one without humic compounds (*Protein* in Figure 4) and another with 17 mg/L of commercial humic acid (Fluka, ref. 53680), (*Prot+Humic* in Figure 9). Absorbance values of the Lowry reactions for protein determination with correction ($A_{protein}$ calculated with equation 3) and without correction (direct reading of A_{withCu}) for the two sets of standards are shown in Figure 9.

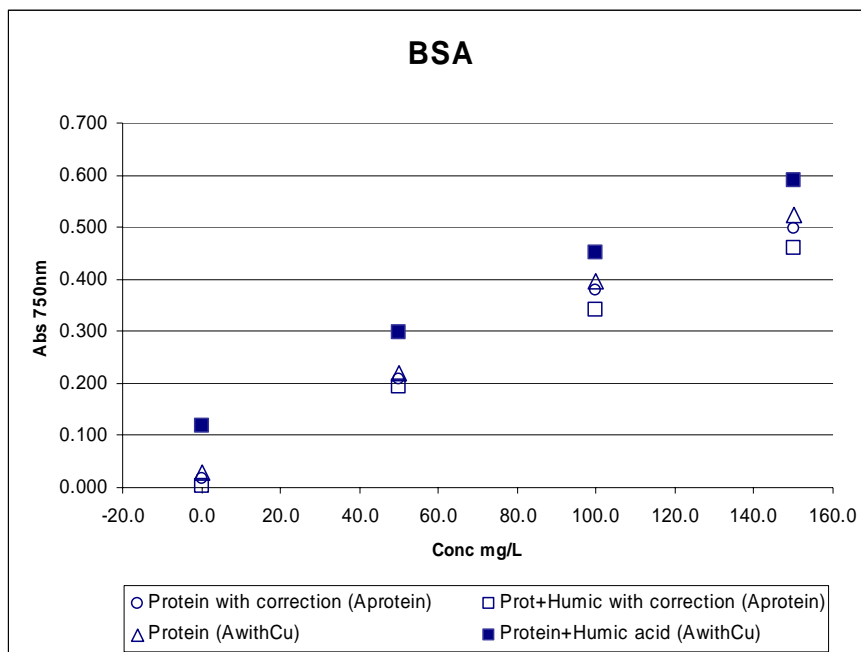


Figure 9 - Protein standards versus Abs at 750nm. Corrections were made using equation (3).

It was expected that corrected absorbance values (A_{protein}) obtained for both sets of standards (*Protein* and *Prot+Humic*) would be equal to the values of BSA standards with no humic acid (blanc, *Protein* - A_{withCu}), since their colour interference due to humic acids is supposed to be deduced through equation 3. However, both sets of corrected values present a deviation in relation to the blank (Figure 9), suggesting that the correction overestimates the interference of humic acids in the Method of Lowry, particularly for higher protein concentrations.

Hence, a new correction factor was calculated in order to make A_{protein} and A_{withCu} coincide. In this experiment colour development by BSA standards in the absence of CuSO_4 decreased to 23.9% instead of 20%. Using 0.239 instead of 0.2 in equation 2, the new correction equations were calculated as:

$$A_{\text{protein}} = 1.314(A_{\text{withCu}} - A_{\text{withoutCu}}) \quad (5)$$

$$A_{\text{humic}} = 1.314A_{\text{withoutCu}} - 0.314A_{\text{withCu}} \quad (6)$$

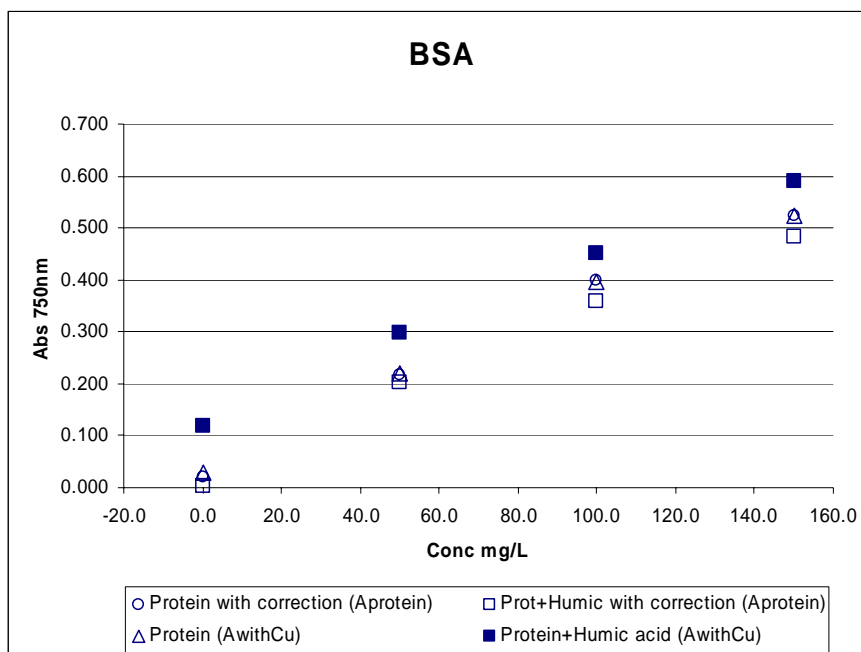


Figure.10 - Protein standards versus Abs at 750 nm. Corrections were made using equation 5.

Figure 10 shows that even with a specific correction, corrected absorbance values (A_{protein}) for samples with protein and 17 mg/L of humic acid are still deviated from the equivalent value for a sample with the same amount of protein but without humic acid. This seems to indicate that correction depends on humic compounds concentration. However our results are only for two different concentrations of humic acid (0 and 17 mg/L), which is not enough to prove this correlation.

Other interferences in the modified method of Lowry for determination of both protein and humic acid content were previously reported. Frolund *et al.*, (1995) tested two types of commercial humic acids, from different suppliers, and found a difference of 12% in the slope of both standard curves. The same authors stated that determination of humic compounds using the Method of Lowry has been subject of debate due to differences in sensitivity. Also in EUROMBRA Deliverable Report – D2, it was highlighted that humic acid corrections can vary depending on the type of spectrophotometer and standard protein used. In that document, a different correction (correspondent to 26% of protein absorbance in the absence of CuSO_4) was found by TU Berlin (AMEDEUS Project) for their specific working conditions.

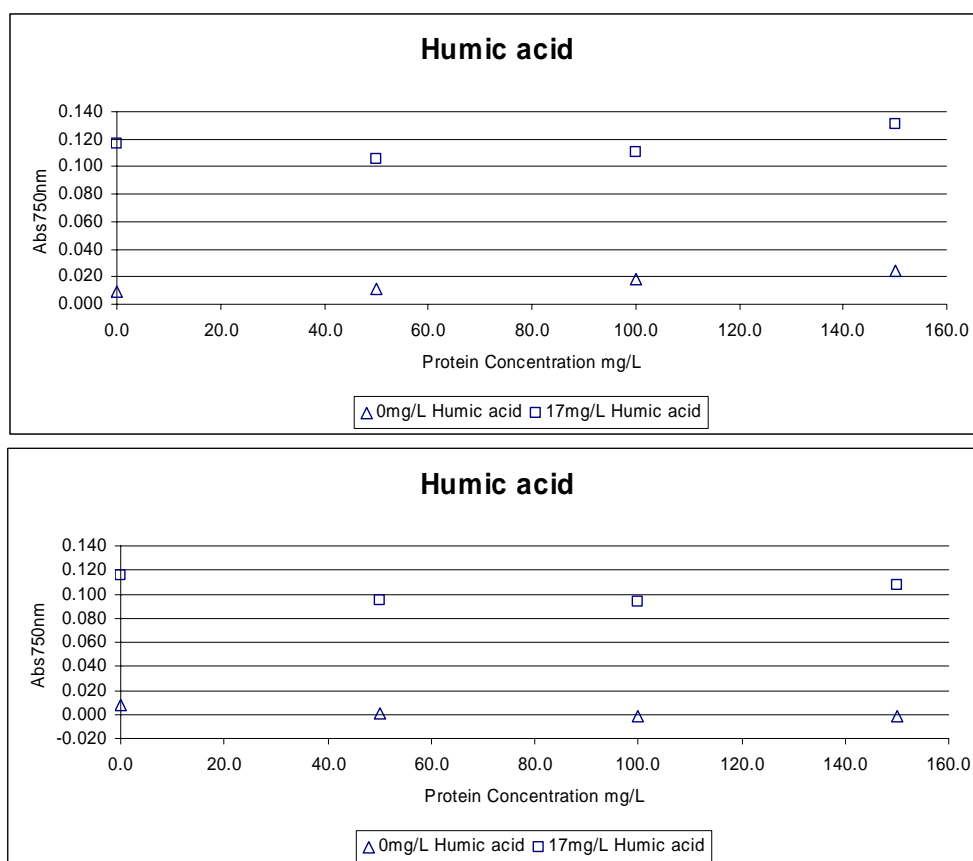


Figure 11 - Calculated humic acid absorbance (A_{humic}) versus protein concentration of each sample. The first graph was calculated using equation 4, and the second was calculated using equation 6.

Figure 11 shows the variation of the calculated humic acid absorbance (A_{humic}) with protein concentration for the two sets of BSA standards, with 0 and 17 mg/L of humic acid. In the first graph, the humic acid absorbance values were calculated with equation 4, while in the second graph, equation 6 was used. Results with the specific correction seem to have less deviation with protein concentration than with equation 4, which means better accuracy. This suggests that **mutual interferences in humic compounds and proteins determination can be decreased with a specific correction for each case. However the main problem consists in doing a proper protein absorbance correction and humic acid calibration using standards, when samples have humic compounds in unknown concentrations and nature.**

3.4. Monitoring of MBR performance with particle size distribution analysis - NTNU

The analytical tools and recommendations outlined in the report will be employed for the analysis and assessment of the BF-MBR concept being studied at NTNU. A laboratory scale pilot plant is currently being operated where various operating conditions and membrane filtration unit design and geometries are being investigated (see WPs 2, 3, 4 and 7 for schematics). The characterization and monitoring techniques covered in this WP are being applied to these studies. Literature reports have identified colloidal material and EPS as dominant and significant foulants in MBR systems. Initial studies with the BF-MBR have shown that **membrane fouling correlates well with particle size distribution (PSD)** analysis where the differential number percent of the submicron particles appears to be a good indicator of fouling potentials. Figure 12 illustrates results where **“Low rate” conditions were found to have lower fouling rates compared to the operating at “High rate” conditions. A question remains though as to what the submicron particles are and how these relate to extracellular polymeric substances (EPS).**

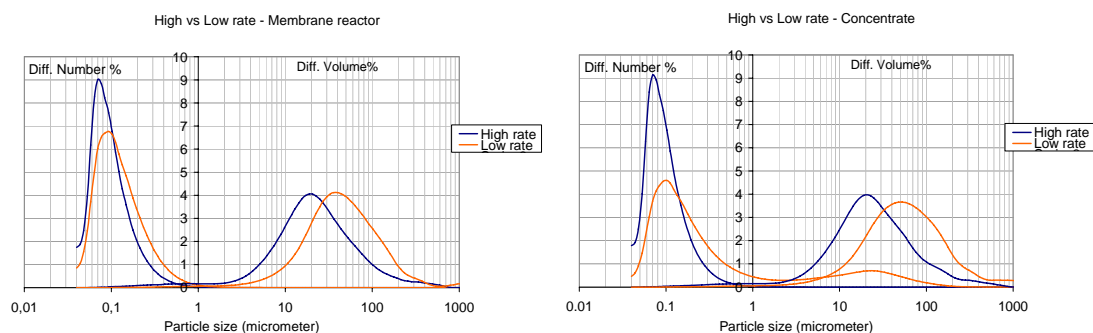


Figure 12. A: Effluent from biofilm reactor B: Concentrate in membrane reactor

Studies are currently being conducted to investigate the effect of EPS on membrane performance and how this relates to PSD analysis results. Initial results where polysaccharides and proteins have been analyzed are shown in the following graphs. Analysis has been done on samples from the inlet water to the treatment train, the effluent from the biofilm reactor, in the membrane reactor (*i.e.* concentrate), and in the permeate. Analysis has been done on filtered samples using a fraction of particles $< 1.2\mu\text{m}$ and $< 0.1\mu\text{m}$ diameters. The distribution of the submicron particles for the samples measured is illustrated in the included graph.

