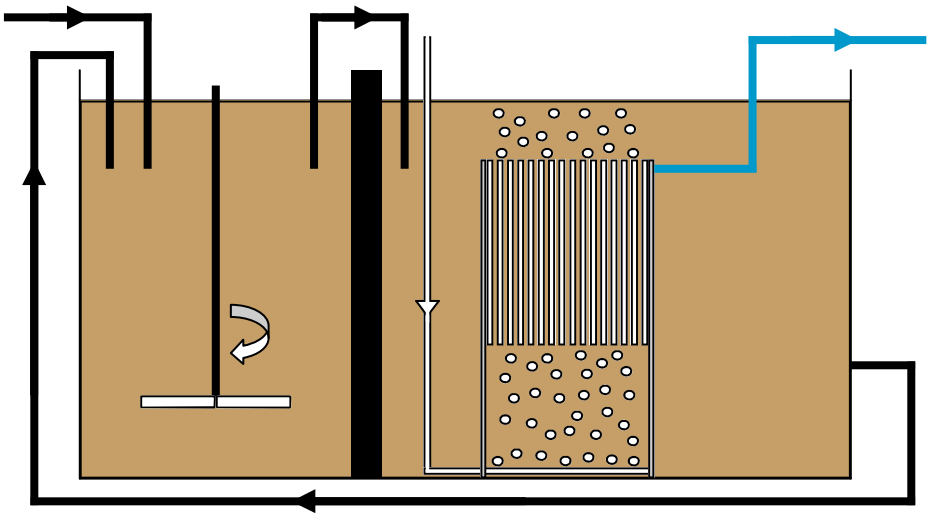
AALBORG UNIVERSITY
Department of Biotechnology, Chemistry and Environmental Engineering

Characterization of Sludge in a Submerged Membrane Bioreactor



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Preface

This report covers a master thesis project in chemical engineering at the Faculty of Engineering, Science and Medicine at Aalborg University. The project was done between 1st of September 2008 and 4th of June 2009.

The project is addressed mainly to people with a similar or higher educational level in chemistry or chemical engineering.

References have been done using the Harvard System as described in [Holland 2004].

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Abstract

The membrane bioreactor (MBR) technology for wastewater treatment has been of growing interest since the late 1980s due to increasing needs for water reuse and stricter environmental demands. However, lacking understanding of the fouling problems is still an obstacle that needs to be cleared before the MBR technology is fully competitive with conventional wastewater treatment processes.

This project focuses on the characteristics of sludge flocs in MBR systems since these are found to be the main origin of foulants. The sludge flocs in MBR systems are typically exposed to higher shear rates than in the conventional wastewater treatment plants, and this is expected to affect the sludge floc characteristics. Therefore, the characteristics of MBR sludge were compared to conventional activated sludge (CAS).

Two submerged MBR systems were used during this project, i.e. a pilot MBR placed at a municipal wastewater treatment plant and a labscale MBR placed at Aalborg University. The labscale MBR was build to be comparable to the pilot MBR.

Sludge flocs from both MBR and the corresponding CAS were analyzed in terms of macroscopic and microscopic changes during the operation periods. Macroscopic analysis involved size and floc strength measurements and were both found to be dependent of the shear level in the MBR systems. Microscopic analysis involved determination of carbohydrates, proteins and humus, hydrophobic interaction chromatography and size exclusion chromatography. These measurements showed some changes of EPS composition, however none that could be ascribed directly to the shear levels in the MBR systems.

The two MBR systems were found to be comparable in terms of the analysis results, even though some main parameters were not the same.

Dansk resume (Danish Abstract)

Interessen for membran bioreaktor (MBR) teknologien til spildevandsrensning har globalt set været stigende siden slutningen af 1980'erne som følge af øget behov for genanvendelse af vand og skærpede miljøkrav. Dog er manglende viden om fouling problemer stadig en forhindring, der skal fjernes før MBR teknologien bliver fuldt konkurrencedygtig i forhold til konventionel spildevandsrensning.

Dette projekt fokuserer på karakteristika for slamflokkene i MBR systemer, da disse er hovedkilden til fouling. Slamflokkene i MBR systemer er typisk udsat for højere shear rater end i det konventionelle spildevandsrensningsanlæg, og dette forventes at påvirke slamflokkenes karakteristika. Derfor er karakteristika for MBR slamflokkene sammenlignet med samme værdier for konventionelt aktiveret slam.

To nedsænkede MBR systemer blev brugt gennem dette projekt; en pilot MBR placeret på et kommunalt spildevandsrensningsanlæg og en labskala MBR placeret på Aalborg Universitet. Labskala MBR'en blev opbygget så den var sammenlignelig med pilot MBR'en.

Slamflokke fra både MBR og den tilhørende konventionelle aktive slam blev analyseret i forhold til makroskopiske og mikroskopiske ændringer under driftsperioden. De makroskopiske analyser inkluderede flokstørrelse og -styrke målinger og begge parametre ændredes som følge af shear niveauet i MBR systemerne. De mikroskopiske analyser omhandlede bestemmelse af sukker, protein og humus, hydrofob interaktionskromatografi og størrelseseksklusions-kromatografi. Disse målinger viste ændringer af slamkarakteristika, men disse kunne ikke direkte tilskrives shear niveauet i MBR systemerne.

De to MBR systemer viste sig at være sammenlignelige i forhold til forsøgsresultaterne selvom nogle vigtige parametre ikke var ens.

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1 Introduction

The membrane bioreactor (MBR) is a wastewater treatment technology, which combines the use of microorganisms for biological degradation of organic pollutants and membrane filtration for solid-liquid separation [Le-Clech *et al.* 2006]. Thus, the area demanding settling tanks of conventional wastewater treatment plants are replaced by membrane filtration.

The technology was introduced in the late 1960s by Dorr-Oliver Inc., who applied a bioreactor combined with a crossflow filtration loop for ship-board wastewater treatment [Judd 2006; Le-Clech *et al.* 2006]. Other similar MBRs were developed at about the same time, but they all suffered from poor economics due to high costs of membranes and use of high fluxes which implied a need for high, energy demanding crossflow velocities [Le-Clech *et al.* 2006]. Hence, these early MBR systems were primarily applied in areas with special needs; e.g. isolated ski resorts or trailer parks [Le-Clech *et al.* 2006].

However, in the late 1980s an important step in the development of the MBR technology was taken as Yamamoto and co-workers submerged the membranes into the bioreactor [Judd 2006]. This implied a significant decrease in the energy demands as the need for aeration of the microorganisms in the bioreactor could be combined with the need of a crossflow over the membranes [Le-Clech *et al.* 2006]. Further process optimization and reduction of membrane costs led to an exponential increase in existing MBR plants throughout the 1990s [Judd 2006].

Since then, the global market for MBRs has been further developing mostly within municipal wastewater treatment but also in industrial applications [Atkinson 2006]. The global market is expected to increase from being $216 millions in 2006 to $363 millions in 2010 [Atkinson 2006]. This results from an increasing need for water reuse and generally stricter environmental demands, which makes the MBRs advantageous due to high effluent quality [Atkinson 2006; Shannon *et al.* 2008]. Other advantages compared to conventional wastewater treatment processes include small footprint, higher load of microorganisms, smaller reactor volume requirements and less production of excess sludge [Le-Clech *et al.* 2006].

The market for MBRs is however still limited due to high costs of membranes and lacking fouling control under varying process parameters [Meng *et al.* 2009]. Fouling refers to deposition of materials either on the membrane surface or in the membrane pores resulting in an increasing filter resistance and thus a declining flux [Mulder 1996]. This phenomenon is very difficult to handle in the wastewater treatment process since the activated sludge is very complex, consisting of many

potential fouling materials (foulants) of both inorganic and organic origin [Le-Clech *et al.* 2006]. Besides, the sludge characteristics change e.g. under varying weather conditions and also depend on the origin of the wastewater [Wilén *et al.* 2003; Wilén *et al.* 2008].

The fouling issues in MBRs have been studied for many years but have been of increasing interest lately with as many as 400 articles dealing with varying aspects of fouling in MBR systems in 2007 alone [Meng *et al.* 2009]. However, many different setups and operation conditions have been used and a lot of empirical correlations have been proposed which are often only valid under specific conditions. Hence, it is still very difficult to get an overview of the subject and to set up some general favorable conditions to limit fouling [Meng *et al.* 2009].

