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WP 2 RESULTS REPORT
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SPECIFIC TARGETED RESEARCH PROJECT

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Nomenclature

A	membrane surface area	m²
c	concentration	kg/m³
dV/dt	permeate flow	m³/s
ΔP	transmembrane pressure	Pa
HRT	hydraulic retention time	h
η	dynamic viscosity	Pas
J	flux	L/(m²h)
$k_L a$	oxygen mass transfer coefficient	1/s
$MLSS$	mixed liquor suspended solids concentration	g/L
OUR	oxygen uptake rate	kg/(m³s)
R	resistance	1/m
t	time	s
T	temperature	°C
TMP	transmembrane pressure	bar
SRT	sludge retention time	d
V	volume	m³

Subscripts

c	cake
$crit$	critical
e	equilibrium
end	endogenous
exo	exogenous
f	fouling
i	internal fouling
irr	irreversible
m	membrane
rev	reversible
tot	total

Abbreviations

CAS	conventional activated sludge process
EPS	extracellular polymeric substances
MBR	membrane bioreactor
MBR-VFM	VITO Fouling Measurement
PAC	powder activated carbon
PES	polyether sulphone
PVDF	polyvinylidene fluoride
RSD	relative standard deviation
SD	standard deviation
SIA	sequential injection analysis
SMP	soluble microbial compounds
TS	Total suspended solids
VFM	VITO Fouling Measurement

Abstract

The objective of this research work was on the one hand the development of an on-line analysing system in order to determine the organic fouling compounds and on the other hand fouling mitigation by using different chemicals (e.g. flocculants, starch, PAC) in membrane bioreactors.

For the first purpose two different sensors have been developed. First a photometrically based sensor, developed by TU Berlin, relies on the direct measurement of fouling active compounds - proteins and polysaccharides - in the mixed liquor. These substances are very important components of EPS/SMP. The second approach, developed by VITO, is a physically based sensor which measures reversible and irreversible filtration resistances of the mixed liquor under well-defined and reproducible conditions – and is thus independent of the current age or fouling state of the MBR plant membrane module. In lab-scale experiments and in technical installations the effectiveness of the measuring instruments was investigated. Good results in terms of reproducibility and sensitivity in different matrices have been obtained for both sensors. A comparison of both sensors is presented.

In a comprehensive and impartial screening 30 substances have been investigated. SMP elimination potential, effects on filterability in small scale, respiration, oxygen transfer, nitrification and denitrification, as well as shear stability, dewaterability and costs were taken into account. Also the impact of biomass concentration, calcium concentration and temperature on flocculation and adsorption was tested. To confirm their applicability at technical scale, two identical MBRs were constructed and operated for several months (one for tests, other as reference). Two polymers and a starch have been selected from pre-screening tests and studied in a MBR pilot plant. The tested additives showed no negative impact on the biological performance in terms of COD- and N-elimination. While both polymers showed a positive effect on membrane performance, the starch led to an accelerated fouling behaviour.

Furthermore dynamic operation of MBR pilot plant has been conducted by simulating of rain events and of influent peaks. The purpose of this test was to evaluate the impact of dynamic operation on fouling and fouling compounds (proteins, polysaccharides) measured online as well as on biological performance.

1 Introduction

In recent years, membrane bioreactors (MBRs) have been traded as a high quality effluent alternative to conventional activated sludge processes. However, their more widespread application is still restricted by membrane fouling which reduces permeate yield and increases investment and operation costs. Recently, many MBR studies have identified extracellular polymeric substances (EPS) in either bound or soluble/colloidal form (also called soluble microbial products – SMP) as the most significant biological factor responsible for fouling (Rosenberger et al., 2006). This group of polymers mainly consists of polysaccharides, proteins and other large biopolymeric substances that can be measured by different means. Several and as yet non-standardised methods are in use which result in contradictory findings.

Recently, several compounds have been identified as additives with a potential to reduce fouling one way or another, e.g. by binding EPS/SMP (e.g. Fang et al., 2006; Yoon and Collins, 2006). However, no comprehensive and impartial screening of such substances has been published yet and currently their dosing is still uncontrolled which is likely to lead to either elevated costs and/or adverse effects through over- or underdosing.

The aim of this study is therefore to identify the best suited flux enhancing compound and to develop appropriate sensors which allow on-line fouling monitoring and subsequent feedback-controlled dosing of flux enhancers. For flux enhancers screening, their EPS elimination potential, their influence on filterability and filtration resistances, their biotoxic effect and costs are considered. For fouling sensor development, two approaches are followed: a chemically based protein and polysaccharide (i.e., EPS) sensor and a physically based one which measures re-

versible and irreversible filtration resistances of the mixed liquor under well-defined and reproducible conditions – and is thus independent of the current age or fouling state of the MBR plant membrane module. In lab-scale experiments and in technical installations the effectiveness of the measuring instruments and flux enhancing additives are investigated.

2 State of Art

2.1 Fouling and EPS/SMP

The composition of EPS/SMP varies in dependence on the microbial sludge community and of the environmental conditions; major components are polysaccharides and proteins (Flemming and Wingender 2001). Several factors like the type of wastewater, sludge loading rate, sludge age, MLSS concentration, and mechanical or temperature stress are thought to influence the concentration of EPS/SMP and their fouling propensity in one way or other (Chang et al., 2002, Grelier et al., 2006, Rosenberger et al., 2006, Trussell et al., 2006). Due to the differences in membrane or plant configurations and especially in analytical methods, findings are often inconsistent or even contradictory.

Several authors (Rosenberger et al., 2006) have reported clear correlations between the concentration of SMP and membrane fouling. It is mostly agreed that soluble EPS (SMP) are of higher importance than the bound form. Even if under certain conditions like high sludge age or strong fluctuations the dependence of fouling on SMP concentration is questionable (Drews et al., 2006), removing these substances is thus thought to reduce the fouling of the membrane.

2.2 Flux Enhancers

2.2.1 Substances

A literature review was conducted in order to find promising additives for fouling reduction. In Table 1 respective additives for SMP elimination and flux enhancing are presented. Activated carbons, metal salts, resins, chitosan (biopolymer made of chitin), other polymers (cationic or anionic) and special enzymes might come in question for this task. Some substances have so far only be used for NOM removal in drinking water or other application and have not yet been tested in activated sludge systems. Possible advantages and disadvantages are also listed in Table 1. As large scale feasibility and economics are interesting for a low cost product, difficult to handle or very expensive substances have been excluded from the analysis. Therefore no further investigations were conducted with resins. All other chemicals and also another very cheap and common biopolymer – starch – were tested excessively instead. A list of the 30 screened substances can be found in Table 2.

Table 1: Flux enhancers – Literatur survey

Component	Form / Producer	Removal	Advantage	Disadvantage	Coupling	Authors
Activated Carbon	Powder Macroporous Microporous	NOM DOM Protein Humics Acids	• Possibility of deposit layer • Modification of structure of biological flocs • Removal of toxics • More stable elimination	• Deposit layer? • Possibility of abrasion?	Iron Aluminium Resin	(Fang et al., 2006, Ying et al., 2006, Munz et al., 2007, Lesage et al., 2008, Sagbo et al., 2008, Remy et al., 2009, Satyawali et al., 2009)
Aluminium	Alum Poly Aluminum Chloride	NOM Protein Humics Acids	• Modification of structure of biological flocs • Enhanced nutrient removal • Removal of supernatant compounts	• Deposit of cake layer? • pH < 7 • disturbance of biological process	PAC Resin	(Holbrook et al., 2004, Kim et al., 2005, Wu et al., 2006, Ji et al., 2008, Song et al., 2008)
Iron	Ferric Chloride Ferric Sulphate (PFS) HFO Ferric Nitrate	NOM Protein Humics Acids REE	• Possibility of deposit layer • Modification of structure of biological flocs • Enhanced nutrient removal • Removal of supernatant compounts	• Deposit layer? • pH < 7 • disturbance of biological process	PAC Resin	(Zhang et al., 2004, Jung et al., 2005, Meyssami et al., 2005, Wu et al., 2006, Ji et al., 2008, Song et al., 2008)
Resin	MIEX (Orica) Dow Chemical Rohn & Haas CER	NOM Protein Humics Acids EPS	• Adsorption of components refractory to coagulation & flocculation • pH ~ 7	• Trials in drinking water (2 to 8 mg/l) • Expensive	Iron Aluminium PAC Polymer	
Chitosan	Chitosan salts Powder	Protein Humics Acids Cells debris	• Formation of very large flocs • pH 6-7 • Removal of supernatant compounts	• Influence of ionic strength		(Bratskaya et al., 2004, le Roux et al., 2005, Meyssami et al., 2005, Tiwari et al., 2005, Ji et al., 2008)
Polymer	Nalco MPE 50 DMMC-AN		• Formation of larger flocs • Effects on filter cake • Removal of supernatant compounts	• Formation of gel layer? • Influence of dosage / ionic strength		(Yoon et al., 2006, Hwang et al., 2007, Thiemig et al., 2008)
Enzyme	Lumafast™	Protein Lipid	• pH >= 7	• Temperature > 35°C		

Table 2: Flux enhancers – Substances tested within WP2

Category	Supplier	Abbrev.	Product	Other information
Activated carbon	Norit	PAC-1	SA Super	-
	PICA	PAC-2	Picahydro LP27	-
Metal salt	Merck	FeCl3	-	Ferrum chloride
	Ciba	PACl-1	Magnasol 5113	Alum
	Ciba	PACl-2	Magnasol 5108	Alum
Chitosan	France Chitine	Chit-1	Chitosan 221	Flakes
	France Chitine	Chit-2	Chitosan 652	Powder
	France Chitine	Chit-3	Chitosan 342	Powder
Polymer	Ciba	Poly-1	Zetag 7878FS40	cationic polymer
	Ciba	Poly-2	Zetag 8846FS	cationic polymer
	Nalco	Poly-3	MPE-50	-
	Kurita	Poly-4	M H 260	-
	Kurita	Poly-5	MP H 30	-
	Kurita	Poly-6	MP 252	-
	Kurita	Poly-7	MP L 30	-
	Adipap	Poly-8	Adifloc KD 450	-
	Adipap	Poly-9	Adifloc KD 451	-
	Adipap	Poly-10	Adifloc KD 452	-
	Adipap	Poly-11	Adifloc KD 453	-
	Diagonal	Poly-12	Diafloc CH100	Biopolymer
Enzyme	Novozymes	Enz-1	Alcalase 2.5 L	Protease
	Novozymes	Enz-2	Viscozyme L	Beta-Glucanase
Starch	Rhodia	Sta-1	Rheozan	Succinoglucane gum
	Rhodia	Sta-2	Jaguar C162	Guar gum
	Rhodia	Sta-3	Rhopodol 23	Xanthan gum
	Tate&Lyle	Sta-4	Mylbond 163	Corn starch
	Tate&Lyle	Sta-5	Mylbond 168	Corn starch
	Tate&Lyle	Sta-6	Mylbond 149	Corn starch
	Roquette	Sta-7	Vector SC 20157	Cationic starch
	Roquette	Sta-8	Vector SC 27216	Cationic starch

2.2.2 Mechanisms

For flux enhancement in MBR different kinds of chemicals come into consideration as already shown in Table 2. These can generally be divided into two classes: cationic flocculants which cause charge neutralisation of the negatively charged sludge flocs and thus lead to larger flocs or adsorbents. The latter accumulates fouling causing solutes at the surface; this task is normally realised by the addition of activated carbon / powdered activated carbon

(PAC) into the MBR. While flocculants are thought to cause macroscopic flocs and a particular aggregation, PAC causes a kind of molecular aggregation. Nevertheless it is reported, that PAC can also lead to a structuring effect of the sludge floc and thus probably to a better filterability (Remy et al., 2009).

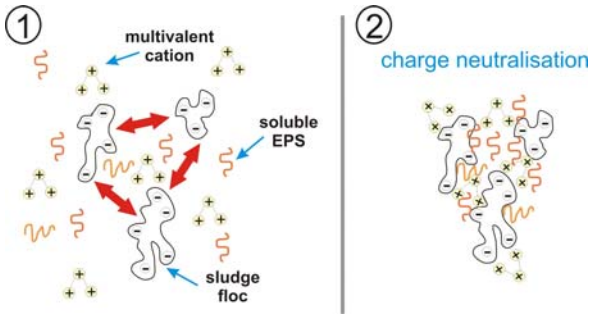


Figure 1: Flocculation of sludge by multivalent cations

Although a wide range of studies on different additives in MBR is available in literature these mostly focus on the effect of one or two additives. The use of additives is mostly based on conventional water treatments (e.g., elimination of colloids in water bodies) and each additive might play a different role in solutes and colloids removal, so it is of high importance to select more pertinent coagulants/adsorbents that can effectively increase filterability e.g. by SMP removal in MBR sludge. It is also of particular significance to understand in what way the additives eliminate SMP substances.

On the other hand these changes of sludge characteristics might have a negative impact on the nutrient removal due to different transport phenomena through the liquid and the floc. For conventional flocculants it is commonly known that overdosing sometimes leads to adverse effects. Therefore, dosing beyond a certain “optimum” concentration might not only lead to higher costs for the additive but also to disturbances in the elimination performance and/or to increased fouling as the additive might not be bound into the flocs anymore but remains in the solution and causes fouling itself.

2.2.3 Dosing Strategies

So far constant additive dosing was applied ignoring changing system and sludge conditions. Yoon and Collins (2006) describe a dosing step (0 mg/L to the found optimum concentration of 300 ppm) at the start of the experiments, followed by a daily addition to compensate for the losses due to excess sludge removal. Other authors describe similar approaches (Ying et al., 2006). If the addition of a flux enhancer can be controlled by the concentration of fouling causing compounds or the fouling propensity, operation costs and chemicals can be saved. In addition, operational problems through under- or overdosing can be reduced. When flux enhancing chemicals are dosed, it must be considered if these have an negative effect on the activated sludge or inhibiting biological processes (Wolborska et al., 2006).

2.3 Fouling Monitoring/Sensors

Currently, no specific sensor for a continuous monitoring or control of fouling phenomena in MBR exists. For this reason it was intended within AMEDEUS to develop two different measuring approaches. The first approach is the direct measurement of fouling active compounds by TU Berlin: a photometrically based sensor, which analyses the concentration of proteins and polysaccharides - two most important components of EPS – directly in sludge. The second approach by VITO is a physically based sensor which measures reversible and irreversible filtration resistances of the mixed liquor under well-defined and reproducible condi-

tions – and is thus independent of the current age or fouling state of the MBR plant membrane module. In lab-scale experiments and in technical installations the effectiveness of the measuring instruments was investigated.

2.3.1 MBR-VFM sensor

It is well known that pressure driven membrane filtration efficiency is determined by the feed's fouling characteristics. This is also the case in MBR practice since the fouling characteristics of the MBR mixed liquor dictate membrane functioning. From the lack of specific sensors and measurement methods with respect to monitoring the mixed liquor fouling propensity, it was planned within AMEDEUS to investigate two types of fouling monitoring devices.

A very concise and up to date review with over 300 references on MBR fouling can be found in Le-Clech et al. (2006). The critical flux determination by the flux-step method should be mentioned in particular (Le-Clech et al. 2003). However, the approach of the flux-step method has some drawbacks. Firstly, there is no standard protocol which standardizes the level of the flux steps and the duration of one step. As a result, unambiguous comparisons between different researchers are not feasible. Secondly, the flux step method introduces a cumulative fouling at the membrane surface from all previous steps. From protocol differences, the step-wise depositing of fouling layers will differ in layer thickness and possibly also in degree of fouling layer compaction. Protocol differences in flux step values are obviously also linked to different transmembrane pressures during testing and therefore possibly induce different pressure gradients across the fouling layer from one protocol to another.

Accordingly, from the unclear situation revealed by the literature, VITO aimed to develop a fouling measurement method and sensor which moreover evaluates both the reversible and irreversible fouling propensity of membrane bioreactor mixed liquor. Fouling is extremely complex and a mathematical fouling model is mostly unfeasible or impractical. For aqueous matrices, aspecific fouling characterization methods do exist, such as the index methods (e.g. SDI and MFI) which in fact try to grasp the complex fouling behaviour in a single number fouling "model" which can be considered as an oversimplified model "F=a" (F=fouling ; a = index value). Index methods risk to a large extent to loose crucial information from within the original fouling data and also risk to give information about a fouling interval outside the operational window of the actual application. Therefore, an alternative and pragmatic fouling measurement method (VFM; VITO Fouling Measurement) was developed at VITO several years ago. This was based on dead-end filtration and preserved the information from all measured data as a VFM fingerprint curve or table. The VFM approach has been described in detail in several publications over the last years by Brauns et al. (2002a, b). Since membrane bioreactors (MBR) are based on cross-flow filtration, the application of the VFM principle within an MBR system needed an adapted version of the VFM, which will be further called the MBR-VFM.

2.3.2 Photometrical EPS fouling sensor

The chemically based EPS fouling sensor conducts continuously automated measurement of the concentration of polysaccharides and proteins in mixed liquor. The determination relies on photometric analysis. The polysaccharides are measured according to the method of Dubois et al. (1956) and the proteins according to method of Lowry et al. (1951). The boundary conditions of a successful implementation and automation of an analytical assay are simplicity, reproducibility and robustness of the method. The automation of the photometric assays is in general realised by the help of Flow Injection Analysis techniques (FIA) (Ruzicka & Hansen, 1988). For this purpose, its advanced development, i.e. the Sequential Injection Analysis (SIA) technique, was used for set-up of the on-line sensor. SIA has been successfully applied to the determination of various compounds in food and bioprocess monitoring, pharmaceutical, industrial and process analysis (Lenehan et al., 2002). For the extraction of a representative sludge sample and the subsequent analysis of polysaccharide and protein

concentration an appropriate sample pre-treatment device was developed. As a pre-treatment step the filtration was applied that has the same separation properties as the manual sample preparation done by the paper filtration.

Important aspect of the development of an analytical EPS sensor is the contribution to the standardisation of the determination techniques for proteins and polysaccharides. Up to now the determination protocols are carried out by different analytical methods (Raunkjær et al., 1994). All of them rely on the photometric measurements, however the protocols utilise different reagents and treatment steps therefore different reaction mechanisms related to the proteins and polysaccharides can be expected. The consequences are hardly comparable results of different research studies.

3 Materials and Methods

3.1 Analysis

3.1.1 Total suspended solids (TS) and total volatile suspended solids (oTS)

The biomass concentration was determined as total suspended solids (TS) and total volatile suspended solids (oTS). The mixed liquor sample was dried at 105°C, in order to obtain the total dried weight (TS) before heating to 550°C in a muffle furnace to obtain the ash content.

3.1.2 Capillary suction time (CST), mean particle size, turbidity

The dewaterability of the different mixed liquor samples was evaluated by measuring the CST (Triton Model 200, Allied Colloids GmbH, Hamburg, Germany) with CST papers and the cartridge for good dewaterable sludge.

The mean particle size was determined with a Mastersizer S (Malvern Instruments GmbH, Herrenberg, Germany) with sample presentation unit, pump, stirrer and UV for a more homogenous mixture of the sample. The 300RF lens was used, allowing the measurement of particle sizes ranging from 0.05 – 900 µm.

The turbidity was measured in the sludge supernatant after paper filtration (Whatmann, 589/1 black ribbon, 125 mm, Maidstone, Kent, GB) with analyzer 2100N Hach (Düsseldorf, Germany).

3.1.3 Extracellular polymeric substances (EPS), soluble microbial products (SMP)

EPS and SMP were determined as the sum of polysaccharide- and protein concentration in the extracted fraction (EPS) and in the sludge supernatant (SMP). The extraction of EPS from sludge flocs was performed with a strongly acidic cation exchange resin (Na-form, Dowex) according to the method of Frolund et al. (1996). Sludge supernatant was prepared by filtering the mixed liquor with filter paper (Whatmann, 589/1 black ribbon, 125 mm, Maidstone, Kent, GB).

Polysaccharide (PS) concentrations were analysed according to the photometric method proposed by Dubois et al. (1956). D-Glucose-Monohydrate was used for calibration. For manual measurement of the pilot plant samples (influent, sludge filtrate, permeate) the influence of nitrite or nitrate on carbohydrate measurement was corrected according to the method proposed by Drews et al (2007c). The concentrations have not been corrected that were determined in batch tests and in on-line measurements with SIA.

Protein (PR) concentrations, expressed in equivalent of bovine serum albumin, were determined according to Lowry et al. (1951). All samples were analysed in duplicate and the re-

sults are given as average values. It has to be taken into account that this method is not protein specific but also responds to humic substances. No modification of the method has been applied in order to minimise the influence of humics like propose e.g. by Frolund et al. (1996).

For on-line measurement of proteins with EPS sensor, the analytical method was slightly modified by adding a chelating agent NTA (nitrilo-tri-acetate) to the alkali reagent in order to prevent a formation of salt precipitation that occur in real samples and strongly disturbed the automated method. (Mehrez et al. 2010a)

3.1.4 Size exclusion chromatography – LCOCD

To characterise the DOC composition of sludge supernatant size exclusion chromatography with continuous organic carbon and $UV_{254\ nm}$ detection (LC-OCD system by DOC-Labor Dr. Huber, Karlsruhe/Germany; SEC column: Toyopearl® HW-55S by Tosoh Bioscience, Tokyo/Japan) was used according to Haberkamp et al. (2007). During transporting through the column the sample components are separated according to their size: (1) biopolymers (including extracellular polymeric substances, i.e., mainly PS and PR; $t_{elution}$ = 35-55 min), (2) humic substances ($t_{elution}$ = 55-64 min), (3) low molecular weight acids and neutrals ($t_{elution}$ > 64 min). For quantification of the results, the peaks of the chromatogram were integrated obtaining the equivalent amount of organic compounds. In this paper only two fractions were considered: peak (1) biopolymers and peaks (2)+(3) humic substances and low molecular weight acids and neutrals together. Typical chromatogram of sludge supernatant is presented in the Figure 2.

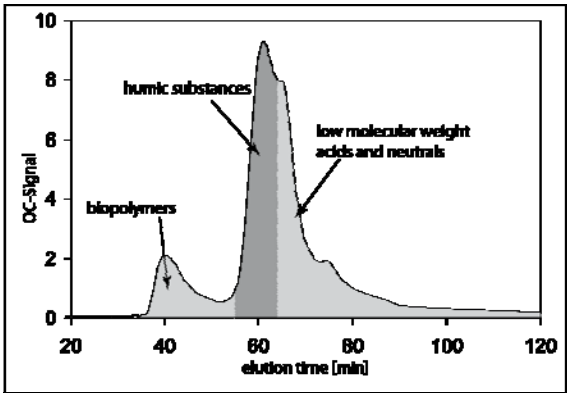


Figure 2: LC-OCD chromatogram, sludge filtrate.

Prior to the measurement with the LC-OCD system, the samples were filtered through 0.45 µm cellulose nitrate filter (Sartorius, Göttingen, Germany) and sludge supernatant samples were diluted 5-fold and the raw wastewater 40-fold (measurement range: 1-5 mg $L^{-1}C$).

3.1.5 Ions, N-total, COD

All samples were analysed directly after sampling. Samples were pre-filtered with a cellulose acetate filter (pore size: 0.2 µm, Sartorius Stedim Biotech GmbH, Germany), before measuring NO_2-N, NO_3-N, NH_3-N, PO_4-P, according to standardised methods (DIN (1996, 1999) with ion chromatography (DIONEX, DX-100 Ion Chromatograph). The samples for the COD and N-total measurement where analysed by Hach Lange kits and spectrophotometry (Hach Lange, ISIS 9000 MDA Photometer) without pre-treatment.

3.2 Experimental Set-Ups and Procedures

3.2.1 Pre-screening tests

3.2.1.1 Shakers

Shaker batch experiments were performed in order to investigate the effect of different additives on the sludge supernatant and its components (particles, DOC fractions, SMP) and to find out the optimal concentration in regard to SMP elimination. The SMP (assumed to be the sum of polysaccharides and proteins) elimination performance was determined as a degree for the effectiveness of an additive. Determination of optimum dosage can be seen in Figure 3. SMP elimination was determined as the difference between the SMP concentration without additive dosing and that achieved with a certain concentration of the respective additive. The optimum additive concentration is therefore the additive concentration which should be adjusted in the sludge, it does not relate to the amount of wastewater treated.

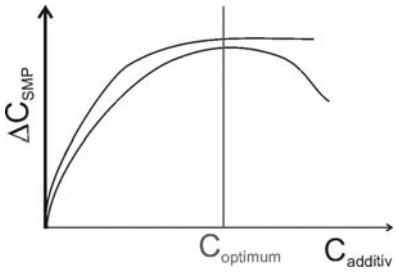


Figure 3: Determination of optimum dosage

Shaker trials were performed in Erlenmeyer flasks (500 mL). 10 mL of dissolved / wetted additive was added to 150 mL activated sludge to achieve the desired concentration. The flasks were agitated for 1 h at 130 Hz with a reciprocating or horizontal shaker at 20°C. Every concentration was tested in duplicate. In each shaker experiment two sludge samples were tested without any additives (blank). Prior to paper filtration, samples were centrifuged for 12 minutes at 3000rpm to avoid retention of SMP in the accumulating filter cake. Afterwards the clear phase was paper filtered (Schleicher & Schuell - Black Ribbon 589/1) to guarantee the absence of particles.

For selected additives (ADIPAP KD 452, NALCO MPE 50, TATE & LYLE Mylbond 168, NORIT SA Super, PICA Picahydro LP27) removal (improvement) of further parameters from the supernatant has been measured in additional experiments: suction time (CST), turbidity and DOC fractions (with size exclusion chromatography).

The removal of measured supernatant compounds was calculated by the following equation:

$$removal\ [\%] = \frac{c_{blank} - c_{treat}}{c_{blank}} \cdot 100\,\%$$

Equation 1

c_{blank}: concentration of measured parameter in the sludge supernatant without additive (blank)

c_{treat}: concentration of measured parameter in the sludge supernatant after additive treatment

Furthermore the influence of total suspended solids concentration (TS), the Ca^{2+}-Ion concentration and the temperature on the efficiency of selected additives has been investigated. The tested substances were ADIPAP KD 452 (30 and 70 mg/L) and NALCO MPE 50 (100 and

500 mg/L), and the starch TATE & LYLE Mylbond 168 (200 and 1000 mg/L), which were applied on the MBR pilot plant. The experiments were conducted with two different dosages: at optimal and low concentrations.

For the examination of TS the shaker experiments have been conducted in sludge with different TS. The sludge was diluted with permeate in relation: 1:3, 1:1.5, no dilution; the received TS concentration was ~2.5, 5, 7 mg/L respectively. Furthermore the sludge has been adjusted to three different concentrations of Ca^{2+}-Ion. The sludge was treated at first with cation exchanger CE (Dowex, Marathons, mixed 1 h 4.9 g equilibrated CE in 3 L sludge) and the [Ca^{2+}] was decreased to ~40 mg/L. Then the concentration was adjusted by addition of $CaCl_2$ salt to ~40, 180, 280 mg/L in the supernatant. For investigation of the influence of the temperature on the additives efficiency the Erlenmeyer flasks have been shaked in the water bath at 10, 20, and 30°C±1°C. In the experiments with different [Ca^{2+}] and temperature the TS of the used sludge was between 7-8 g/L.

3.2.1.2 Filtration test cell

Small scale filterability tests under defined and representative conditions were conducted in a cross flow filtration test cell designed at the department of Chemical Engineering, TU Berlin (Rosenberger et al., 2001), simulating the cross flow conditions between two flat sheets in a submerged MBR plate and frame module. A flow sheet of the test cell can be seen in Figure 4. Channel height was 5mm for all tests conducted, but can be varied. Effective membrane area is 88cm². The test cell can be operated under constant flux or constant pressure conditions while TMP and flux are monitored. The cell can be aerated with different aeration intensities and a second circuit can be operated to switch easily between water and sludge tests.

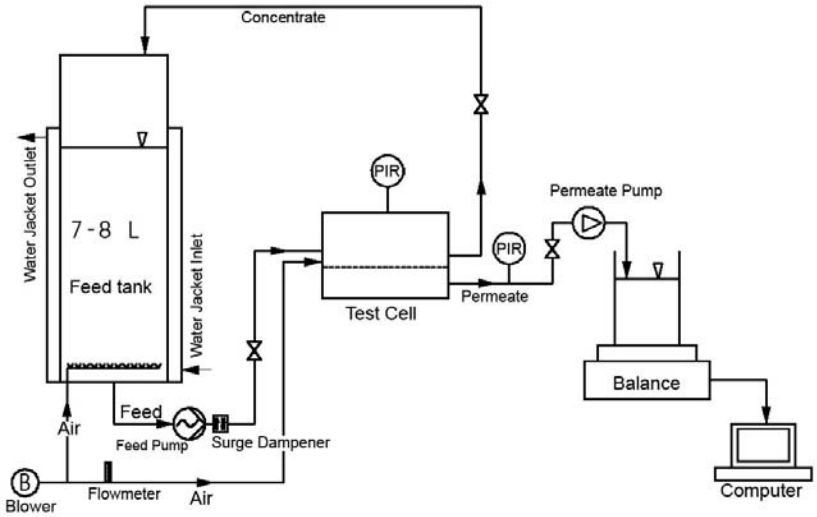


Figure 4: Flow sheet of the filtration test cell

Four sets of tests have been conducted:

1. Aerated filtration of untreated/treated (optimal concentration) sludge according to the protocol shown in Figure 5 with a constant flux, J = 22-25 L/(m²h), this enables measurement / calculation of the different resistances
2. Filtration of water with 5% optimal additive concentration for 2h at J = 60L/(m²h)
3. Filtration tests with 4 different additive concentrations (no additive, optimum concentration, slightly lower than optimum concentration and slightly higher) for 2h at constant flux, J = 27L/(m²h), 10min puls/2min pause

4. Flux stepping tests in order to determine the critical flux Jcrit, the flux was increased incrementally by steps of 3 L/(m²h) between 10 L/(m²h) and 61 L/(m²h) with permeation pauses and flux backsteps.