Initial results show the relative distribution of the polysaccharide and protein concentrations measured is quite stable within the operating period in which the sampling and analysis was done, indicating very stable operating conditions. As this is at an initial stage of the investigation there are not sufficient results to draw any conclusive results or interpretations of the findings.

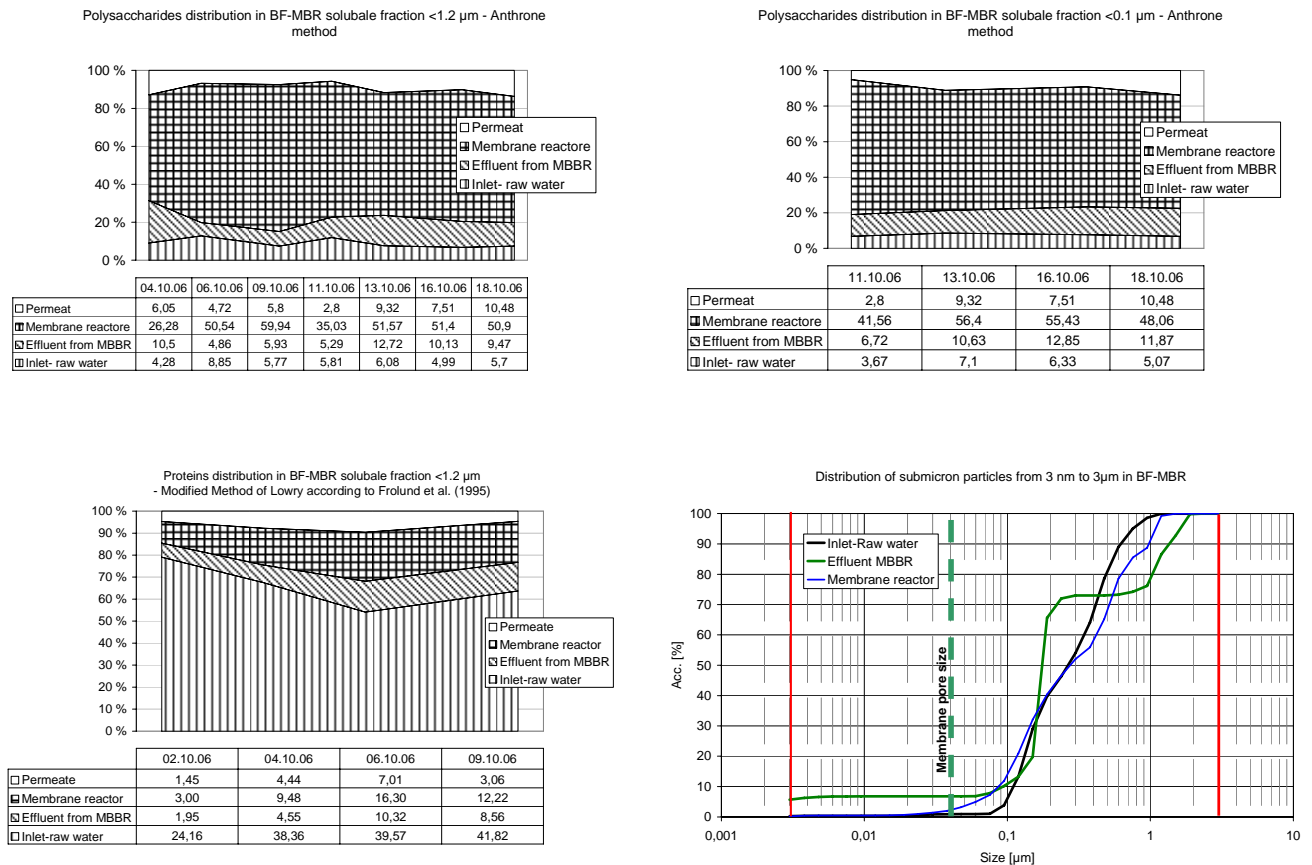


Figure 13. Graphs illustrating initial results of analysis of polysaccharides and proteins during an operating cycle of the BF-MBR process.

3.5. Respirometry as a tool for monitoring feedwater characteristics and biological activity - UNITN

Respirometry was applied at UNITN as a technique to fractionate the feedwater COD and to monitor heterotrophic and autotrophic bacteria in a pilot plant placed at the full scale plant of Lavis (Trento). The pilot facility is equipped with a Huber VRM 20/36 membrane immersed in a suitable membrane tank and fed with pre-screened real sewage (2 mm) pumped from the grit chamber of the full scale plant.

The COD fractionated using respirometric methods (see D3 and D7) gave the results shown in Table 5.

Table 5. Results from COD fractioning by respirometric methods and Standard Methods

PARAMETER	UNIT	VALUE
Total COD	g m^{-3}	562 ± 245
Soluble biodegradable COD	%	19.3
Soluble unbiodegradable COD	%	5.0
Particulate biodegradable COD	%	49.9
Particulate unbiodegradable COD	%	25.8

Once achieved steady state conditions, sludge samples have been collected from both the biological tank and the membrane one and respirometry has been used to investigate the maximum growth rate of heterotrophs and autotrophs, after addition of sodium acetate and ammonia (or nitrite) respectively. Respirometric tests were performed at $20^\circ\text{C} \pm 0.5^\circ\text{C}$ with the experimental setup described in D7 and D3. **Trials on the heterotrophic biomass always showed very evidently the storage phenomenon of biodegradable substrates;** this confirms previous observations on other MBR sludges and will be further investigated and modelled in a deterministic way by means of the ASM3 model, which assumes biomass growth to take place only on stored substrate.

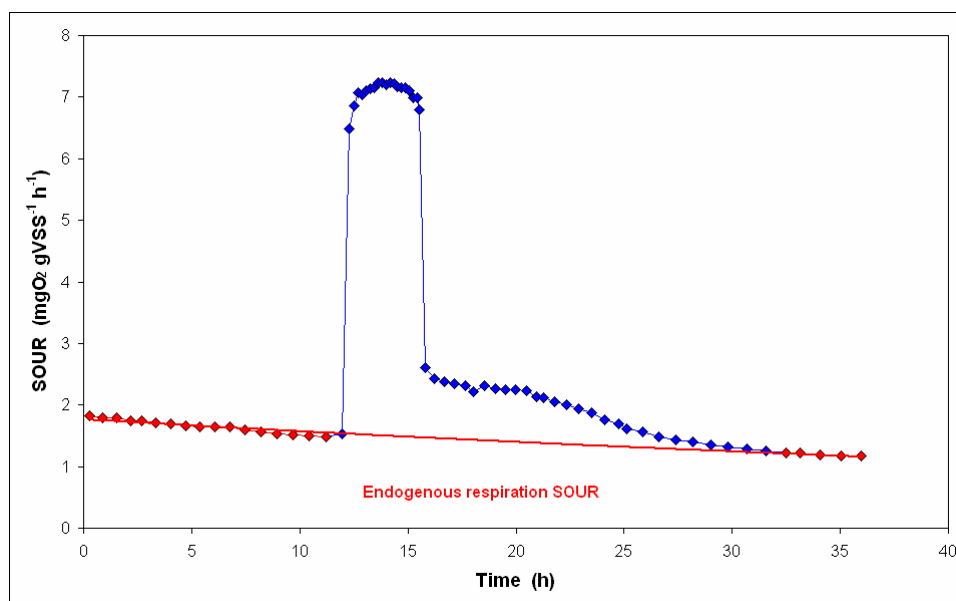


Figure 14. Endogenous and exogenous SOUR of heterotrophic biomass ($S_0/X_0 = 0.07 \text{ gCOD gVSS}^{-1}$; ATU added)

Tests carried out on autotrophic biomass gave maximum growth rate of nitrifiers lower than the conventional activated sludge, with values of μ_{n20} ranging between 0.07 and 0.15 d^{-1} . Furthermore, **the respirometric tests on sludge sampled always presented a measured value of the dissolved oxygen consumption significantly lower than the stoichiometric one, thus suggesting that non-conventional ammonia nitrogen removal mechanisms could have taken place in the membrane**

tank, where a lower dissolved oxygen concentration was achieved due to the lower oxygen transfer efficiency of the coarse bubble aerator. This is also confirmed by the nitrogen mass balance data shown in the WP4 yearly report. If further confirmed, such aspect could play a crucial role in process optimisation in terms of energy saving for biological process aeration.

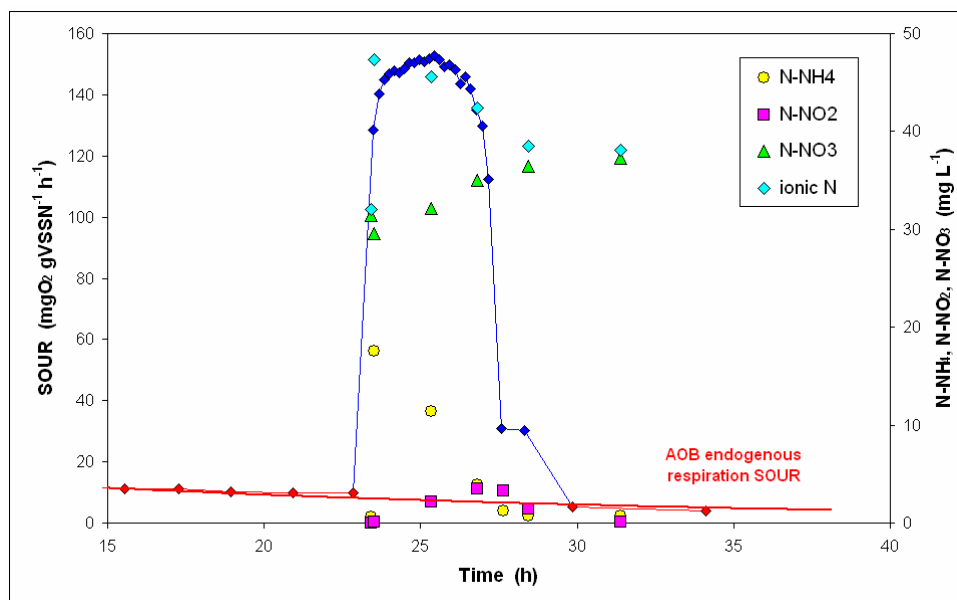


Figure 15. Autotrophic SOUR with track-study for ionic forms of nitrogen (19.2 mg of $\text{NH}_4\text{-N}$ added in the 1.4 L respirometer with biomass concentration of 2.2 gVSS L^{-1})

Since October 2006 a new large scale pilot plant (overall volume: 15 m^3) equipped with a 3-modules array Zenon ZW500d has been started up at the same full scale facility in Trento; differently than the above-described, pre-denitrification process is available.