Therefore, the overall objective of this project is to build up a more detailed understanding of general characteristics of activated sludge flocs in a MBR system for municipal wastewater treatment as the sludge flocs are the main origin of the potential foulants [Meng *et al.* 2009]. The next sections include a more thorough overview of MBR systems and operation parameters, followed by a description of relevant aspects of sludge characteristics. Finally, both the general fouling phenomenon and fouling in MBR systems will be introduced.

2 General Principles of Membrane Bioreactors

As introduced previously, both MBR configurations with an external filtration loop (side-stream MBR) and with a membrane unit submerged in the bioreactor (submerged MBR) exist. However, only the submerged systems will be described here, since they are the most common [Le-Clech *et al.* 2006]. Fig. 1 is a simple schematic presentation of a submerged MBR outlining important operation parameters for a typical MBR system.

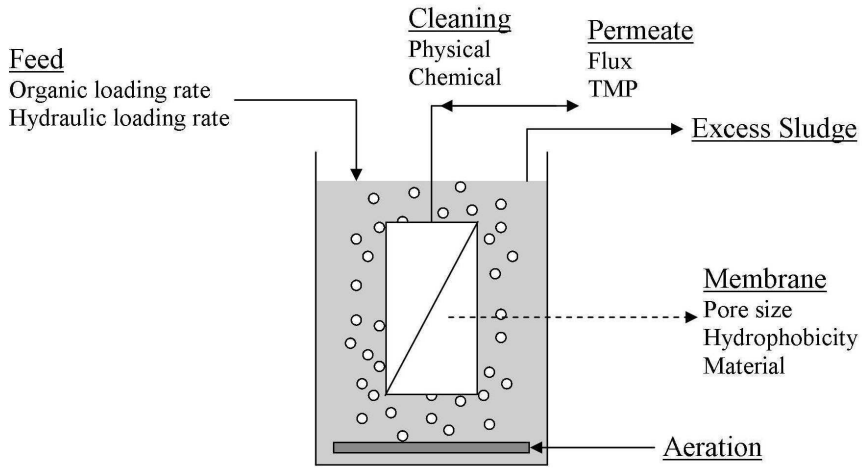

Fig. 1: A schematic design of a MBR system outlining important operation parameters. Adapted from [Le-Clech *et al.* 2006].

In the submerged MBR, the membrane unit is placed inside the bioreactor with an aeration setup which creates a crossflow "scrubbing" the membrane surface and furthermore supplies the oxygen needed for the microbial processes [Judd 2006]. The permeate flux is driven by a transmembrane pressure (TMP) and either the flux or the TMP is kept at a constant level [Le-Clech *et al.* 2006]. The permeate line is normally also used in cleaning of the membrane [Judd 2006]. The feed is continuously loaded at the same flow as the permeate [Judd 2006]. The next sections will describe some of the operation parameters introduced in Fig. 1.

2.1 Membrane Configuration and Material

The most common membrane configurations are hollow fiber and flat sheet, and the majority of membranes applied in MBR systems are polymeric membranes, e.g. polyethylene (PE) or

polyvinylidene fluoride (PVDF) [Le-Clech *et al.* 2006]. The pore sizes typically range from coarse ultrafiltration to fine microfiltration [Le-Clech *et al.* 2006]. Ceramic membranes generally perform better than polymeric-based membranes, but these membranes are much more expensive to produce, and hence the overall cost makes them unfavorable [Le-Clech *et al.* 2006].

2.2 Aeration

As earlier mentioned, the aeration combines oxygen demand from the microorganisms and the need for a crossflow along the membrane surface. The bubble size, the flow rate and the aerator area are the main aeration characteristics that influence the system [Judd 2006]. The bubble size must not be too large, since smaller bubbles give a higher mass transfer of oxygen from the bubbles into the liquid phase [Judd 2006]. On the other hand, the so called “scrubbing” is also dependent on the size of the bubbles, since larger bubbles create more turbulence [Judd 2006]. The flow rate of the aeration is also important both for sufficient oxygen levels and for the velocity of the crossflow [Judd 2006]. The aerator area influences the area of membrane that has a crossflow at the desired velocity [Judd 2006]. The aeration results in shear levels in the aerated area of the MBR systems, which are mostly higher than the shear in conventional wastewater treatment processes [Çiçek *et al.* 1999]. These higher shear levels might result in undesired changes of sludge floc characteristics, but the shear rate is important to diminish the need for membrane cleaning [Judd 2006].

2.3 Membrane Cleaning

Some degree of cleaning of the membranes is necessary to ensure a high filtration performance of the membranes over long-term filtration. Cleaning of membranes can be divided in two categories; i.e. physical and chemical cleaning [Le-Clech *et al.* 2006].

Physical cleaning is a part of the continuous process and includes either relaxation or backwash. Relaxation is a period of time without TMP, e. g. ten minutes of filtration and two minutes of relaxation [Le-Clech *et al.* 2006]. Backwash is cleaning with water from permeate side of the membrane with a reversed flux [Le-Clech *et al.* 2006].

Chemical cleaning is normally done as a clean in place or line (CIP or CIL) process. In the CIP process, the membrane stays in the reactor but the sludge is removed during the cleaning procedure [Le-Clech *et al.* 2006]. CIL is the supply of cleaning chemicals from the permeate side of the membrane without removing sludge from the reactor [Le-Clech *et al.* 2006]. In both cases, two

different chemicals are used; one for removal of organic foulants, e.g. hypochlorite, and another for inorganic foulants, e.g. citric acid [Le-Clech *et al.* 2006]. Different approaches have been taken to chemical cleaning from once a day to once a year [Le-Clech *et al.* 2006]. The problem with chemical cleaning is that the chemicals are harmful to the microorganisms and shortens the lifetime of polymeric membranes [Le-Clech *et al.* 2006].

In general, the cleaning processes are expensive, especially the use of chemicals and backwash. Hence, it is desired to reduce the need for cleaning or at least improve the efficiency of the applied cleaning procedures, and this leads to a need for more understanding of fouling in MBR systems.

2.4 The Biological Processes

The microorganisms in the bioreactor metabolize dissolved and suspended organic components of the feed wastewater and this process is advantageous due to high chemical conversion efficiency [Judd 2006]. The aerobic processes are ideally capable of converting large organic molecules into CO_2, H_2O and inorganic nitrogen products [Judd 2006]. However, some amounts of extracellular polymeric substances (EPS) will also be produced depending on the conditions in the reactor as well as the feed composition [Judd 2006]. The ideal end product of the anaerobic processes is methane and the combination of both aerobic and anaerobic treatment results in biological nutrient removal (removal of nitrogen) [Judd 2006]. The efficiency of the processes and the production of byproducts depend on various operation parameters, both physical and biological [Judd 2006].

2.5 Biological Operation Parameters – Feed and Activated Sludge

This section will present the most important parameters used to describe the feed and the activated sludge in MBR processes. The total substrate concentration of the feed is normally described by either biological or chemical oxygen demand, BOD or COD (kg/m^3) [Judd 2006]. Combined with the feed flow rate (m^3/d), BOD or COD yields the organic loading rate, OLR (kg/d) [Judd 2006]. The mixed liquor suspended solids, MLSS (kg/m^3), is a measure of the amounts of microorganisms in the reactor which depends on the COD, the decomposition of feed by microorganisms and the excess sludge taken out of the reactor [Judd 2006].

OLR can be used to calculate the feed to microorganism ratio, F/M ratio (kg COD/kg MLSS · days), as shown in Eq. 1.

$$F/M = \frac{OLR}{V \cdot MLSS} \qquad \textbf{Eq. 1} \qquad \text{[Judd 2006]}$$

Where V is the volume of the reactor in m^3.

The solid retention time, SRT (days), is a measure of the retention time of the microorganisms in the bioreactor. For the MBR systems, the retention time is equal to the average lifetime of the microorganisms as they are retained by the membrane. SRT can be calculated from the F/M ratio (see Eq. 2).

$$\frac{1}{SRT} = Y(F/M)\frac{E}{100} - k_e \qquad \textbf{Eq. 2} \qquad \text{[Judd 2006]}$$

Where Y is the biomass yield which is the mass of cells formed per mass of substrate consumed (kg MLSS/kg COD). E is the process efficiency in % and k_e is the death rate constant ($days^{-1}$).