For sludge trials a PVDF membrane by Microdyn-Nadir was used, as this is the material A3 Water Solutions used for the modules in the pilots. For the residual tests different membranes were used in order to see the impact of the additive that is not bound within sludge flocs on membrane material. Properties of the different membranes are listed in Table 3.

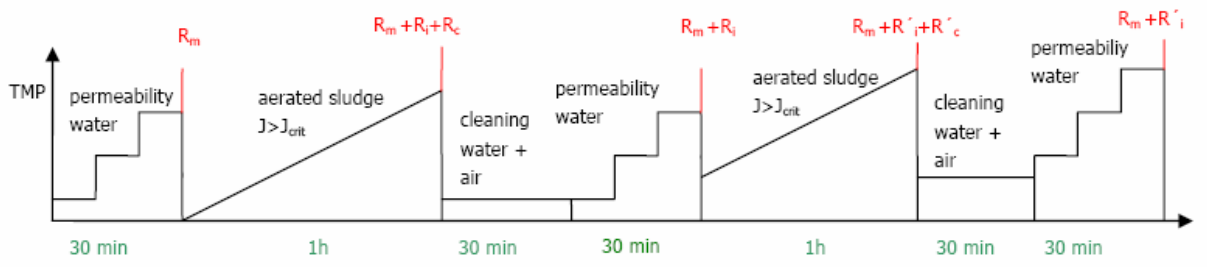


Figure 5: Protocol for the determination of the filterability of treated/untreated sludge

Table 3: Membranes used for the residual tests

Name	Microdyn-Nadir MF	Millipore	Microdyn-Nadir UF
Material	PVDF	PVDF	PES
Pore size	0.2 µm	0.2 µm	MWCO: 150 kD

3.2.1.3 Oxygen uptake and transfer rate

In order to investigate whether a substance has a negative impact on biomass activity (biotoxicity) or oxygen transfer respiration tests were conducted in accordance with Rosenberger (2003). These tests were carried out with sludge treated by the optimal additive concentration as well as with untreated sludge as a reference. Activated sludge was sampled from a conventional wastewater treatment plant in Berlin since the MBR pilots were not available yet at the time. As sludge properties change from day to day, an untreated reference sludge sample taken on the same day was investigated for each substance.

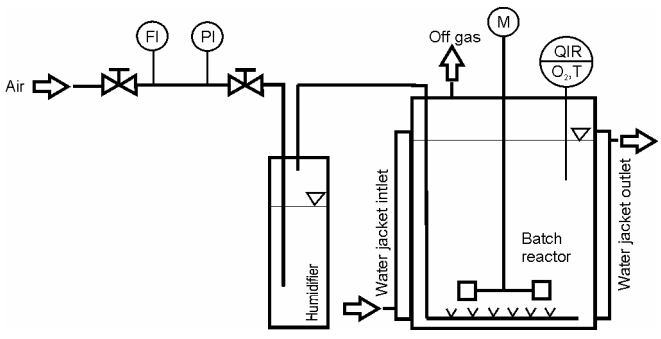


Figure 6: Experimental setup of the respirometer

The experimental setup is shown in Figure 6. To provide sufficient mixing and oxygen transfer, the 1L batch was equipped with a Rushton turbine and baffles and was constantly temperated at 20°± 2°C. Fine bubble aeration was employed through a porous tube; aeration was set to 40L/h; in order to avoid evaporation the air was humidified prior to the reactor inlet. Dissolved oxygen (DO) and temperature were measured with an oxygen probe, type CellOx 325, which was calibrated before each measurement and placed close to the stirrer. Data were recorded every second. The experiment was executed as shown in Figure 7.

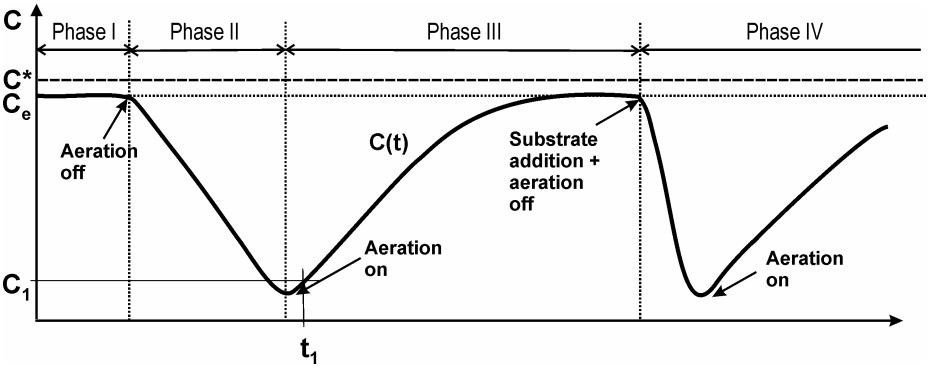


Figure 7: Procedure for the determinatio n of OUR and kLa (Rosenberger, 2003)

In biological systems oxygen is consumed continuously due to respiration activity of the microorganisms. Therefore, the steady state DO concentration ce is lower than the saturation DO concentration c*. After reaching the stationary value (phase I), aeration was turned off. When a DO concentration of <1mg/L was reached (phase II), aeration was turned on until equilibrium was reached again (phase III). For reproducibility reasons, phase II and III were repeated once. In a last step, substrate was added to determine exogenous respiration. Endogenous and exogenous oxygen uptake rates (OUR) were determined from the slope in phase II and phase IV while oxygen mass transfer coefficient (k_La) was calculated according to:

$$k_L a = \frac{\ln\left[\frac{c_e - c}{c_e - c_1}\right]}{t_1 - t}$$ **Equation 2**

The data were evaluated between $c_1(t1)$ = 1 mg/L and $c(t) = 0.8c_e$.

3.2.1.4 Nitrification and Denitrification

Batch tests were conducted in order to determine the effects of flux enhancing chemicals on the activity of nitrifying/denitrifying bacteria in the mixed liquor. Two cylindrical tanks with water jackets (temperated to 20±0.5 °C) were used for these parallel tests. One of the tanks contained mixed liquor with the additive and the other contained the untreated reference sludge. Then, required volumes of acetic acid, sodium nitrate and ammonium chloride solutions were added to both reactors in order to obtain 100 mg/L acetate, 10 mg NO_3^--N/L and 30 mg NH_4^+-N/L in the reactors. Mixing speed was 200/min.

Similar to the contact times in the pilot plant from which the sludge was sampled, both reactors were operated under anoxic conditions for 1 h. Fine bubble nitrogen gas sparging was employed through a porous tube, in order to strip all molecular oxygen and to achieve similar hydrodynamic conditions as in the aerated period. Dissolved oxygen and temperature were measured with an oxygen-meter (WTW CellOx 325, Germany). After one hour, air was

sparged instead of nitrogen to start the nitrification process. Samples from both tanks were taken every twenty minutes.

All samples were filtered through a cellulose acetate filter (pore size: 0.2µm, Sartorius Stedim Biotech GmbH, Germany), before analysis. Concentration of anions (NO_3^--N, NO_2^--N) and ammonia (NH_4^+-N) were measured in a Dionex DX 100 ion chromatograph equipped with an IonPac AS 4a or an IonPac CS12a column, respectively.

3.2.1.5 Particle size distribution

Shearing of the sludge

Activated sludge was freshly sampled daily from the pilot described in chapter 3.2.2 without additive dosing described above. Each day the untreated reference mixed liquor was measured in comparison to mixed liquor spiked with one of the additives shown in Tab.1. The samples were then sheared at defined shear rates between 0 and 4000s-1 for 5 to 60min in a rotational viscometer (Type Viscotester VT 550, Haake GmbH, Karlsruhe, Germany) with a gap width of 0.35mm. Approximately 10mL of sludge were sheared in the viscometer. From own measurements and approximations a representative shear rate in a plate and frame module can be expected to be in the range of 2000s-1. The temperature of the mixed liquor was between 24 and 29°C with a temperature of 26°C for most samples. The values presented here are not temperature corrected. After shearing the particle size distribution and the dewaterability were determined according to chapter 3.1.2.

3.2.1.6 Combination of flocculants

Activated sludge samples were obtained from a pilot-scale MBR operated within this WP (see chapter 3.2.2). FeCl3, the polymer NALCO MPE 50 and the PAC SA Super (see Table 2) were combined with each other in various dosages as shown in Table 4. The respective optimum concentrations were determined in previous shaking flask tests for single additives (see Chapter 3.2.1.1).

Table 4: Combinations of additives and dosage (optimum dosage according to Table 6)

sample no.	1	2	3	4	5	6	7	8	9
additive (s) (dosage)	reference sludge	A1 (od)	A2 (od)	A1 (od) + A2 (od)	A1 (od) + A2 (0.5)	A1 (0.5) + A2 (od)	A1 (0.5) + A2 (0.5)	A1 (1.2) + A2 (od)	A1 (od) + A2 (1.2)

(A1: additive 1, A2: additive 2, od: optimum dosage, 0.5: 50% of optimum dosage, 1.2: 120% of optimum dosage)

Shaking flask tests (comparable to these described in Chapter 3.2.1.1) were conducted for each combination to determine the extent of SMP removals and the effect on sludge dewaterability. Afterwards concentrations of protein and polysaccharide fractions of SMP were determined in the supernatant (see chapter 3.1.3). The dewaterability of the different sludge samples was evaluated measuring the capillary suction time (CST) according to chapter 3.1.2.

3.2.2 Pilot plant

In order to investigate the effect of flux enhancing chemicals in MBRs, two identical pilot plants were set up (Figure 8). Each plant consists of two 1 m³ tanks with a working volume of approx. 0.8 m³. The pilot units are located in a 20' sea container next to a pumping station of Berliner Wasserbetriebe thus drawing combined municipal wastewater from Berlin city centre as influent. Characteristics of the pre-settled wastewater can be found in Table 17.

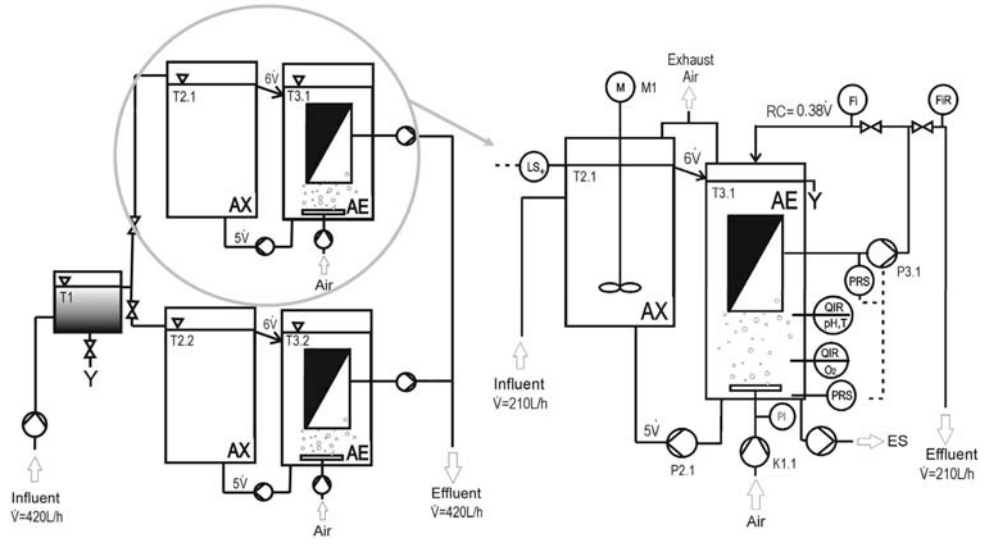


Figure 8: a) Set-up of the parallel pilot plants b) Flow sheet of one system

After the settler used as sand trap for the removal of larger particles, the wastewater flows into a stirred anoxic chamber. The following tank is aerated and equipped with a membrane module. Transmembrane pressure (TMP), flux, dissolved oxygen (DO), temperature (T) and pH in the membrane chamber are registered on-line.

The two identical filtration units (module M20; A3 Water Solutions GmbH, Germany) are submerged in tank T3.1 and T3.2. They consist of 26 plates and compromise a total filtration area of 22 m² each. The membrane is made from PVDF with a nominal pore size of 0.2 µm. The applied flux was approx. 16 L $m^{-2}h^{-1}$ (12 min filtration / 3 min relaxation) during normal operation. A higher flux of 20 L m^{-2}h-1 was applied during high flux operation The membrane aeration was maintained at 16-18 m^3h^{-1} (specific aeration demand = 0.8 $m^3m^{-2}h^{-1}$, superficial air velocity = 0.028 m s^{-1}) for all experiments.

The systems have been in operation since October 2006. While different flocculants were dosed into one system, the other pilot served as a reference. Each additive was tested for 2-3 month with a subsequent membrane cleaning

3.2.2.1 Operating conditions

Before the trials with the flocculant started, the plant had been in operation for more than 350 d. So the microbial population was assumed to be adapted well to the treated wastewater. Due to the seasonal changes, the sludge temperature varied between 15 - 28°C. The system was operated with a hydraulic retention time of 7 h and a sludge age of 13 d. Three different additives for flux enhancement were tested in pilot system 2. The initial dosing for each additive was done in one step in order to reach the optimum dosage according to Table 6 Then the additive was re-dosed twice per week according to the amount lost with the excess sludge withdrawal.

The duration of each period, the tested additives and concentrations are shown in Table 5. At the end of each period the flux was increased by about 20%. Afterwards nearly three sludge ages passed without any additive dosing (drive out time) to remove approximately 95% the additive before the membrane was cleaned chemically and the sludges from both systems were mixed to achieve identical initial conditions for the next dosing period.

Table 5: Pilot plant operation periods for the three tested flocculants

	concentration [mg L^{-1}]	operation time with FE [d]	high flux [d]	drive out time [d]	cleaning [d]
NALCO MPE 50	500	0 – 74	66 – 70 AE1 66 – 74 AE2	75 – 108	on day 108/109/116 in AE1/AE2/AE1 on day 149/151 in plant AE2/AE1
Mylbond 168	1500	158 – 174	201 – 208 AE1	206 – 238	
	2000	175 – 192	201 – 208 AE2		day 238/239/241 in AE1/AE2/AE2
	1500	193 – 208			
ADIPAP KD 452	70	246 – 309	301– 309 AE2	310-345	

3.2.2.2 Membrane cleaning

A chemical cleaning was conducted when a TMP of 200 mbar was reached (approx. after three to four months). For a chemical cleaning the sludge was taken out of the filtration chamber. The chamber was then filled with a citric acid solution with a pH of 2.7 to 3 and soaked for one hour. After the acidic cleaning the module and chamber were rinsed with water and the module was soaked in a 2000 ppm chlorine solution (sodium hypochlorite) for another 3 hours. After a careful rinsing, the membrane chamber was filled with sludge and normal operation re-started.

If the described cleaning protocol did not lead to a sufficient permeability, the cleaning steps were extended and the acidic cleaning was repeated after the soaking in the chlorine solution (2 hours acid solution, 48 hours 2000 ppm chlorine solution, 2 hours acid solution).

3.2.2.3 Dynamic operation

To investigate the influence of dynamic operational modes, over a period of six month peaking events were conducted. The flux was increased to 130% respectively 160% for 6 hours. Afterwards the flux was decreased to 90% respectively 80% for 18 hours to ensure a constant net flux over the investigated period of 24 hours. Peaking events were conducted twice a week. In order to determine the effect of rain water events, tap water was added according to the increased flux into the anoxic chamber. I.e. HRT increased while organic loading remained constant. To investigate the effect of influent peaks, the same protocol was applied but waste water instead of tap water added.

To evaluate possible effects on the membrane performance, the TMP evolution was observed and compared to earlier investigated periods where the pilot was operated with constant parameters.

Samples (influent, effluent, sludge) were taken at least twice a week. Total COD and total nitrogen N_{tot}, but also NH_4-N, NO_2-N, NO_3-N concentrations (according to chapter 3.1.5) were determined to evaluate effects on the microbiologic activity. Additionally total suspended solids (TS), filterability (TTF) and dewaterability (CST) were measured (as described in chapters 3.1.1 and 3.1.2.)

3.2.3 MBR-VFM sensor

3.2.3.1 Set-up

The MBR-VFM approach is based on the widely accepted resistances-in-series model of the membrane resistance and the total additional fouling resistance R_f.

$$\frac{dV}{dt} = \left(\frac{A \cdot \Delta P}{\eta}\right) \cdot \frac{1}{(R_m + R_f)}$$ **Equation 3**

dV/dt = permeate flow (m³/s)

A = membrane surface area (m²)

ΔP = transmembrane pressure (Pa)

η = absolute viscosity (kg/m.s)

R_m = membrane resistance (m^{-1})

R_f = all additional fouling resistance (m^{-1})

A module (sensor) was designed which holds one tubular membrane as illustrated in Figure 9 (part A). The sensor can be placed directly in a MBR or within a separate tank, which is fed by a sampling device and (dis)continuously delivers a representative sample of the MBR mixed liquor. The MBR-VFM measuring apparatus (Figure 9, part B) is a software controlled and fully automatic filtration device which extracts permeate from the sensor while storing all relevant filtration data. The control, data-acquisition by automatic sampling and MBR-VFM related standard calculations are performed within the proprietary software MeFiAS® which was developed at VITO under LabVIEW® and adapted towards the specific set-up.

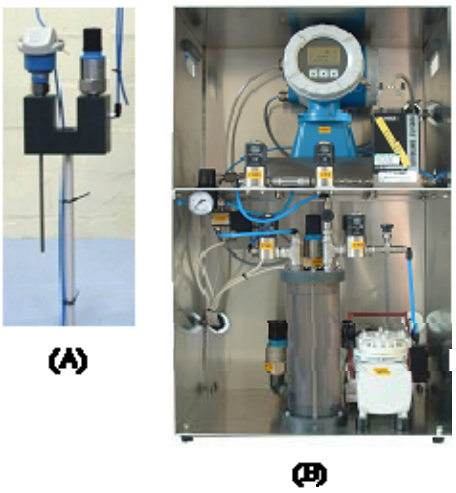


Figure 9: MBR-VFM set-up (A= sensor ; B= measuring device)

Another important aspect of a fouling measurement method is the fact that the total fouling resistance R_f which adds to the membrane resistance R_m in reality is to be considered as consisting of two completely different fouling components : the reversible fouling component and the irreversible fouling component. As a result, the equation (3) can be modified into:

$$\frac{dV}{dt} = \left(\frac{A \cdot \Delta P}{\eta}\right) \cdot \frac{1}{(R_m + R_{f,rev} + R_{f,irr})} = \left(\frac{A \cdot \Delta P}{\eta}\right) \cdot \frac{1}{R_{tot}} \qquad \textbf{Equation 4}$$

In equation (4) the total resistance R_{tot} is assumed to be the result of three resistances in series and is therefore the sum of the membrane resistance, the reversible fouling resistance and the irreversible fouling resistance. Reversible fouling can be overcome relatively easy by shear forces induced by a mechanical action, such as backwashing, air bubble action, etc. For fouling phenomena which are related to much stronger binding forces and can only be removed by chemical membrane cleaning actions, it has become common to use the term irreversible fouling. It was judged to be absolutely necessary to implement within the MBR-VFM measuring protocol the possibility to determine and distinguish both the reversible and irreversible fouling characteristics. Therefore a cyclical protocol was elaborated which envisages to measure the reversible part in a first cycle under low cross-flow mode resulting from a low slug aeration flow. The irreversible fouling is then measured in a high cross-flow mode in the next cycles (see Figure 10).

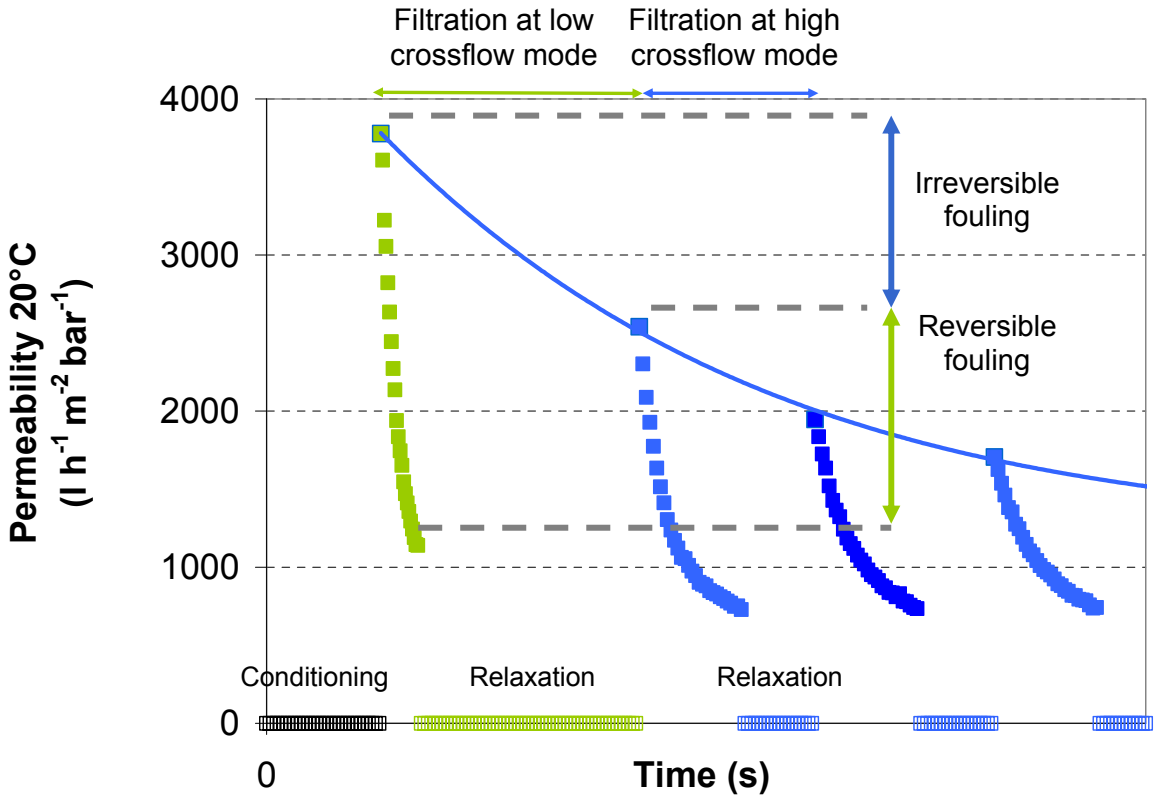


Figure 10: Schematic representation of MBR-VFM measurement protocol.

From the basic MBR-VFM measurement data (as in Figure 10), MBR-VFM fingerprints can be calculated in which V/A (m^3 of permeate volume per m^2 of membrane) is respectively plotted against $R_{tot,rev}/R_m$ (ratio of total reversible hydraulic fouling resistance versus membrane resistance) and $R_{tot,irr}/R_m$ (ratio of total irreversible hydraulic fouling resistance versus membrane resistance). As a result two MBR-VFM fouling graphs are produced: the reversible and the irreversible one. Through image recognition, this information can be translated into a reversible and irreversible fouling number, ranging from 0 to 100%.

More details on set-up, protocols and calculations, are described in Huyskens et al. (2008).

3.2.3.2 Reproducibility and influence of membrane type

MBR-VFM measurements were either performed with 5 mm tubular membranes provided by the former *Millenniumpore Ltd. (UK)*. These were made of surface modified polyethersulphone (PES), had a nominal pore size of 0.1 µm and a length of 65 cm. The second membrane type had a similar internal diameter of 5.2 mm, a nominal pore size of 0.03 µm, a length of 68 cm and was provided by *X-Flow (the Netherlands)*. This tubular membrane was made of polyvinylidene difluoride (PVDF) supported by a mechanically robust polyester substrate. Only membranes with a clean water permeability within a selected range were selected for fouling measurements. Membranes were chemically cleaned in between measurements with 500 mg l^{-1} hypochlorite during 2h.

Mixed liquor samples were collected at MBRs treating municipal wastewater, aerated and prescreened at 2 mm to prevent clogging before the measurement took place. During off-line fouling measurements on mixed liquor samples an extra coarse bubble aeration was provided to prevent sedimentation of the sludge. pH and dissolved oxygen concentration (DO) were measured for each MBR-VFM measurement.

3.2.3.3 Tests on lab-scale MBR systems

In a first test, one lab-scale MBR was operated on municipal wastewater. In a second experiment, two lab-scale systems were run in parallel. In each case, the MBRs were equipped with Kubota membranes and were inoculated with activated sludge from a full-scale municipal wastewater treatment plant. Both units of the second test were operated at the same hydraulic retention time (HRT) and sludge age. The only difference was the organic loading, as MBR1 was fed with diluted wastewater and MBR2 with undiluted wastewater. In both experiments, membrane fouling was monitored through permeability and MBR-VFM measurements. In addition, other parameters related to membrane fouling were analyzed frequently, such as EPS. Routine chemical measurements were performed to check biological treatment efficiency.

3.2.4 Photometrical EPS sensor

The sensor for continuous online determination of carbohydrates and proteins in the supernatant of MBR activated sludge consists of two main components: a newly developed sample pre-treatment device Mehrez et al. (2007a) and the Sequential injection analyser (SIA). In Figure 11 the scheme of the whole sensor is presented.

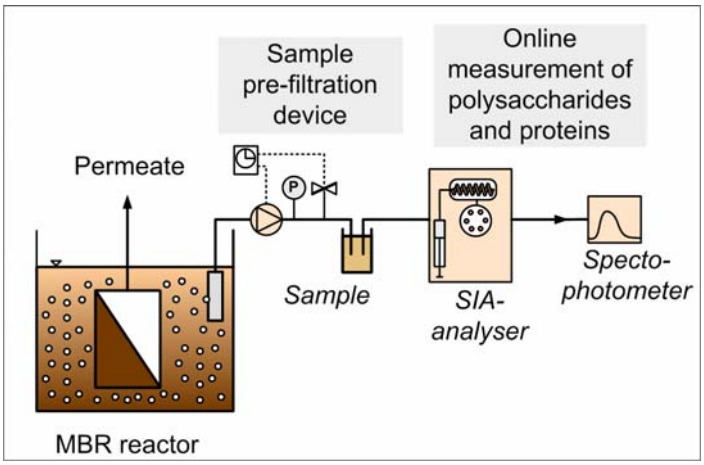


Figure 11: Scheme of EPS sensor system.

3.2.4.1 Sample pre-treatment device

A stainless steel filter with a nominal pore size of 1 µm and surface of about 50 cm^2 is utilized. The filter is submerged in the activated sludge; the sludge is filtrated continuously with a flux between 10 and 17 $Lm^{-2}h^{-1}$. In order to prevent the filter clogging the filtration (10 min) is intermitted by a relaxation interval of 2 min, than peristaltic pump stops, a valve opens allowing the air to come into the tube system. The pump and the valve are controlled by a clock-pulse generator. Additionally sludge turbulence and air souring in the membrane reactor prolong the filtration time until the cleaning of the filter is necessary (every other week). The filter is cleaned with 1 % sodium hypochlorite for at least two hours followed by the treatment with 10 % concentrated sulphuric acid for at least two hours.

The filtrated supernatant is pumped into the small sample vessel equipped with an overfall, which discharge the surplus filtrate. Subsequently the SIA analyser aspirates the necessary sample volume from the sample vessel and measures the concentration of polysaccharides or proteins in the supernatant.

3.2.4.2 SIA analyser

Sequential Injection Analysis (SIA). SIA technique was used to develop and to perform the analysis of polysaccharides and proteins in continuous on-line mode. The apparatus (Figure 12) was purchased from FIAlab Instruments, USA (MicroSIA). It consists of a syringe pump (1 mL) driven by a stepper motor, a 10-port selector valve, the holding coil, the flow cell with 20 mm optical path (internal volume 8 µL), the tungsten-halogen lamp (LS1, OceanOptics, USA) and a spectrometer (USB2000, OceanOptics, USA). Fiber-optic cables are used to connect the flow cell to the light source and the spectrometer. Experimental data were collected by FIAlab for Windows (versions 5.9.292-306).

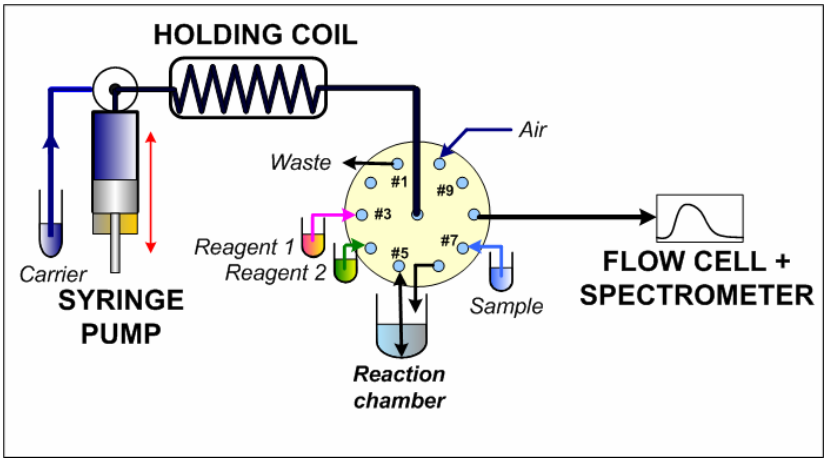


Figure 12: Scheme of SIA analyser.

Procedure of automated assays For the determination of polysaccharides concentration the reagents were prepared according to Dubois et al. (1956). The carrier fluid was degassed ~82 % (w/w) concentrated sulphuric acid. The absorption measurement was performed at 490 nm by a reference wavelength of 750 nm.