4. Implementation of characterisation and monitoring techniques on MBR installations

4.1. Delft University of Technology Filtration Characterisation InstallationTM for determination of relationships between filterability characteristics and full-scale permeability data of MBR Varsseveld

4.1.1. Introduction

For research into membrane fouling and feedwater characterisation, the section of Sanitary Engineering from the Delft University of Technology has developed the Delft University of Technology Filtration Characterisation InstallationTM (DFCITM) and an accompanying measuring protocol. This deliverable for workpackage V of the EUROMBRA project focuses on the introduction of the DFCITM and its application for feedwater and activated sludge characterisation in MBR fouling research.

4.1.2. Material and Methods

Delft University of Technology Filtration Characterisation Installation™

The Delft University of Technology Filtration Characterisation Installation™ is represented schematically in Figure 16 and described in detail by Evenblij (2003). The DFcI™ is not intended as a lab-scale MBR installation aiming at imitating the MBR process on a long term, but is a measuring tool for short-term assessment of the fouling properties of a given MBR activated sludge sample. The heart of the installation is formed by a single sidestream ultrafiltration membrane tube (X-flow, diameter 8 mm, nominal pore size 0.03 µm). A peristaltic pump is used to recirculate the sludge; cross-flow velocity is adjustable. Permeate is extracted with another adjustable peristaltic pump, so the flux can be regulated. With the DFcI™ it is possible to filtrate MBR activated sludge samples collected under different circumstances or from different MBR installations under identical circumstances.

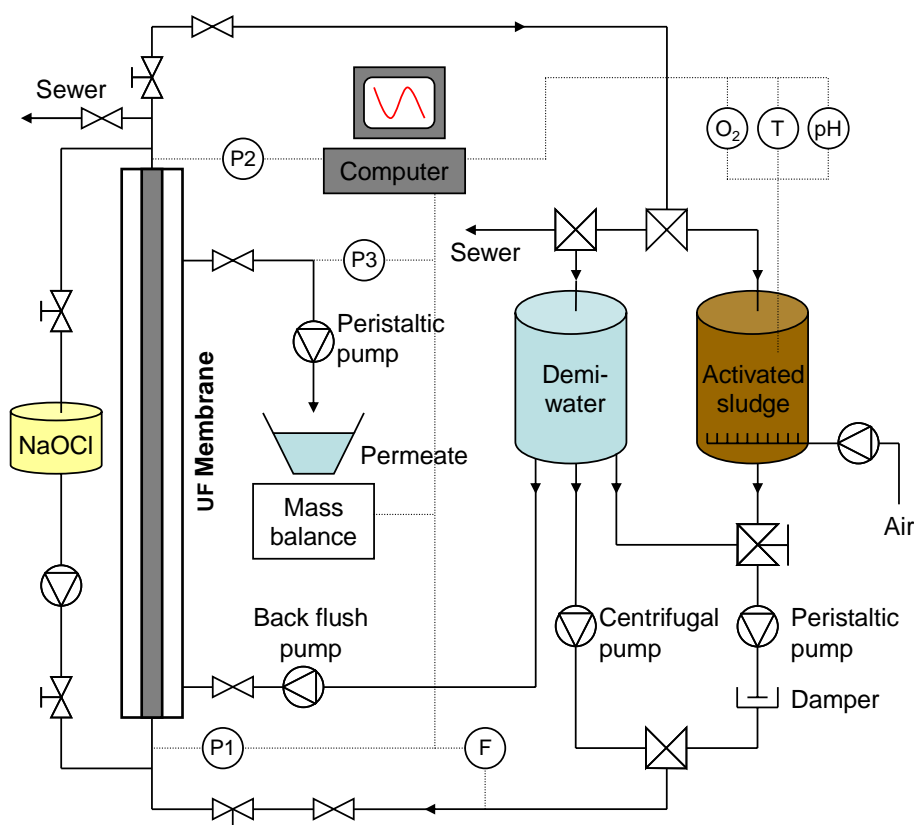


Figure 16 - Schematically representation of the Filtration Characterisation Installation (DFcI™)

An important issue is how the filterability measured with the DFcI™ relates to the functioning of the membranes of the full-scale installation in practice. A number of factors which could complicate the interpretation of the filtration characteristics produced by the DFcITM can be pointed out:

- The membrane configurations of the DFcI™ and MBR Varsseveld are different; while the DFcI™ is equipped with an external (inside-out) membrane the full-scale MBR has submerged (outside-in) membranes. This implies that the shear created tangential to the membrane surface to prevent fouling is different for both systems. In the full-scale installation shear is

created by coarse bubble aeration, while in the DFcI™ shear is created by means of cross-flow velocity of the fluid in the membrane tube.

- To prevent fouling in the full-scale installation the membranes are usually operated under low subcritical fluxes, while in the DFcI™ fouling is forced by applying high supercritical fluxes.
- The difference between the type/brand of the membranes in the DFcI™ and the full-scale MBR can be mentioned. However, the difference between the pore size of the membranes in the DFcI™ (X-flow, nominal pore size 0.03 µm) and the full-scale installation (Zenon, nominal pore size 0.04 µm) is negligible.

In previous research it was presumed that the difference between external and submerged membrane configuration are comparable, because for both configurations the fouling mechanisms are identical. This is confirmed in research by Le-Clech c.s. (2005).

Also the difference between the full-scale installation and the DFcI™ concerning the applied fluxes does not seem problematic. Experiments with the DFcI™ show that increasing the flux quickens the fouling process, but not essentially changes it. To analyse the potential effect of different types of membranes in future research other membranes besides X-flow membranes could be fitted in the DFcI™.

Filtration characterisation experiments

In the period between January 31st and October 25th of 2005 twelve feedwater characterisation experiments were conducted with activated sludge sampled from the full-scale MBR of WWTP Varsseveld (Rhine and IJssel Water Board, The Netherlands) to gain understanding of the fouling properties of the sludge.

In order to obtain unequivocal results a standard measuring protocol was formulated, consisting of three steps:

- a) Clean Water Resistance determination. Demineralised water is recirculated with a cross-flow velocity of 1.0 m/s and filtrated with a flux of 80 L·m⁻²·h⁻¹ to verify whether the membrane is clean prior to sludge filtration. Clean water resistance should be around 0.5·10¹² m⁻¹.
- b) Sludge filtration, the actual experiment. A sample of about 30 L is poured into the activated sludge vessel en kept in suspension by stirring and/or aeration. The sample is recirculated with a cross flow velocity. Permeate can be extracted with any desired flux in between 0 and 160 L·m⁻²·h⁻¹.
- c) Membrane cleaning. After sludge filtration the membrane has to be cleaned. For this (a combination of) three methods can be used:
 - Forward flush with demineralised water (cross-flow velocity about 4 m·s⁻¹, 30 seconds)
 - Back flush with demineralised water (TMP about -0.6 bar, 30 seconds)
 - Chemical cleaning, soaking with NaOCl (500 ppm, 20 minutes)

After the membrane cleaning the clean water resistance is determined again to verify whether the membrane has been cleaned properly. If not supplementary more intensive chemical cleaning (1,500 ppm NaOCl) will be applied.

In previous research (Evenblij, 2005) the DFcITM was stationed at four different WWTPs with operational MBR pilots (Maasbommel, Hilversum, Beverwijk, Varsseveld). The results of the filtration characterisation experiments proved significant differences between the four MBRs. Besides this, according to the findings of the operators, it appeared that when the pilots (permeability) functioned well also the results with the DFcITM showed good filterability of the sludge. On the other hand, when problems with the filterability in the pilot installation were encountered, also the filterability in the DFcITM was bad. However, attention for relating full-scale permeability to the filtration characterisation results has been limited so far and is therefore desirable.

4.1.3. Results

Filtration Characteristics

All sludge samples have been filtrated with the DFcITM under identical circumstances, namely with a cross-flow velocity of $1.0 \text{ m}\cdot\text{s}^{-1}$ and a flux of $60 \text{ l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$. Figure 17 represents the additional resistance induced by the occurring fouling versus the produced permeate volume (litre per square meter membrane surface).

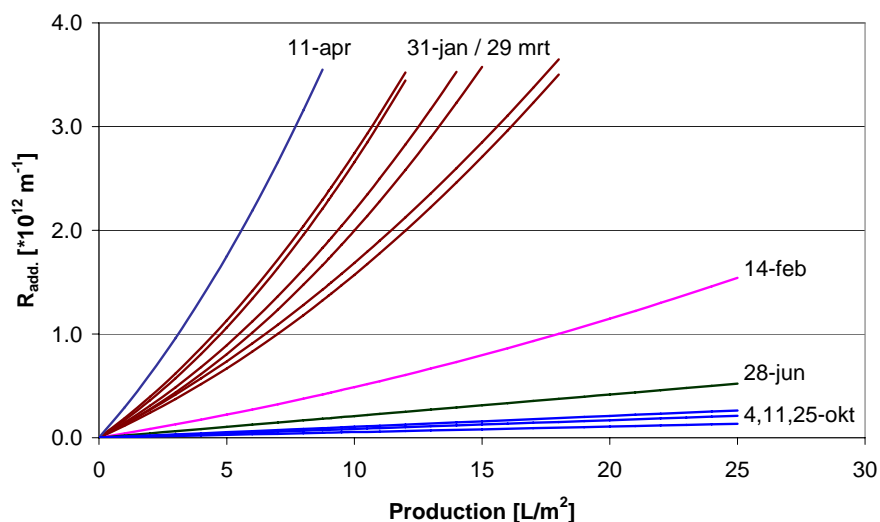


Figure 11 - Filtration curves sludge membrane tank, $J=60 \text{ l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ en $\text{CFV}=1.0 \text{ m}\cdot\text{s}^{-1}$

In the first months after the biological start-up of the full-scale MBR major problems were experienced concerning the filterability of the sludge/water mixture. Soon it was suspected that the wastewater stream from a local cheese factory was the malefactor. This wastewater contained the substance PVA (poly vinyl acetate), which is used to cover the cheese with a plastic-like coat. It appeared that this PVA was responsible for sticking of the membranes. To put a stop to the PVA problems it was decided to uncouple the cheese factory from the sewer (as from the 9th of May) and to transport this wastewater to another (conventional) WWTP.

The uncoupling of the cheese factory resulted in a tremendous improvement of the filterability measured with the DFcITM. In the period between the end of January until mid April (January 31st, February 21st and 28th, March 8th, 14th, 29th and April 11th) filterability is quite constant and can be qualified as “bad”. The only relatively positive exception is formed by the measurement of February 14th. The first experiment after uncoupling the cheese factory (June 28th) shows a major

improvement of the filterability. This improvement perseveres in the following period, given the results from the three measurements in October.