The hydraulic retention time, HRT (days), is the retention time of the fluids in the system [Judd 2006]. In the conventional wastewater treatment process this is related to SRT, but in the MBR system, a high SRT but low HRT is possible since the solids are retained by the membrane [Judd 2006].
General differences of the biological parameters in conventional wastewater treatment and in MBR systems are presented in Table 1 below.

Table 1: Comparison of biological parameters in conventional wastewater treatment and in MBR systems [Judd 2006].

	Conventional wastewater treatment	MBR
SRT [days]	~8	~40
MLSS [kg/m^3]	~2.5	8-12
F/M [kg COD/kg MLSS·day]	>0.12	<0.12

The higher SRT in MBR systems results in a higher decomposition of substrate and thereby a lower amount of excess sludge and is also the reason for the higher MLSS [Judd 2006]. These factors will all affect the characteristics of sludge flocs in the MBR system [Judd 2006].

3 Characteristics of Sludge Flocs

In the literature, it is difficult to find specific descriptions of sludge characteristics in MBR systems, and therefore this section will describe sludge characteristics mainly based on the conventional activated sludge processes.

Most of the microorganisms in conventional activated sludge processes self-aggregates in complex sludge flocs which mainly consist of bacterial colonies surrounded by a network of extracellular polymeric substances (EPS) [Wilén *et al.* 2008]. Besides, the flocs include organic fibres and particles and inorganic components as presented in Fig. 2.

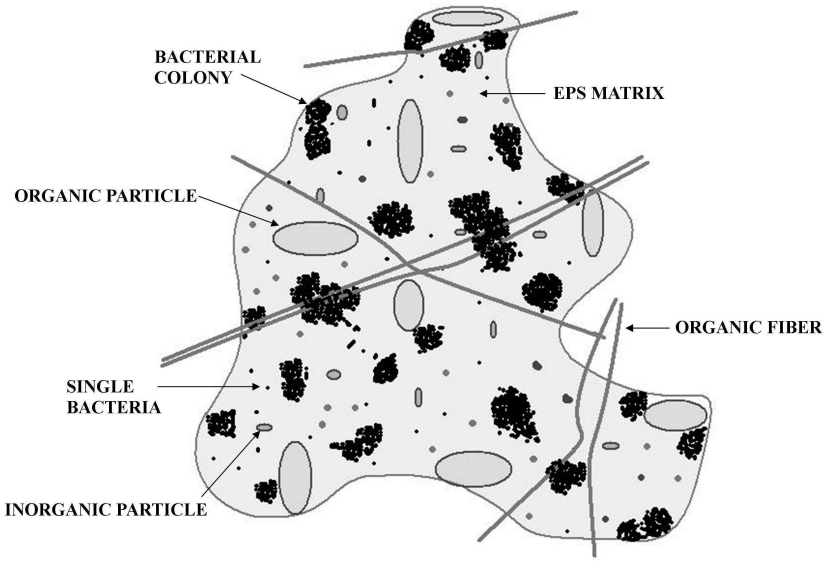

Fig. 2: Schematic example of the structure of an activated sludge floc including single bacteria, bacterial colonies, absorbed organic and inorganic particles and organic fibres surrounded by the EPS matrix. Adapted from [Mikkelsen 1999].

The most important component with regards to stability and structure of the sludge floc is EPS, which typically constitute 50 to 60 % of the organic fraction of sludge flocs whereas the cell biomass only constitutes 2 to 20 % of same [Wilén *et al.* 2003]. EPS either originate from metabolic byproducts secreted from the cells or from cell lysis [Le-Clech *et al.* 2006]. Aside from the self-aggregation function, the EPS also serve as a protective barrier around the bacteria and ensure retention of water and adhesion to surfaces [Le-Clech *et al.* 2006]. Fractions of EPS are water soluble and might end up as dissolved polymers in the aqueous phase due to surface erosion from the flocs.

The main constituents of EPS are proteins, carbohydrates and humus, but also presence of lipids and nucleic acids are reported [Wilén *et al.* 2008; Meng *et al.* 2009]. In the EPS matrix, relative high amounts of multivalent cations (Ca^{2+}, Fe^{3+}, Mg^{2+}) are present and these are important since they are needed to stabilize the negative charged surfaces introduced by proteins and humus [Wilén *et al.* 2008].

3.1 Size and Strength of Sludge Flocs

Sludge typically has a bimodal size distribution, and this has been observed in MBR systems as well [Le-Clech *et al.* 2006]. The smaller fraction is primary particles, e.g. single bacteria and colloids, and the larger fraction is the sludge flocs, respectively [Mikkelsen and Keiding 1999]. The bimodal distribution results from an equilibrium between flocculation and deflocculation [Mikkelsen and Keiding 1999], i.e. aggregation of new flocs or incorporation of primary particles into existing flocs and erosion of particles from the surface of existing flocs or large scale fragmentation of flocs, respectively (see Fig. 3) [Jarvis *et al.* 2005]. The state of this equilibrium depends on the strength of the forces involved in the interaction within the sludge flocs and the external shear forces applied on the flocs [Jarvis *et al.* 2005].

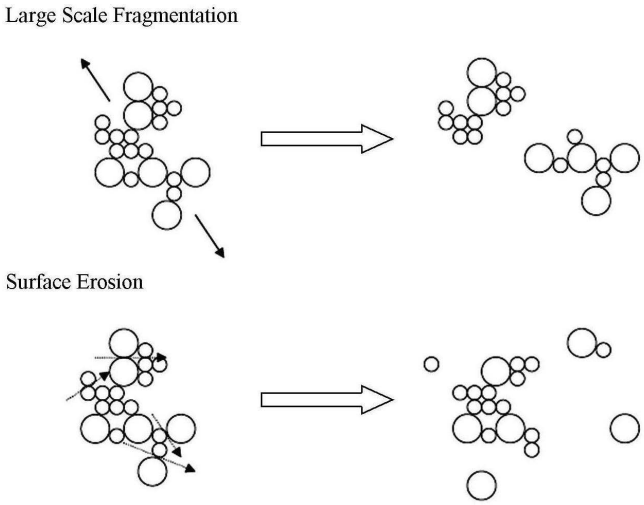

Fig. 3: Floc breakage involves either large scale fragmentation or surface erosion. Adapted from [Jarvis *et al.* 2005].

The forces involved in the interaction between the surfaces in the EPS matrix are comparable to those involved for non-living colloids, including electrostatic forces (DLVO), hydrophobic

interaction, polymer entanglement and bridging by multivalent ions etc. [Wilén *et al.* 2008]. Based only on DLVO forces, the interaction energy is the sum of attractive van der Waals forces and repulsive electrostatic forces as presented in Fig. 4.

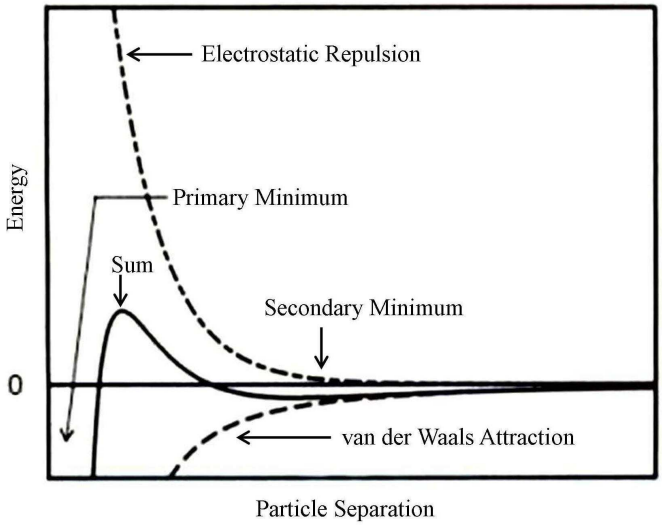

Fig. 4: The potential energy curve resulting from DLVO forces between colloids. The repulsive forces are electrostatic whereas the attractive are van der Waals forces. The minimum refers to the sum curve. Adapted from [Israelachvili 1991].

The strength of the repulsive forces is governed by the surface charges and the ionic strength of the medium and here multivalent ions are important since they both reduce surface charges by adsorption to the stern layer and increase the ionic strength [Mikkelsen 1999]. The attractive forces depend on the composition of the surfaces and the medium. The DLVO forces involve two minima in the potential energy, as shown in Fig. 4 [Israelachvili 1991]. The primary minimum represents short range and irreversible contact between surfaces, where a very high kinetic energy is needed for breaking up the contact [Mikkelsen 1999]. The secondary minimum exists at a longer range between the surfaces and represents lower energy interaction [Israelachvili 1991]. Hence, the secondary minimum is the interactions important in terms of the flocculation/deflocculation equilibrium [Mikkelsen 1999].