For the determination of protein concentration with SIA the reagents were prepared according to Salerno et al. (1985). Different methods have been successively developed: Method I (Mehrez et al., 2007b), Method II (Mehrez et al. 2008), Method II improved; thereby the reagents and the reaction performance changed slightly (Mehrez et al. 2010, b). In order to prevent formation of precipitates in sludge filtrate samples during the analysis chelating agent nitrilotriacetic acid trisodium salt monohydrate (Merck, Germany) was added to the alkalic reagent in the concentration of 3.21 g/L (Method II), no influence on measurement

sensitivity was observed. Solution made of 4.4 g/L NaOH and 4.9 g/L Na_2CO_3 was used as carrier. The absorption was measured at 730 nm by a reference wavelength of 500 nm.

In the Method I the reaction (mixing of the sample and the reagents) took place in the holding coil. To support this process the injected sample and the reagents were aspirated in small sequences and moved forwards and backwards several times in the holding coil.

In the Method II and II improved the reaction between the sample and reagents took place in a newly developed reaction chamber in similar way to the manual assay. The reaction chamber is made from analytical glass. The form is conic, what enable complete removal of fluids from the chamber. The chamber is covered by a PVDF cover fixing the inlet tubes. The sample and first reagent is pumped into the chamber; mixing is performed by introducing air and resulting turbulence. The second reagent is added through an additional port. After the mixing 200 µL of formed coloured reaction products are aspirated and transferred to the flow cell (optical path 2 cm) were absorption is measured. The aspiration of larger volumes minimizes the dilution of reaction products on the way to the detector with the carrier fluid which increases the method sensitivity (comparing with Method I). Cleaning of the reaction chamber is performed by removal of remaining fluid following the introducing of 1 mL (for carbohydrates analysis) or 2 mL (for protein analysis) of carrier solution and subsequently air mixing for intensifying the cleaning procedure.

The measurement of polysaccharides and proteins can theoretically be performed in parallel by the SIA analyser. However the reagents used for the determination of polysaccharides interfere with the measurement of proteins largely, so that the parameters can only be measured subsequently. After the measurement of polysaccharides the SIA analyser has to be rinsed by the cyclo-hexane to remove the residues of the phenol reagent.

3.2.4.3 On-line measurements on the pilot plant

Figure 13 presents the sensor installed in the MBR pilot plant. The concentration of polysaccharides and proteins were measured in the filtrate of the sludge from aerobic chamber. Polysaccharides and proteins were analysed subsequently in 1 to 3 weeks interval. For proteins analysis the Method II (see 4.2.2.2) were applied.

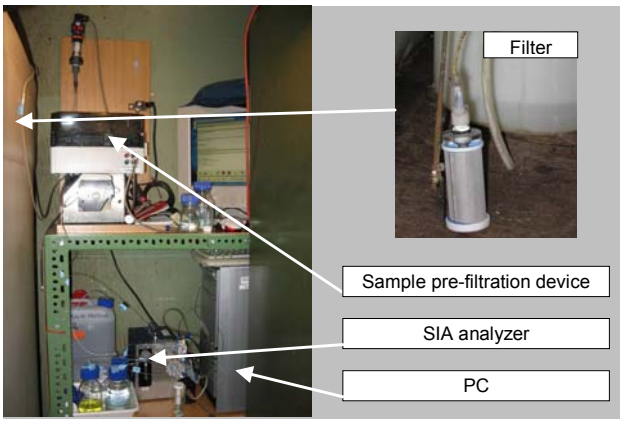


Figure 13: EPS sensor installed in the pilot plant.

During the continuous measurements standard solution was measured about 3 times per day (BSA 25 mg/L for proteins, glucose 10 mg/L for polysaccharides) in order to verify the accuracy and the sensitivity of online analysis. The received values were averaged for one day and used then for the recalculation of measured concentrations in sludge filtrate calculated according the equation derived from calibration curves (Figure 32 a and Figure 38 a).

Parallel to continuous analysis with SIA sensor manual measurements have been performed 3-4 times a week in order to verify the on-line measured concentrations.

Due to low temperature in the winter (~10°C and below), the carrier used for polysaccharides analysis was warmed in water bath. It was necessary because the temperature influences the density of the carrier (82 % w/w H_2SO_4) what have an influence on the shape of measured peaks and with it on the method accuracy.

Furthermore, because of aggressive reagents (conc. H_2SO_4) caution and frequent maintenance of the sensor was required when polysaccharides were measured. E.g. one time the SIA apparatus was not leak-proof causing the entrance of the acid into the SIA components damaging the selector valve and syringe pump like showing the Figure 14. Furthermore the abrasion of PVDF rotor caused that the particles came into the SIA system (tubes, flow cell), what may restrict the accuracy of the method with time. Otherwise the PVDF rotor was necessary because of the used conc. H_2SO_4.

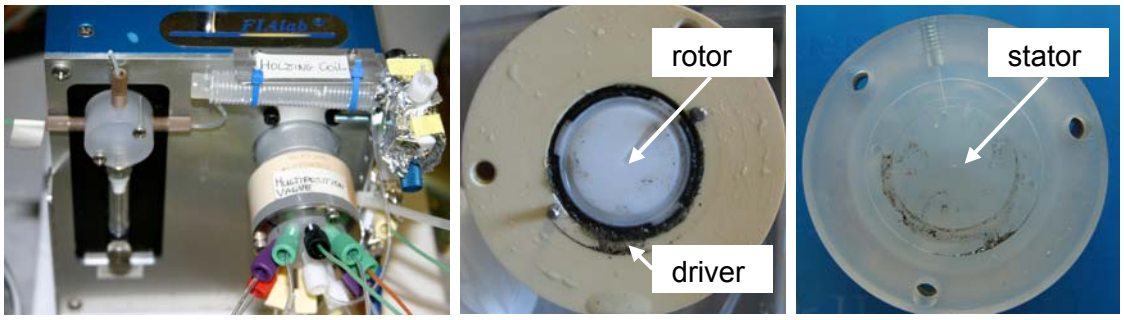


Figure 14: a) SIA apparatus with syringe pump and selector valve; b) open selector valve with white PVDF rotor and visible damaged driver (metal part) due to contact with acid; c) pollution of stator.

3.2.5 Comparison MBR-VFM and EPS sensor

Mixed liquor samples were collected at the Berlin MBR pilot plant and transported to the laboratory in closed vessels (transportation time approximately 20 min). The MBR-VFM measurement was started immediately after arrival. The applied membrane was a tubular PVDF membrane (diameter 8 mm, pore size 30 nm), provided by *X-Flow, the Netherlands*. During the MBR-VFM measurement, an extra coarse bubble aeration was used to prevent sedimentation. At the beginning and at the end of the measurement, small subsamples were taken for analyses. As the SIA sensor was defective at the time of the test period, parallel EPS measurements were performed manually.

4 Results and Discussion

4.1 Flux Enhancers

4.1.1 Determination of the optimum concentration

A series of well-defined jar tests were carried out prior to the filtration performance experiments on the pilot-scale MBR plant in order to select the most effective chemicals with regard to the removal of fouling-causing compounds (polysaccharides and proteins). The results of jar tests indicate the effect of tested chemicals only under these lab conditions. Presumably not all of the effects can be observed in pilot-scale MBRs because of different experimental conditions like physical-chemical parameters, changing composition of the influent, microbiological processes, mechanical shear stress of flocs caused by the aeration in the filtration tank etc.

As can be seen from Figure 15, chitosan shows the best ability to bind SMP in supernatant. But also the polymer KD452 seems to be very effective. Starch, activated carbon and the Nalco polymer which was especially developed for MBR application are just in a medium range. The optimal dosage in terms of EPS elimination found in shaking flasks is listed in Table 6.

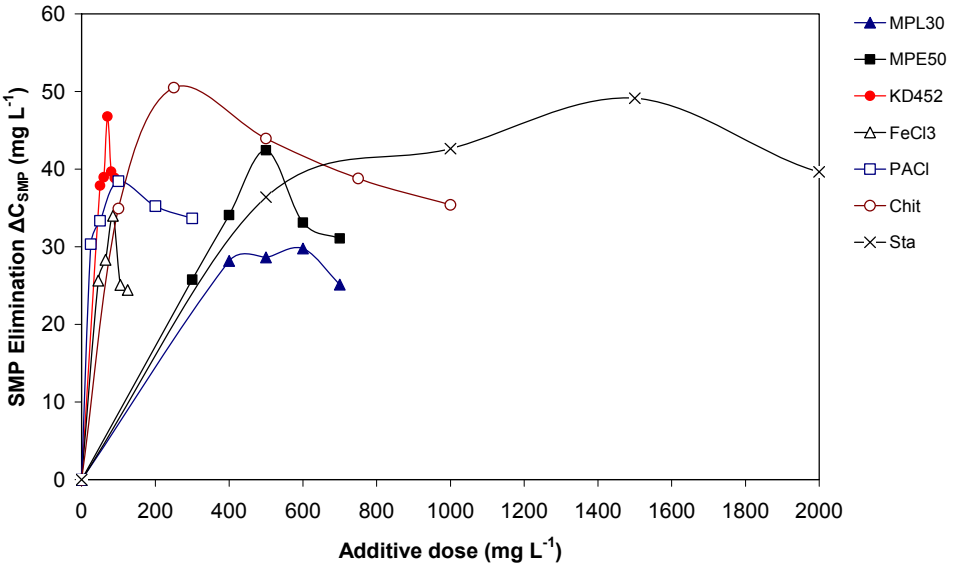


Figure 15: Impacts of additives on SMP removals (SMP removals are averages of duplicate measurements) (Koseoglu et al., 2008)

Table 6: Optimal dosing in terms of SMP elimination for tested additives

Category	Supplier	Abbrev.	Product	Optimal dosage in mg/L
Activated carbon	Norit	PAC-1	SA Super	450
	PICA	PAC-2	Picahydro LP27	5000
Metal salt	Merck	FeCl3	-	85
	Ciba	PACl-1	Magnasol 5113	-
	Ciba	PACl-2	Magnasol 5108	100
Chitosan	France Chitine	Chit-1	Chitosan 221	250
	France Chitine	Chit-2	Chitosan 652	200
	France Chitine	Chit-3	Chitosan 342	200
Polymer	Ciba	Poly-1	Zetag 7878FS40	-
	Ciba	Poly-2	Zetag 8846FS	-
	Nalco	Poly-3	MPE-50	500
	Kurita	Poly-4	M H 260	500
	Kurita	Poly-5	MP H 30	500
	Kurita	Poly-6	MP 252	500
	Kurita	Poly-7	MP L 30	500

	Adipap	Poly-8	Adifloc KD 450	70
	Adipap	Poly-9	Adifloc KD 451	70
	Adipap	Poly-10	Adifloc KD 452	70
	Adipap	Poly-11	Adifloc KD 453	70
	Diagonal	Poly-12	Diafloc CH100	150
Enzyme	Novozymes	Enz-1	Alcalase 2.5 L	-
	Novozymes	Enz-2	Viscozyme L	-
Starch	Rhodia	Sta-1	Rheozan	-
	Rhodia	Sta-2	Jaguar C162	300
	Rhodia	Sta-3	Rhopodol 23	-
	Tate&Lyle	Sta-4	Mylbond 163	1500
	Tate&Lyle	Sta-5	Mylbond 168	1500
	Tate&Lyle	Sta-6	Mylbond 149	1500
	Roquette	Sta-7	Vector SC 20157	1000
	Roquette	Sta-8	Vector SC 27216	1000

Further information can be found in (Iversen et al., 2007, Koseoglu et al., 2008).

4.1.2 Influence on SMP and other fouling relevant parameters reduction

Figure 16 depicts the removal of different supernatant components by selected additives: flocculants ADIPAP KD 452 and NALCO MPE 50, starch TATE & LYLE Mylbond 168 and PACs NORIT SA Super and PICA Picahydro LP27.

The results derived from these tests show that in most cases the tested additives removed the considered compounds from the supernatant. However, the optimal additive concentration was difficult to determine because the highest additive efficiency was almost always observed at the highest tested concentration. These findings were contradictory to those described in previous experiments (see chapter 4.1.1), what might be explained by the fact that the sludge used in these contained higher concentration of suspended solids TS (~13.5 g L^{-1}) than used before (8-10 g L^{-1}) which might shift the optimal dosage to higher additive concentration. The influence of TS concentration on the efficiency of the tested additives has been observed in additional experiments (see 4.1.3): the higher the TS concentration was, the higher the amount of additive was necessary to obtain the same removal efficiency.

This Figure 17 summarizes the effects of five flux enhancing additives on MBR mixed liquor.

The cationic polymers (ADIPAP KD 452 and NALCO MPE 50) showed positive impacts on almost all of the tested parameters. They removed efficiently biopolymers, polysaccharides and proteins and reduced CST to a very high extend. During the flocculation the charge neutralisation of sludge flocs takes place that promotes the sludge dewatering (reduced CST). Furthermore flocculants form bridges between the flocs that increase the floc size (Iversen et al., 2008c). It seems that larger molecules like biopolymers are better trapped into the floc during the flocculation process than the rest of DOC irrespective of the much smaller biopolymers concentration. As well the turbidity of the supernatant is effectively reduced by the polymer. The flocculants seem to be recommendable for application in MBR, since they removed specifically larger molecules that are presumably the fouling causing substances.

PAC (NORIT SA Super, PICA Picahydro LP27) showed no distinct selectivity for the removal of macromolecules, and removes DOC throughout the whole range of macromolecular

weight. Also SMP (polysaccharides, proteins) was removed quite effectively. By this, sludge dewaterability (CST) could be improved slightly. However at high concentrations PAC particles remained in the supernatant what may amplify the particulate fouling during the filtration performance and cause abrasion of the membrane. Since the described experiments are only short time experiments, aspects like adsorption capacity of PAC during time and possible enhanced microbiological degradation of specific wastewater compounds could not be examined and have to be taken into account for by long-time filtration tests.

The tests with starch revealed that this additive has positive and negative impacts on the supernatant. It eliminates efficiently the biopolymers and reduces CST; however the concentration of polysaccharides increases due to residues in the supernatant (photometric analysis, SEC).

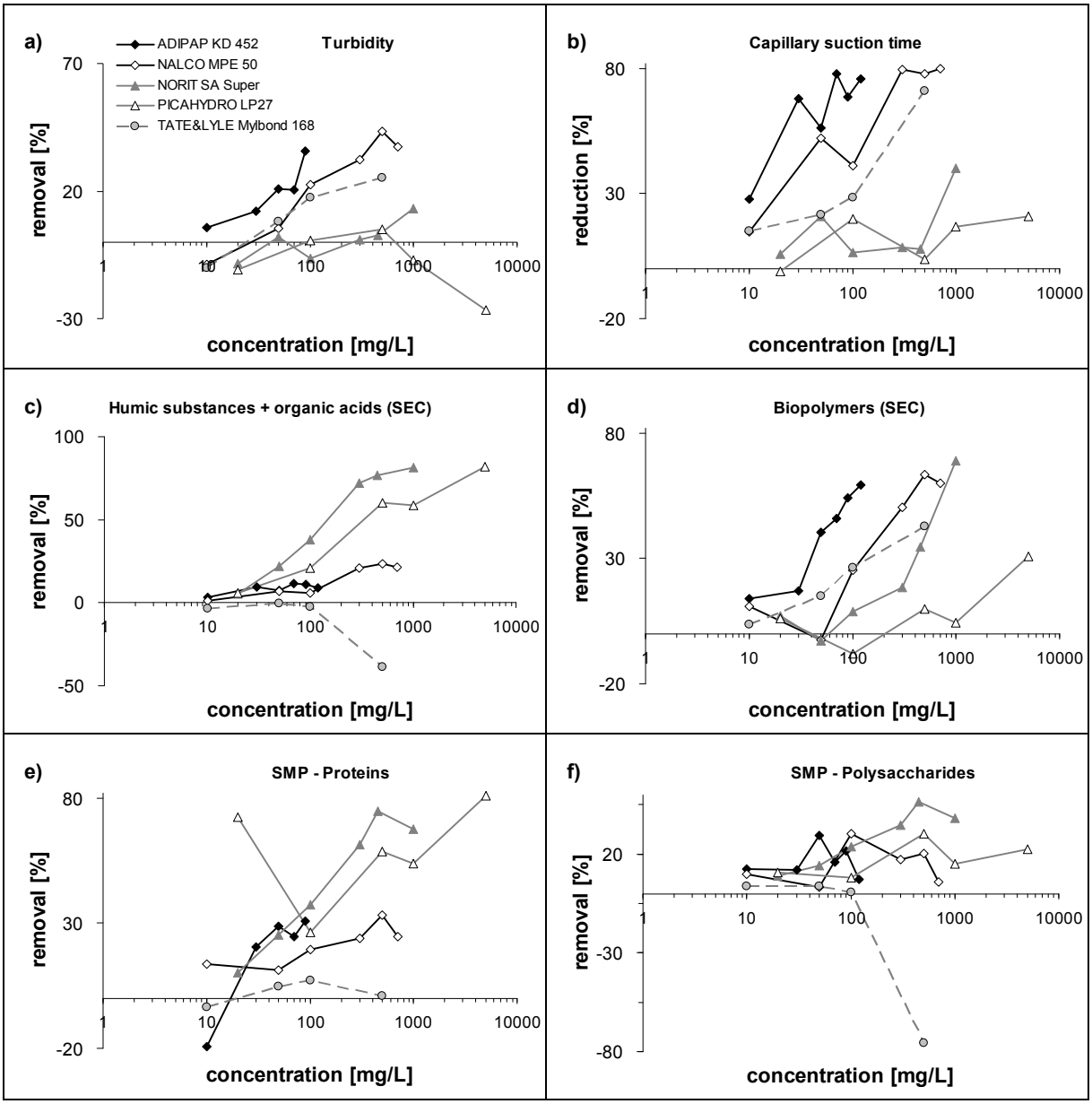


Figure 16: Removal of different fractions of sludge supernatant (a, c-f) and improvement of CST (b) by flocculants (ADIPAP KD 452, NALCO MPE 50), by starch (TATE & LYLE Mylbond 168) and by PACs (NORIT SA Super, PICA Picahydro LP27). (Iversen et al. 2009)

	Adipap KD 451	*Nalco MPE50*	*Norit SA Super*	*Picahydro LP27*	*Tate&Lyle Mylbond168*
Turbidity	+	++	o / --	--	+ / o
DOC	+	+	++	++	o / --
Biopolymers	++	++	++ / +	o / +	++
Humics+Acids	o	+	++	++	--
Proteins	+	+	++	++	o
Carbohydrates	+	+	++	+	--
CST	++	++	+ / o	+	++

++	very good effect (removal > 40%)
+	good effect (removal 15-40%)
o	small / no effect (removal 0-15%)
--	negative effect (removal < 0)

Figure 17: Effect of selected additives on different fractions of supernatant and CST classified in four groups. (Iversen et al. 2009)

The SEC measurement revealed that some residues of ADIPAP KD 452 remain in the liquid phase (Figure 18 a): the emergent peak at 90 min shows probably the monomers or the impurities contained in the flocculant. The same was observed for the starch TATE & LYLE Mylbond 168: the concentration of humic and low molecular weight substances increased with increasing concentration of starch (Figure 18 b). It seems that the applied starch contained a large amount of small molecules that remain in the supernatant that may amplify the membrane fouling during the long term filtration.

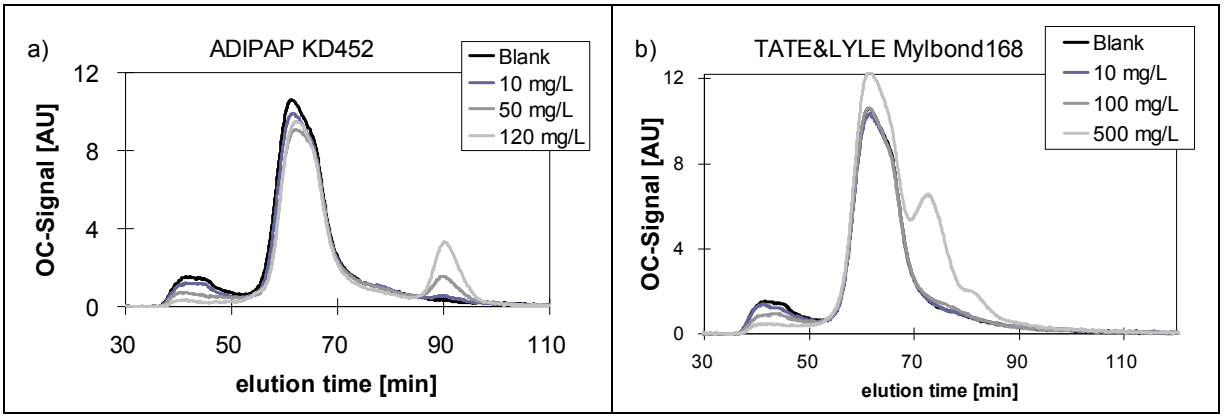


Figure 18: LC-OCD chromatogram of sludge supernatant measured during shaker batch tests with different concentrations of ADIPAP KD 452 (a) and of TATE & LYLE Mylbond 168 (b). (Iversen et al. 2009)

4.1.3 Influence of TS, Ca^{2+} Ions and temperature on the efficiency of additives

Figure 19 displays the results for investigated parameters exemplary for one additive (other results see Figure 54, Figure 55, Figure 56).

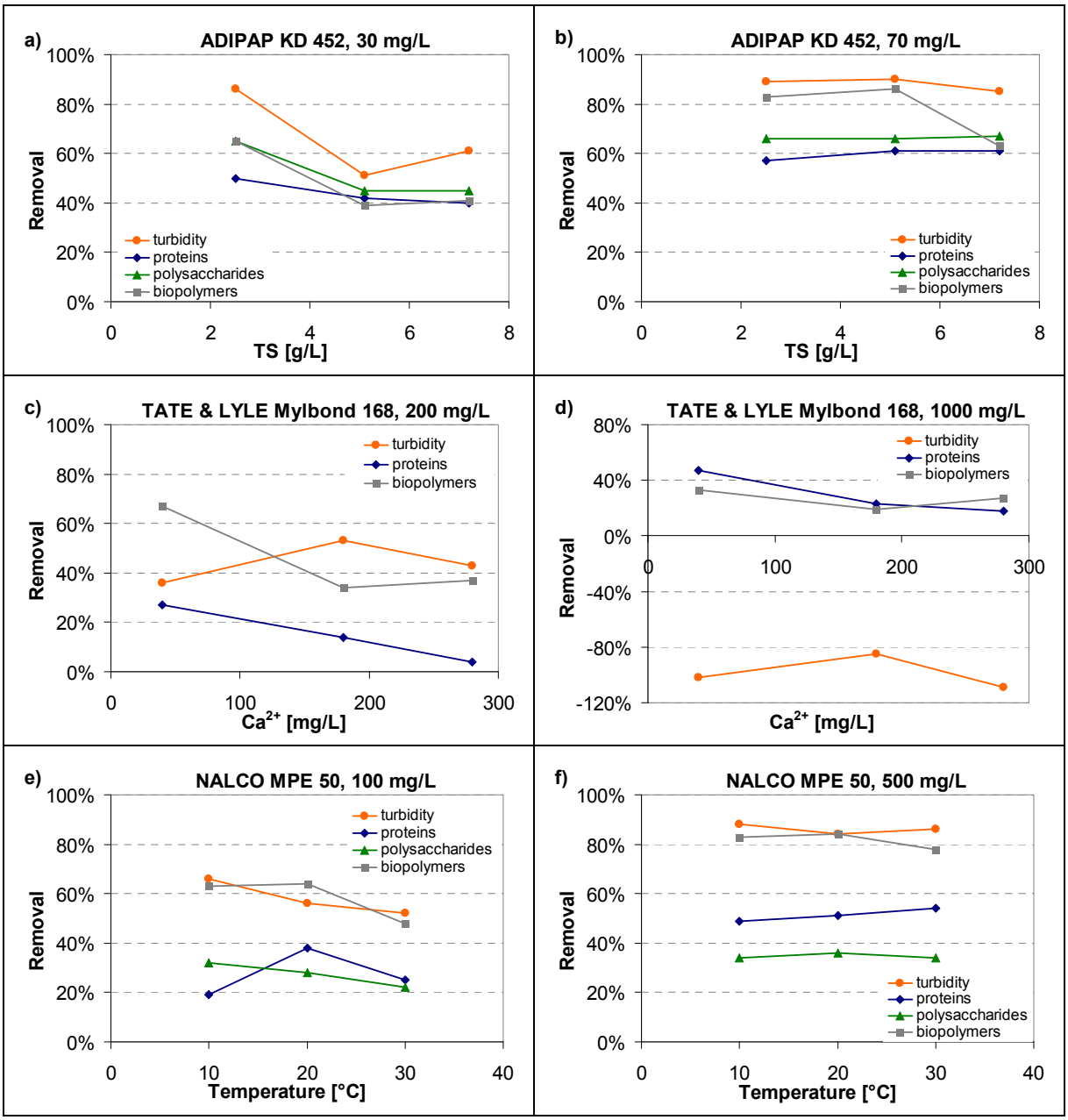


Figure 19: Influence of TS (a-b), Ca^{2+}-Ions (c-d) and temperature (e-f) on removal of different fractions of sludge supernatant at two additive concentrations for ADIPAP KD 452 (a-b), TATE & LYLE Mylbond 168 (c-d) and NALCO MPE 50 (e-f).

The experiments with three different TS sludge concentrations revealed that TS has no influence on the additives efficiency when optimal concentration of additive is applied (Figure 19 b, example ADIPAP KD 452). However if small quantity of ADIPAP KD 452 was used, the efficiency of flocculent decreased with increasing TS concentration (Figure 19 a). Equivalent observations have been done with NALCO MPE-50, TATE & LYLE Mylbond 168.

Ca^{2+} concentrations have not shown any impact on the efficiency of ADIPAP KD 452 and NALCO MPE-50 in the tested range. In the case of the starch, higher Ca^{2+} concentration decreased the removal of proteins and biopolymers from supernatant; stronger for smaller concentration of applied TATE & LYLE Mylbond 168 (Figure 19 c-d). No impact on removal efficiency of particles (turbidity) has been observed. The polysaccharides concentration increased several times in the supernatant, when the starch was applied. The same effect was observed for the turbidity at 1000 mg/L application dosage.

The temperature has slightly influence on the removal of dissolved substances and particles. If the dosage of NALCO MPE-50 is too small, the removal efficiency decreased (Figure 19 e) with increasing temperature for all parameters except for proteins. At optimal concentration no effect of temperature has been seen. Similar results have been found for ADIPAP KD 452 (Figure 56). TATE & LYLE Mylbond 168 had no impact on the removal efficiency of soluble organics but on the removal of particles: with increasing temperature the turbidity of the supernatant decreased.

4.1.4 Influence on filterability

Four sets of tests have been conducted:

1. Aerated filtration of untreated/treated (optimal concentration) sludge according to the protocol shown in Figure 5 with a constant flux, J = 22-25 L/(m²h), this enables measurement / calculation of the different resistances

Figure 20 shows results of the filtration tests with sludge which were carried out. At first sight it seems that the addition of chitosan and the polymers MPE 50, MPL 30 and KD 452 strongly enhances the filtration performance while an addition of the starch Mylbond 168 worsens the performance in comparison to the reference sludge. At second sight it must be said that the trials on the 15/9 with addition were done first while the test with untreated sludge was done afterwards. For all other tested substances the reference sludge was tested first. In comparison to the reference sludges on the other days the resistance one the 15/9 is very small. It is therefore possible that fouling causing substances like EPS are eliminated within the 4-5h between the start of the first and the second experiment. These results further stress the importance to conduct parallel tests like the ones with the two pilot MBRs and not to store the sludge.

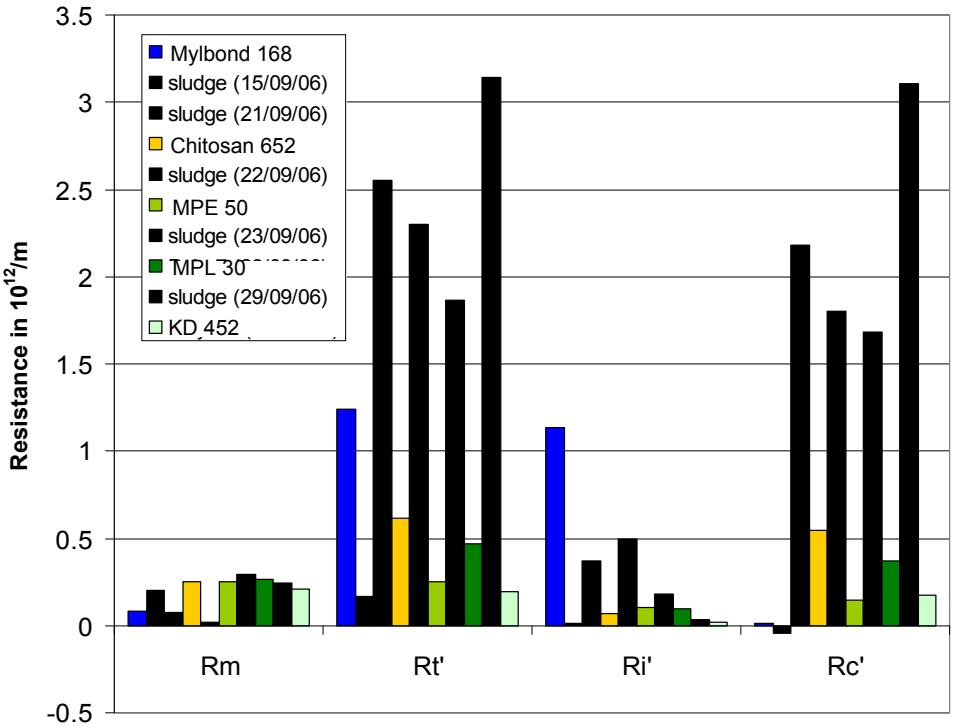


Figure 20: Effect of additive on filtration (black bars: reference sludge) (Iversen et al., 2007)

2. Filtration of water with 5% optimal additive concentration for 2h at J = 60L/(m²h)

Water with 5% of the optimal additive dosing was membrane filtered for two hours in the cross flow filtration test cell. With the difference between the TMP at the start and the end of filtration, the fouling rate (ΔTMP/Δt) was calculated in comparison to pure water filtration. Results are shown in Figure 21. The starch Jaguar has a quite strong effect on filtration performance and seems therefore not advisable for membrane filtration. This is not astonishing as carbohydrates and polysaccharides are thought to be strong foulants in MBR systems. The filtration performance of starch flocculated sludge, however, might be different. Also the two tested polymers have a strong impact especially for membrane PVDF-2. The astonishingly high fouling rates are probably due to the experimental setup and procedure: no soothing of adsorption effects due to a cake on the membrane, quite high fluxes (which was selected on purpose to accelerate potentially slow fouling) and no interactions between the flocculant and sludge. It is also possible that 5% of optimum dosing overrate the fraction of additive that is not bound into flocs. This residual rate was set up arbitrary in absence of information in the literature. In any case, these results exemplify the risk of overdosing when resorting to chemical flux enhancers: the appropriate dosage of these chemicals is of paramount importance to avoid detrimental counter effects on membrane fouling. Results also indicate that when choosing the most suited additive, not only sludge characteristics but also the membrane type must be taken into account.