Analysis permeability data

The filtration characterisation results are evaluated more in detail in combination with the permeability data of the full-scale plant. Permeate extraction in the full-scale MBR takes place in four separate membrane tanks; permeability is monitored separately for each tank. In practice permeability values differ somewhat between the tanks, caused by slight differences concerning the hydraulic circumstances in the tanks. Nonetheless, over the whole period the permeability trend in the four tanks can be considered equal. Differences between the membrane tanks are left out of consideration, because the (filtration) quality of the sludge can be considered similar for all membrane tanks.

Figure 18 represents the permeability trend in one of the four membrane tanks (MT2) over a period of about 11 months. Besides the permeability also the moments of membrane cleaning and sampling for the DFcITM experiments are indicated. A clear difference manifests between the period before and after the cheese factory was uncoupled from the system. In the first months the permeability shows a decreasing trend. After the uncoupling of the cheese factory permeability stays more or less stable.

It is important to mention that the permeability itself does not necessarily provide information about the filterability quality of the sludge. A high permeability of the membrane does not imply good filterability of the sludge, because besides the sludge quality also the cleaning measures and operation of the membranes are affecting the permeability. A good example of this is the situation on April 11th. As a result of an intensive cleaning the permeability recovered from about 300 to 450 l·m⁻²·h⁻¹·bar⁻¹. However, this IC did of course not change the sludge quality. Ergo, the decreasing trend in the permeability encountered in the first months endures.

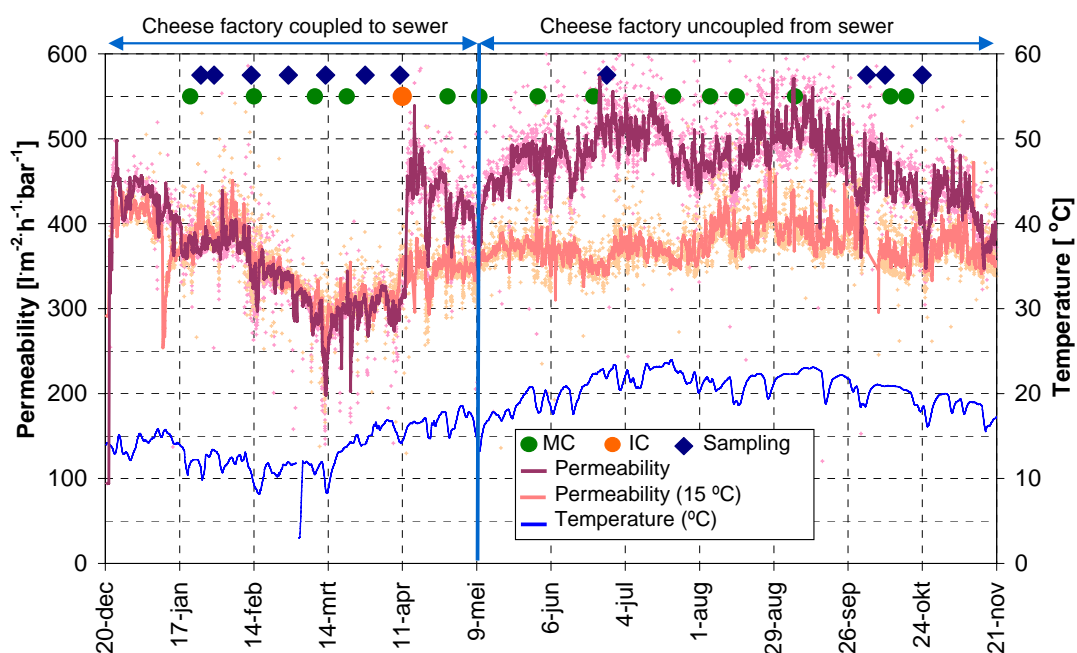


Figure 18 - Permeability, cleaning- and sampling moments: 20 December 2004 to 21 November 2005

January 31st

The first filterability experiments took place on January 31st. As can be derived from Figure 17 the filterability that was measured with the DFcITM can be qualified as “bad”. The permeability data of the full-scale MBR also indicate a troublesome filterability, see Figure 19. In the first few hours of the day permeate was extracted with a relatively modest flux of 20 l·m⁻²·h⁻¹. Nevertheless, even with this flux clear decrease of the permeability occurs. In the evening hours a flux of about 33 l·m⁻²·h⁻¹ was applied which immediately results in an even faster decrease of the permeability.

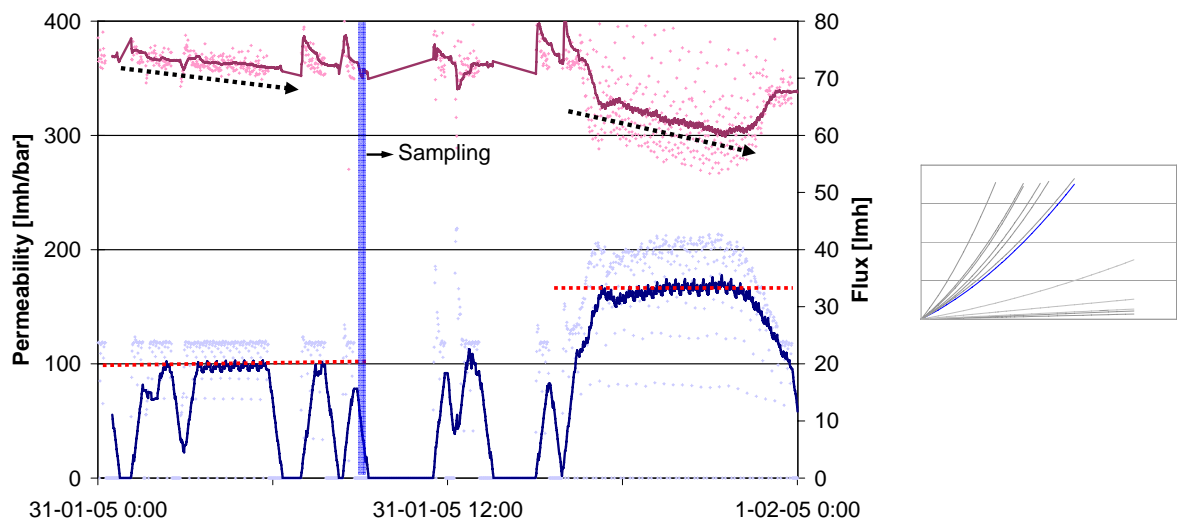


Figure 19 - Permeability and flux on January 31st 2005 (MB4)

February/March

The problems with the filterability persist during the first months after the start-up of the full-scale MBR. Also the filterability measured with the DFcITM stays bad. As an example the situation in the period around March 14th is analysed, see Figure 20. Permeability is low and when the flux is increased to values over 20 l·m⁻²·h⁻¹, permeability immediately drops.

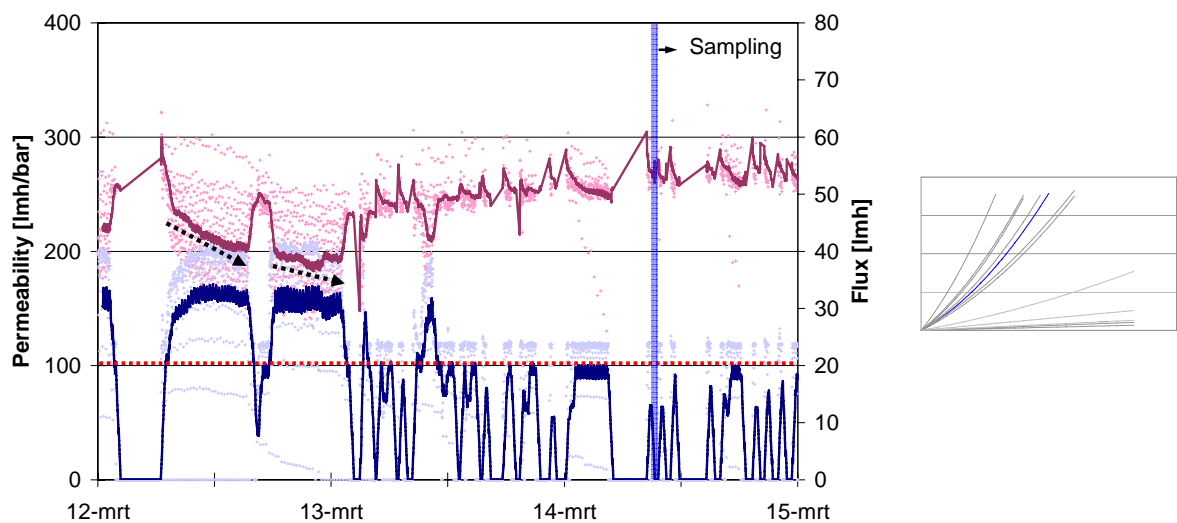


Figure 20 - Permeability and flux in the period around March 14th (MB1)

April 11th

The worst filterability measured with the DFcI™ was encountered on April 11th 2005, see Figure 17. The major problems with the filterability also come to light in the full-scale permeability data. Permeability values in all tanks have dropped to disconcerting levels (<400 lmh/bar). In this period under DWF conditions all four membrane tanks are in operation continuously to cope with the required flow. The applied flux is very low, approximately 17 l·m⁻²·h⁻¹. Increasing the flux directly leads to a severe permeability drop, see Figure 16. During this period the entire functioning of the MBR was in danger, as it was not possible anymore to cope with RWF conditions. This measurement was the last one before the cheese factory was uncoupled from the sewage.

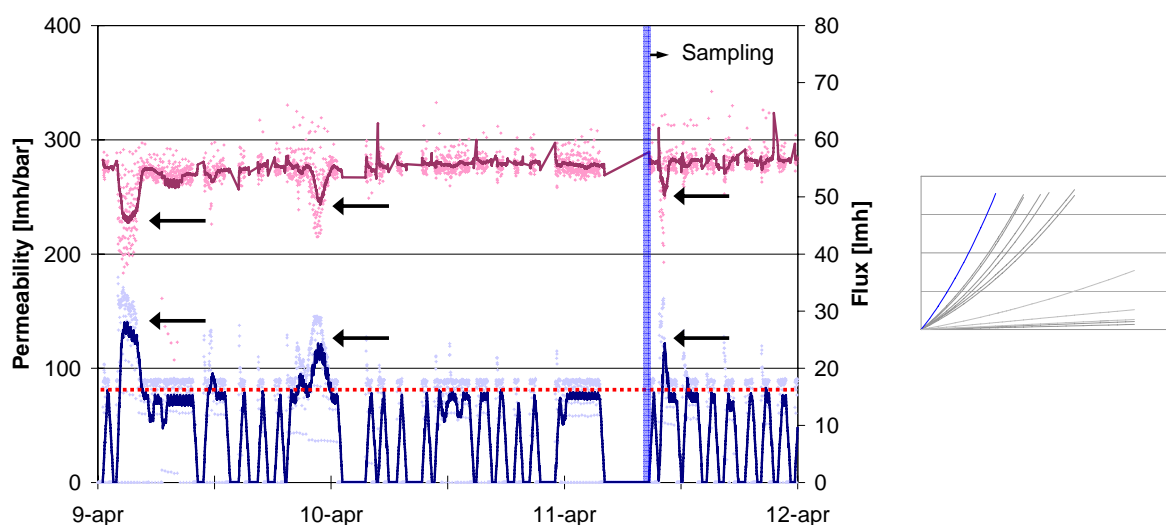


Figure 16 - Permeability and fluxes in the period around April 11th (MB3)

February 14th

As can be derived from Figure 17 the filtration curve of February 14th forms a relatively positive exception compared to the other filtration curves gathered in the first few months after the start-up. Figure 17 represents the permeability and the applied flux in one of the tanks in the period around February 14th. Data for the other tanks are comparable with this tank. On February 13th almost continuously permeate was extracted with high fluxes (± 35 l·m⁻²·h⁻¹). As a result of this the permeability shows a decreasing trend.