The non DLVO forces, which according to both Mikkelsen [1999] and Axelos *et al.* [1994] might be even more important for the total interaction between biopolymers, will affect both the distance and the depth of the secondary minimum [Mikkelsen 1999]. Hence, even though an increasing amount of EPS within the floc increase the surface charge, it has still been shown to result in an increased floc strength, indicating that non DLVO forces are more important for the sludge floc stability [Mikkelsen 1999].

As stated in the aeration section, the shear forces are typically higher in MBR systems compared to the conventional wastewater treatment process. Hence, a higher rate of deflocculation must occur in these systems. Neglecting that a changed shear also might change the rate of flocculation, it is assumed that the equilibrium between flocculation and deflocculation must be adjusted, so that more single particles (e.g. single bacteria, dissolved EPS) are present at higher shear, which might lead increasing fouling problems.

4 Fouling Mechanisms

As stated in the introduction, fouling refers to deposition of matter on the membrane surface or in the membrane pores resulting in an increased resistance to permeate flux [Mulder 1996]. The permeate flux at a given TMP depends on the membrane resistance and fouling resistance as described in Eq. 3.

$$J = \frac{\text{TMP}}{\mu\left(R_m + R_f\right)} \qquad \textbf{Eq. 3} \qquad \text{[Mulder 1996]}$$

Where J is the flux in $m^3/(s \cdot m^2)$, TMP is the transmembrane pressure in Pa, μ is the dynamic viscosity of the solution in Pa/s and R_f and R_m is the resistances in m^{-1}.

4.1 Fouling Types

In the literature, many different types of fouling have been introduced; i.e. cake formation, biofilm formation, gel formation, pore blocking, adsorption to the membrane surface and concentration polarization [Mulder 1996; Judd 2006]. In most cases, more than one of these are important for the fouling resistance. However, some of the resistances are negligible in systems where other forms of fouling have a much greater impact on the flux. To understand these types of fouling it is important to know what their driving forces are.

Cake formation refers to a fouling layer on the membrane surface created by drag forces towards the membrane resulting from the TMP [Mulder 1996].

A *biofilm* is a network of biopolymers on the membrane surface formed by microorganisms, which secrete the polymers forming the network [Judd 2006].

Gel formation also refer to the formation of a polymeric network on the membrane surface, but gels are created by polymers in the bulk and hence not actively by microorganisms. Formation of a gel on the membrane surface can result both from TMP and concentration polarization [Mulder 1996].

Pore blocking is when matter penetrates into the membrane and gets stock in a pore and thereby blocks it [Mulder 1996].

Adsorption can happen either at the membrane surface or in the pores of the membrane. This is controlled by attractive forces between the matter near the membrane and the membrane surface [Mulder 1996].

Concentration polarization is created by retained molecules near the membrane surface. These retained molecules influences on the mass transfer through membrane by increasing the resistance

through the area of concentration polarization [Mulder 1996]. This is however mostly a problem in systems with low or no TMP, since the TMP in most cases yields much more fouling than the concentration polarization [Mulder 1996].

4.2 Critical and Steady State Flux

The main reason for fouling in MBR systems is the drag forces towards the membrane resulting from TMP. As stated earlier, the aeration in MBR systems creates turbulence at the membrane surface comparable to crossflow systems. The effect of TMP on the flux for a crossflow filtration system is shown in Fig. 5.

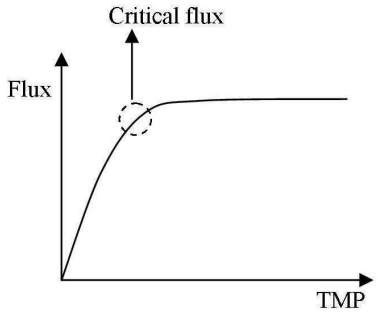

Fig. 5: Flux vs. TMP. Below critical flux, the relation is proportional. Above critical flux, increases in TMP do not increase the flux.

The critical flux is the highest obtainable flux in a membrane system before the fouling resistance increases rapidly. This means that the flux increases proportional to TMP under critical flux, but over critical flux increasing TMP does not lead to an increase in flux [Judd 2006].
If the flux is below critical flux at a constant TMP in a crossflow system, it reaches a stable level over time, i.e. steady state flux (see Fig. 6) [Mulder 1996].

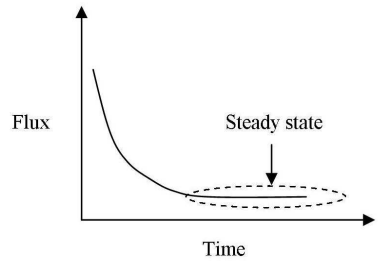

Fig. 6: Flux vs. time at constant TMP. Over time steady state flux is obtained. Adapted from [Judd 2006].

The steady state flux is obtained when the fouling resistance reaches a stable level. This depends on the balance between the amounts of new foulants adhering to the fouling layer and amounts of foulants ripped off the fouling layer [Christensen *et al.* 2009]. The flux creates a force towards the membrane surface due to a difference in pressure whereas the crossflow creates a force away from the membrane surface due to a difference in velocity [Christensen *et al.* 2009]. Attractive forces towards the membrane or other foulants due to charge or hydrophobicity as well as repulsion due to the same forces will also affect the steady state flux [Christensen *et al.* 2009].

4.3 Fouling in Membrane Bioreactors

The fouling in general is normally categorized as either reversible or irreversible, however in MBR systems these categories are not sufficient since there are two general types of cleaning (see section 2.3). Therefore, the reversible fouling can be split into removable and irremovable [Meng *et al.* 2009]. The removable fouling is the fouling that can be removed by physical cleaning, while irremovable fouling is the fouling that cannot be removed by physical cleaning but only by chemical cleaning [Meng *et al.* 2009]. The irreversible fouling can neither be removed by physical nor chemical cleaning (see Fig. 7) [Meng *et al.* 2009].

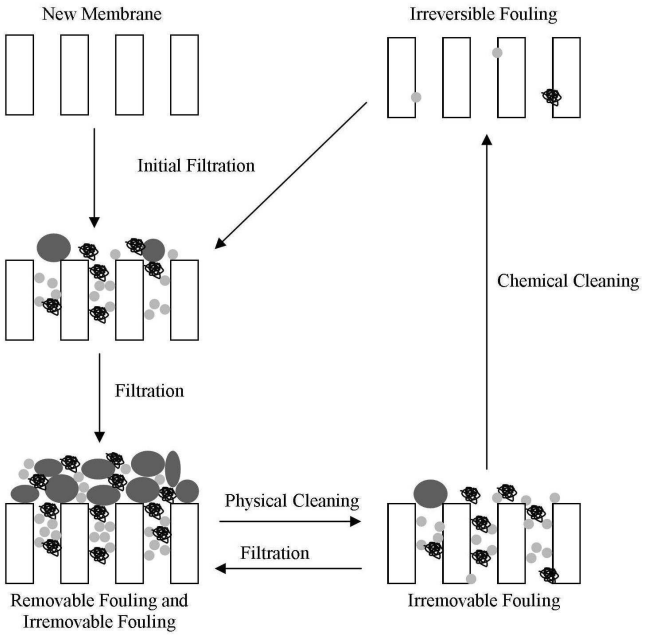

Fig. 7: Removable and irremovable fouling needs physical or chemical cleaning, respectively. Irreversible fouling cannot be removed by any of the cleaning procedures. Adapted from [Meng *et al.* 2009].

In MBR systems operated at constant TMP and with regular physical cleaning, the flux will decline rapidly during the initial filtration due to the formation of irremovable and irreversible fouling [Le-Clech *et al.* 2006]. It then reaches a more stable level, but as little fouling still occurs, the flux will slightly decrease over time and chemical cleaning will be needed [Le-Clech *et al.* 2006]. This description is analogous to the general description of steady state flux above.

Most reported MBR systems are though carried out at constant flux, and therefore more thorough descriptions of the development of the fouling resistance are found for such systems. In these systems, comparable stages occur but the TMP needs to be gradually increased to maintain constant flux and this accelerates the fouling formation.