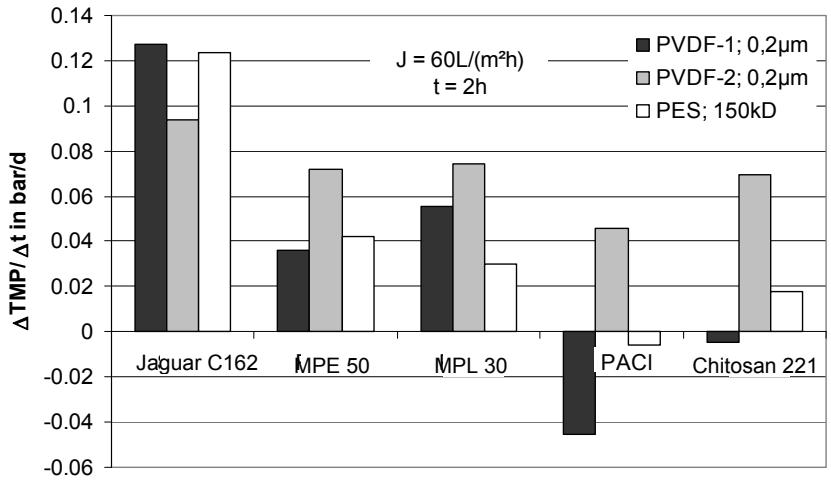


Figure 21: Results of the residual tests (Iversen et al., 2008a)

3. Filtration tests with 4 different additive concentrations for 2h at constant flux, J = 27L/(m²h), 10min puls/2min pause

Table 7 summarizes the results of short term filtration tests in terms of average TMP, fouling rate (ΔTMP/Δt), total resistance, and permeability values. Such values were calculated using the TMP data of the last 10 min of filtration since both raw and additive dosed sludges generally exhibited maximum TMP values and fouling rates at the last filtration interval.

When compared with the filterability of the reference (untreated) sludge, the best performance was achieved by starch Mylbond 168 in terms of TMP_{ave} and fouling rates in the short term filtration tests. The starch at a dose of 1500 mg L^{-1} decreased TMP_{ave} and fouling rate by 3 and 10 times, respectively. Similar to starch, chitosan was also successful in fouling control. At its optimum dosage, chitosan, provided 90% reduction in fouling rate. All tested cationic polymers significantly reduced fouling rates and increased permeability values, similar to starch and chitosan. At their optimum dosages, MPE50, MPL30 and KD452 provided 96, 80 and 74% reductions in fouling rates, respectively.

Table 7: Short term filtration performances of raw sludges and additive-dosed sludges (Koseoglu et al., 2008)

Sample	TMP_{ave} (bar)	$\Delta TMP/\Delta t$ (mbar/min)	R_t (10^{11} m^{-1})	K (L m^{-2} h^{-1} bar^{-1})
Untreated Sludge	0.023	0.070	2.92	1174
400 mg L^{-1} MPE50	0.008	0.002	1.02	3375
500 mg L^{-1} MPE50	0.010	0.003	1.27	2700
600 mg L^{-1} MPE50	0.008	0.007	1.02	3375
Untreated Sludge	0.016	0.040	2.03	1688
500 mg L^{-1} MPL30	0.009	0.010	1.14	3000
600 mg L^{-1} MPL30	0.008	0.008	1.02	3375
700 mg L^{-1} MPL30	0.007	0.007	1.02	3375
Untreated Sludge	0.013	0.030	1.65	2077
60 mg L^{-1} KD452	0.009	0.008	1.14	3000
70 mg L^{-1} KD452	0.008	0.008	1.02	3375
80 mg L^{-1} KD452	0.009	0.003	1.14	3000
Untreated Sludge	0.024	0.100	3.05	1125
100 mg L^{-1} Chit	0.015	0.010	1.90	1800
250 mg L^{-1} Chit	0.015	0.010	1.90	1800
500 mg L^{-1} Chit	0.021	0.090	2.67	1286
Untreated Sludge	0.028	0.090	3.56	964
65 mg L^{-1} $FeCl_3$	0.024	0.080	3.05	1125
85 mg L^{-1} $FeCl_3$	0.017	0.060	2.16	1588
100 mg L^{-1} $FeCl_3$	0.024	0.070	3.05	1125
Untreated Sludge	0.023	0.080	2.92	1174
50 mg L^{-1} PACl	0.019	0.040	2.41	1421
100 mg L^{-1} PACl	0.019	0.050	2.41	1421
200 mg L^{-1} PACl	0.027	0.080	2.43	1000
Untreated Sludge	0.032	0.100	4.06	844
1000 mg L^{-1} Sta	0.015	0.030	1.90	1800
1500 mg L^{-1} Sta	0.011	0.010	1.40	2455
2000 mg L^{-1} Sta	0.011	0.020	1.40	2455

PACl and $FeCl_3$ exhibited the least improvement in fouling rates and TMP_{ave} values among tested additives. At their optimum dosages, reductions in fouling rates with respect to their reference sludge were 33 and 37% for $FeCl_3$ and PACl, respectively. On other hand, higher dosages of PACl in fact exhibited higher TMP_{ave} value than that of raw sludge highlighting the importance of a controlled dosing. The finding that PACl and $FeCl_3$ exhibited the least improvement in fouling control can be linked to the weak structure or smaller sizes of flocs formed by these metal salts. Apparently cationic polymers, chitosan and starch triggered the flocculation mechanism better than the metal salts.

4. Flux stepping tests in order to determine the critical flux J_{crit}

Critical flux is one of the most important parameters for the MBR operations and membrane fouling rates are generally exponentially proportional to the flux especially above the critical flux.

Figure 22 shows critical flux values of raw and additive-dosed sludges. The critical values found for MPL30, MPE50, KD452, FeCl3, PACl, Chit, and Sta were 51, 54, 51, 42, 42, 36, 45 L m^{-2} h^{-1}, respectively. Based on the average critical flux value of 37 L m^{-2} h^{-1} for the raw sludge, such values correspond to critical flux enhancements of 38, 46, 38, 14, 14, 0, and 22 %. Except chitosan, all additives increased the critical flux value of the raw sludge and such increases were statistically significant. The result for chitosan was unexpected since this additive showed good SMP removal (see Chapter 4.1.1) at its optimum dosage and improved subcritical filtration performance in 2 h tests. Therefore, this result again indicated that removal of SMP and further improvement in filtration performance does not always correlate. Consistent with short term filtration tests, cationic polymers performed well and increased critical flux values to above 50 L m^{-2} h^{-1} levels. The enhanced filterability of the MBR sludges with cationic polymers can be attributed to a charge neutralization mechanism, further increase in floc size and decrease in concentration of soluble foulants due to entrapment/sorption onto flocs. The results obtained in this work for cationic polymers are consistent with the work of Yoon and Collins (2006) in which MPE50 polymer enhanced the flux by 39 %.

FeCl3 and PACl enhanced the critical flux of the raw sludge only by 14 %. Similarly, they had exhibited the least improvement in fouling rates in short term filtration tests.

Although not as effective as cationic polymers, starch also increased critical flux up to 45 L m^{-2} h^{-1} levels. Overall, enhancements in critical flux values by the additives may be linked to sludge flocculation and thus subsequent floc size enlargement and increase in hydraulic diameter in the cake layer.

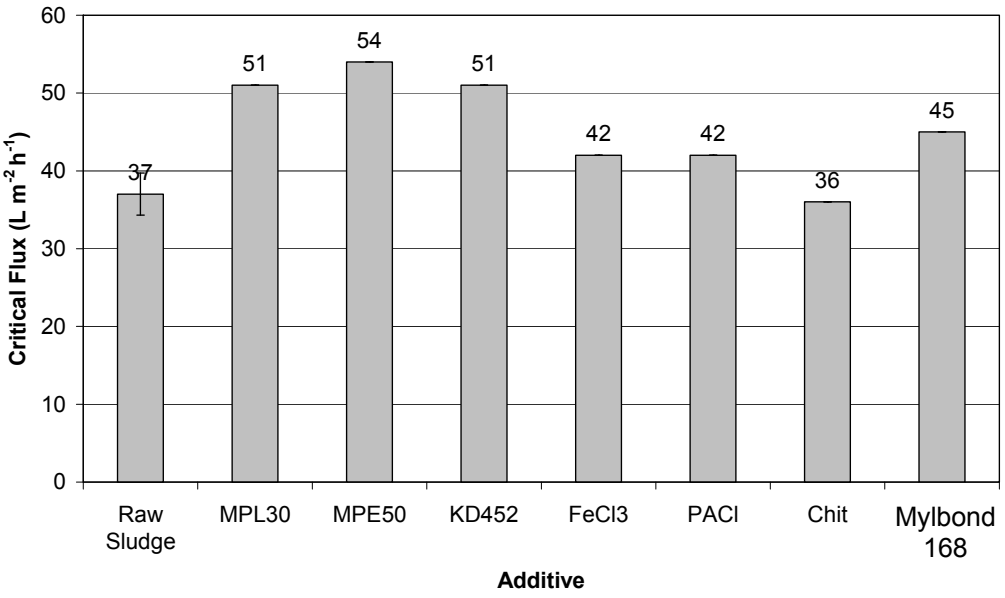


Figure 22: Critical flux values for the different flocculants (Koseoglu et al., 2008)

These results are also reported in more detail in (Iversen et al., 2007, Iversen et al., 2008a, Koseoglu et al., 2008)

4.1.5 Biotoxicity

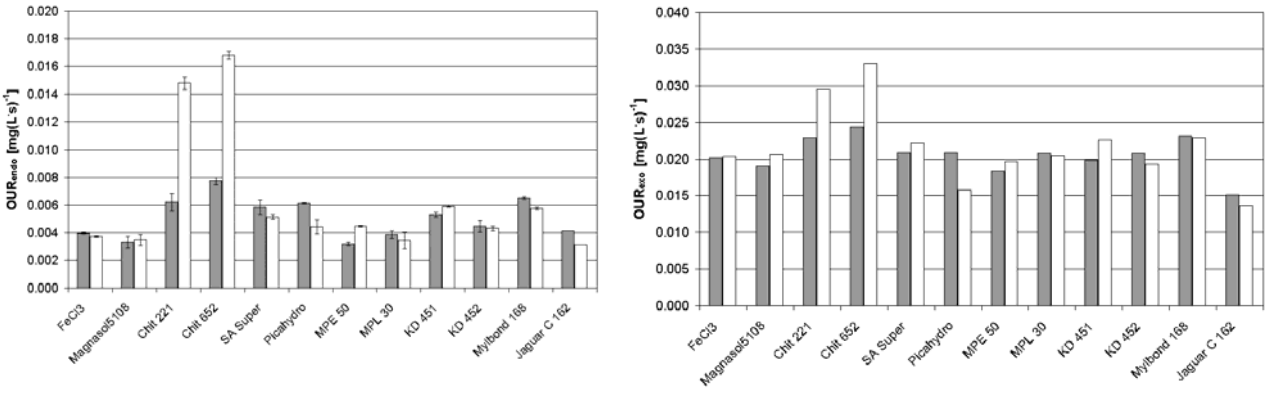
While filterability is certainly an important parameter in MBRs, the impacts of the tested substances on the biomass and the degradation mechanisms must further be evaluated. Also, it would be detrimental if these substances are readily biodegradable. As nitrification is considered to be the most sensitive biological mechanism concerning toxicity or inhibition in activated sludge processes, the impacts of selected additives on nitrification were evaluated in this study. Investigations were also performed on denitrification and respiration activity.

Further information can be found in (Iversen et al., 2008a, Iversen et al., 2009)

4.1.5.1 Respiration and oxygen transfer

In Figure 23 a, the endogenous oxygen uptake rate is depicted. The high rates for the chitosans indicate the biodegradable nature of the tested chitosans. Approximately 50-80% of this increase is due to the acid in which the chitosan is dissolved, as measured in separate batch tests (data not shown). However, the chitosan is biodegradable, and thus an excess dosing would be necessary. Considering the relatively high costs of this product, an application for wastewater does not seem to be advisable. Furthermore, the filtration tests showed that although the SMP in the supernatant were effectively removed, the critical flux did not alter much with the chitosans (Figure 22). The addition of the PAC Picahydro LP 27 causes a 28% decrease in respiration. This phenomenon can be explained by the significant pH shift of the treated sludge (pH: 5.7 in comparison to 7.1 for the reference) compared to neutral reference sludge. Nevertheless, it must be stressed that the dosing of 5 g L^{-1} for this additive is very high.

A similar trend was found for the exogenous oxygen uptake, as shown in Figure 23 b. The lactic acid accounted for 59% of the increase in OURexo (Chit 221) and for 9% (Chit 652). For the PAC Picahydro LP 27 treated sludge, a 25 % decrease in oxygen uptake was noticed, which can again be explained by the change in pH.



a) b)

Figure 23: a) Endogenous and b) exogenous oxygen uptake rate for flux enhanced sludge (white bars) in comparison to the reference sludge (grey bars) (Iversen et al., 2009)

Figure 24 shows the results of the k_La measurements. A decrease of 13 % in oxygen transfer was found for sludge treated with the PACl Magnasol 5108, which might be due to the highly viscous nature of this additive. While the supernatant viscosity was not changed when PACl was added, the viscosity of the mixed liquor increased slightly (Iversen et al., 2008b). Also other important parameters for oxygen transfer (surface tension, bubble diameter, viscosity, diffusion coefficient, and rise velocity) might change if this PACl is added. The transfer coefficients for also shifted if Chitosan 221 (-14%) and the starch Jaguar C 162 (+20%) were added to the sludge. The chitosan also lead to a worse settleability of the spiked sludge in

comparison to the reference (Iversen et al., 2009). This might be attributed to changed sludge characteristics like particle size and form or mixed liquor viscosity that might also have caused the decreased kLa value. A slight decrease (-10%) of the oxygen transfer coefficient was observed when the PAC Picahydro LP 27 was added to the system. This might be due to the change of pH and thus effects on sludge characteristics. Yoon et al. (2006) found an increase in oxygen transfer rate of 10-20% for the polymer MPE 50. For this polymer a slight increase of 6% was found in kLa in this study. For all other additives the changes in k_La values are not significant (± 10%) and remain in the range of the natural time variability of the mixed liquor. Indeed, as shown in Figure 24, the k_La values of the reference mixed liquors also vary from 0.004-0.006 s^{-1}. Such a wide range stresses the importance to conduct parallel tests, as wrong quantifications and effects will be implied otherwise.

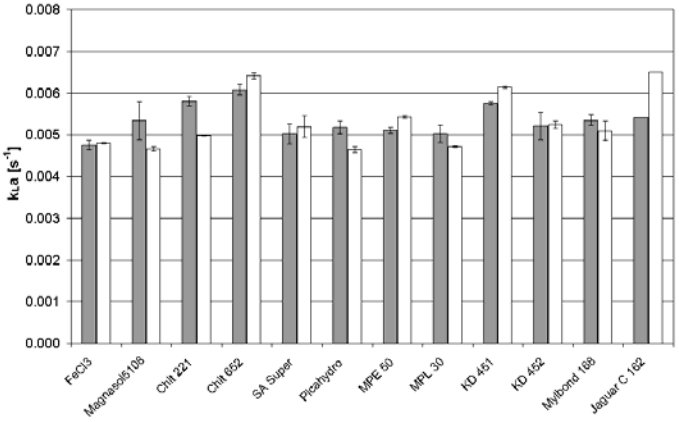


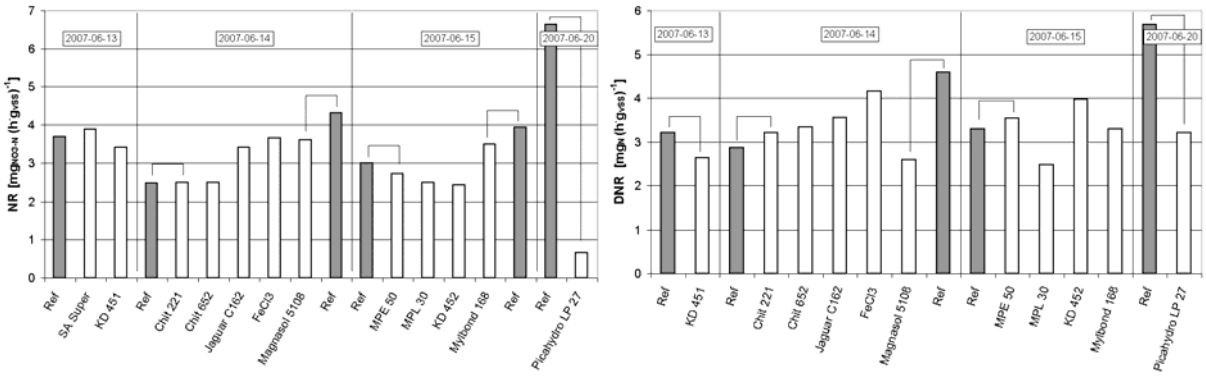
Figure 24: Oxygen transfer coefficient k_La for flux enhanced sludge (white bars) in comparison to the reference sludge (grey bars) (Iversen et al., 2009)

4.1.5.2 Nitrification and Denitrification

Figure 25 a shows the denitrification rates (DNR) of treated sludges in comparison to the reference sludge measured on the same day. The DNR of the reference sludge varies between 2.9 and 5.7 mgN h^{-1} $gVSS^{-1}$ on different days, which is in a medium range for an easily biodegradable substrate like acetate (Kraume et al., 2005).

The denitrification rate with polyaluminumchloride was reduced by 43 %. The reduction in nitrification was only 16 %, while at the same time the nitrification rate also varied strongly within the same day. Wolborska et al. (2006) also found a reduction in the activity of nitrifying microorganisms when alum was added. Song et al. (2008) did not observe an influence of alum on nitrogen removal (2.2-50 mg L^{-1} alum), but stated that this topic was not thoroughly investigated in their study.

Also, the addition of activated carbon Picahydro LP27 strongly disturbed the denitrification process (- 43 % in DNR). As both nitrification and denitrification processes optimally take place at a neutral to slightly alkaline pH (Focht et al., 1975), this can be explained by the shift in pH when this activated carbon is added.



a) b)

Figure 25: a) Nitrification and b) denitrification rates for treated MBR sludges and respective references (grey bars); parallel measurements indicated (Iversen et al., 2009)

The nitrification rates (NR) for the same experiments are shown in Figure 25 b. The values for the untreated sludge correspond to values found in literature for nitrification rates in MBRs (Bracklow et al., 2007, Vocks et al., 2007). It can be seen that except for activated carbon Picahydro LP27 the values are well within the expected range and no inhibition effects on nitrification were found. The addition of this activated carbon strongly impacted on nitrification (reduction of nitrification rate by 90%) during these short term jar tests. As said before, nitrification is enhanced at slightly higher than neutral pHs. A pH of 7.5 to 9 is ideal for nitrifying bacteria. As this PAC is activated by an acid, it significantly decreases the solution pH, as indicated in Table 1. This is surely some short term effect as the acidity will wash out with time. Thiemig et al. (2008) found a slight decline in NR when Nalco MPE50 was added but no effect on denitrification similar to the slight decrease (10%) in nitrification found in this study.

4.1.6 Particle size distribution vs. shear stress

Table 8 shows the CST, viscosity and medium particle size for different shearing rates. The increases for the most significant parameters were calculated for the treated mixed liquors in comparison to the reference.

4.1.6.1 Impact on CST

From Table 8 it can be seen that most additives have a positive effect on the dewaterability of the mixed liquor. This improvement did not always correlate with an increase in particle size. It should be noted that the manufacturer of the CST apparatus generally states that the accuracy of this measurement is not very high and only a deviation of more than 20 % is significant. Nevertheless, the agreement for the duplicate samples was generally very good (medium standard deviation of 5 %). Shearing at 4000 s^{-1} for 5 min decreased the CST in most cases slightly, for the PAC Picahydro LP 27 and the polymer KD 452 the deviation was higher with about 33 % and 26%, respectively.

Table 8: Effect of additive on CST, dynamic viscosity and medium particle (v – volume, n – number based) size for 5min shearing at rates of 0, 718 and 4000 s^{-1} (Iversen et al., 2008b)

	CST0 in s	Increase in %	CST4000 in s	µ718 in mPa s	µ4000 in mPa s	d(v 0.5)0 in µm	Increase in %	d(v 0.5) 4000 in µm	d(n 0.5)0 in µm	Increase in %
Ref	50		51.5	6.02	3.18	66.8		66.2	0.628	
$FeCl_3$	43.8	12	50.5	5.07	3.28	66.9	0	67	0.629	0.2
Ref	40.2		44	3.88	2.87	65.5		64.2	0.629	
Magnasol 5108	40.7	-1	39.6	4.14	2.85	65.1	-1	65.2	0.625	-0.7
MPE 50	10.7	73	12.5	4.41	2.9	100.6	54	96.22	0.616	-2.2
Ref	39.9		40.7	3.84	2.99	63.3		61.5	0.627	
Chit 221	10.6	73	10.6	2.07	3.24	143.6	127	135.8	0.601	-4.4
Chit 652	13.7	66	15.4	4.18	2.93	78.6	24	80.2	0.594	-5.5
Ref	43.2		47.1	4.32	3.1	63.1		62.4	0.661	
MPL 30	24.6	43	24.5	3.87	2.93	65.4	4	64.5	0.649	-1.7
KD 451	11.4	74	12.05	3.06	2.86	88.5	40	84.2	0.627	-5.4
KD 452	12.9	70	16.3	3.49	3.01	93.9	49	77.5	0.628	-5.3
Ref	33.3		33.3	2.97	2.69	64.9		62.5	0.656	
Jaguar C162	24.1	28	22.6	3.17	2.75	70.1	8	61	0.688	4.7
Mylbond 168	26.1	22	23.9	3.36	2.74	58.5	-10	57.8	0.712	7.9
Ref	33.1		31.4	3.06	2.76	67.7		67	0.821	
SA Super	31.8	4	36.1	2.77	2.54	68.5	1	68.6	0.839	2.2
Picahydro LP 27	24.3	27	32.3	3.22	2.73	63.2	-7	62.4	0.918	10.6

4.1.6.2 Impact on particle size

The particle size distribution and the medium particle size (volume based distribution) were generally not or only slightly changed if shear stress was applied. Except for the polymer KD452 and the starch Jaguar C162 were a 17 % and 13 % reduction in the mean volume particle size was observed. It should be noted that the sheared sludge volume was quite small (10 mL). Replicate measurements have therefore been conducted, which did not show any significant differences.

While $FeCl_3$ and alum were not effective for the formation of larger flocs at the applied concentration, especially the tested polymers and chitosan strongly increased the volume based particle size. Chitosan 221 had the strongest effect with a median volume based particle size of 143µm. The tested activated carbons did not change the volume based particle size distribution.

Oppositional to the volume based results are the data for the number based distributions. Except the activated carbons and the starches the additives did not increase or even decreased the mean number based particle size.

4.1.6.3 Impact on viscosity

The dynamic viscosity of all samples decreased with increasing shear rate as expected, due to the pseudoplastic rheology of activated sludge. The viscosity measured at 718 s^{-1} was either decreased (up to 46 %) or increased (up to 13 %) if the sludge was spiked with an additive. These differences were diminished at the higher shearing rate of 4000 s^{-1}, where the deviation was only up to ± 8 %.

For further information, please refer to (Iversen et al., 2008b)

4.1.7 Combination of flux enhancer

Three different additives and their combinations were tested on their effects on SMP removal and sludge dewaterability (see Table 9). While the activated carbon is most efficient on SMP removal with up to 90% of protein concentration and 45% of polysaccharide concentration, the cationic polymer is most suitable to increase the dewaterability, in this case up to 40%. Therefore the combination of these two additives can be stated the best to use.

Table 9: Results – Combination of flux enhancers.

combination / Parameter	$FeCl_3$ – SA Super	MPE50 + SA Super	$FeCl_3$ – MPE50
SMP-P Elimination	Up to 88%	Up to 89%	Up to 76%
SMP-PS Elimination	Up to 49%	Up to 55%	Up to 48%
Dewaterability	Not measured	Up to 40%	

4.2 Fouling Sensors

4.2.1 MBR-VFM

4.2.1.1 Reproducibility and sensitivity to fouling relevant parameters

Within AMEDEUS, the MBR-VFM sensor was developed and the measurement protocol defined and optimized. In a first series of experiments, the reproducibility of the method was demonstrated. Then, the influence of the membrane material (PES versus PVDF) on reversible and irreversible fouling of mixed liquor was studied as well as the sensitivity for various parameters which are implied in MBR membrane fouling, such as MLSS, EPS concentration, etc. These have all been extensively reported in Huyskens et al. (2008). Here, we only give an indication on the reproducibility and the impact of the applied membrane material on the results.

The reproducibility of the MBR-VFM was tested by off-line measurements with PES- and PVDF-membranes on mixed liquor samples from MBRs treating municipal wastewater. As indicated in Figure 26, the MBR-VFM reversible and irreversible fouling graphs for duplicate measurements overlapped which demonstrates the reproducibility of the MBR-VFM method.

The applied membrane material (PES and PVDF) clearly had an influence on reversible and irreversible fouling measurements. Figure 27 shows that reversible MBR-VFM graphs for a specific mixed liquor and measured with both membranes practically overlapped, but that the PES-membrane had a much higher irreversible fouling propensity. This may be related to its larger pore openings which make it less prone to reversible fouling, but more susceptible to irreversible fouling. As reversible fouling was similar for both membranes, a possible explanation is that irreversible fouling of the PES-membrane occurred during the early stages of filtration. This caused a fast reduction in effective pore size, which then became comparable to the pore size of the PVDF-membrane. Eventually, this resulted in the formation of a reversible cake layer with a similar filtration resistance.

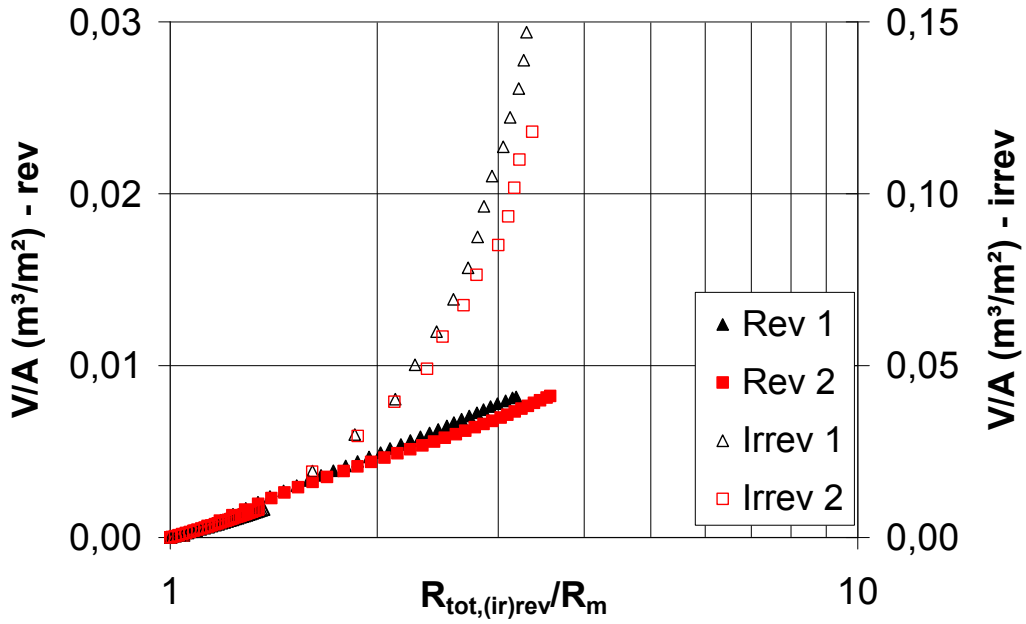


Figure 26: Reproducibility of MBR-VFM measurements with PES membranes (after Huyskens et al., 2008)

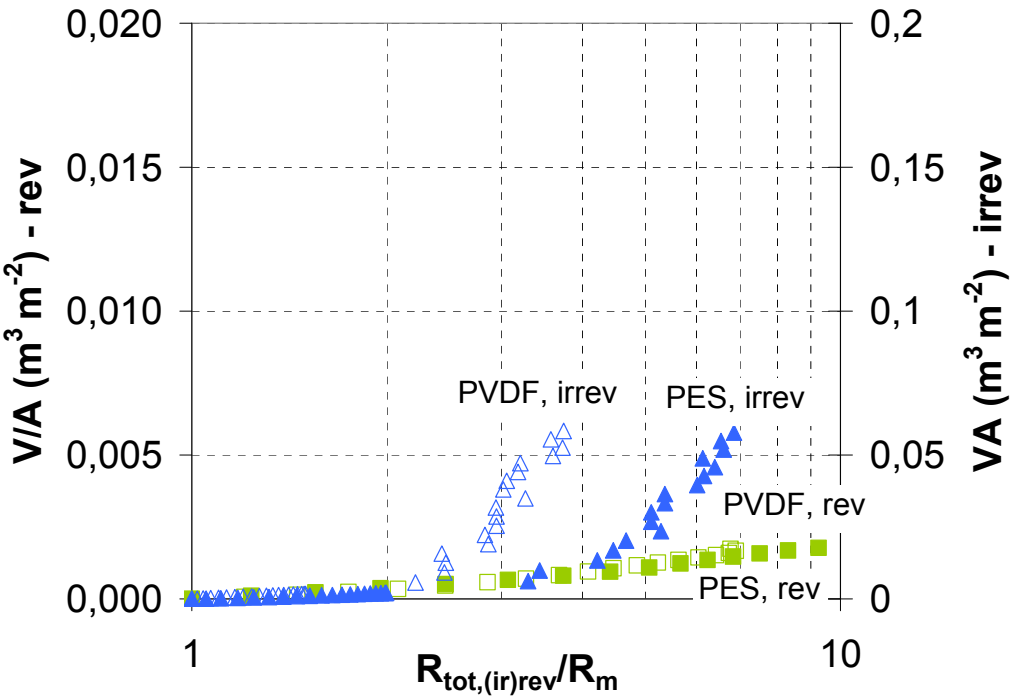


Figure 27: Influence of membrane material on MBR-VFM fouling measurement (after Huyskens et al., 2008)

4.2.1.2 Some validation studies

After reproducibility and fouling sensitivity studies, the MBR-VFM was validated in lab-scale MBR tests.