In the night and early morning of February 14th an interesting event takes place. For unknown reasons during this period hardly any permeate extraction takes place; sludge and wastewater are only recirculated within the system. After resumption of the filtration some momentarily recovery of the permeability occurs, even while the applied flux is still relatively high. This would mean that as a result of the extension of the hydraulic retention time the sludge/water mixture obtains a relatively good (filterability) quality. After a few hours the decreasing trend of the permeability returns.

By coincidence sampling for the experiments with the DFcI™ took place directly after the long period in which no permeate had been extracted. The relatively good filterability measured with the DFcI™ is likely the direct result of the internal recirculation period in the full-scale installation.

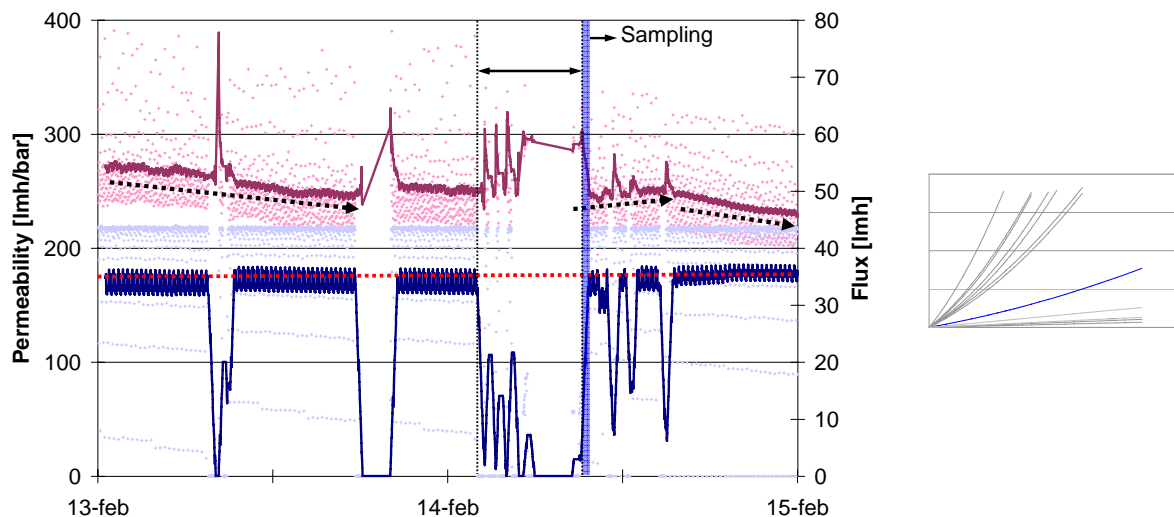


Figure 17 - Permeability and flux in the period around February 14th (MB2)

June 28th

The first experiments after uncoupling the cheese factory from the sewer took place on June 28th. Filterability measured with the DFcI™ showed a spectacular improvement compared to the previous experiments, see Figure 17. The period around June 28th is characterised by dry weather circumstances with high temperatures. Total flow of the MBR is low in this period ($130 \text{ m}^3 \cdot \text{h}^{-1}$ on average). The applied fluxes in the full-scale MBR are low so not much interesting information can be derived from the permeability data.

Figure 18 represents the permeability and flux for one of the tanks during the period around June 28th. Permeability has recovered ($>400 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1} \cdot \text{bar}^{-1}$) since the uncoupling of the cheese factory. Only on one occasion the flux was temporarily increased to a value higher than $20 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ (June 27th, 12 'o clock). This is already an indication that permeability is recovering; the flux increase to a value over $30 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ has no effect on the permeability.

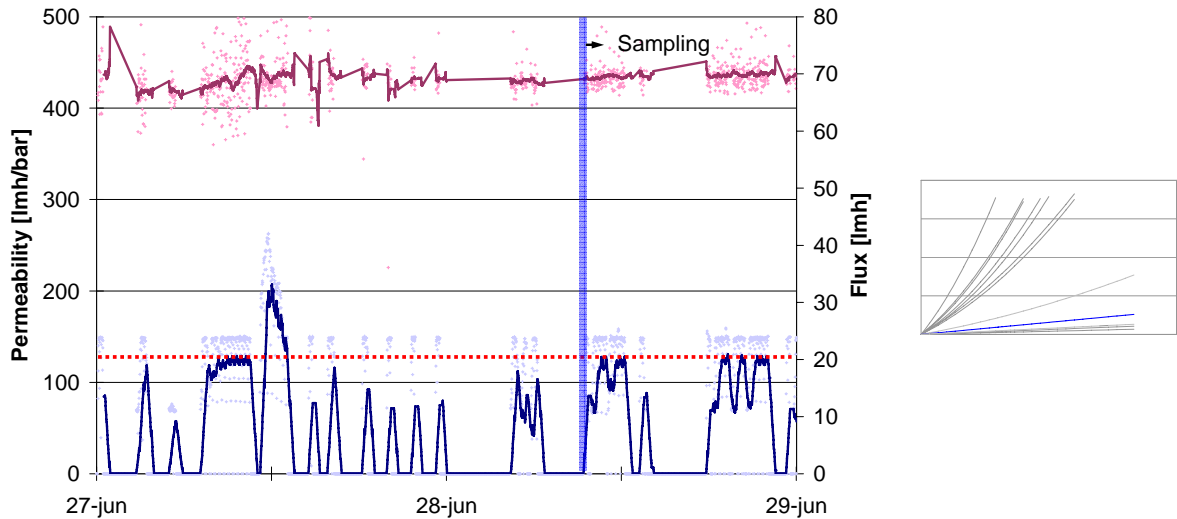


Figure 18 - Permeability and flux in the period around June 28th (MB3)

October 4th

The last experiments took place in October. Filterability measured with the DFcITM is excellent, and has even improved somewhat compared to the experiment at the end of June. By chance at the moment of sampling the Water Board was executing a capacity test with one of the membrane tanks. Figure 19 represents the applied flux and the permeability in the concerned tank. Results show that the permeability is hardly influenced by the continuous high flux (approximately 35 l·m⁻²·h⁻¹). The excellent filterability that was measured with the DFcITM is again in accordance with the permeability data monitored for the full-scale installation.

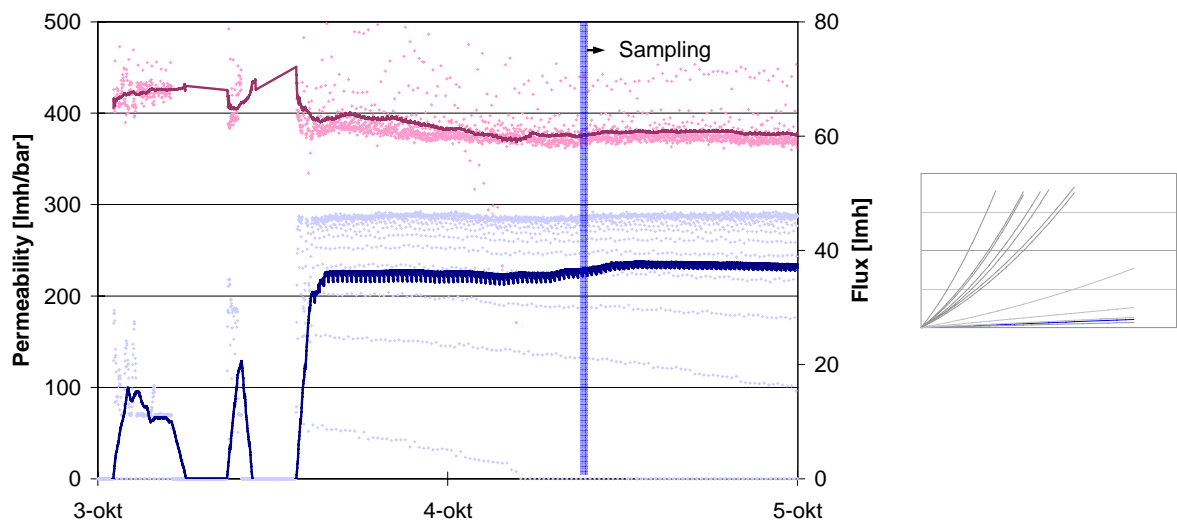


Figure 19 - Permeability and flux in the period around October 4th (MB3)

4.1.4. Conclusions

The filterability measurements conducted with the Filtration Characterisation Installation (DFCI™) show a clear split in filterability before and after the uncoupling of a local cheese factory from the sewage discharging to MBR Varsseveld. This split is in accordance with the permeability data monitored by the Water Board Rhine and IJssel.

Besides this, also more subtle changes in the sludge quality seem to be demonstrated by Filtration Characterisation. On February 14th the usual operation of the membranes was interrupted. This resulted in an improvement of the permeability of the full-scale installation. This improvement was clearly backed up by the results of the filtration characterisation experiments.

It can be concluded that Filtration Characterisation offers a good method to assess the filterability of a given activated sludge sample tapped from a pilot- or full-scale MBR installation with a short term experiment.

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ANNEX A

Methods for EPS extraction compared in this study

Method 1 - Sperandio et al, 2005

Chemicals and reagents

- Buffer: 2 mM Na₃PO₄, 4 mM NaH₂PO₄, 9 mM NaCl, 2 mM KCl
- Cation exchange resin (CER): Dowex^RMarathon^R (91973 Sigma Aldrich) Na⁺ form, 20-50 mesh

Extraction protocol

- Centrifuge sludge sample at 4500 rpm for 15 minutes at 4°C
- Remove the supernatant (= soluble EPS)
- Re-suspend the sludge pellet to its original volume using the buffer solution
- Disperse this sludge solution by ultrasound at 37 W for 20 s at 4°C
- Transfer the sludge sample to an extraction vessel and add the cation exchange resin in a proportion of 70 g per VSS
- Stir the suspension for 45 min at 500 rpm and 4°C
- Allow the CER to settle for 5 min and centrifuge the supernatant at 20,000 g for 15 minutes at 4°C
- Remove the supernatant ('extract' = bound EPS)

Method 2 - Frolund et al., 1995 and 1996

Chemicals and reagents

- Buffer (pH 7): 2 mM Na₃PO₄, 4 mM NaH₂PO₄, 9 mM NaCl, 1 mM KCl
- Cation exchange resin (CER): Dowex^RMarathon^R (91973 Sigma Aldrich) Na⁺ form, 20-50 mesh

Extraction protocol

- Weigh CER : 70-75 g per VSS of the sludge sample
- Equilibrate the CER for 1 h in the buffer solution
- Centrifuge a 200 mL sludge sample at 10,000 g for 15 minutes at 15°C
- Remove the supernatant (= soluble EPS)
- Re-suspend the sludge pellet in 200 mL of buffer solution
- Centrifuge again at 10 000 g for 15 minutes at 15°C
- Remove the supernatant ('washings' = loose-bound EPS)
- Re-suspend the sludge pellet to their original volume in buffer solution
- Transfer 200 mL of the washed sludge sample to an extraction vessel and add the equilibrated cation exchange resin (70-75 g per VSS)
- Stir the suspension for 2 hours at 600 rpm
- Allow the CER to settle and centrifuge the supernatant at 10,000 g for 15 minutes at 15°C.
- Remove the supernatant ('extract' = cell-bound EPS)

ANNEX B

Methods for protein and polysaccharide measurement used in this study

Method of Lowry

Equipment

- Spectrophotometer

Reagents

- Solution A: Na_2CO_3 , 2g + Tartrate of NaK, 0,02g + NaOH (0.1 N), 100mL.
- Solution B: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.5g) + H_2SO_4 (1 drop) + H_2O (10mL).
- Solution C: Reagent A (50 mL) + Reagent B (1 mL).
- Solution D: aqueous solution of Folin with a dilution 1:2.