Operation at constant flux typically results in increases in TMP as shown in Fig. 8.

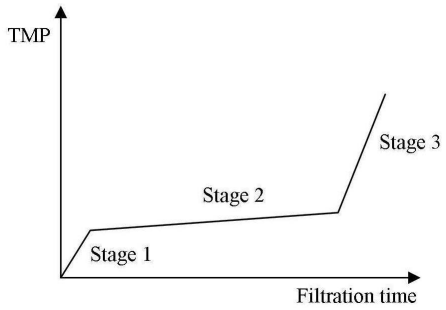

Fig. 8: TMP as a function of filtration time. The TMP rises in three stages. Adapted from [Meng *et al.* 2009].

Stage 1 involves conditioning fouling at a low TMP and is therefore more dependent on the interactions between the membrane and components of the sludge [Judd 2006]. This stage is often negligible compared to the fouling occurring later in the process [Judd 2006].

Stage 2 has a long, weak rise in TMP created by cake formation, biofilm growth and/or pore blocking. As long as the distribution of fouling is regular over the membrane surface, the TMP will be kept at a rather low level thereby keeping the flux under critical flux. However, the fouling will be distributed irregularly over time [Judd 2006].

In *Stage 3*, the fouling is irregularly distributed resulting in an increased flux in local areas of the membrane. This leads to areas with a flux higher than the critical flux and thereby a high increase in fouling, resulting in the TMP jump seen in Fig. 8 [Judd 2006]. However, recent studies have also shown that the TMP jump might result from changes in the cake layer with an increase in EPS concentration at the bottom of the cake layer [Meng *et al.* 2009].

4.4 Foulants in Membrane Bioreactors

The fouling components in MBR systems can generally be defined in three categories; i.e. biofouling, organic fouling and inorganic fouling [Meng et al. 2009]. Biofouling refers to fouling involving microbial cells. The cells are much larger than the membrane pores, and therefore the microbial cells will typically be involved in removable cake formation on the membrane surface [Meng *et al.* 2009]. However, the cells might adhere and grow on the membrane surface forming a biofilm, but often this step first involves deposition of EPS or at least an increased secretion of EPS [Meng *et al.* 2009]. Hence, it might be considered as a gel formation and not a biofilm. *Organic fouling* involves EPS, and these foulants will typically easier adhere to the membrane surface since they are smaller, and therefore less affected by the lift forces at the membrane [Meng *et al.* 2009]. Besides, they might also partially penetrate the membrane pores and end up as pore blocking. *Inorganic fouling* refers to fouling involving inorganics such as $CaCO_3$, and this type of fouling is generally considered insignificant compared biofouling and organic fouling [Meng *et al.* 2009]. However, the presence of multivalent cations such as Ca^{2+} and Mg^{2+} are important with regards to the formation of fouling layers since e.g. deposited proteins needs stabilization of their negative charges [Meng *et al.* 2009]. Thus, the cations are needed for formation of gel layers and since they are dissolved in the water passing the membrane, high concentrations of cations can be build up in the organic and biofouling layers [Meng *et al.* 2009].

5 Problem Statement

Based on the sections describing fouling in MBR systems, an understanding of the characteristics of MBR sludge must be important since sludge is the main origin of potential foulants. Assuming that higher shear forces in the MBR systems compared to the CAS process will affect the sludge floc characteristics, led to the following problem statement of this project.

How do sludge floc characteristics change resulting from shear forces in a MBR system compared to the conventional activated sludge processes?

6 Experimental Approach

The analysis of the sludge flocs focused both on macroscopic characteristics including floc size and strength and on microscopic characteristics in terms of EPS composition. The effect of shear forces on floc size and strength was described in the literature, and hence it was expected that these parameters would change in the MBR systems due to higher shear forces. These characteristics are also interesting with regards to fouling, mainly for the fouling resistance resulting from cake formation (see section 4.4).

The EPS composition was studied both for dissolved and extractable EPS fractions. The hypothesis for these studies was that higher shear forces change the composition of the EPS both in the bulk and in the sludge flocs as loosely bound EPS would be dispersed whereas strongly bound EPS would remain. Therefore, the contents of the three major EPS constituents (i.e. proteins, humus and carbohydrates) were determined. However, methods that detect changes in the polymeric composition in terms of properties such as size, charge or hydrophobicity might be more interesting both with regards to stability of sludge flocs and fouling in MBRs.

Hydrophobic interaction chromatography (HIC) is a method typically used for separation of proteins and other large complex biopolymers with hydrophobic parts [Bradshaw 2006]. In the known literature, this chromatography method has not been applied for EPS analysis. Hence, it was assumed that this method could serve as a measure of changes in EPS composition in terms of hydrophobicity. Size exclusion chromatography (SEC) has been shown to yield fingerprints of the EPS composition, which change resulting from variations of operation parameters [Garnier *et al.* 2005; Lyko *et al.* 2007]. For a complex mixture of polymers such as EPS samples, not only size exclusion mechanisms will affect the retention times since some of the polymers might interact with the column material. However, by further characterization of the separated polymer fractions, specific information of the changes in polymeric composition was obtained in these studies. Therefore, these two chromatography methods were also used in the studies of changes in EPS composition.

The analysis methods were first applied on sludge samples from initial experiments. In these short term experiments, sludge was treated with varying shear forces assumed comparable to the shear forces in MBR systems (shear turbulence values from 500 to 1700 s^{-1}).

The analysis methods were applied on sludge samples from a pilot MBR placed at Lundtofte WWTP. The sludge was regularly analyzed over a period of 57 days from the start-up with

conventional activated sludge (CAS) with an MLSS of about 3 g/L. To obtain the desired F/M ratio, MLSS was increased with 9 g/L as the final aim. The objective of analysis was to follow changes in sludge characteristics from the start up and till this final MLSS level was reached, but since this period was longer than expected, the whole period was not covered within this project. As reference, CAS samples with the same feed were analyzed.

Finally, a labscale MBR was build up, making it possible to follow the changes of the sludge characteristics in the initial phase more thoroughly. The object was to make a setup as close as possible to the setup of the pilot MBR, so that comparison of results was possible. For the labscale MBR, sludge samples were analyzed over a period of 14 days but with smaller intervals between the samples compared to the pilot MBR.

7 Experimental Setup

7.1 Setup for Initial Experiments

Sludge was gathered at Aalborg East municipal wastewater treatment plant (WWTP). The sludge was concentrated to 10 - 11 g/L dry matter contents and stored for maximum 24 h before the shear experiments.

Mikkelsen and Keiding [2002] have calculated turbulent shear levels (G) using a certain baffled reactor and a certain single bladed paddle from the stirring speed in rpm (see Table 2).

Table 2: Relations between G and stirring speed in a baffled reactor (cylindrical, d: 105 mm, h: 120 mm) with a single bladed paddle [Mikkelsen and Keiding 2002].

G [s^{-1}]	Stirring speed [rpm]
500	670
800	900
1100	1150
1400	1350
1700	1560

To test the shear influence on the sludge, three turbulent shear levels were chosen for the experiments; i.e. 500, 1100 and 1700 s^{-1}. The experiments with the three shear levels were conducted simultaneously and with sludge from the same batch. The sludge was kept cool in an ice bath.

The changes of the sludge flocs were followed during the experiments by measurements of supernatant turbidity, which is a measure of the amounts of dispersed mass in the aqueous phase. This was done by taking out a sample of 6 mL of sludge, which was centrifuged for 2 min at 3000 rpm (Sigma Laboratory Centrifuges, Model 3-15). The turbidity of the supernatant was measured in a spectrophotometer at 650 nm (Thermo Spectronic, Helios Epsilon). The experiments were stopped when the supernatant turbidity had reached a level with only slight increases, and the sludge was then immediately used for further analysis. The further analysis is described in section 8.

7.2 Setup and Operation Parameters for Pilot MBR at Lundtofte WWTP

The pilot MBR produced by Alfa Laval A/S was placed at and operated by Lundtofte WWTP. It was a submerged MBR system with a flat sheet membrane configuration. The setup included an aerobic tank (for nitrification) containing the membrane unit and an anaerobic tank for denitrification. TMP was controlled by the difference of water levels in the aerated tank and the permeate tank, and was ~15 mbar during the analyzed period yielding a flux of ~7.5 L/ h·m^2.
Physical and biological operation parameters are listed in Table 3 and 4, respectively. Since the MLSS was increasing in the operation period, the listed biological values are approximate values.