In a first test with a lab-scale MBR, MBR-VFM measurements were performed and compared to on-line permeability data. Figure 28 shows how the increase in TMP (measured on-line) corresponded with the higher reversible fouling propensity measured by the MBR-VFM. Interestingly, the fouling fingerprint had already started to decrease on day 22 while a clear TMP increase only became visible a few days later in the on-line TMP data.

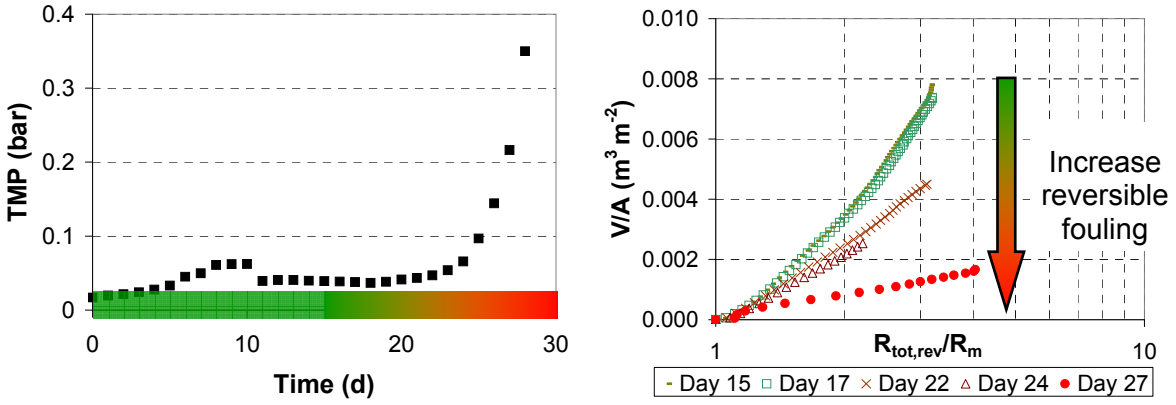


Figure 28: Evolution in on-line TMP measurement and reversible fouling fingerprints for a lab-scale MBR.

In a second test, two MBRs were operated at different organic loading rates. Figure 29 shows that TMP evolution was very stable in MBR1 which received a more diluted wastewater. In MBR2 with the higher organic loading, fouling was observed through TMP increase from day 17 onwards. At the timepoint when TMP was low in both MBRs (green arrow), the corresponding MBR-VFM measurements showed a similar fouling behavior. At a later stage, when on-line TMP was high in MBR2 and low in MBR1, the fouling fingerprints had shifted towards higher fouling in MBR2 and towards lower fouling in MBR1.

These results demonstrate that the MBR-VFM is a good indicator of fouling propensity and can even detect fouling earlier than can be seen from the on-line filtration data of the lab-scale systems themselves.

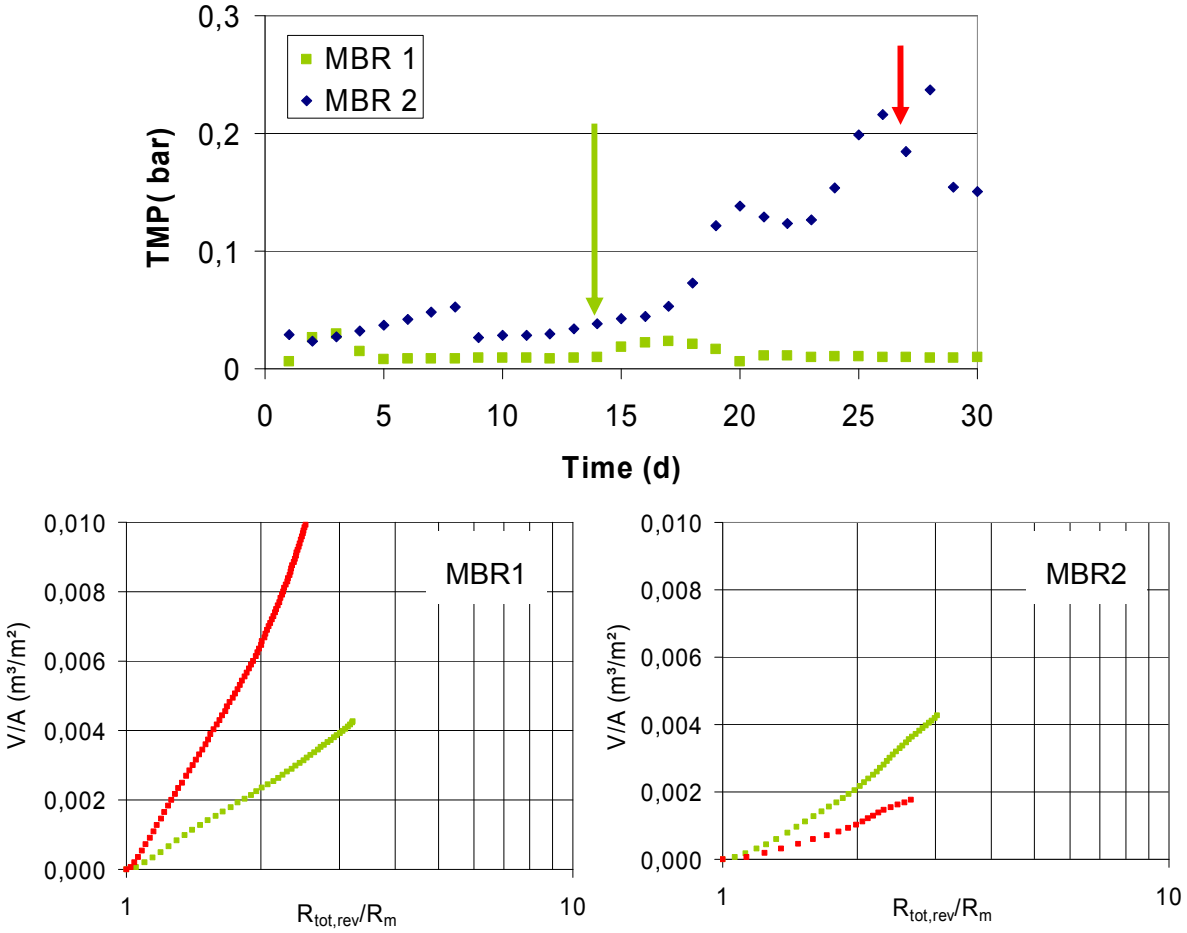


Figure 29: Evolution in on-line TMP measurement (top) and reversible fouling fingerprints (bottom) for two lab-scale MBRs running in parallel. The green and red arrow indicates two timepoints for which the corresponding MBR-VFM measurements are shown.

In conclusion, our studies proved that the MBR-VFM method enables the (on-line) evaluation of the reversible and the irreversible fouling propensity of MBR mixed liquor in a reproducible way. Under the specific operational conditions applied in a laboratory-scale MBR for method validation purposes, the measurements corresponded with the actual fouling behavior of the MBR. The MBR-VFM thus has an important potential to characterize the fouling propensity of MBR mixed liquors.

The distinction between irreversible and reversible fouling components can be used in principle as input for an advanced control system (ACS) to optimize the mechanical membrane cleaning actions related to reversible fouling and the chemical membrane cleaning actions related to irreversible fouling. Results on 1.5 year of pilot testing with the MBR-VFM as input for an ACS are reported in deliverable D51.

4.2.2 Photometrical EPS sensor

4.2.2.1 Sample pre-treatment device

Filter run time. In preliminary short-time filtration tests the most appropriate filtration protocol with regard to the longest filter run-time and to the highest flux were determined (Mehrez et al., 2007a). The filtration experiments were carried out in a small reactor filled with 1.9 L or 0.8 L of activated sludge from MBR pilot plant. The activated sludge was filtrated continuously with flux between 10 and 21 L/m²h and the filtration intervals were changed from 20 to 10 min. Municipal wastewater was continuously added to the reactor with the same flow rate as the activated sludge was filtrated.

The results of these experiments showed that the length of the filtration interval had the most important influence on filter run-time. When the filtration interval of 20 min was set, the filter was clogged already after 3 days while in the experiment with filtration interval of 10 min and the same flux the filter run until 10th day. Different flux values showed no high effect.

For on-line analysis of polysaccharides and proteins on the MBR pilot plant, the following filtration protocol was applied: filtration/relaxation interval of 10 min/2 min, flux between 10-17 L/m²h. As an example pressure loss of the filter running at the pilot plant is shown in the Figure 30 d. The typical filter-run time was between 10 and 20 days.

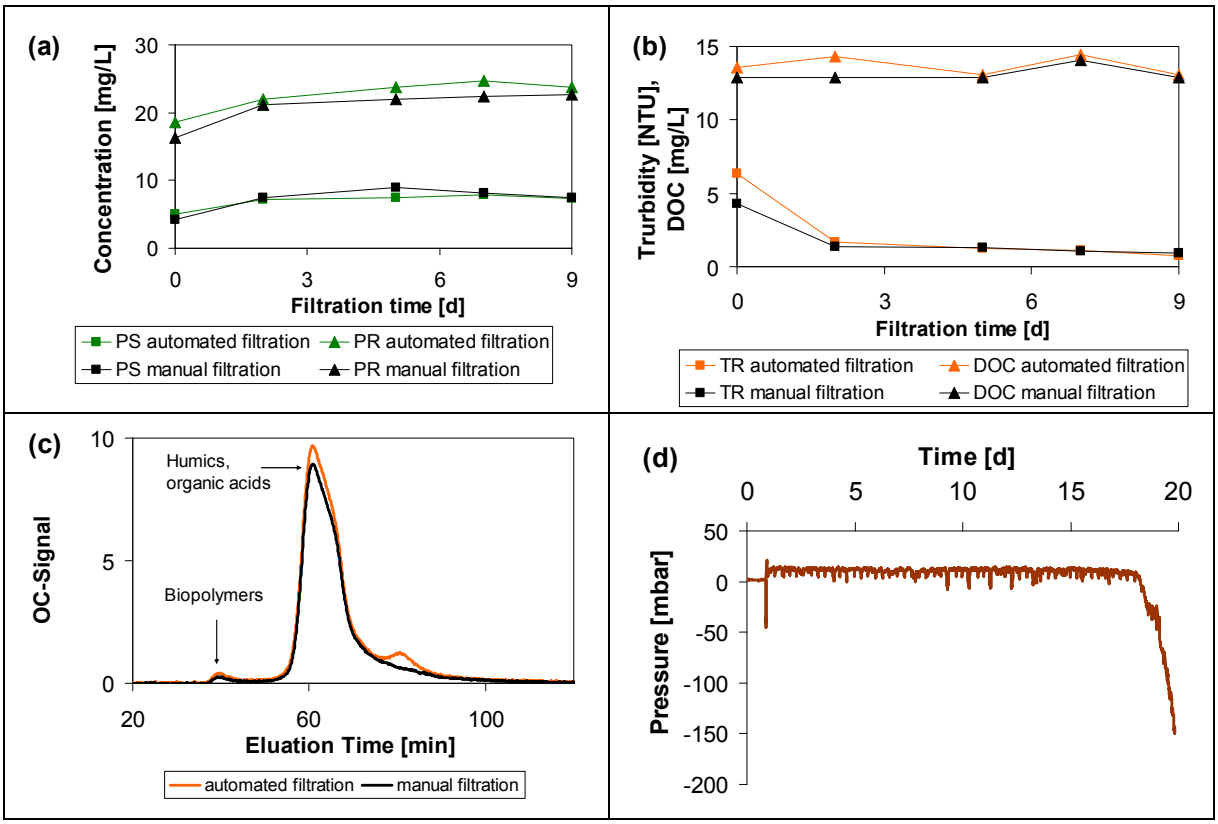


Figure 30: Separation characteristic of stainless steel filter during sludge filtration experiments in comparison with manual filtration in regard to (a) polysaccharides (PS) and proteins (PR), (b) turbidity (TR) and DOC, (c) biopolymers and humics. (d) Example of pressure loss during filtration in MBR sludge. (Mehrez et al., 2009)

Separation characteristics. Characterisation of separation properties of the stainless steel filter with regard to parameters like turbidity, DOC, concentration of polysaccharides and proteins were one of the objectives of different preliminary experiments in the lab (Mehrez et al., 2007a).

The stainless steel filter allows passage of carbohydrates and proteins and retains only suspended solids and bacterial flocs in a very effective way. Produced filtrate is not completely particle-free (similar to the paper filtration used as a reference), however the turbidity is very low (<2 NTU) (Figure 30 b). Separation characteristics of stainless steel filter with respect to the concentration of polysaccharides and proteins are comparable to that of the paper filter (Whatmann, Schleicher and Schuell filter - 589/1 black ribbon, 125mm) used for the manual sample preparation. Figure 30 a shows obtained concentration of proteins and polysaccharides measured on different days in the filtrate produced by the developed pre-filtration device during continuous filtration. As reference manual filtrated sample was measured at the same time. Separation properties of stainless steel filter remain the same over the time; this guarantees representative sample pre-treatment during the whole filter run-time for subsequent measurement of proteins and polysaccharides in the SIA system. The same was observed for DOC (Figure 30 b) and biopolymers and humics+organic acids (Figure 30 c) measured with SEC chromatography (LCOCD).

Determination of critical flux and the filter run-time. In order to determine a sustainable flux for the filtration with stainless steel filter and to guarantee a long filter run-time, critical flux tests were determined with different sludges and on different days. The procedure has been carried out according to Le Clech et al. (2003). The results confirm that the critical flux depends strongly on the sludge propensities. The obtained values vary largely - critical flux were found ranging from 10.6 and 18.4 $Lm^{-1}h^{-1}$. This observed finding result from the properties of the stainless steel filter, which surface is not smooth. After longer operation time particle or colloid deposition can not be removed effectively only by the air souring or by the relaxation time and chemical cleaning is necessary. For continuous analysis of carbohydrates and proteins the filter is operated with the flux in the range of the determined critical flux.

4.2.2.2 SIA - Development of analytical methods

Method screening

Some essential aspects have to be taken into account when transferring the manual protocols to the automated SIA technique. Firstly the chemistry of reagent based assays is important e.g. the kinetic of reaction (reaction time), pH value, temperature, number of reagents. Complex treatment steps like cooking or precipitation would make the automation difficult or even impossible. Moreover the sensitivity of the applied assay has to be high. During the automation the reaction response often decreases because of the fact that the coloured reaction products underlay a dispersion process during the transport through the SIA apparatus or the reaction is not finished at the moment of the detection. Aspects like toxicity, availability and costs of the reagent may play also a role by choosing the right method for its automation.

Method screening experiments were carried out to identify the boundary condition for different protein or polysaccharide determination assays with respect to their adaptation as automated measurement. For proteins determination two assays were applied – the Lowry- and Bradford- Assay – and for the polysaccharides determination – the Dubois- and Anthron-Assay (Mehrez et al. 2007b). The experiments were conducted with different protein and sugar standard solutions in order to determine the method sensitivity and the reaction rate (kinetic experiments) of tested assays. The screening tests suggested (results not shown) that the most adequate protein method for the automation is the Lowry-Assay. Although the Bradford-Assay requires only one reagent and the reaction kinetic is quite fast, the big disadvantage of the method is that the coloured reagent tends to stick on glass walls and tubes, which will cause problems with regard to the reproducibility of the reaction and the measurement of absorption, especially within the tube system of SIA. The Dubois-Assay was chosen as the most adequate polysaccharide assay for the automation. This assay is characterised by fast reaction kinetic and high sensitivity. In comparison, the Anthron-Assay has the disadvantage that the used reagent is chemically not stable and it has to be prepared directly before the measurement (Raunkjær et al., 1994). Another difficulty is the treatment of the

sample at elevated temperature. Although the heat treatment can be realised with FIA and SIA technique, it seemed to be complex and difficult for the automation.

Measurement of polysaccharides with automated Dubois-Assay

Difficulties during method development Decisive factors for successful adaptation of polysaccharides method with SIA were:

- use of reaction chamber for reaction occurrence
- dosage of conc. H_2SO_4 reagent with high flowrate (650 µl/s)
- use of degassed carrier with 82 % (w/w) of H_2SO_4 (equal to sample)
- use of holding coil with greater diameter (13 mm; before 7.4 mm)
- choice of appropriate reference wavelength (750 nm)

In preliminary tests the addition of the sulphuric acid to sample-phenol mixture was identified as the most important and critical step of the reaction. The central factor was very fast addition of the reagent in view of a complete and reproducible signals (Figure 31 a). At higher dispensing velocities higher peaks and thus higher sensitivity of the method has been obtained. Thereby the heat of the reaction, formed by H_2SO_4 addition, is important for the development of the colour. By the use of reaction chamber a reproducible and intensive reaction could be realised that was comparable with manual procedure.

Another problem to over come was the formation of density trails during the contact between the carrier and reaction product The trails occurred due to the high density of sulphuric acid (1.8 g/cm³) and caused refraction effects (so called “Schlieren effects”) during the measurement of the absorption. This effect had as a consequence an appearance of very high blank signal and poor reproducibility of the measured absorption peaks. An appropriate carrier has been searched by tests with different concentrated H_2SO_4 solutions (Figure 31 b). The smallest blank and the best shape have been measured by using 82 % concentrated and degassed H_2SO_4.

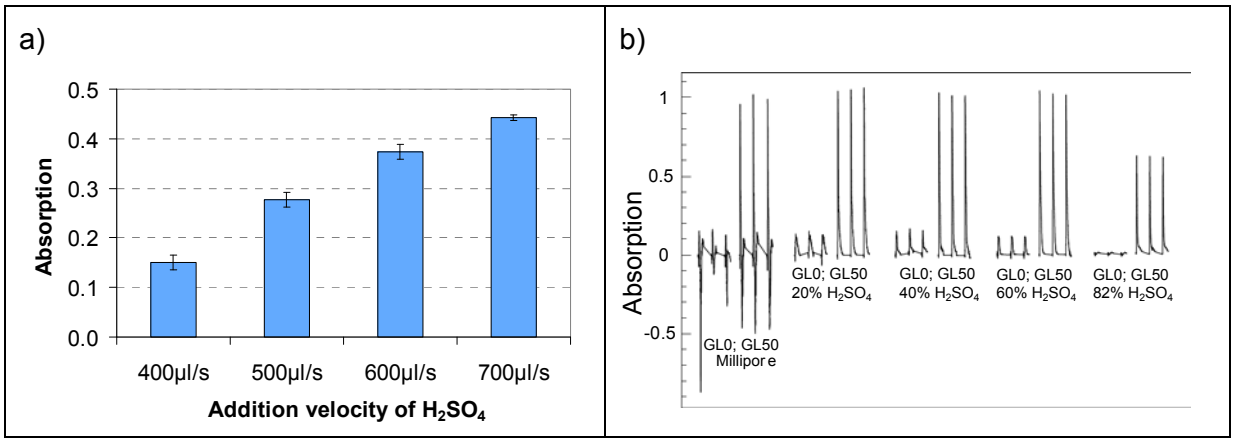


Figure 31: a) Influence of dispensing velocity of reagent 2 (conc. H_2SO_4) on absorption height. b) Influence of the carrier concentration (diluted H_2SO_4) on blank height and peak shape (tests with dest. water GL0 and glucose 50 mg/L GL50), (Aust et al., 2008)

Method sensitivity and method validation. In Figure 32 a the calibration curve made with different concentration of glucose are shown for the developed automated method and as comparison for the manual assay. The sensitivity of the SIA method, represented by the slope of the curve is higher (1.3E-2 L $mg^{-1}cm^{-1}$/2 cm) then for manual polysaccharides method (1.0E-2 L $mg^{-1}cm^{-1}$/1 cm). The limit of detection LOD and of quantification LOQ calculated according to German DIN32645 is very similar for both methods and is even a little bit smaller for the developed method. LOD and LOQ were determined to 0.9 and 3.4 mg/L for automated and to 1.2 and 4.2 mg/L for manual assay respectively.

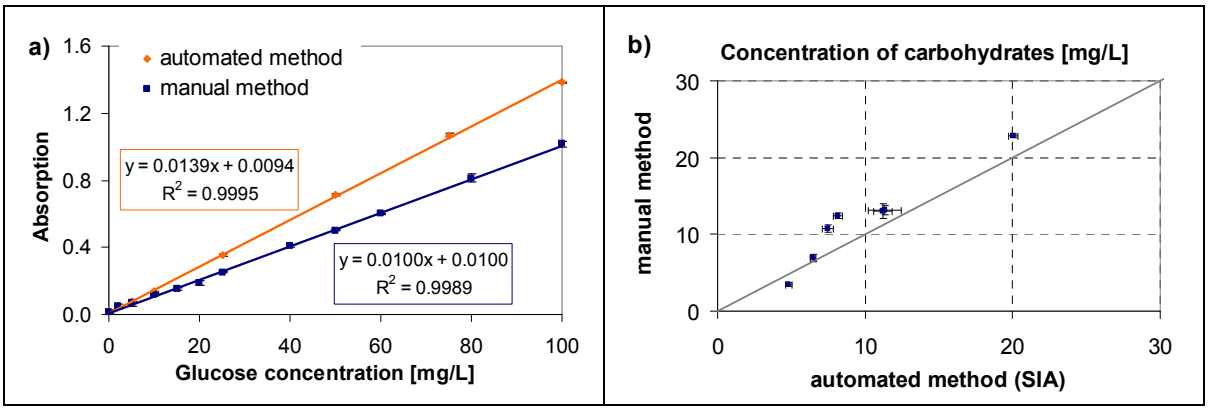


Figure 32: Dubois assay: (a) measurement of glucose as standard solution with automated (n=4) and manual method (n=2); (b) validation of automated method; (error bars: SD), (Mehrez et al., 2008)

In order to validate the accuracy of the developed method the concentrations of polysaccharides were measured in different samples of filtrate of activated sludge and of wastewater with manual and developed automated assay. The results displayed in Figure 32 b show good agreement between these two methods in the measured concentration range of 5-20 mg/L. Small differences between the two methods can be caused by the fact, that the samples were not measured at identical days.

In order to assess the precision of the developed method repeatability tests were carried out, where glucose solution in the concentration of 10 mg/L and the filtrate of activated sludge were measured in ten replicates. The results summarized in the Table **10** show very good repeatability of SIA method that is even better then that of the manual assay. The relative standard deviation (RSD), which describes the spread of data related to the mean value, is smaller for developed (SIA) method (5.1 % and 6.6 %) then for manual standard method (5.4 % and 11.8 %). This improvement can be linked to the "human factor", which is excluded in the SIA analysis. The critical step of the addition of sulphuric acid (second reagents) is carried out in more reproducible way by the machine then by laboratory staff.

Table 10: Repeatability test: absorption (abs.) and resulting concentration (conc.) values, standard deviation (SD) and relative standard deviation measured (RSD) in different samples with automated and manual method (n=10), (Mehrez et al., 2008)

	AUTOMATED ASSAY		MANUAL ASSAY	
	10mg/L glucose	filtrate of sludge	10mg/L glucose	filtrate of sludge
Mean (abs./conc.)	0.150 / 10.1 mg/L	0.108 / 9.8 mg/L	0.103 / 9.3 mg/L	0.102 / 9.2 mg/L
SD (abs./conc.)	0.0072 / 0.52	0.0065 / 0.65	0.0069 / 0.50	0.0011 / 1.1
RSD (abs./conc.)	4.8% / 5.1%	6.0% /6.6%	6.7% / 5.3%	10.7% / 11.8%

Robustness of the method and of the SIA analyser To verify the robustness and reliability of the developed method and of the SIA analyser standard solution glucose 10 mg/L has been measured continuously during 3 days (N=33) (Figure 33). After each 4-fold measurement 90 min delay were made in order to simulate the frequency of on-line analysis in MBR reactor. The measurement of the glucose shows very good repeatability. The relative standard deviation RSD during three days can be calculated to 2.6 %, what corresponds to the stan-

dard deviation SD of 0.26. The single four-fold measurements show very good repeatability as well (mean RSD=2.7 %, mean SD=0.30). SD has not increased during the measurement time, what confirms the robustness of the method and the measurement device. Since for this experiment the same reaction chamber was used, no contamination of the reaction chamber could be observed. The dispersion of the detected concentrations is in mean about 10 mg/L during the whole measurement time and no increase of the concentration is observed. This can be expected, because the cleaning of the chamber is carried out with the carrier solution, which is in the case of carbohydrates measurement 82 % w/w H_2SO_4. This acid has a strong ability to hydrolyse the impurities what ensured the efficient cleaning of the reaction chamber.

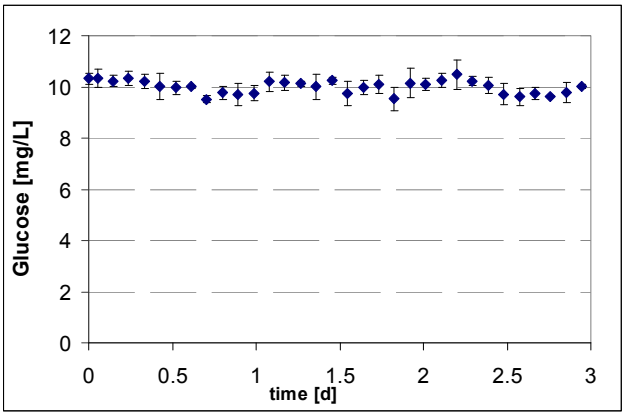


Figure 33: a) Dispersion of values during continuous measurement of glucose 10 mg/L (N=33; error bars: SD, n=4), (Mehrez et al., 2008)

Measurement of proteins with automated Lowry-Assay

Development of the automated method for protein detection occurred in several steps. Firstly the reaction took place in the holding coil (Method I), what had as advantage small reagent consumption but unfortunately a small sensitivity causing high measurement error. To improve method sensitivity another approach has been tested: a self constructed reaction chamber was used for the reaction occurrence (Method II). At the end of the project one more attempt has been tried to lower the measurement error. In several experiments different method settings of Method II have been improved that influenced the reaction intensity and repeatability (Method II improved).

Method I – reaction in the holding coil

In the first method the reaction (mixing of the sample and the reagents) took place in the holding coil as in measurements that usually applied the SIA technique [Rudzicka&Marshall, 1990]. To support this process the injected sample and the reagents were aspirated in small sequences and moved forwards and backwards several times in the holding coil. The developed measurement protocol was optimized with regard to different parameters like the volumes and number of the stacked zones of the sample and the reagents, the number of reversed flows as well as with regard to the delay (reaction) time. From the fact that the pH value of 10 is important for the occurrence of the reaction, the effect of different carrier solutions on the reaction within the SIA system was examined. Pure water and three different concentrations of Na_2CO_3 were tested. It could be observed that the carrier did not have any effect on the reaction, but only on the blank value. With decreasing concentration of Na_2CO_3 the blank value increased, which can be explained by the increasing difference of density between the carrier solution and the reaction product that causes higher refraction of the light during the absorption measurement.

Method sensitivity Figure 34 displays the calibration curve of the developed method for a standard protein solution (bovine serum albumin – BSA) and SIA. The curves can be fitted as linear function. The slope of the curve presents the sensitivity of the method. For the SIA assay, the slope is 6.8E-4 L/(mg*2cm) (optical path: 2 cm), which is ten times smaller than the manual method (slope= 6.7E-3 L/(mg*cm); optical path: 1 cm). This discrepancy can be attributed to the different principles of the manual and automated measurement (Mehrez et al., 2007b):

1. The formed reaction products undergo dispersion (dilution) phenomena during the transport through the SIA tubes to the detector. The relative contribution to the decrease of the method sensitivity could be calculated to about 52 %.
2. Absorption is measured by the SIA technique as the difference between absorption at the measurement wavelength (730 nm) and at the reference wavelength (500 nm). This results in sensitivity decrease of 32 %.
3. The absorption measurement of the automated method is performed when the steady state of the reaction is still not reached (i.e. the coloured products are not completely formed). The sensitivity decrease can be estimated to 16 %.

The detection limit was calculated for the SIA method according to German DIN32645. The lowest concentration of analyte that can be detected was determined to be 10 mg/L for the automated assay.