Procedure

1 mL of sample is mixed with 3 mL of reagent C and allowed to react for 10 minutes. 0.3 mL of solution D is then added to this mixture. After 30 minutes, absorbance of this solution is measured with a spectrophotometer at 750 nm.

Bovine serum albumin (BSA) was used as standard for method calibration. The concentration range used was 0 to 200 mg/L. The absorbance/concentration curve was found to be non-linear for protein concentrations higher than 100 mg/L.

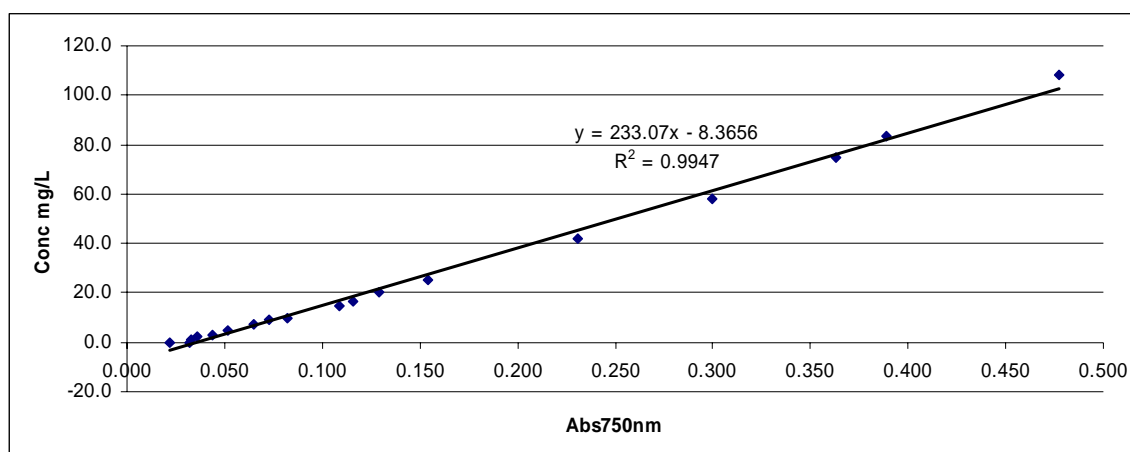


Fig.B.1- Method of Lowry for protein analysis: calibration curve using bovine serum albumine (BSA).

Method of Dubois

Equipment

- Spectrophotometer

Reagents

- Phenol 5% (w/w) in water
- H₂SO₄ conc.

Procedure

1 mL of 5% phenol in water is added to 1 mL of sample. Then 5 mL of concentrated sulphuric acid is added rapidly, on the liquid surface. The mixtures are allowed to react for 10 min, then mixed again and allowed to react for another 30 min at room temperature. Absorbance is measured at 490 nm. The colour is stable for several hours.

D-glucose monohydrate was used as a glucose standard for method calibration, in a concentration range between 0 and 200 mg/L.

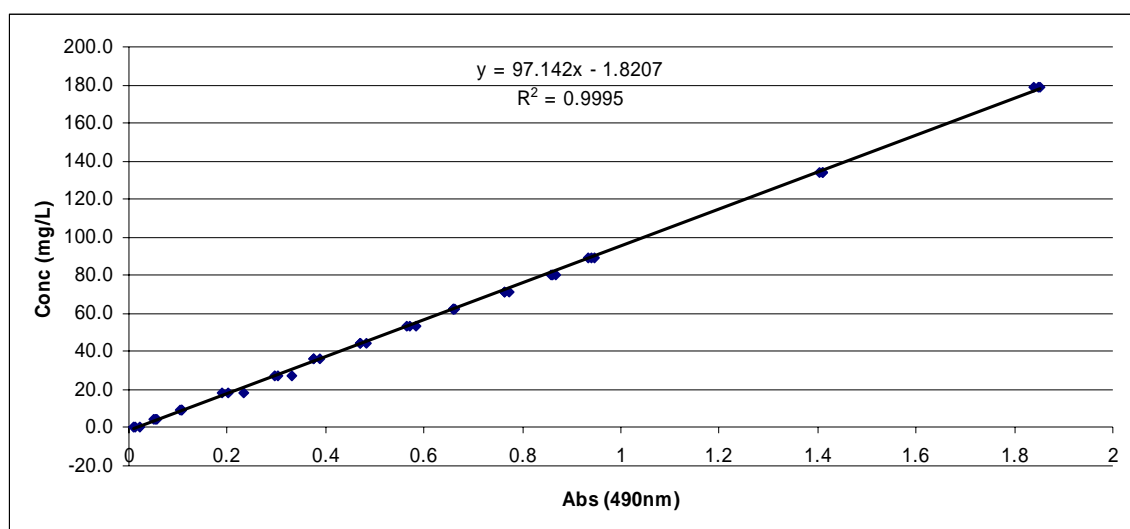


Figure B.2- Method of Dubois for polysaccharide analysis: calibration curve using D-glucose monohydrate.

Method of Anthrone

Equipment

- Spectrophotometer
- Sonicator
- Centrifuge

Reagents

- Anthrone solution: 0.2 g of Anthrone and 100 mL of H₂SO₄
This solution is prepared 4 hours in advance.

Procedure

1 mL of sample is added to 2 mL of Anthrone solution in a tube which is kept in ice. The tube is heated to 100°C for 10 minutes, after which the reaction is stopped by putting the tube back in the ice. Polysaccharides are then measured by analysing the absorbance at 625 nm with a spectrophotometer.

D-glucose monohydrate was used as standard to calibrate the method, in a concentration range of 0 to 100 mg/L.

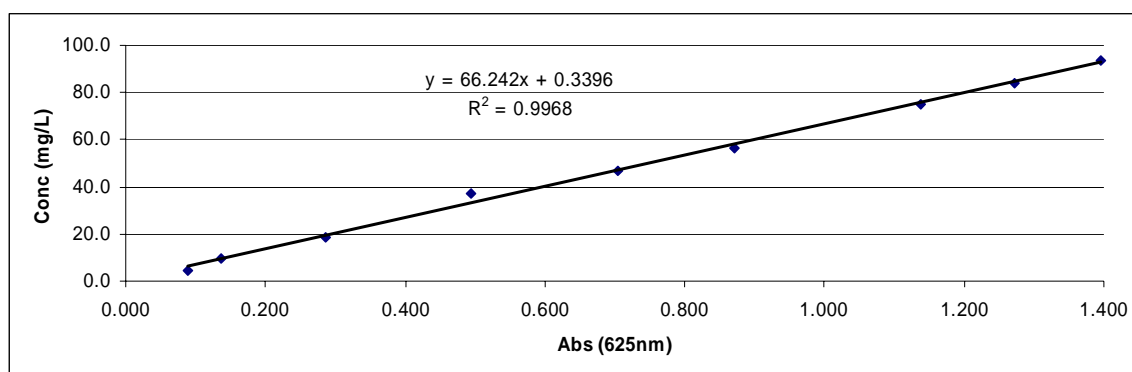


Figure B.3- Calibration curve for polysaccharide analysis using the method of Anthrone. Glucose was used as standard.

ANNEX C

Delft Filtration Characterisation Unit

C.1. INSTALLATION MAJOR PURPOSES

The mobile test Unit developed by Delft University of Technology is a tool which characterizes sludge fouling tendencies. Filtrations of different full scale MBR sludges with the test Unit enable a database formation.

This database can be used as a comparison starting point.

The test unit can also be combined with batch tests. Dynamic changes can be investigated by manipulating the sludge (30L sample) and by monitoring differences in sludge fouling behaviour. Impacts of substrate, pH, aeration, dilution factor were already examined.

The Unit can also be useful during starting up or optimization of full scale MBR plants. Indeed monitoring the sludge characteristics during a long term period can provide information about the sludge behaviour and evolution. Then actions can be taken quickly.

C.2. REQUIREMENTS ON SITE

There is not any specific requirement depending on the location of the MBRs (pilots or full scale plants). The Test Unit only needs some 220V power supply and a sewage discharge.

However, water analysis facilities should be supplied by the host / partner university (EPS, MLSS, SVI, turbidity).

C.3. MAJOR RESULTS

Fouling tendencies observed during batch test experiments are presented below.