Table 3: The physical parameters for the pilot MBR system.

V aerobic tank [m^3]	4.1
V anaerobic tank [m^3]	4.2
TMP [mbar]	~15
Membrane material	PVDF
Membrane area [m^2]	40
Average pore size [µm]	0.2
Recirculation between tanks	300 %
Relaxation	2 min every 10 min
Aeration [L/(min·m^2)]	8

Table 4: Approximate values of the biological parameters for the pilot MBR system.

Feed COD [mg/L]	~ 300
COD load [kg/day]	~ 2.2
F/M [kg COD/kg MLSS·day]	~ 0.06
MLSS (g/L)	3 – 6
HRT [h]	17

Sludge samples of 1 L were taken from the aerobic tank of the MB, and as reference sludge samples of 1 L were also taken from the CAS process with same feed. The two samples were transported (approximately 24 h) in a cool box, which kept the samples at approximately 5 °C. After transport, the samples were handled as described in section 8. Samples were taken out on day 1, 4, 11, 15, 22, 25, 29, 32, 36, 43, 50 and 57.

7.3 Setup and Operation Parameters for Labscale MBR

Fig. 9 is a schematic presentation of the labscale MBR, which was built up as a part of this project. The aim of the setup was to have biological and physical parameters comparable to those of the pilot MBR, but due to limited resources and time within this project, this was not achievable in all aspects.

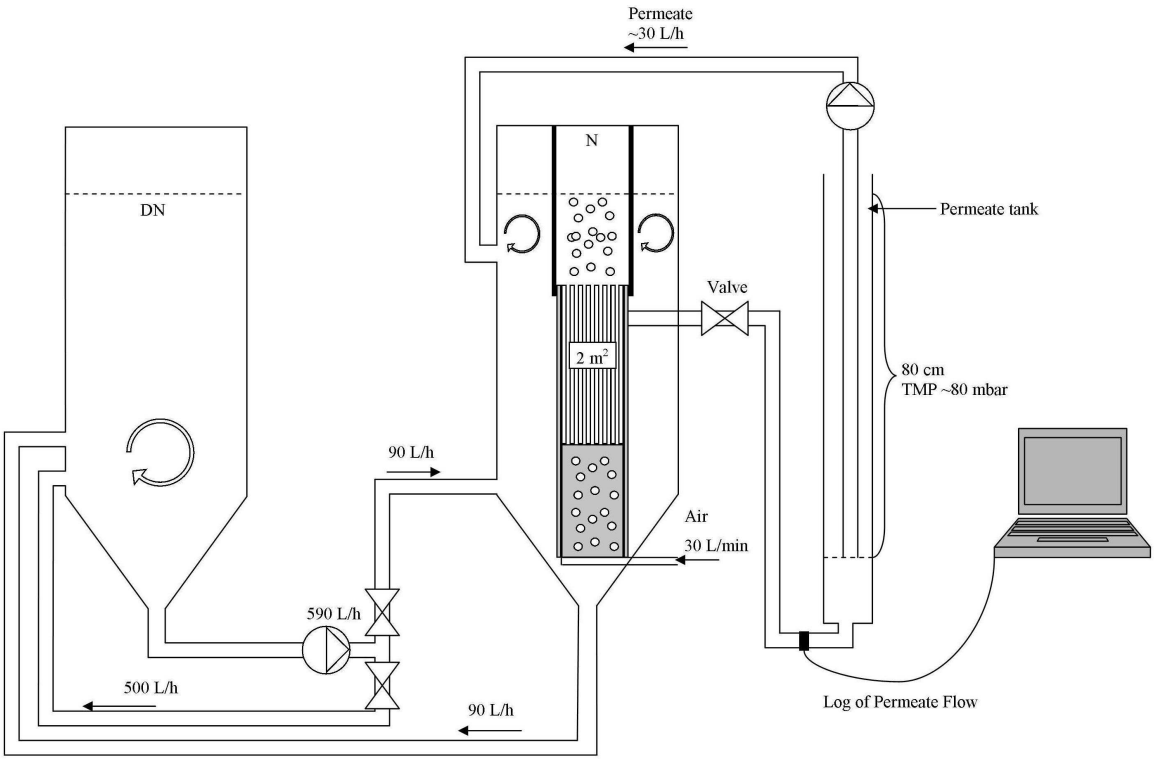

Fig. 9: Schematic presentation of the labscale MBR system.

The setup included an aerated tank (nitrification) in which the membrane unit (flat sheet membranes supplied by Alfa Laval A/S, see Appendix for dimensions) was submerged and an anaerobic tank (denitrification) of same volume. The TMP was controlled by the difference in water levels between the aerated tank and the permeate tank, but due to high resistance of the flowmeter, a higher TMP was applied to obtain a flux similar to that of the aim for the MBR pilot.

Relaxation was applied by using a programmed valve (Bürkert) placed in the flow line before the permeate tank. The permeate flow was continuously logged using a flowmeter and a PMD (1208 LS) connected to a computer with a datalog software programmed in Delphi (ver. 6.0, Borland Software Corporation). The output of the flowmeter was converted to a flow in the software using a calibration curve.

Aeration was applied from a “cage” under the membrane unit, ensuring that the aeration created a crossflow at the membrane surfaces. To avoid settling in the anaerobic tank, the sludge of this tank was recirculated by a pump which also ensured recirculation between the aerated and anaerobic tank.

All physical operation parameters of the labscale are listed in Table 5.

Table 5: The physical parameters for the labscale MBR system.

V aerobic [m^3]	0.35
V anaerobic [m^3]	0.35
TMP [mbar]	~ 80
Membrane material	PVDF
Membrane area [m^2]	2
Average pore size [µm]	0.2
Recirculation between tanks	300 %
Recirkulation in anaerobic tank [L/h]	~ 500
Relaxation	2 min every 10 min
Aeration [L/(min·m^2)]	15

The sludge was gathered at Aalborg East municipal WWTP, and had an initial MLSS of approximately 9 g/L. The feed was dog food (Trim Fullkost, Carbohydrates 58%, Proteins 18%, Fat 6%) which was grinded and mixed with water and added in amounts yielding an F/M ratio corresponding to the desired F/M ratio of the pilot MBR. The biological operation parameters are presented in Table 6.

Table 6 shows the biological parameters for the labscale MBR system.

Feed COD [g COD/g dog food]	1
F/M [kg COD/kg MLSS·day]	0.06
Amount of dog food per day [g/day]	420
MLSS (g/L)	~9
HRT [h]	28

Sludge samples of approximately 1.4 L were taken from the aerobic tank and handled as described in section 8.

Samples were taken at the same time on following days: 0, 1, 3, 6, 9, 12 and 14. The sample from day 0 was taken out before the MBR system was started and hence not affected by the shear of the MBR system.

The system was fed with 24 h intervals throughout the period and always after sampling.

To control the permeate quality, permeate samples of 0.2 L were taken out with same frequency as sludge samples and the turbidity of permeate samples were measured at 650 nm (Thermo Spectronic, Helios Epsilon). Furthermore, the dry matter contents and MLSS were measured.

8 Experimental Methods

For all sludge samples from the initial experiments, pilot MBR, CAS and labscale MBR, measurements of conductivity, pH, dry matter contents and MLSS were carried out immediately.
Floc size distribution and extraction of EPS were also carried out immediately in the initial experiments and for the labscale MBR, while they were carried out after the 24 h of transport for pilot MBR and CAS samples. Floc strength measurements were carried out immediately but only for pilot MBR, CAS and labscale MBR samples.

8.1 Determination of Conductivity and pH

Conductivity of the samples was measured using a conductivity meter (Radiometer Analytical, CDM 210). pH was measured with a pH meter (Radiometer Analytical, PHN 220) equipped with a pH electrode (Schott Instruments, Blueline 11 PH).

8.2 Determination of Dry Matter Contents and Mixed Liquor Suspended Solids

For determination of dry matter contents, 10 mL of sample was dried in a pre-weighed alumina tray in a furnace at 105 °C for 24 h and then weighed again in accordance with Dansk Standard 204 [1980]. For determination of MLSS, 10 mL of sample was filtered on a pre-weighed glass fibre filter (Advantec, GA55, 1.6 µm). Filter and filtercake was dried in a pre-weighed alumina tray in a furnace at 105 °C for 24 h and then weighed again in accordance with Dansk Standard 207 [1985].