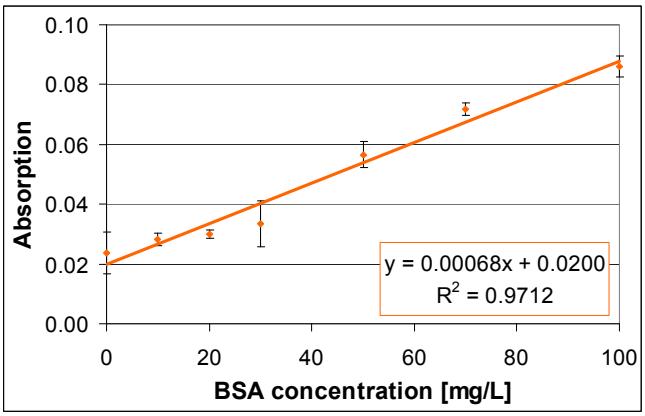


Figure 34: Calibration curve of method for protein determination done with SIA (Mehrez et al., 2007b)

Method II – reaction in reaction chamber

Modification of the manual Lowry-method During the measurement of proteins in real samples (like sludge supernatant, permeate, raw wastewater) formation of small precipitates occurred (~1.2 µm). The precipitates caused an overestimation of protein concentration of about 25 % by the manual method. The proteins measurement in real samples with the automated method (using the reaction chamber) was not possible because the formed hazed increased the absorption signal not only at the measurement wavelength (730 nm) but also at the reference wavelength (500 nm). The consequence was that no peak occurred since the signal is calculated by difference of absorptions at measurement and reference wavelength (Figure 35).

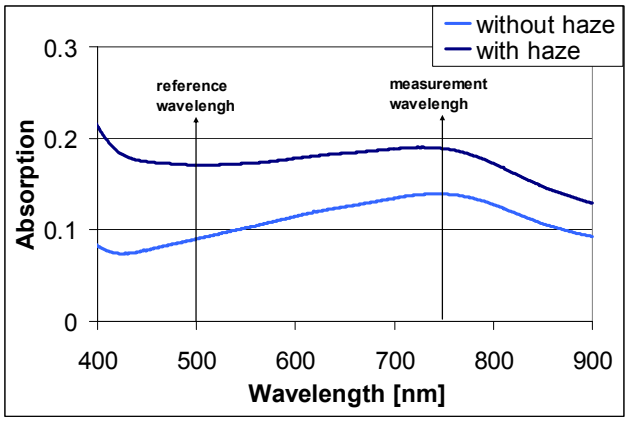


Figure 35: Absorption spectrum of the reaction product (sludge sample) with and without haze (after centrifugation), (Mehrez et al., 2010a)

Firstly the manual Lowry method was modified in order to prevent the haze formation. Different possibilities have been proved like change of SIA specific parameters (e.g. reference wavelength), online separation of the particles (filtration and sedimentation), change of the concentration of used reagents, etc. The experiments showed that finally the addition of chelating agents (EDTA, Triphosphate, NTA) to the sample could avoid the precipitate formation and support the hypothesis that the calcium ions are mainly responsible for the haze formation (Figure 36 a). NTA (nitrilo-tri-acetate) has been chosen as the most promising substances. In further experiments, derived by tests with manual method, optimal concentration (Figure 36 b), influence of NTA on the method sensitivity, combination with alkaline reagent, calibration, validation (Figure 37 a) were carried out. The application of the chelating agent slightly decreases the sensitivity of the manual method; however the detection and quantification limit, and the measurement error are not affected by NTA addition.

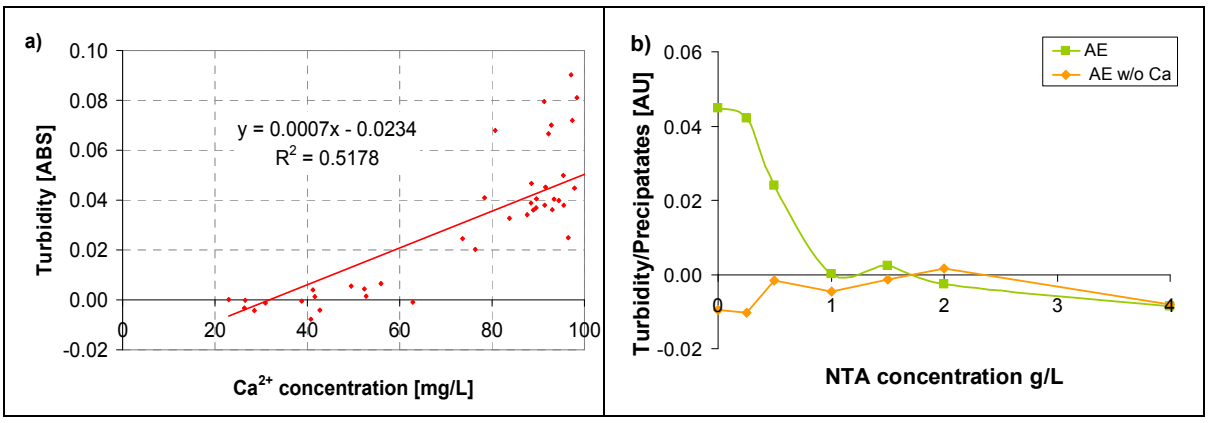


Figure 36: a) Correlation of the Ca^{2+} concentration contained in the sample and the intensity of precipitate formation; b) Influence of NTA concentration in reagent 1 and the formation of precipitates (manual method); AE: filtrate of sludge (Mehrez et al., 2010a)

The modified method was transferred as SIA automated method. The sensitivity of the automated method (SIA) did not change (Figure 37 b) as well the detection and quantification limit and the measurement error.

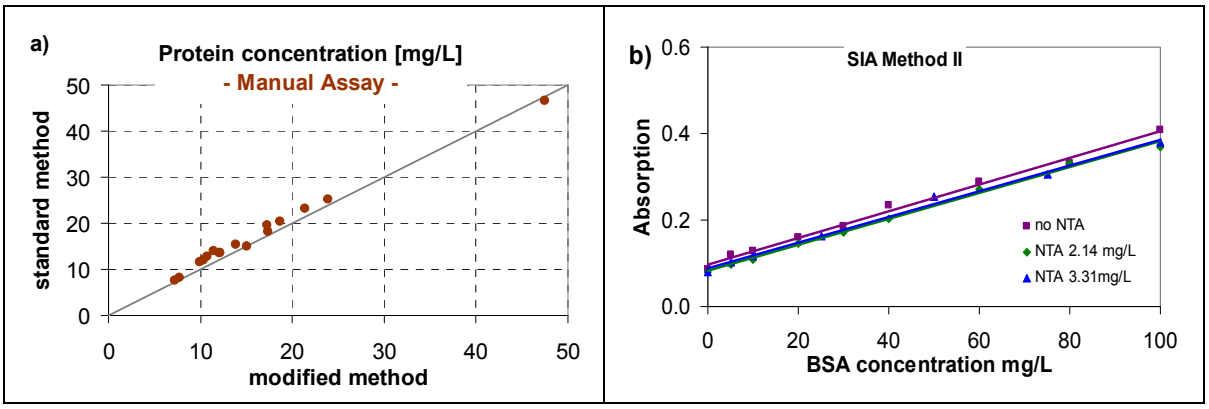


Figure 37: a) Validation of the modified manual method (using NTA) with standard manual method (without NTA) by measuring of proteins in real samples (Mehrez et al., 2010a); b) calibration curves made with BSA and automated SIA method by using different concentration of NTA (Mehrez et al., 2010b)

Method sensitivity and method validation In the Figure 38 a the calibration curve made with different solutions of BSA are shown for the developed automated method (with NTA 4 g/L in reagent 1) and as comparison for the manual method. Sensitivity of the developed method, represented as a slope of the calibration curve is two times smaller then of the manual Lowry protein method, 3.0E-3 and 5.1E-3 respectively. Limit of the detection (LOD) and quantification (LOQ) calculated according to German DIN32645 (1996) was determined to 3.9 and 13.5 mg/L for automated and to 1.0 and 3.3 mg/L for manual assay respectively.

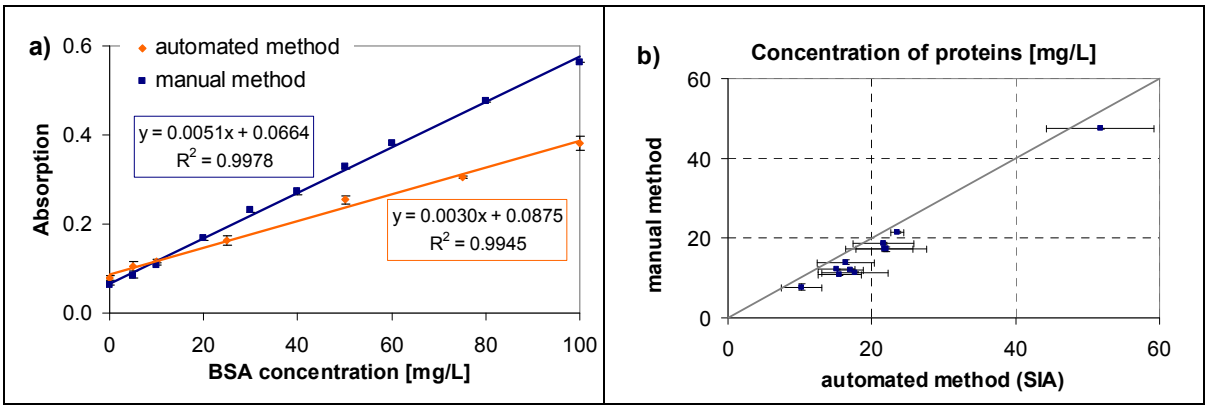


Figure 38: Lowry assay: (a) measurement of BSA as standard solution with automated (n=4) and manual method (n=2); (b) validation of automated method (error bars: SD), (Mehrez et al., 2008)

To validate the accuracy of the developed method the concentrations of proteins were measured in different samples (filtrate of activated sludge and of raw waste water) with manual and automated assay. The results are displayed in Figure 38 b. They show good correlation between these two methods in the measured concentration range of 10-50 mg/L. The error bars representing the standard deviation are partly higher then calculated in other repeatability tests due to minor problems with the detector at this time.

The precision of both methods was examined by repeatability tests, where BSA 20 mg/L and the filtrate of activated sludge were measured in ten replicates. The results are summarized in the Table 11. The manual method for protein determination seems to have a smaller measurement error (RSD) compared to the automated SIA method. The smaller sensitivity of automated method and a slightly higher standard deviation of absorption values (BSA

20 mg/L: 0.0059, 0.0020 respectively for automated and manual method) results in a relatively high SD and RSD of calculated concentration values.

Table 11: Repeatability test: absorption (abs.) and resulting concentration (conc.) values, standard deviation (SD) and relative standard deviation measured (RSD) in different samples with automated and manual method (n=10). (Mehrez et al., 2008)

	AUTOMATED ASSAY		MANUAL ASSAY	
	20mg/L BSA	filtrate of sludge	20mg/L BSA	filtrate of sludge
Mean (abs./conc.)	0.1473 / 22.6 mg/L	0.1317 / 17.4 mg/L	0.1885 / 19.4 mg/L	0.1577 / 14.2 mg/L
SD (abs./conc.)	0.0059 / 2.0	0.0074 / 2.5	0.0020 / 0.32	0.0019 / 0.30
RSD (abs./conc.)	4.0% / 8.8%	5.6% /14.4%	1.0% / 1.6%	1.2% / 2.2%

Higher limit of detection and smaller precision of the automated Lowry assay for proteins are caused by the smaller sensitivity of the developed method. Nevertheless in comparison with the automated method I (Mehrez et al., 2007b), the sensitivity of method II was improved by four times with resulting reduction of LOQ and error values.

Robustness of the method and of the SIA analyser To verify the robustness and reliability of the developed method and of the SIA analyser standard solution of BSA 20 mg/L has been measured continuously for almost 6 days. After each 4-fold measurement 60 to 120 min delay were made in order to simulate the frequency of this analysis under real conditions. The results are shown in the Figure 39, the number of four-fold measurements N was 53. The relative standard deviation RSD during the whole experiment of averaged four-fold measurements can be calculated to 7.6%, what corresponds to the standard deviation of 1.6. The single four-fold measurements show similar repeatability as in repeatability tests - in average RSD (conc.) was 10.6% and SD (conc.) 2.2. The standard deviation has not increased during the measurement time, what shows good robustness of the SIA device and of the method.

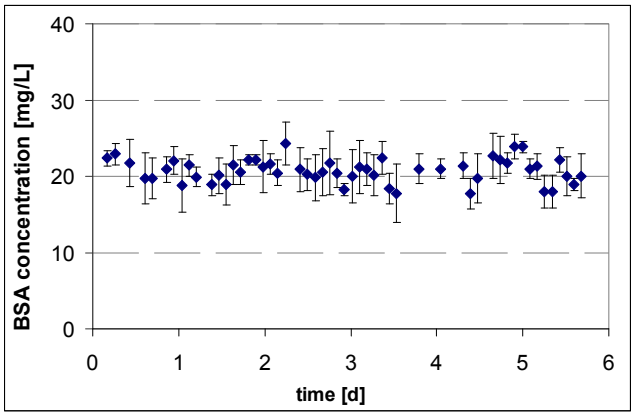


Figure 39: Dispersion of values during continuous measurement of BSA 20 mg/L (N=53; error bars: SD, n=4), (Mehrez et al., 2008)

For the whole experiment the same reaction chamber was used. It could be observed that the reaction chamber has been contaminated with time, since the blank value increased rapid during the first 12 hours from 0.0882 to 0.1018 (corresponds to the concentration of 4.5

mg/L). After that time only a slow contamination has been measured, after 5 days the mean blank was 0.1120 that corresponds to the concentration of 7.9 mg/L. It can be conclude that the reaction chamber has to be changed every 3-4 days; however increasing blank values have no influence on the spread of the measured values

Improvement of Method II

The purpose of these experiments was the improvement of the protein method with regard to the lower measurement error and higher method sensitivity. In experiments with standard solutions different method parameters of Method II were optimised (concentration and volume of reagents, volume and relation of sample and reagents, velocity of aspiration and dispensing of sample and reagents, carrier velocity during measurement, reaction time). Addition of the second reagent (Folin) at high velocities and at higher concentration was identified as the most important factor. Figure 40 displays the influence of these parameters on the reaction intensity and repeatability expressed as standard deviation SD and relative standard deviation RSD. Based on these result the best method settings were chosen with regard to absorption level and the standard deviation for a new modified protein method: the Folin-Reagent dispensing velocity was changed from 50 µL/s (Method II) to 200 µL/s, and the concentration from 1:6 (Method II) to 1:5.

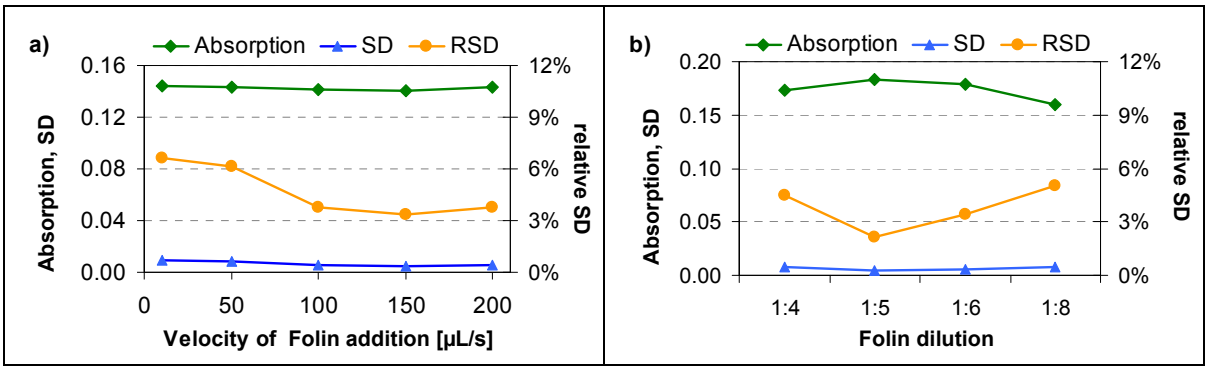


Figure 40: Lowry assay: influence of Folin-Reagent addition velocity (a) and concentration (b) on reaction intensity (absorption) and repeatability (SD, RSD; n=10), (Mehrez et al., 2010b)

Another important factor for the improvement of the method accuracy might also be the change of the selector valve mounted in SIA apparatus. In the new selector valve the rotor was made by the mechanically more stable PPS (polyphenylene sulphide) material. PVDF rotor that was used before was necessary for measurement of polysaccharides, because the analysis needs caustic reagents (conc. H_2SO_4). Due to the high abrasion of the PVDF rotor, small particles came into the SIA system (tubes, flow cell, reaction chamber), what may decrease the measurement precision.

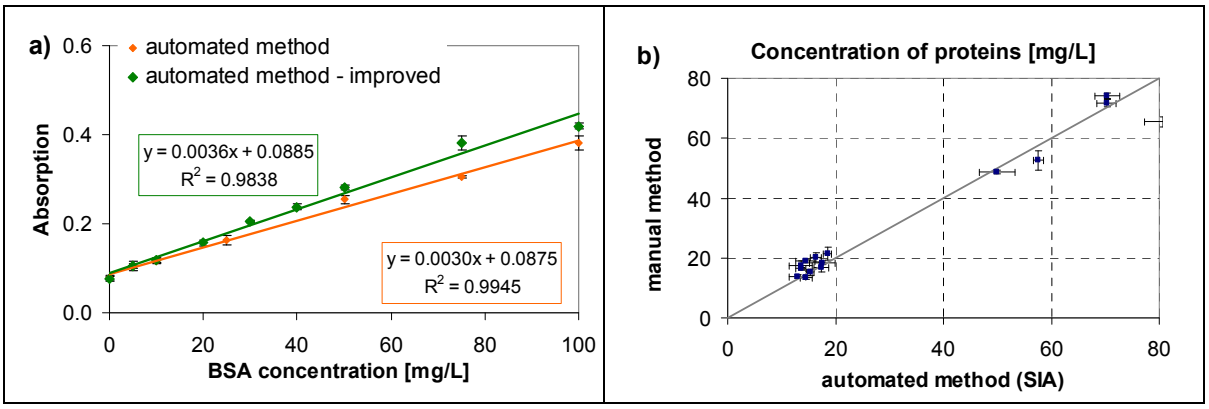


Figure 41: Lowry assay: (a) measurement of BSA as standard solution with automated and improved automated (n=4); (b) validation of improved automated method (error bars: SD), (Mehrez et al., 2010b)

In the Figure 41 the calibration curve made with different solutions of BSA is shown for the improved automated method. Sensitivity of the developed method, represented as a slope of the calibration curve could be improved by 20 % comparing with Method II. Limit of the detection (LOD) and quantification (LOQ) calculated according to German DIN32645 (1996) was determined to 1.3 and 4.8 mg/L, what was three times lower then for the Method II.

To validate the accuracy of the improved method the concentrations of proteins were measured in different samples (filtrate of activated sludge and of raw waste water) with manual and improved automated assay. The results (Figure 41 b) showed good agreement between these two methods in the measured concentration range of 10-70 mg/L.

Analogically to previous methods, the precision of the improved method was examined by short time repeatability tests, where BSA 20 mg/L and the filtrate of activated sludge were measured in ten replicates. (Table 12). The measurement error could be decreased by 50 % (SD conc. of BSA 20 mg/L: 2.0 for Method II and 1.0 for improved Method II). In comparison with the manual method, the measurement error is still higher; however the improved method measures the proteins concentration with very acceptable error (RDS conc. 4.9 %).

Table 12: Repeatability test: absorption (abs.) and resulting concentration (conc.) values, standard deviation (SD) and relative standard deviation measured (RSD) in different samples with automated and manual method (n=10)

	IMPROVED AUTOMATED MATHOD II	
	20mg/L BSA	filtrate of sludge
Mean (abs./conc.)	0.1556 / 21.9 mg/L	0.1338 / 15.8 mg/L
SD (abs./conc.)	0.0036 / 1.0	0.0028 / 0.8
RSD (abs./conc.)	2.5% / 4.8%	2.1% /4.9%

To verify the robustness and reliability of the improved method for proteins in on-line mode, standard solution BSA 20 mg/L has been measured continuously during 4 days (N=22) (Figure 42 a). After each 4-fold measurement 160 min delay were made. The measurement of the BSA shows very good repeatability and reveals an improvement of the method with regard to long term accuracy comparing with previous method (Figure 39). The relative standard deviation during four days can be calculated to 4.4 %, what corresponds to the standard deviation of 1.0. The single four-fold measurements show very good repeatability as well (mean RSD=3.8 %, mean SD=0.85). The standard deviation has not increased during the

measurement time, what confirms the robustness of the method and the measurement device.

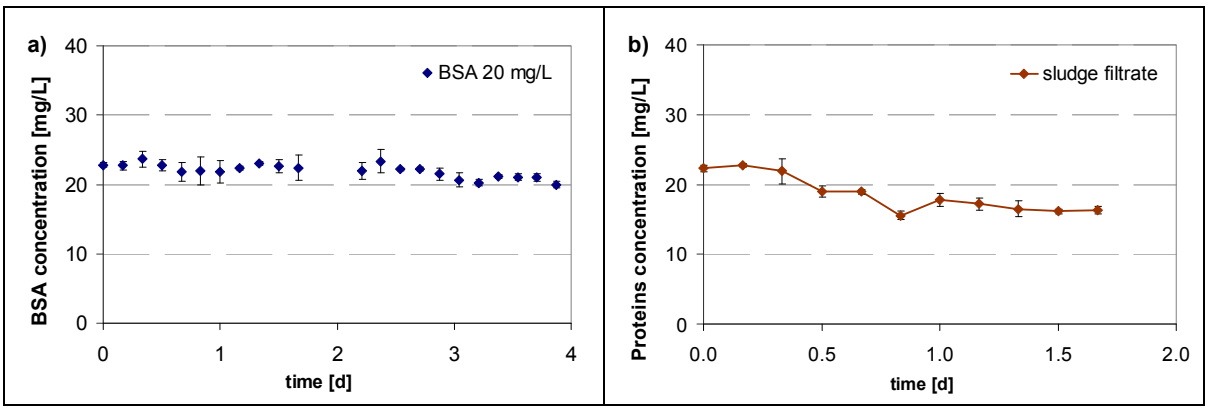


Figure 42: Dispersion of values during continuous measurement of (a) BSA 20 mg/L and of (b) sludge filtrate; error bars: SD, (n=4).

Figure 42 b shows results of continuous measurement in a single sludge filtrate sample during almost 2 days at ambient temperature. This measurement in a real sample showed good robustness as well (mean SD / RSD of four-fold measurement: 0.77 / 4.2 %). The protein concentration decreased from 22.8 to 15.6 mg/L, probably due to the biodegradation of proteins in sample that was not cooled during the measurement time.

In Table 13 the most important characteristics of all developed methods are summarised. In the course of the project the detection and quantification limit as well as the measurement error of automated protein method have been decreased.

Table 13: Sensitivity, detection and quantification limit, measurement error of different methods for protein determination.

	SIA (Method I)	SIA (Method II)	SIA (Improved Method II)	Manual method
Sensitivity (slope cal.)	6.8E-4 L/(mg*2cm)	3.0E-3 L/(mg*2cm)	3.6E-3 L/(mg*2cm)	6.7E-3 L/(mg*1cm)
Detection limit [mg/L]	10.5	3.9	1.3	1.0
Quantification limit [mg/L]	32.8	13.5	4.8	3.3
Measurement error [mg/L]	2.8	2.3	0.9	0.3

4.2.2.3 Implementation of EPS online sensor in the pilot plant

Preliminary considerations on measurement frequency and EPS concentrations

The implementation of the EPS online sensor and the selection of a suited sampling frequency require the knowledge about the concentration of the polysaccharides and proteins and its variation during the day. Table 14 summarise the measured concentration of proteins and polysaccharides in the MBR pilot plant during the project time.

Table 14: Concentration of proteins and polysaccharides measured manually in operated MBR pilot plants; concentration±SD, in brackets number of measurements

	Proteins	Polysaccharides
	[mg/L]	[mg/L]
Raw water	87.6 ± 16.6 (26)	15.7 ± 5.0 (45)
Activated sludge, filtrate, line 1	15.8 ± 2.8 (11)	6.8 ± 3.0 (30)
Activated sludge, filtrate, line 2	15.6 ± 4.5 (25)	6.8 ± 3.0 (44)
Permeate, line 1	11.1 ± 1.8 (11)	3.0 ± 1.5 (30)
Permeate, line 2	11.5 ± 2.9 (25)	3.1 ± 1.7 (44)

The concentrations of proteins and polysaccharides measured in sludge filtrate are higher than the detection and the quantification limits of online methods performed with EPS sensor, showing the suitability of online analysis for monitoring of these substances in the MBR.

With respect to the sampling frequency required for continuous monitoring by the SIA system, the variation of proteins and polysaccharides during daily operation was analysed (08.12.09). Figure 43 confirms that the concentrations are not rapidly changing vs. operation time of the MBR system. In addition to the pilot tests, small bench scale MBR experiments have been conducted to examine the SMP reaction in stress situation (results not shown, see Ernst et. al., 2007). The lack of oxygen led to the production of proteins and the polysaccharide concentration decreases gradually. Results of these experiments suggest a slower reaction of the polysaccharide content in comparison with the proteins during MBR operation. On basis of the pilot and bench scale results a sampling frequency of 3 hours for proteins and 4 hours for polysaccharides was considered to be sufficiently for monitoring of the change during MBR operation.

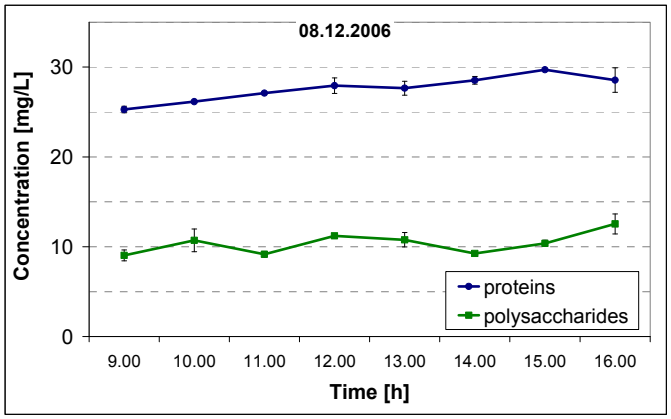


Figure 43: Variation of polysaccharides and proteins concentration versus time in MBR pilot plant (line 2), (Ernst et. al., 2007)

Stability of reagents and of standard solutions

All reagents except the alkaline copper solution (reagent 1 for proteins) used for determination of polysaccharides and protein concentrations are chemically very stable for several weeks.

The reagent 1 for proteins as well the standard solution are described in the literature that they have to be prepared freshly. Since they were not cooled during the continuous measurements on the pilot plant their stability has been proved in additional experiments. Figure

44 shows BSA 100 mg/L measured with reagents 1 prepared at different days and stored at ambient temperature. The results show very good stability of reagent 1 within first 5 days storage and after that slow decrease of its reactivity. Since the reagent 1 was replaced by a fresh one each 2-3 days, the decrease of the sensitivity can not be expected higher then 5 %.

Figure 44: Stability of reagent 1 measured with BSA 100 mg/L and SIA method (error bars: SD, n=5)

Similar experiment was conducted with three different concentrations of glucose (10, 20, 50 mg/L) and BSA (20, 50, 100 mg/L), which were stored at ambient temperature within 7 or 14 days and measured with manual methods frequently. Glucose 10 mg/L and BSA 25 mg/L were used as standard solutions for continuous measurement of polysaccharides and proteins. The results showed that the standard solutions are stable especially for low concentrations that are also used for the online measurements in the pilot plant. The glucose is stable until 7th day (Figure 45a). After 14 days the concentration decreases at measured conditions about 1.4, 1.8 and 2.3 mg/L in 10, 20 and 50 mg/L standard solution, respectively. For the BSA solution it could be observed that it changes rapidly after its preparation, particularly for the high concentration of 100 mg/L (Figure 45b). After the third day 1.0 and 1.2 mg/L BSA was reduced for 20 mg/L and 50 mg/L respectively. The standard solutions were replaced by fresh one each 2-3 days.

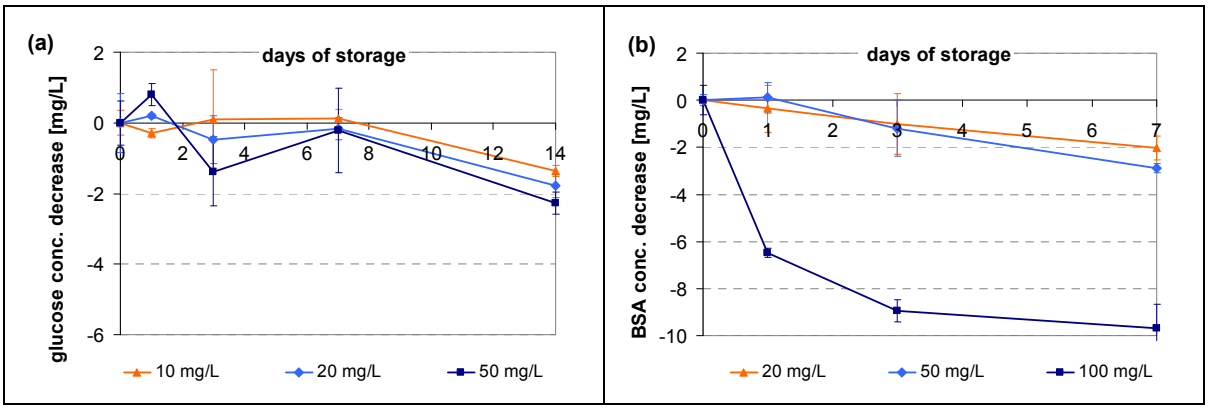


Figure 45: Stability of glucose (a) and BSA (b) solutions; measurement with manual methods (error bars: SD, n=3) (Mehrez et. al., 2008).

On-line measurement of polysaccharides and proteins in sludge filtrate of MBR pilot plant

Online measurements of polysaccharides and proteins in sludge filtrate have been conducted under different conditions (Mehrez et al., 2010b):

- Standard conditions
- Simulation of rain water events
- Simulation of influent peaks.

Operation of MBR under standard conditions

Polysaccharides Figure 46 depicts measured concentration of polysaccharides in sludge filtrate from 5th to 14th September 2008. During the monitoring time of 9 days the concentration decreased from 9.8 mg/L to 4.2 mg/L (average 6.6 mg/L). This decrease is hardly to elucidate and can not be explain by the TS concentration that was quite stable in this time and even augmented by 1.1 g/L. TMP increased slightly during the observation time, showing that there is no direct correlation between polysaccharides concentration and TMP of MBR.