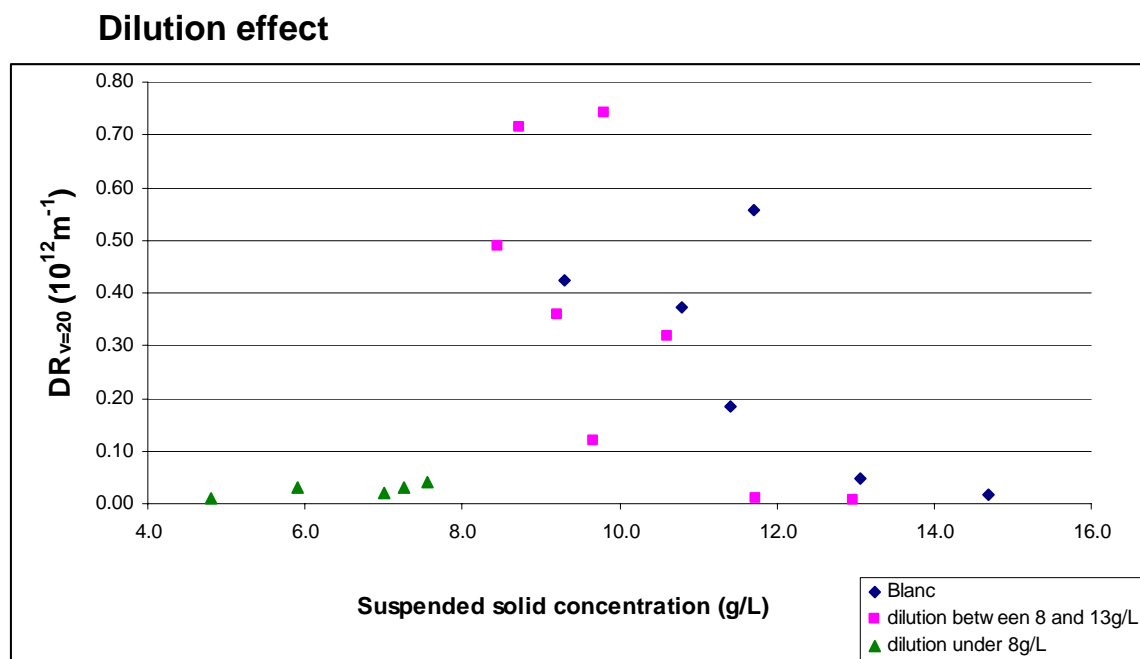


Figure C. 1: Impact of the dilution effect on filterability

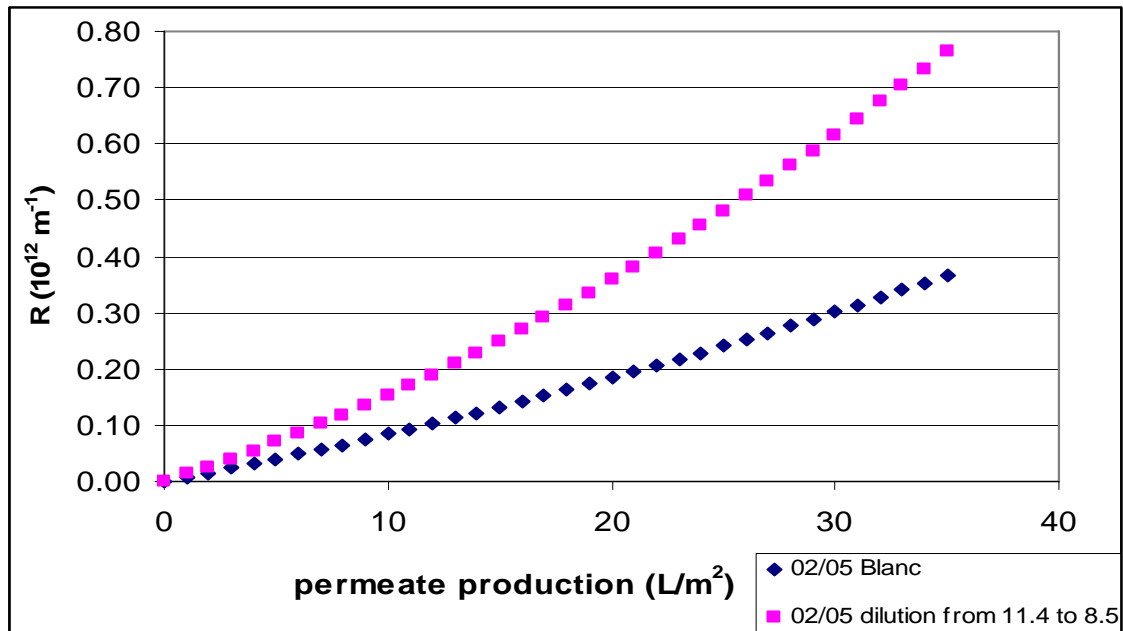


Figure C. 2: Example of filterability curve

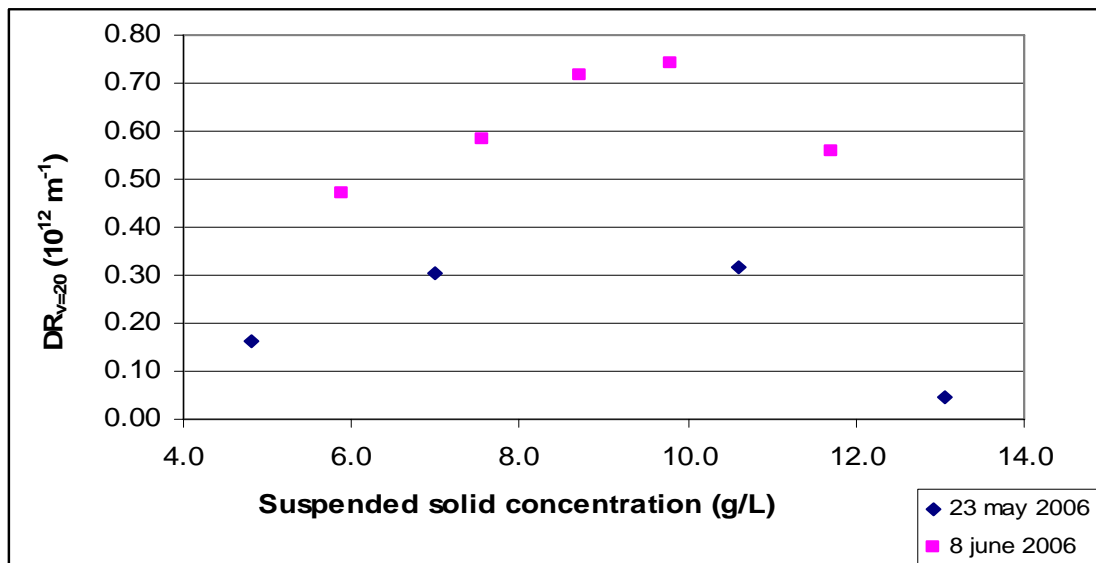


Figure C. 3: Typical behaviour obtained during dilution

Figure C.1 represents the filterability ($DR_{v=20}$ is the value of the membrane total resistance after $20L/m^2$ of permeate extraction) depending on the suspended solid concentration. From blanc measurements (without dilution samples), resistance seems to decrease when the suspended solid concentration increases, Moreover figures C.1 to C.3 show that the filterability gets worse when the final dilution concentration is between 7 and 11g/L. Links with other parameters (EPS, temperature, particle size distribution) will be developed in further studies.

pH effect

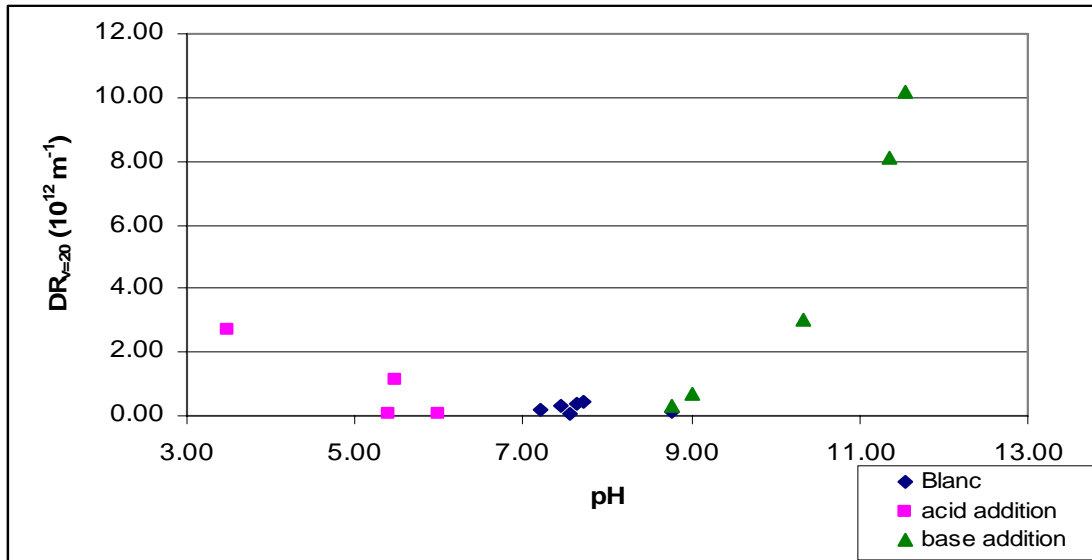


Figure C. 4: Impact of pH change on filterability

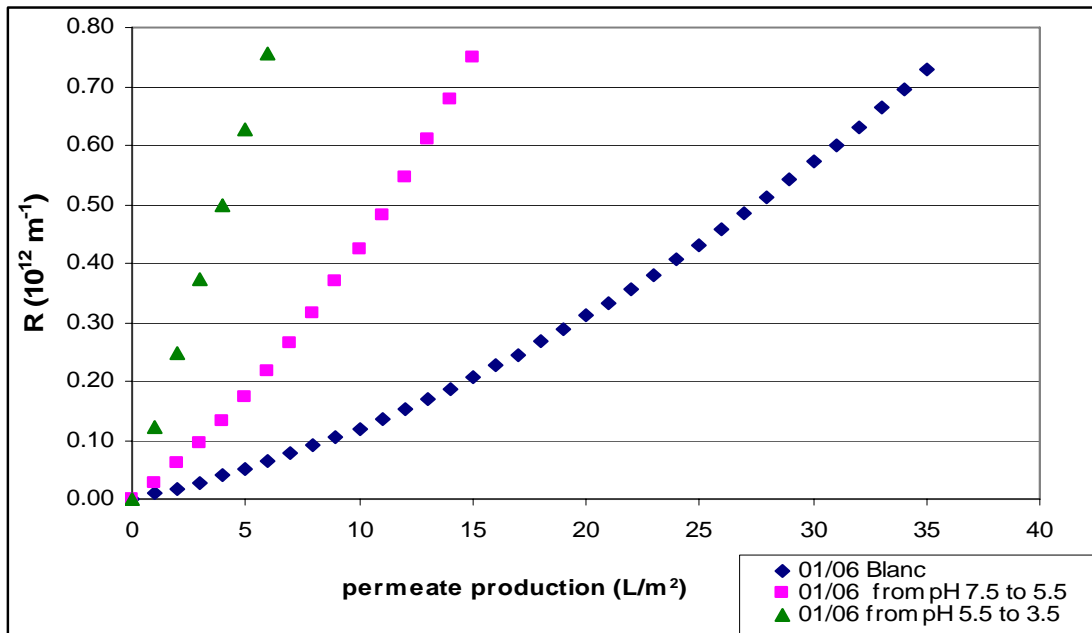


Figure C. 5: Example of filterability curve

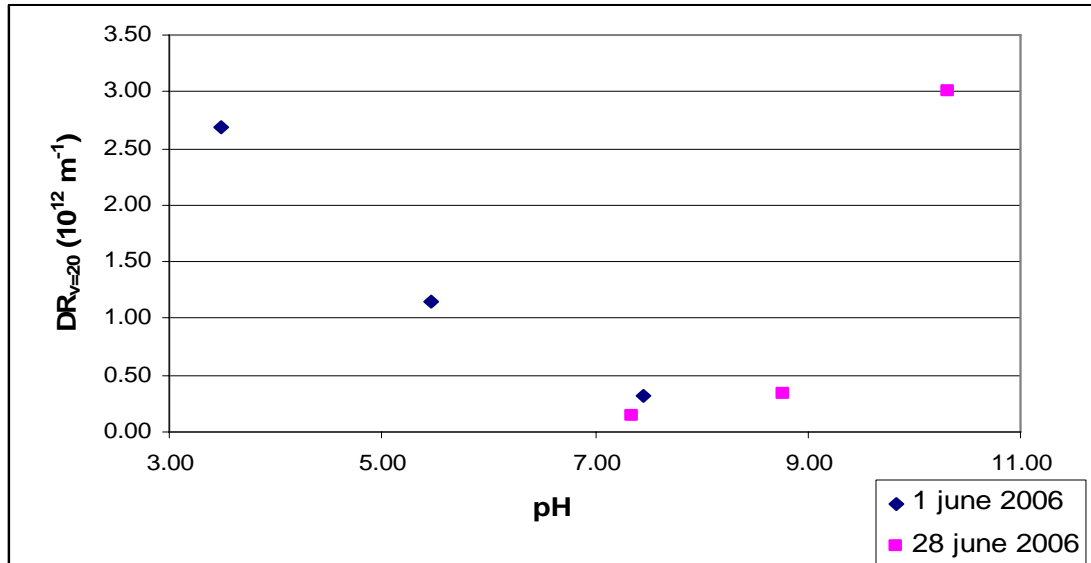


Figure C. 6: Typical behaviour obtained during pH tests

Figure C.4 represents the filterability depending on pH. Figures C.4 to C.6 show that the filterability is worse when the pH is out of the 6.5 - 8 frame. Links with other parameters (EPS, temperature, particle size distribution) will be developed in further publications.