8.3 Measurements of Floc Size Distribution

The sludge samples were analyzed using a Microtrac (Microtrac II, Model 7997-10, Leeds & Northrup). The samples were diluted until the laser attenuation was within the range from 0.8 to 0.85. Two measurements of 20 seconds were conducted for each sludge sample.

8.4 Measurements of Floc Strength

The floc strength measurements were carried out by the method introduced by Mikkelsen and Keiding [1999]. The sludge sample (700 mL) was stirred for 5 hours with a single bladed paddle at 900 rpm ($G = 800\ s^{-1}$) (Heidolph, RZR 2041) in a baffled reactor (cylindrical, d: 105 mm, h: 120

mm). The reactor was kept in an iced water bath ensuring a constant temperature of 4 °C. The amounts of dispersed mass were followed throughout the 5 hours by measuring the turbidity of sludge supernatant. 6 mL of withdrawn sludge sample was centrifuged (Sigma Laboratory Centrifuges, Model 3-15) for 2 minutes at 3000 rpm. The turbidity of the supernatant was determined by measuring absorbance at 650 nm (Thermo Spectronic, Helios Epsilon).
The floc strength measurements of the pilot MBR and CAS samples were carried out by DHI. The same method was used but the supernatant turbidity was measured in NTU units and the samples were only stirred for 2 hours.

8.5 Extraction of Extracellular Polymeric Substances

The extraction of EPS was carried out using the method described by Frølund *et al.* [1996]. 250 mL sludge was centrifuged (Sigma Laboratory Centrifuges, 6-16K) at 2000 G for 15 minutes at 4 °C. A sample of the supernatant was stored (4 °C, 0.005 % NaN_3) for further analysis, and this sample will be referred 'dissolved EPS' in the remaining sections.
The pellet was resuspended in tap water to a volume of 250 mL and mixed with 75 g cation exchange resin (DOWEX, Marathon C) per total g dry matter contents. The mixture was stirred at 900 rpm (G = 800 s^{-1}) for 2 hours in a baffled reactor (cylindrical, d: 105 mm, h: 120 mm) at 4 °C followed by centrifugation at 12000 G for 1 minute. The supernatant was further centrifuged at 12000 G in two steps of 15 minutes and finally stored (4 °C, 0.005 % NaN_3). This sample will be referred to as 'extracted EPS' in the remaining sections.
The dry matter contents of both the dissolved and extracted EPS samples were determined by the same method as the sludge samples (see section 8.2).

8.6 Determination of Protein and Humus Contents in EPS samples

The determination of humus and protein contents in the samples of extracted and dissolved EPS was carried out using the modified Lowry method [Frølund *et al.* 1996; Lowry 1951]. 500 µL sample was mixed with 700 µL of an alkaline copper reagent and 10 minutes later 100 µL of Folin reagent was added. After 45 minutes, the absorbance of the mixture was measured (750 nm, Shimadzu UV-1601). The absorbance of a blind with the same amount of sample and reagents but without copper was also measured. The exact description of the used reagents and procedures can be found in Appendix B.

Bovine serum albumin (BSA) and humic acid (Fluka Chemika) were used to obtain standard curves for protein and humus, respectively.

8.7 Determination of Carbohydrate Contents in EPS samples

The determination of carbohydrate contents in the samples of extracted and dissolved EPS was carried out using the anthrone method [Gaudy 1962]. A mixture of 1 mL sample and 2 mL of a sulphuric acid solution with anthrone reagent as well as a blind with same volume of sample and sulphuric acid solution but without anthrone was boiled for 14 minutes in a water bath at 100 °C. Afterwards the absorbance of sample and blind was measured (625 nm, Shimadzu UV-1601). The exact description of the used reagents and procedures can be found in Appendix C. Glucose was used to obtain a standard curve.

8.8 Hydrophobic Interaction Chromatography of EPS samples

The hydrophobic interaction chromatography (HIC) was carried out using a HPLC setup (Dionex chromatograph) with a Phenomenex Jupiter C18 column. Detection was carried out with an UV detector (Dionex UVD170U) at 225 and 275 nm and an evaporative light scattering detector (ELSD, Varian 380-LC). The mobile phase was an aqueous solution (milliQ water, degassed) of 0.005 M NH_4HCO_3 (AppliChem, p.a.) at pH 8.3 and the applied flow rate was constant at 1.0 mL/min.

The samples were filtered using a 0.45 µm syringe filter (PVDF, Millipore) and the injected sample volume was 50 µL for samples of extracted EPS.

BSA (Applichem, Fraction V, 98%) was applied as protein standard and humic acid (Fluka Chemika) as standard for humus.

8.9 Size Exclusion Chromatography of EPS samples

SEC was carried out with the same setup, mobile phase etc. and with the same samples as HIC but with a Phenomenex PolySep SEC P4000 column.

9 Results

This section first presents measured operation parameters of the pilot and labscale MBR. Afterwards, the results of the sludge sample analysis from both the initial experiments, pilot MBR, CAS and labscale MBR are presented in sections handling each of the applied analysis methods.
To ensure clarity of the presented figures and the results in general, only the results for chosen samples are included, but the samples have been chosen so that they cover the whole sampling periods and so that no significant trends are hidden. Results which are not presented here and all raw data can be found on the enclosed CD-ROM.

9.1 Operation of Pilot and Labscale MBR

Control parameters such as pH, conductivity and MLSS are handled in Appendix D. The logged permeate flux data for the pilot and labscale MBR are presented in the next sections.

9.1.1 Permeate Flux of MBR Pilot at Lundtofte WWTP

The permeate flux in the analyzed period of the pilot MBR (until day 57) was about 7.5 L/(h·m^2), but the permeate flux data from this period was only available as a rough sketch (see Appendix E). To show a more detailed example of the permeate flux of the pilot MBR, Fig. 10 shows the permeate flux over a period of 8 hours obtained after the 57 day period.

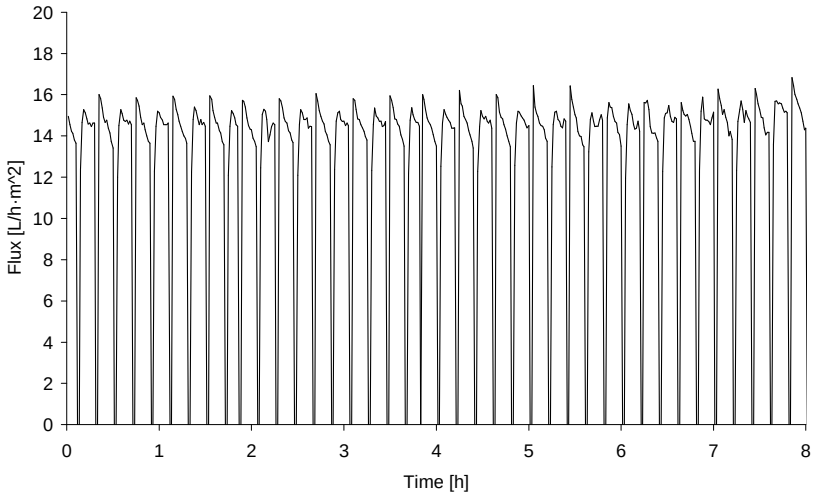

Fig. 10: Logged permeate flux (1 min^{-1}) of the pilot MBR at Lundtofte WWTP over an 8 h interval. Flux was regularly paused for 2 minutes in every 10 minutes. Note that the presented interval was measured after the initial 57 days otherwise included in this report.

In the presented interval, the permeate flux was at a steady state of approximately 15 L/(h·m^2). After the 2 minutes relaxation, the flux was higher than the steady state level, and then declined during the 10 minutes.

9.1.2 Permeate Flux of Labscale MBR

The logged permeate flux for the whole operation period of the labscale MBR is seen in Fig. 11.