The daily fluctuations of the concentration are rather moderate: the highest change was 3.4 mg/L; however in average the concentration varied about 0.8 mg/L (SD ±0.62). Parallel manual measurements agreed well with automatic analysis confirming the accuracy of the online method. The standard deviation of four parallel measurements representing the measurement error (each point displays a mean of 4-fold analysis) was in mean 0.23 that is equivalent to 3.6 % relative standard deviation RSD.

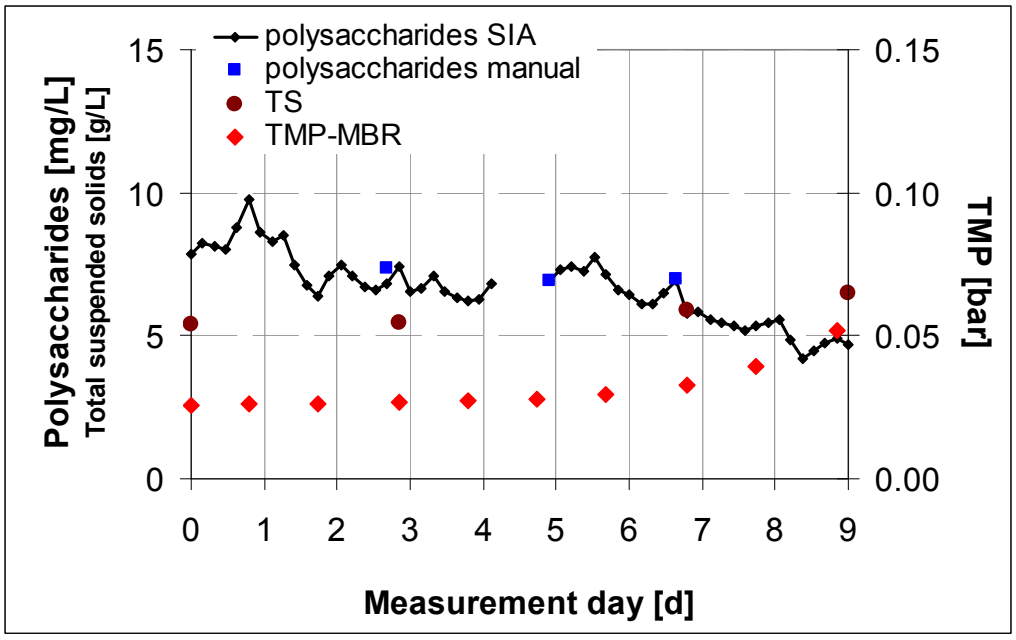


Figure 46: Variation of PS concentration in sludge filtrate of MBR measured continuously under normal conditions (from 5th to 14th Sep. 2008), (Mehrez et al., 2010b)

Proteins Figure 47 depicts measured concentration of protein concentration in sludge filtrate from 22th to 31st August 2008. Because the measurement error of protein analysis was quite high (in average ±2.1), calculation of a mean for 8 h concentration is proposed to assure the reliability of measured concentrations (green line in the Figure 47). Continuous measurements of proteins showed moderate variation during 9 days observation; however the variations are higher than in the case of polysaccharides. The averaged concentration in this period was 16.3 mg/L with minimal and maximal concentration of 13 mg/L and 20 mg/L respectively. The highest daily variation was 3.7 mg/L; however the mean can be calculated to 1.2 mg/L (SD ±0.91).

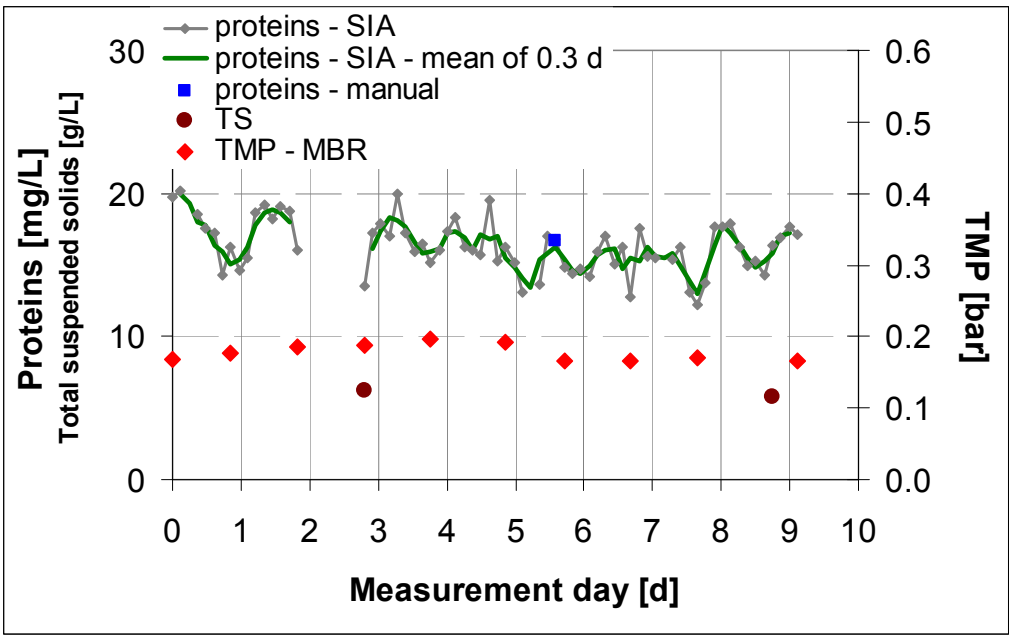


Figure 47: Variation of PR concentration in sludge filtrate of MBR measured continuously under normal conditions (from 22nd to 31st Aug. 2008), (Mehrez et al., 2010b)

Operation of MBR during simulation of rain water events

The investigation of rain water events was carried out in two periods with different amounts of “added rain” (tap water as a surrogate) corresponding to fluxes increased to 130% and 160%, respectively. Flux was increased for 6 hours. Afterwards flux was decreased to 90% respectively 80% for 18 hours to ensure a constant net flux (and thus HRT) over the investigated period of 24 hours.

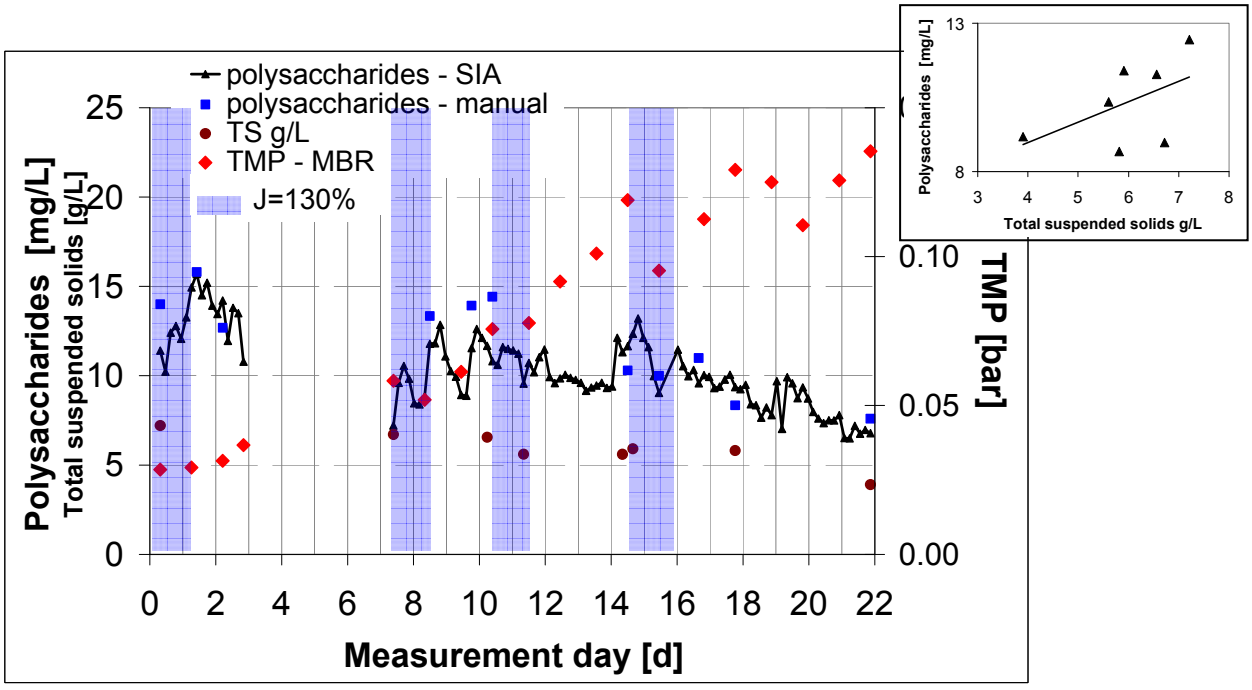


Figure 48: Variation of PS concentration in sludge filtrate of MBR measured continuously during simulation of rain water events (from 20th Oct. to 10th Nov. 2008); small figure: correlation of on-line measured PS concentration and total suspended solids concentration (TS), (Mehrez et al., 2010b)

Polysaccharides During three weeks of monitoring time of polysaccharides in October/ November 2008 high variation of concentrations was observed (Figure 48). Daily variation of the concentrations was between 1.7 mg/L (SD ±1.01), higher then measured in September 2008, what can be presumably ascribed to the colder weather (temperature, rain) during autumn time. During the monitoring period no daily or weekly profiles were observed. The dynamic experiments with rain water events on the MBR pilot plant have not provoked any significant variation of polysaccharides. Furthermore monitoring of polysaccharides during three weeks revealed a decrease of the concentration from ~15.7 to 6.5 mg/L that correlated with the decrease of the total suspended solids concentration in the sludge (decrease from 7.3 to 4.0 mg/L; small Figure 48). Meanwhile the TMP increased in the MBR pilot. The measurement error for polysaccharides (expressed as standard deviation of 4-fold measurements) was in mean 0.79 mg/L. The parallel conducted manual measurements of polysaccharides in sludge supernatant showed good agreement with continuous measurements.

Proteins The proteins concentration was measured on-line during three weeks in Nov./Dec. 2008 (Figure 49). Because of the relatively high measurement error of the protein method (~2.6 mg/L) a mean concentration of 8 h were calculated to improve reliability of results. During the monitoring time of 3 weeks variation of protein concentrations between ~13.4 and 37.4 mg/L (average 22.3 mg/L) was observed. The concentration fluctuation is higher than observed in September (daily fluctuation in mean 4.9 mg/L±4.0), what can be presumably explained by weather change (temperature decrease, rain) during autumn time. No weekly profile or effect of rain water simulation in the MBR plant could be monitored. The transmembrane pressure increased slightly during the monitoring time while the concentration of proteins decreased. The sludge suspended solids concentration increased slightly as well from 6.0 g/L to 9.6 g/L, what is opposite comparing with polysaccharides monitored in the period before, which correlated positively with TS concentration.

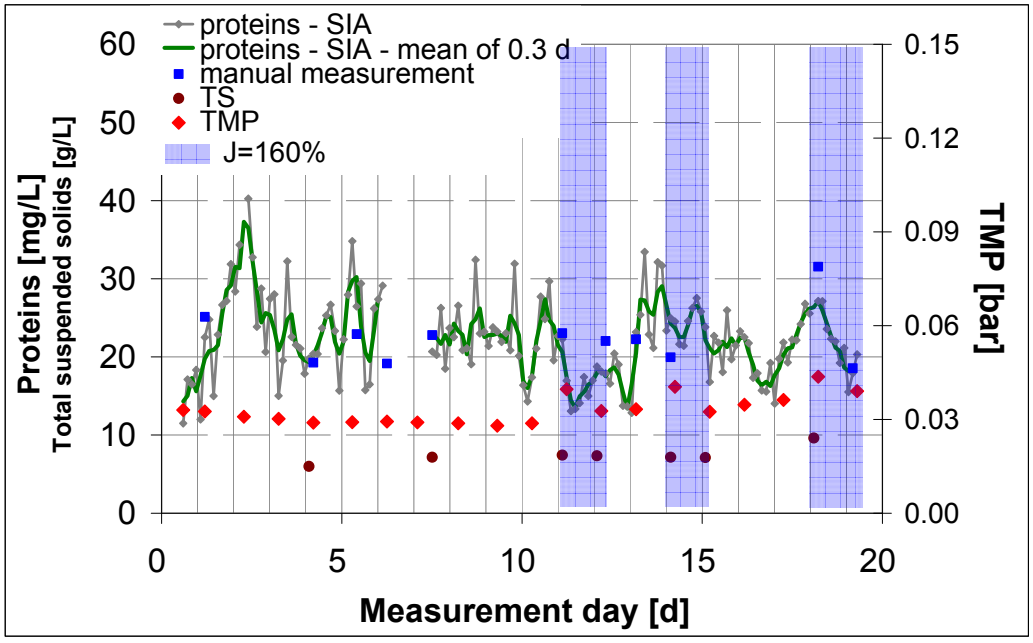


Figure 49: Variation of PR concentration in sludge filtrate of MBR measured continuously during simulation of rain water events (from 13th Nov. to 2nd Dec. 2008), (Mehrez et al., 2010b)

Operation of MBR by simulation of influent peaks

Polysaccharides The variation of polysaccharide concentration (Figure 50) was very high during the investigation of the effect of inflow peaks (March – April 2009). Particularly the

inflow peaks provoked sudden rise of the concentration of about 35 % (average increase of 4.8 mg/L). This increase can be explained by the shortage of HRT during inflow peaks that was probably not enough for the biodegradation of bigger molecules in influent like polysaccharides. The enhanced concentration decreased again within several days after operation was normalised. Particularly after the third and forth inflow peak (14 and 16 measurement day) the polysaccharides concentration decreased, what can be attributed to the biodegradation of them in sludge supernatant. During last inflow peak no rise of polysaccharides can be observed. It is hardly to explain, because no significant differences between experimental days could be found. The TMP increased slightly during the monitoring time; however the results show not direct correlation with polysaccharide concentration.

The measurement error for polysaccharides (expressed as standard deviation of 4-fold measurements) was in mean 0.43 mg/L. The parallel conducted manual measurements of polysaccharides in sludge supernatant showed good agreement with continuous measurements.

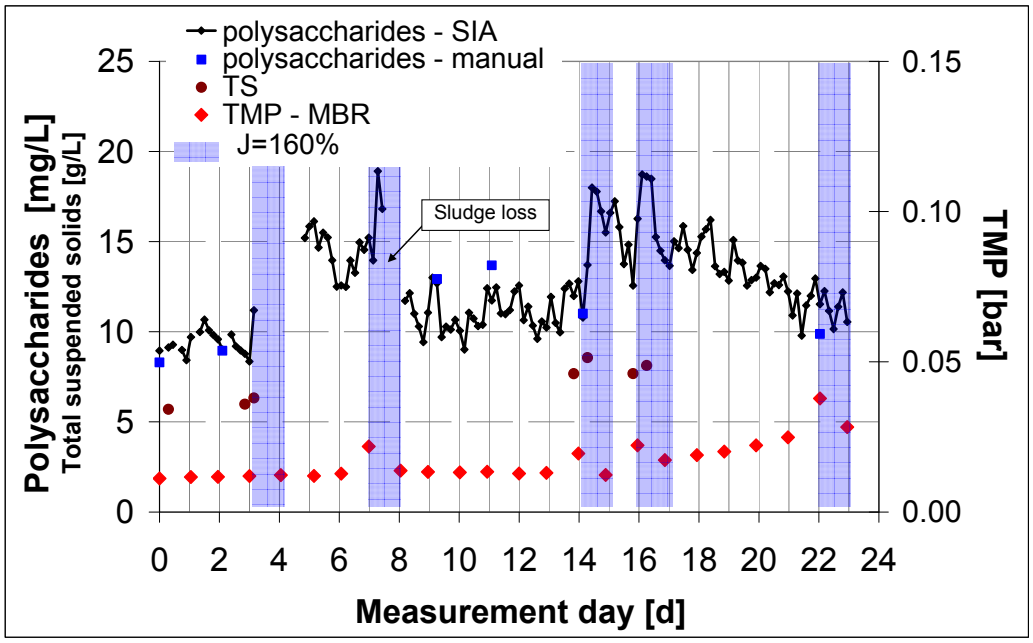


Figure 50: Variation of PS concentration in sludge filtrate of MBR measured continuously during simulation of inflow peaks (from 23rd March to 15th April 2009), (Mehrez et al., 2010b)

Proteins The monitoring of proteins concentration during the simulation of inflow peaks has not revealed any significant fluctuations or increase contrary to the measurement of polysaccharides. However the increase could be expected, because the concentration of proteins in the influent is in average five times higher then in the sludge filtrate and due to shortage of HRT low biodegradation as in the case of polysaccharides could be expected. Apparently the additional proteins coming with influent penetrated the membrane and were not accumulate in the sludge. The higher COD concentration in permeate during the inflow peak (10 to 20 %) confirms this assumption.

Interesting is the sudden decrease of the concentration from 25.5 mg/L to 8.8 mg/L in the 5th measurement day and increase again to the primary concentration (6th day). The decrease is hardly to explain, because no apparent events happed that could provoke so high decline of protein concentration in sludge filtrate.

No correlation between TMP and protein concentration could be observed. TMP increased slightly and the proteins stayed at very stable concentration of in average 20.2 mg/L (SD±2.6).

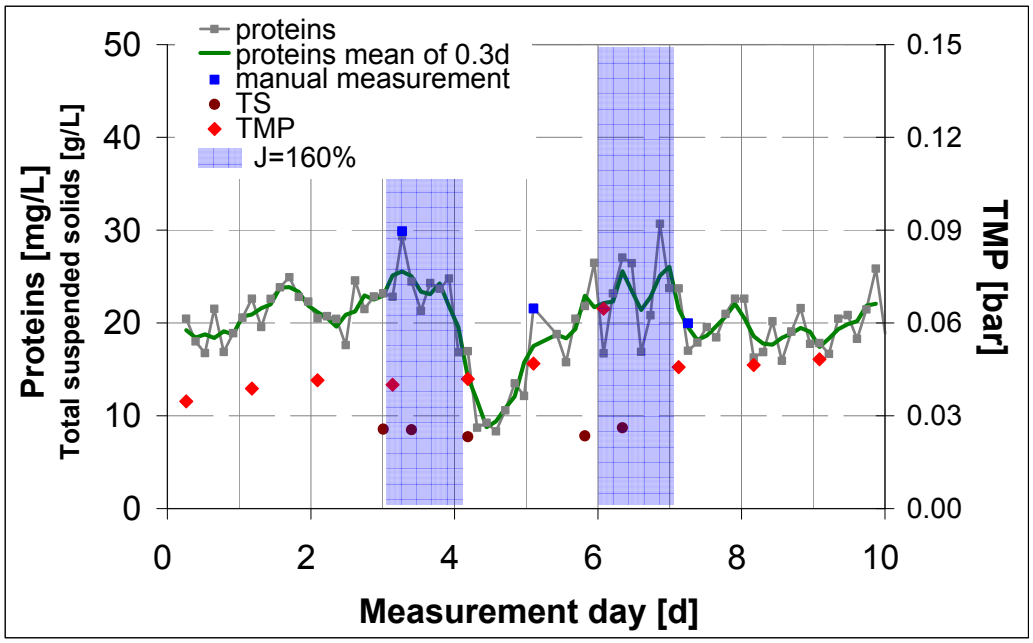


Figure 51: Variation of PR concentration in sludge filtrate of MBR measured continuously during simulation of inflow peaks (from 17thd to 27th April 2009), (Mehrez et al., 2010b)

4.2.3 Comparison MBR-VFM and EPS sensor

The aim was to compare both online fouling sensors during a 4 day-test period in Berlin. Unfortunately, the SIA sensor was defective at the time of the test period. Therefore, EPS measurements were performed manually. However, past experience has shown that these manual measurements correlate well with the automated assay.

The reversible and irreversible fouling propensity measured by the MBR-VFM remained quite stable during the test period. A small increase in reversible fouling propensity could be observed at the end of the period together with an increase in EPS-concentration (Table 15).

Table 15: Evolution of fouling propensity and fouling-related biological parameters

		Day 1 AM	Day 1 PM	Day 2	Day 4
VFM reversible	%	46.1	46.6	48.4	52.7
VFM irreversible	%	5.7	4.7	4.9	4.8
TTF	s	30	27	26	28
Proteins	mg/L	15.7	12.9	14.7	20.3
Polysaccharides	mg/L	12.1	12.4	10.0	13.0
Biopolymers	mg/L	1.91	1.90	1.95	2.34
MLSS	g/L	6.7	5.9	6.2	6.7
pH		7.75	7.56	7.84	7.76
Conductivity	µS/cm	1203	1258	1249	1180

As the test period only comprised 4 days, the obtained dataset was too limited to perform statistical analyses. However, it is clear that the fouling propensity and the EPS-concentration showed a similar course in time.

In Table 16, a comparison between the MBR-VFM and EPS-SIA Sensors is made and measurements principles as well as pros and cons are summarised. Due to the strong complementarities of both approaches, their simultaneous implementation as inputs for an advanced control system could be highly interesting.

Table 16: Comparison of MBR-VFM and EPS-SIA sensors

	MBR-VFM sensor	EPS-SIA sensor
Measurement principle	Physical filtration of mixed liquor	Chemical (photometrical) analysis of foulants in sludge filtrate
Evaluation of fouling potential	Determination of reversible and irreversible fouling characteristic	Determination of the concentration of polysaccharides and proteins as foulants substances
Measurement mode	Automated according to optimized protocol Membrane mounting and cleaning manual until now	Fully automatically, Calculation of concentrations manually until now
Pre-treatment of the sludge sample	Filtration at 1 mm	Filtration with filter (stainless steel, 1µm) in order to remove the solids
Measurement time / frequency	1 h if only reversible fouling number is needed, otherwise 2-3 h daily measurement seems sufficient, but measurement every 3 h is possible	< 1.5 h measurement every 2-3 hours possible
Possible application	Optimisation of both mechanical (relaxation, backwash, etc.) and chemical cleaning actions, based on reversible and irreversible fouling number respectively	Optimisation of operation (e.g. HRT), addition of specific fouling reducers to counteract high foulant concentrations
Pros	• Direct method for determination of fouling propensity • Takes into account all contributing foulants, e.g. MLSS, sludge composition, all EPS fractions, fluctuating wastewater composition, … • Differentiation between reversible and irreversible fouling • Possibility to adjust filtration parameters related to mechanical or chemical cleaning to the measured reversible of irreversible fouling potential respectively	• Full automatic measurement on-site • Monitoring of daily and seasonal variations of fouling substances (polysaccharides, proteins) • Quick identification of changes of the sludge properties • Modular in application: measurement can be extended on other substances that are determined with photometric methods • Reusable filter for sample pre-filtration

		• Standardisation of the determination techniques for proteins and polysaccharides
Cons	• Hydrodynamic conditions in the testing membrane and membrane material of senor should be identical to those of the real operational membranes of the MBR • Execution of consecutive measurements is not automated yet, but automation is possible	• The verification of the direct relation of fouling vs. polysaccharides / proteins could not be finalized yet. Potential risk not to measure fouling potential directly by polysaccharides and proteins. • Calculation of concentrations manually (further development possible) • Parallel measurement of polysaccharides and proteins only possible with two SIA devices

4.3 Pilot plant operation

4.3.1 Flux enhancers

Three additives were tested in the pilot system described in chapter 3.2.2. Their impacts on nutrient removal, permeability and sludge characteristics will be discussed in the following part.

4.3.1.1 Biological performance

The major task of an MBR is to provide an effluent quality that meets the legal requirements. It is therefore important to assure that the biological treatment process is not disturbed by the addition of a flux enhancing chemical. As nitrification is generally the more sensitive process, while COD removal is normally quite robust, attention should be paid especially to N-elimination.

In Table 17, the effluent characteristics for measurement campaigns with three different flux enhancing chemicals are listed. COD elimination was always 96 % on average, the degradation of COD was not hindered by any of the tested additives. It should be noted that the effluent COD in the plant with flocculant increased dramatically directly after the starch addition. Both sampling and re-dosing of starch (to account for the excess sludge withdrawal) took place twice a week, but on different days. The effect of the starch washout is therefore not reflected in Table 17. From a COD balance it was found that most of the added starch was washed out directly with the permeate and did not attach to the sludge flocs.

The Ntot-N elimination was generally 86 % on average; this was also not affected by the addition of any of the three chemicals. The influence of additive dosing on respiration activity and N-removal was tested previously in lab tests (see chapter 4.1.5) and also no influence of these additives on microbiological degradation activity was found.

Table 17: Influent (both plants) and effluent characteristics (grey: pilot plant 1 reference, white: pilot plant 2 with flocculant; mean value ± standard deviation and in brackets number of samples) (Iversen et al. 2009)

		Parameter	Mean	Max	Min
Influent Wastewater		COD [mg L^{-1}]	785 ± 244 (60)	1606	236
		N_{tot}-N [mg L^{-1}]	97 ± 18 (24)	147	71
		NH_4-N [mg L^{-1}]	50 ± 13 (44)	74	27
		PO_4-P [mg L^{-1}]	7 ± 2.5 (57)	14	1
MBR Effluent	NALCO MPE 50	COD [mg L^{-1}]	30.5 ± 5.9 (15)	43.0	18.9
			27.5 ± 6.5 (17)	35.9	15.3
		N_{tot}-N [mg L^{-1}]	21.2 ± 8.4 (4)	31.6	14.0
			18.5 ± 5.8 (4)	25.2	11.8
		PO_4-P [mg L^{-1}]	5.2 ± 2.4 (15)	8.7	0.4
			4.9 ± 2.7 (16)	9.0	0.1
	TATE & LYLE Mylbond 168	COD [mg L^{-1}]	25.3 ± 7.0 (11)	34.7	14.3
			27.8 ± 9.6 (11)	41.3	12.8
		NO_3-N [mg L^{-1}]	9.9 ± 1.9 (11)	12.6	6.6
			8.6 ± 2.1 (11)	11.2	3.7
		PO_4-P [mg L^{-1}]	5.8 ± 2.5 (10)	12.1	3.4
			5.4 ± 1.8 (11)	8.5	3
	ADIPAP KD 452	COD [mg L^{-1}]	30.5 ± 2.0 (8)	33.6	27.6
			31.8 ± 2.4 (8)	34.7	28.9
		N_{tot}-N [mg L^{-1}]	12.9 ± 1.6 (7)	14.8	10.4
			12.8 ± 1.8 (5)	15.9	11.7
		NO_3-N [mg L^{-1}]	10.1 ± 0.8 (8)	11.4	8.6
			9.3 ± 1.8 (8)	15.9	11.7
		PO_4-P [mg L^{-1}]	3.6 ± 1.5 (8)	6.3	1.7
			3.9 ± 1.7 (8)	6.1	1.1

4.3.1.2 Permeability

The TMP evolution for the three tested additives is shown in Figure 52. The TMP curve always shows the typical exponential characteristic with a slow increase in the first 20 to 40 days followed by a rapid steep increase. While the addition of a chemical did not change the initial TMP and the evolution during the first days, the exponential increase and the beginning of it were significantly altered.

When a cationic polymer (ADIPAP KD 452 or NALCO MPE 50) was added to the activated sludge a decrease of fouling was observed in comparison to the untreated reference (Figure 52 a and c). Especially when ADIPAP KD 452 was added to the sludge the time when the exponential increase started was significantly shifted. NALCO MPE 50 also showed quite good results in retarding the fouling. This can especially be seen in the last phase when the flux was increased by about 20 % and during the drive out period while there was still some flocculant in the plant. Here, the recovery after the chemical cleaning was much higher for the NALCO MPE 50 treated plant (72 % for the treated plant vs. 18 % for the reference). The fouling layer seems to be less persistent as in the reference plant. This was also expected

from previous filterability tests in test cell experiments (see chapter 4.1.4), where an increase in critical flux of 46 % for NALCO MPE 50 and of 38 % for ADIPAP KD 452 proved the positive impact of these additives on filtration.

The effectiveness of NALCO MPE 50 has already been validated in several studies (Yoon et al., 2005, Yoon et al., 2006, Lee et al., 2007, Thiemig et al., 2008). It is generally assumed that the cationic polymer entraps SMP into the sludge flocs, increases the size of the sludge flocs and leads to a more porous filtration cake, thus enhancing the filterability. Nevertheless, the astonishing filterability improvements reported elsewhere (Yoon et al., 2005, 2006) were not found in this pilot trials.

A totally different effect was found if the starch TATE & LYLE Mylbond 168 was added to the sludge. Due to the very promising results in test cell trials (see chapter 4.1.4), where the filterability and the critical flux were increased when this starch was added to the sludge and the fact that this chemical is a natural polymer, TATE & LYLE Mylbond 168 was selected for further trials in the pilot plant. Nevertheless, the addition of TATE & LYLE Mylbond 168 to the sludge had detrimental effects on the membrane as can be seen in Figure 52 b). Although the initial TMP was (like for the other trials) around 20 mbar in both plants, the TMP started to differ significantly after 30 days. During the high flux trials the pressure even increased to the limiting value of 200 mbar. Nevertheless, the strong fouling triggered during this period seems to be reversible as the TMP values before and after the high flux trials were fairly similar. Also this observation fits with the results from the shaking flask tests: the observed increase in polysaccharide concentration in the supernatant and humic and low molecular weight substances. The starch is not bound to the sludge flocs but penetrates the membrane and can cause fouling on and inside the membrane. Previous experiments examined the impact of the residual additive concentration that is not bound to flocs (assumption: 5% of the optimum additive concentration dissolved in deionised water) on the membrane (Iversen et al., 2008a). It was shown that starch had detrimental effects on all tested membranes. The contradicting results between the test cell trials and the results from pilot plant operation stress the importance to evaluate possible flux enhancers not only by short term experiments but also in long term and larger scale trials.