Aeration effect

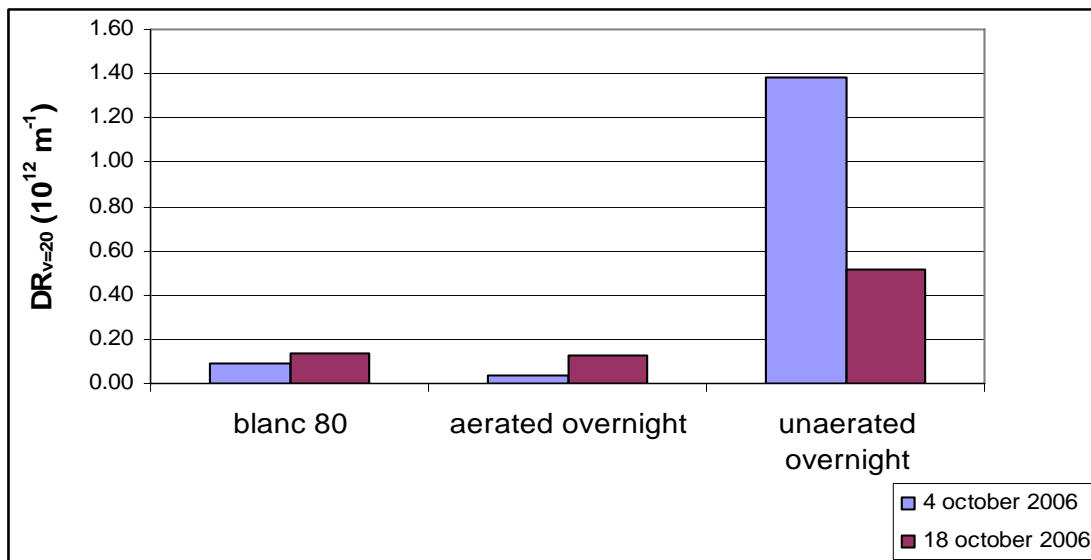


Figure C.7: effect of the aeration on the filterability

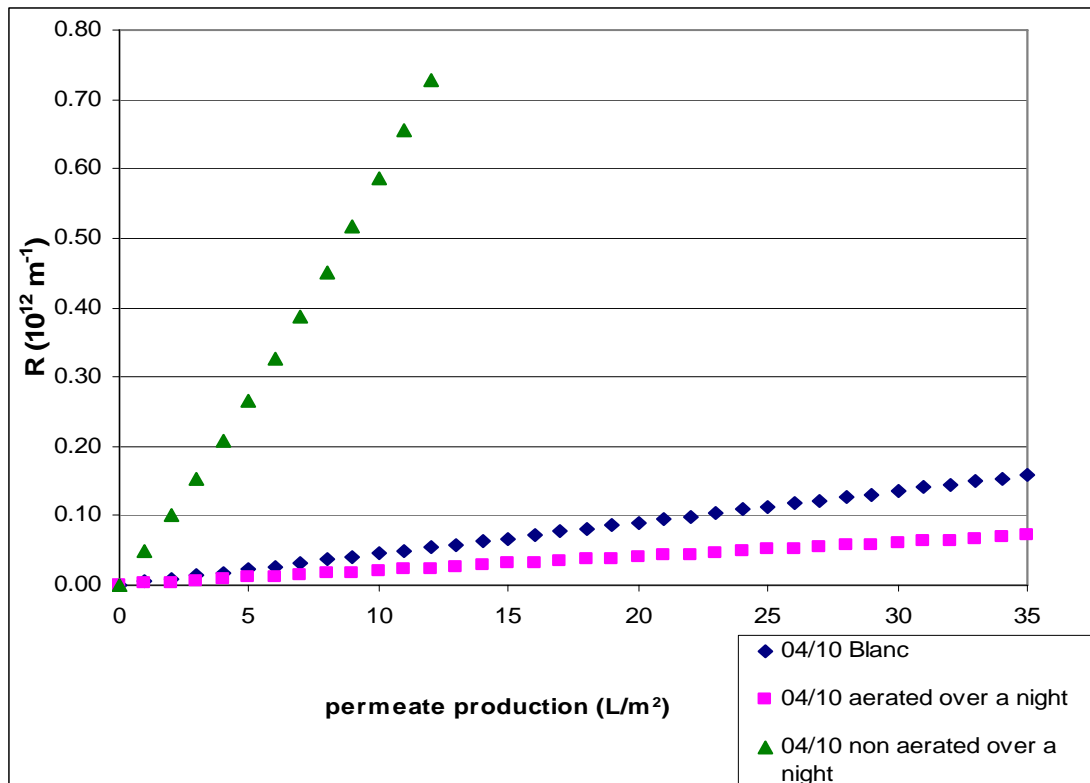


Figure C.8: example of filterability curve

Figure C.7 represents the filterability depending on the aeration. Figures C.7 and C.8 show that aeration/no aeration has a large impact on filterability. Further investigations will be performed to increase the accuracy of these results (step by step increase in O_2 concentration). Links with other parameters (EPS, temperature, particle size distribution) will be developed in further studies.

Substrate addition effect

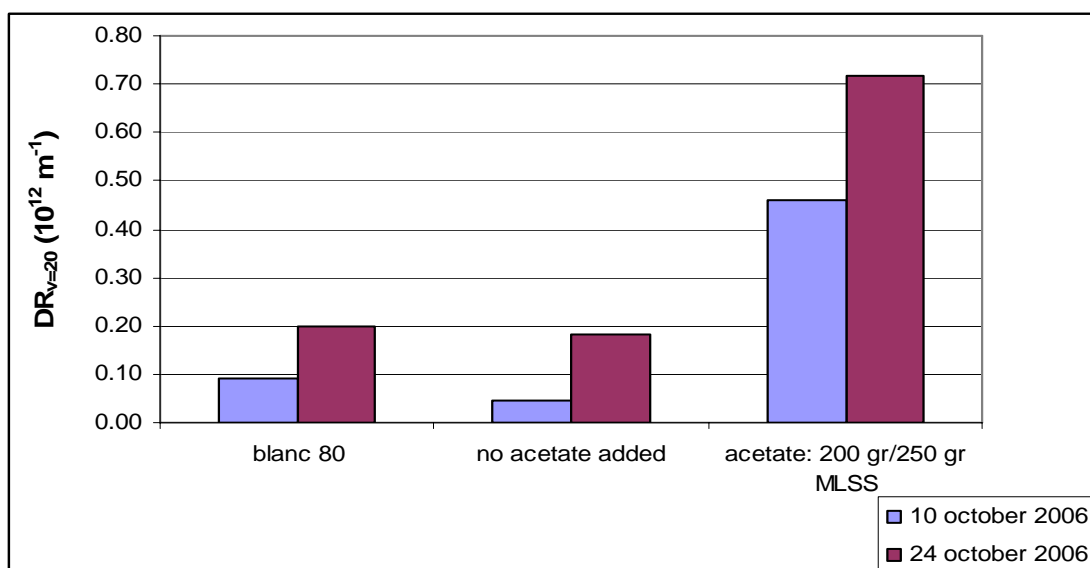


Figure C.9: effect of the substrate addition on filterability after one day

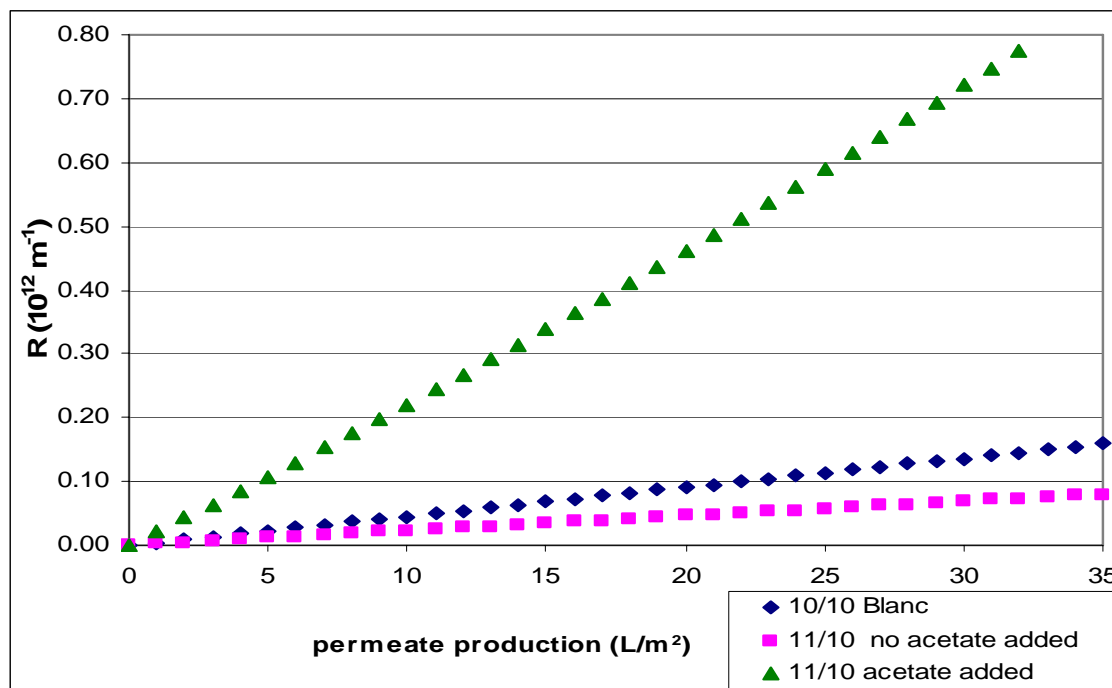


Figure C.10: example of filterability curve

Figure C.9 represents the filterability depending on substrate additions (200g of acetate per 250g MLSS were added). Figures C.9 and C.10 underline the increase in resistance when some carbon source is added to the sludge. Links with other parameters (foulant production, EPS, temperature, particle size distribution) will be developed in further studies.

C.4. PERSPECTIVES FOR THE FUTURE

The major perspective is to gather as much data as possible to extend the database. Therefore, in the next coming research period, several full scale MBRs in Europe will be investigated.

Batch test experiments will be extended as well to temperature and specific compounds.

One of the possible improvements is the full automatisisation of the Unit. Thus 24h - on line measurements can be programmed and the Unit can evolve from short term to middle/long term measurements.