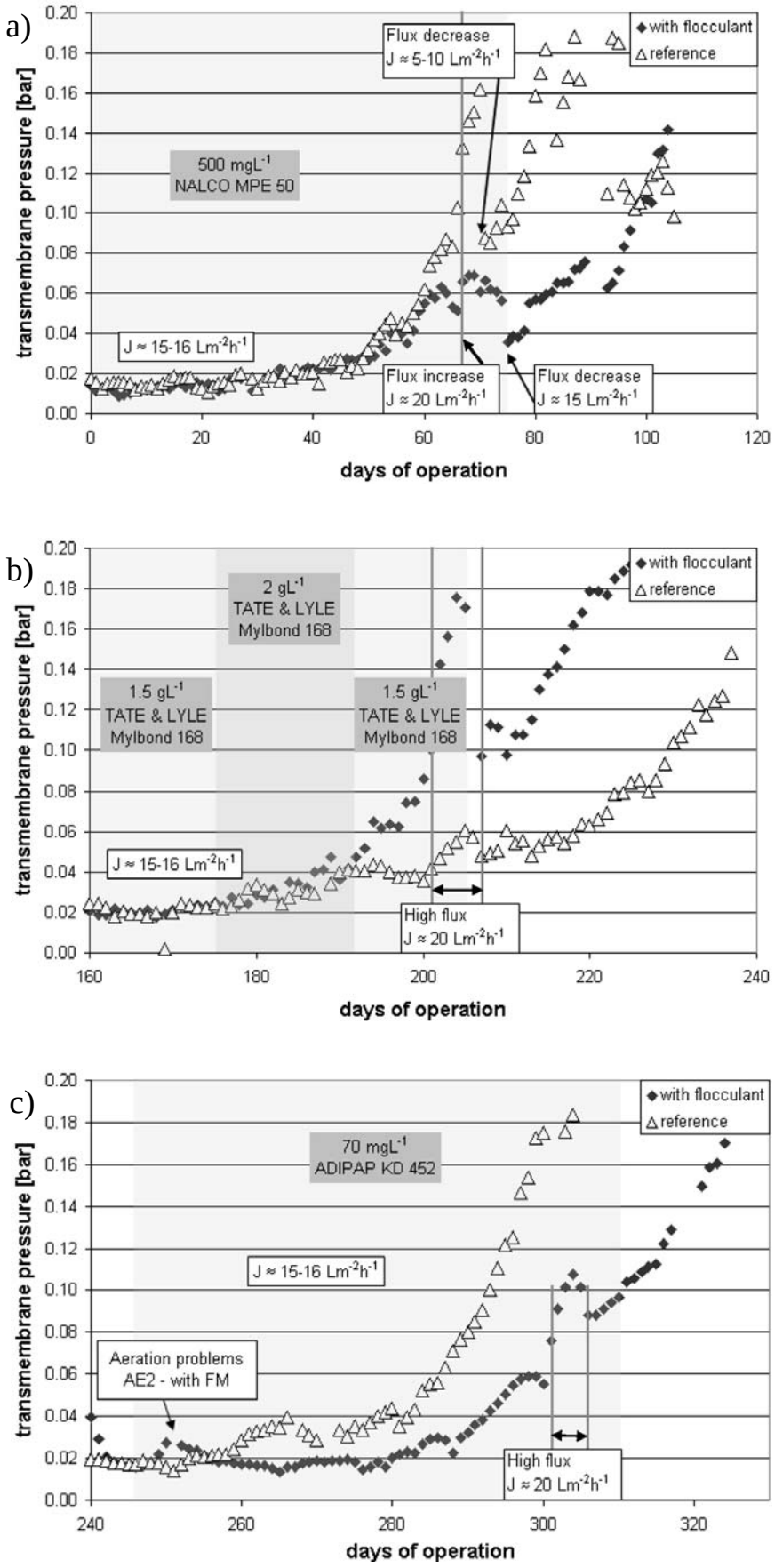


Figure 52: TMP evolution for plant with a) NALCO MPE 50 b) TATE & LYLE Mylbond 168 and c) ADIPAP KD 452 dosing and for respective reference plant operation (Iversen et al. 2009)

4.3.1.3 Comparison of sludge characteristics during flocculant dosing

The mean sludge characteristics during the periods with different flux enhancing chemicals in the plant and the respective values for the reference plant are shown in Table 18.

Table 18: Mean characteristics of the mixed liquor during trials with flux enhancing chemicals (mean value ± standard deviation and in brackets number of samples) (Iversen et al. 2009)

	SMP-PR	SMP-PS	EPS-PR	EPS-PS	Biopoly -mers	CST	d(v 0.5)	TS
	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[s]	[µm]	[g L^{-1}]
Reference (MPE 50)	21.1±8.0 (13)	9.1±3.4 (13)	131±42 (9)	34.2±10.4 (9)	2.11±0.94 (4)	43.9±16.0 (12)	52.5	7.6±2.4 (11)
MPE 50	19.1±6.2 (13)	8.0±2.3 (13)	160±38 (9)	45.8±11.5 (9)	2.56±1.48 (4)	33.8±7.6 (12)	61.5	10.1±1. 1(13)
Reference (Mylbond 168)	20.1±9.1 (6)	13.3±7.1 (6)	242±42 (6)	31.4±7.3 (6)	2.04±0.98 (4)	39.3±11.4 (5)	67.7	7.4±0.6 (6)
Mylbond	20.5±9.9 (6)	10.6±5.5 (6)	196±49 (5)	29.2±9.4 (5)	2.05±1.37 (4)	30.1±12.5 (5)	72.1	7.1±0.9 (6)
Reference (KD 452)	14.2±3.4 (5)	3.4	214±85 (5)	25.8±12.8 (5)	1.71±0.83 (4)	13.9±5.1 (5)	89.4	6.6±0.5 (6)
KD 452	11.9±3.1 (5)	3.1	181±23 (5)	26.4±6.2 (5)	0.70±0.17 (4)	15.0±6.9 (5)	106	7.4±0.6 (6)

During the NALCO MPE 50 dosing a small decrease in SMP concentration (sum of PR and PS) of 10 % was found. At the same time, EPS concentration increased by about 25 %, which confirms the result that SMP are bound into the flocs by the help of the flocculant. No clear effect of NALCO MPE 50 could be observed on the removal of biopolymers although in the shaker experiments, high eliminations of this compound were measured. Furthermore the mean particle size of the sludge flocs increased by about 17 %. Results presented in chapter 4.1.6 showed that the particle size was strongly enhanced (54 %) in lab tests if this flocculant was added to the sludge. These differences between lab and pilot tests can probably be attributed to additional shear stress, the impact of the wastewater and long term effects in the pilot plant. Nevertheless, the CST, which is often taken as a fouling indicator (Wang et al., 2006, Wu et al., 2007) was 23 % lower in the NALCO MPE 50 spiked plant, which is also lower than in the lab (80 % improvement, Figure 16 b).

It should be noted that the TS was much higher in the plant with NALCO MPE 50 dosing during that time compared with the reference plant. Although the uttermost efforts were done to identify the cause for this discrepancy the reason could not be found. As the amount of excess sludge withdrawn and the feeding was similar in both plants the only reason for this could have been different mixing conditions and therefore withdrawal of an excess sludge with a different TS. Also, a little more sludge was taken from the reference plant for further lab experiments; however, this cannot be the reason for this strong variation.

During the tests with TATE & LYLE Mylbond 168, the concentrations of SMP-PR, SMP-PS, EPS-PS and biopolymers are very similar in both plants, as well the biomass concentration (TS). In spite of the results of the shaking flasks no higher PS concentration in the supernatant (SMP-PS) was found. This might be due to the fact that the starch was quickly washed out after dosing and was not present in the samples taken on a different day. Also

the particle size was only changed very slightly (6.5 %). The strong improvement in CST about 23 % cannot be explained, since the fouling behaviour of the membrane was even worse in the plant with TATE & LYLE Mylbond 168 dosing (Figure 52 b).Nevertheless, own studies showed that the CST is not a sufficient parameter for the prediction of the fouling propensity of sludge (De la Torre Garcia et al., 2009).

The addition of ADIPAP KD 452 to the sludge showed the strongest effects on the parameters biopolymer concentration (reduction of 59 %) and mean particle size (increase of 18.6 %), while at the same time, nearly no effect on CST could be found. The SMP-PR and SMP-PS were also eliminated from the supernatant on average by 16 % and 9 %, respectively. The removal is smaller in comparison to lab tests; however, in the shaker experiments higher initial concentrations of these parameters were measured (SMP-PR = 23.5 mg L-1, SMP-PS = 10.0 mg L-1) which could influence the elimination rate.

The filterability data shown in Figure 52 c nevertheless indicates a strong improvement for the flocculated sludge. CST might therefore not be a good fouling indicator for this system.

Also, for this last period it should be noted that the biomass concentration was lower in the reference plant, but not as distinctive as during the MPE50 period. This can be attributed to the higher sludge withdrawal from this plant for further testing and also to some membrane integrity problems which occurred at the end of the trials.

4.3.2 Dynamic operation

Figure 52 a-c showed a characteristic exponential behaviour of the TMP evolution. The TMP only slowly increased in the first 20 to 40 days followed by a rapid steep increase.

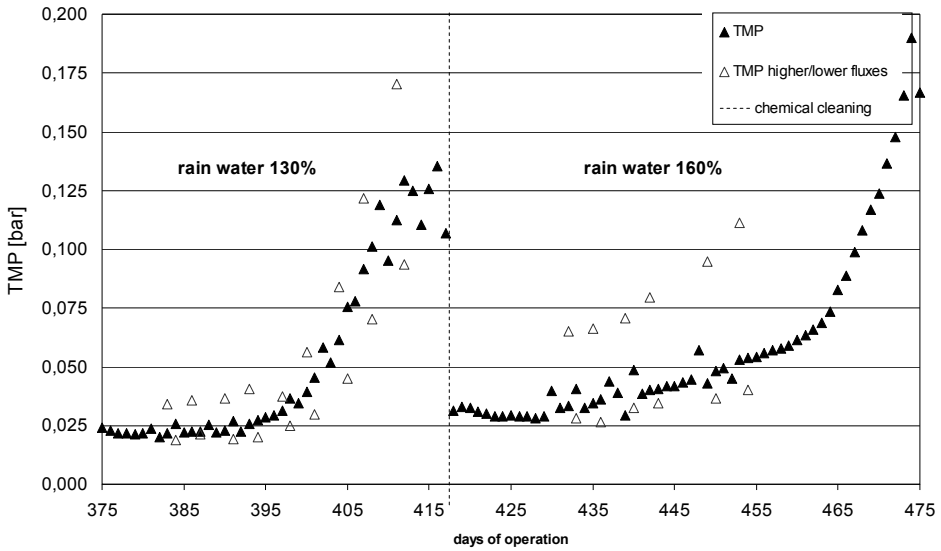


Figure 53: TMP evolution during rain water events

During the periods when the pilot was operated with dynamic fluxes due to rain water events the TMP curves in general showed the described behaviour (Figure 31). In the first period with fluxes increased to 130 % the TMP escalated after approximately 25 days of almost constant TMP. The chemical cleaning of the membrane was therefore needed after approximately 45 days of operation which is according to experience rather a short operation time. Nevertheless the rain water events showed no direct influence on the TMP evolution. Increasing the flux to 160 % in contrast led to a slowly rising flux immediately after the first rain

water event. But the TMP jump only occurred after 40 operation days thus the chemical cleaning was required later. In addition, sludge parameters (Table 19) in this period were worse compared to the period where the flux was increased to 130 %, implicating contrary results. Nevertheless both evolutions in general showed the exponential character with variations well known from experience. The nutrient removal did not change in the period with fluxes increased to 130 %. Permeate quality was good. Values for COD and total nitrogen were quite similar to the values from the earlier investigated periods (see chapter 4.3.1.1). Higher amounts of rain water and therefore higher fluxes (160 %) seemed to lead to a worse nitrification at the beginning. Ammonia (approx. 2-3 mg/L) was found in the effluent and nitrite (1 mg/L) in the sludge supernatant from the aerobic membrane chamber from time to time.

Table 19: Sludge characteristics during dynamic operation

	TS [g/L]	CST [s]	TTF [s]
Reference	7.6 (±2.4)	43.9 (±16.0)	-
Rain water 130%	7.1 (±1.2)	22.7 (±5.1)	25.8 (±6.4)
Rain water 160%	8.6 (±1.3)	56.4 (±17.8)	44.8 (±14.5)
Influent 160%	8.5 (±1.2)	52.8 (±11.3)	-

After the rain water events a sufficient permeability could not be reached with the usual cleaning protocol and the extended protocol, according to chapter 3.2.2.2, was applied. It could not be stated if the permeability problems were caused by the dynamic operational modes, the sludge characteristics or the alteration of the membrane.

Influent peaks (160%) led to a slowly increasing TMP after some peaking events. Nevertheless the TMP curve also showed the well known earlier described exponential profile. Concerning sludge parameters an increased TS after peaking event (up to 1g/L higher than before peaking event) could be determined. Long term effects could thereby not be observed. Due to decreased retention times, ammonia was found in permeate (up to 13 g/L) and in sludge supernatant (up to 12 g/L) after peaking event from time to time. Total nitrogen in permeate was though increased to 20-30 mg/L.

5 Summary and Conclusions

Flux enhancers 30 different additives of different chemical composition (activated carbons, metal salts, chitosans, synthetic polymers, enzymes and starches) were screened concerning their ability to bind SMP in the supernatant. The most promising substances from the different chemical categories were selected for further tests. These tests included experimental investigation of effects on filterability in small scale, on respiration, oxygen transfer, nitrification and denitrification, as well as shear stability, dewaterability. Also the impact of biomass concentration, calcium concentration and temperature on flocculation and adsorption was tested.

Table 20: Summarisation of the different experiments for selected additives

Substance	Product	c_{Add} [mg L-1]	SMP	k_La	OUR	Nitri/ Deni	Particle size V	J_{crit} test cell	Plant
Metal salt	*Magnasol 5108*	100	+	–	+	–	–	+	
	Merck FeCl3	85	+	+	+	+	–	+	
Chitosan	*Chitosan 221*	200	++	–	–	+	++	–	
	Chitosan 652	250	+	+	–	+	+		
Activated carbon	*SA Super*	450	+	+	+	+	–		
	Picahydro LP 27	5000	+	–	–	–	–		
Polymer	*MPE-50*	500	++	++	+/-	+	+	++	+
	MP H 30	500	+						
	MP L 30	500	+	–	+	–	–	++	
	Adifloc KD 451	70	+	++	+	–	+		
	Adifloc KD 452	70	++	+	+	+	(+)	++	++
Starch	*Jaguar C162*	300		+	+	+	–		
	Mylbond 168	1500	+	++	+	+	–	+	– –

Especially the activated carbon Picahydro LP 27 and the PACl Magnasol 5108 showed detrimental effects on the biology. The tested polymers and chitosans showed the best results for the enlargements of floc size. While the polymers were also very effective to increase the critical flux in test cell trials, the chitosan showed negative impacts here. The metal salts and the biopolymers chitosan and starch are also tricky to dose, as over- or underdosing might cause further fouling on the membrane. Especially the overdosing of the PACl Magnasol 5108 can cause accelerated fouling compared to the untreated reference sludge.

The temperature, Ca^{2+} concentration and TS might have an influence on the efficiency of additives if the applied concentration is smaller then the optimal dosage. With increasing TS and temperature the removal of supernatant compounds decreased.

Two synthetic polymers and a starch were then tested in pilot trials in a system of two parallel pilot plants with 1.5m³ working volume each. Although the starch showed promising results in the filtration test cell trials, it had detrimental effect on the membrane in large scale and long term trials. The TMP increased more quickly in the system with starch dosage than in the one without. On the other hand the fouling was retarded if a polymer was added. The additives showed no effects on the permeate quality and only slight differences for the sludge characteristics were found.

A summarisation of the results can be found in Table 20.

Photometrical Sensor On-line sensor for continuous photometric measurement of polysaccharides and proteins (SMP) was developed and tested in MBR pilot (Berlin, Mitte). Through the application of the sensor collection of comprehensive data set of polysaccharides and proteins concentration in MBR sludge filtrate is possible now, allowing the monitoring of daily and seasonal variations of these parameters as well as evaluation of their impact on membrane fouling and flux decrease in the MBR reactor.

The automation of both manual methods for analysis of polysaccharides and proteins was successfully conducted with the SIA technique. The automated method for polysaccharides is characterised by low detection and quantification limit and small measurement error that are comparable with manual procedure. The method showed good robustness during online application at the pilot plant. The variation of the concentration and influence of different events of e.g. influent peaks could be easily recognised. Because of aggressive reagents (conc. H_2SO_4) caution and frequent maintenance of the sensor is required when polysaccharides are measured.

The automated method used for continuous measurement at the pilot plant (Method II) is suitable for monitoring of trends especially at high proteins concentration. The sensitive measurement in MBR is limited due to relative high quantification limit and measurement error. The improved Method II lowers the measurement error to acceptable level, as well as the detection limit. Due to time constraints this more sensitive protein method could not be applied in the real MBR system. The results from the continuous measurement of proteins in the lab with the new method showed its very good robustness for several days.

The continuous measurements could not verify the positive correlation of measured parameters versus TMP neither for proteins nor for polysaccharides that was observed by Rosenberger et al. (2006) in a previous research project. Mostly (for two first analysis periods) the polysaccharides concentration decreased while the TMP increased. During the on-line analysis of proteins no or only slight increase of TMP was observed while the proteins amount in sludge filtrate stayed quite stable.

Monitoring of the concentration of polysaccharides and proteins with EPS sensor showed quite high fluctuation during the week and sometimes within one day. This demonstrates that manual sampling and analysis of considered parameters may not give an appropriate image of the variation since the sampling is less often. Frequent manual sampling is almost not possible and very expensive.

MBR-VFM Sensor. A new fouling measurement method was developed to determine a mixed liquor's fouling propensity. The MBR-VFM uses a specific measurement protocol consisting of alternating filtration and physical cleaning steps, which enables the calculation of both the reversible and the irreversible fouling resistances. The approach proved to be reproducible and sensitive to most parameters relevant for fouling. Furthermore, our experiments indicated that the MBR-VFM can accurately measure fouling and that it can even be detected earlier than can be seen from the on-line filtration data of lab-scale MBR systems. Furthermore, the differences measured in reversible and irreversible fouling seemed to be related to the observed impact of physical and chemical cleaning respectively. Therefore, the application of the MBR-VFM as an on-line sensor in an advanced control system, enabling the deployment of the measured fouling data for the control of membrane cleaning, seemed feasible. Such an Advanced Control System based on MBR-VFM measurements was consequently also developed and tested in AMEDEUS and is reported in deliverable D51.

In the validation experiments, MBR-VFM measurements corresponded well with the actual fouling in the system, despite the fact that different membrane materials and configurations were used in the MBR-VFM and the lab-scale MBRs. Evidently, both have an impact on the extent and mechanisms of membrane fouling. The MBR-VFM is currently equipped with tubular membranes and investigations have been performed with two different membrane materials. Future research may focus on the development of sensor systems with different configurations to ensure compatibility with full-scale MBRs.

The actual MBR-VFM set-up allows for on-line measurements. However, membrane replacement is still performed manually. On the long term, a development towards a fully automated measurement with a fixed sequence of membrane replacements, measurements and membrane cleanings will have to be aimed for.

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Annex

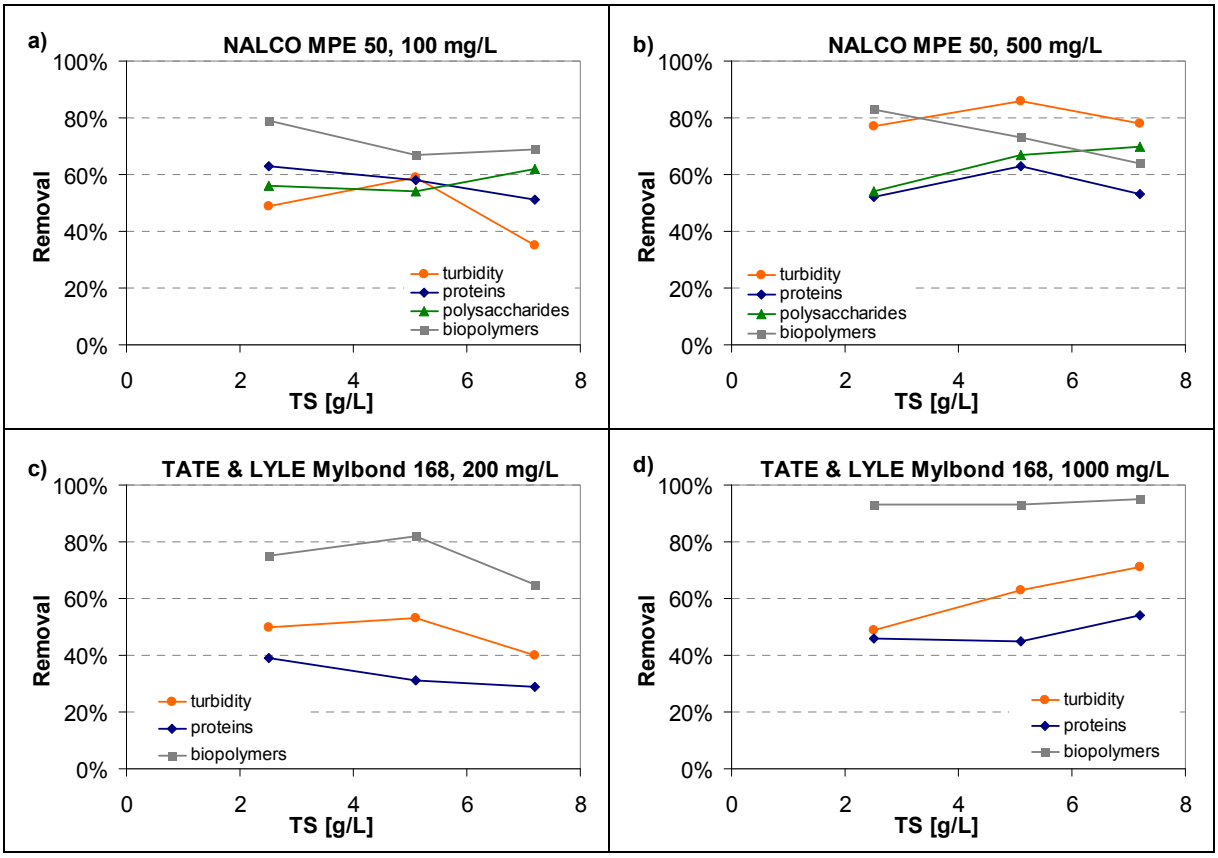
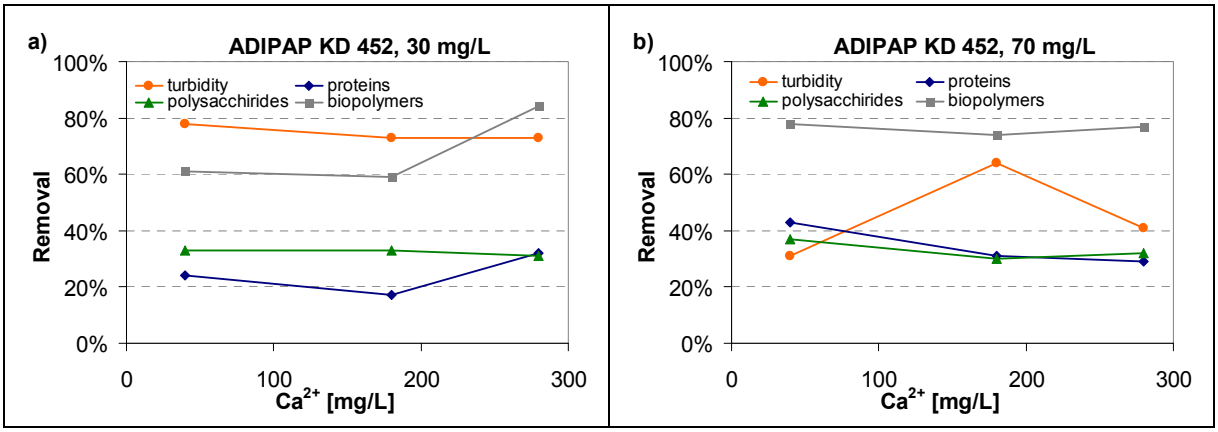


Figure 54: Influence of TS on removal of different fractions of sludge supernatant at two additive concentrations for NALCO MPE 50 (a-b) and TATE & LYLE Mylbond 168 (c-d).



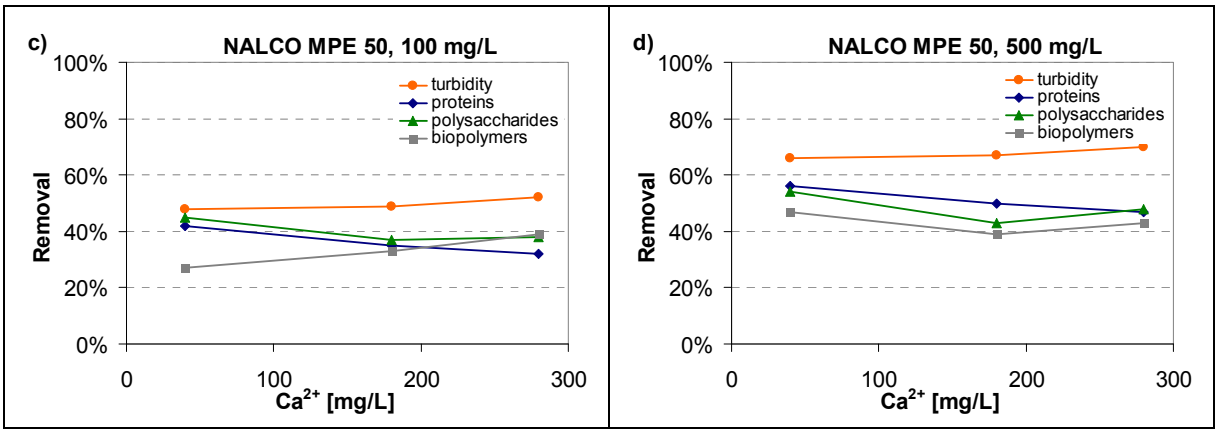


Figure 55: Influence of Ca^{2+}-Ions on removal of different fractions of sludge supernatant at two additive concentrations for ADIPAP KD 452 (a-b) and NALCO MPE 50 (c-d).

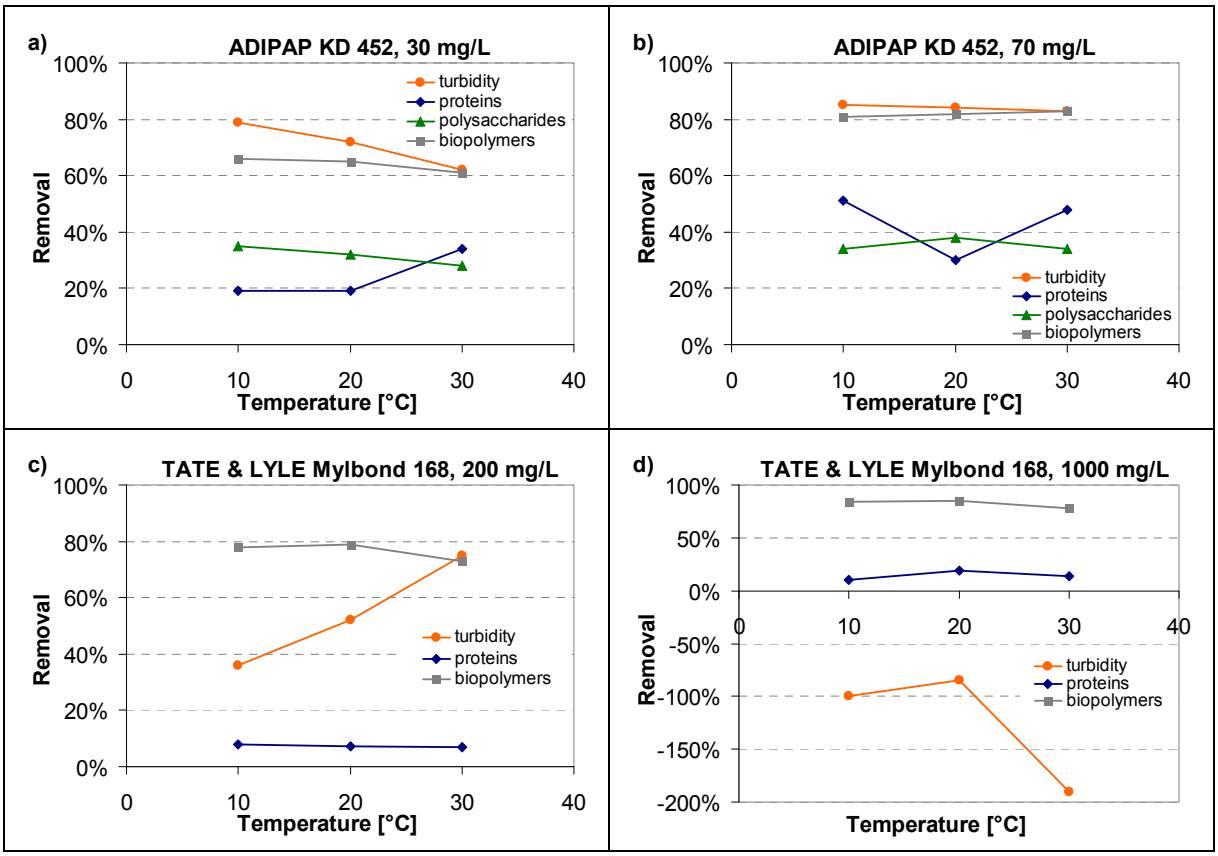


Figure 56: Influence of the temperature on removal of different fractions of sludge supernatant at two additive concentrations for ADIPAP KD 452 (a-b) and TATE & LYLE Mylbond 168 (c-d).