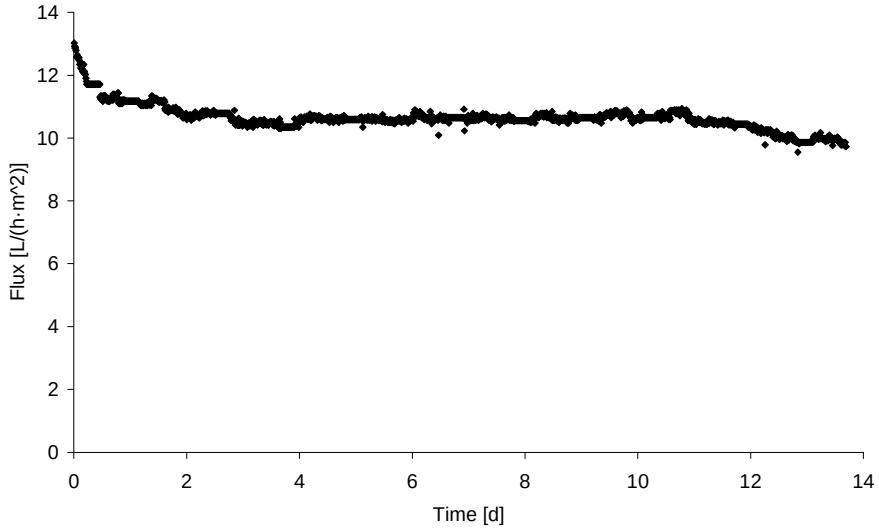

Fig. 11: Permeate flux for the 14 days of operation for the labscale MBR. The flux in the relaxation periods is not included in this figure.

It is seen that the permeate flux decreased initially, reached a steady state after 2 days and then started to decrease slightly again after 10 days and throughout the remaining period of operation.
In Fig. 11, the flux during relaxation is not included and as a result of the logging frequency (2 min^{-1}), the higher flux obtained initially after relaxation was not logged. Therefore, the flux was logged at a higher frequency for 1 hour within the steady state period (see Fig. 12).

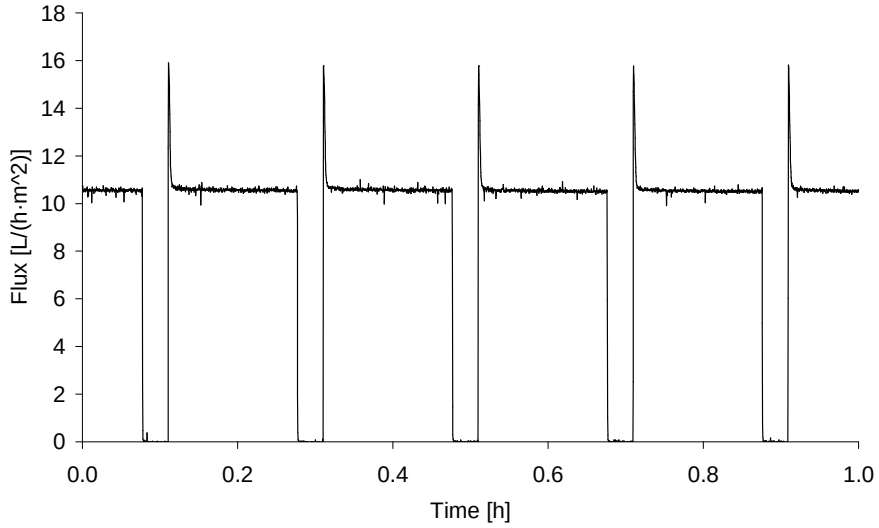

Fig. 12: Permeate flux with a higher log frequency (60 min^{-1}) in a 1 h interval after 7 days of operation for the labscale MBR.

Fig. 12 shows that the flux declined rapidly after each relaxation and reached a steady state level which was stable throughout this 1 hour period.

The resistances of the permeate system (flowmeter), the membrane and the fouling layer build up after 14 days of filtration for the labscale MBR are presented in fig. 13. The resistances were determined by flux vs. TMP measurements and use of Eq. 3, section 4.

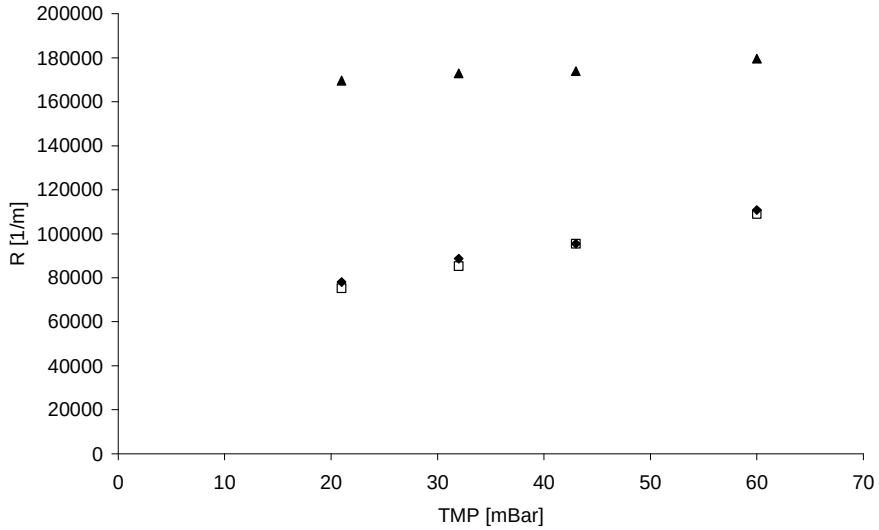

Fig. 13: Resistance (m^{-1}) vs. TMP (mbar). ◆ marks the determined system resistances, □ marks the accumulated system and membrane resistances and ▲ marks the accumulated system, membrane and fouling resistances after 14 days of filtration.

It is seen that the membrane resistance was negligible compared to the system resistance (see Fig. 13). After 14 days of filtration, the fouling resistance had increased the total resistance significantly. By including only fouling and membrane resistance in Eq. 3 (that is subtracting the system resistance from the total resistance), the actual TMP working over the membrane was estimated to be 25 mbar with the total TMP of 80 mbar applied during the 14 days of operation.

9.2 Initial Experiments

This section presents the measurements of supernatant absorbance during the 5 hours of shear exposure in the initial experiments. The results of the sludge analysis carried out after the 5 hours of shear exposure are presented along with the results from the pilot and labscale MBR.

9.2.1 Development of Dispersed Mass

The development of amounts of dispersed mass at the three different shear levels is indicated in Fig. 14, which shows the absorbance of sludge supernatant as function of time.

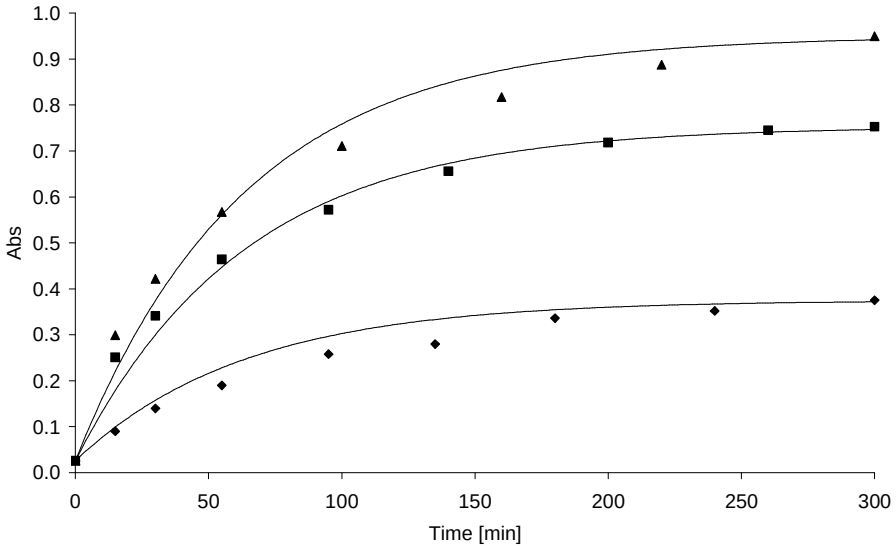

Fig. 14: The absorbance (at 650 nm) of sludge supernatant over time for the three applied shear levels: ◆ marks 500 s^{-1}, ■ marks 1100 s^{-1} and ▲ marks 1700 s^{-1}. The lines are guides for the eyes.

It is seen that the amounts of dispersed mass increased initially but reached a more stable level within the 5 hours of shear exposure. Besides, it is seen that a higher shear yielded larger amounts of dispersed mass.

9.3 Size Distribution of Sludge

9.3.1 Sludge Samples from Initial Experiments

The size distributions of CAS resulting from varied shear levels are shown in Fig. 15.

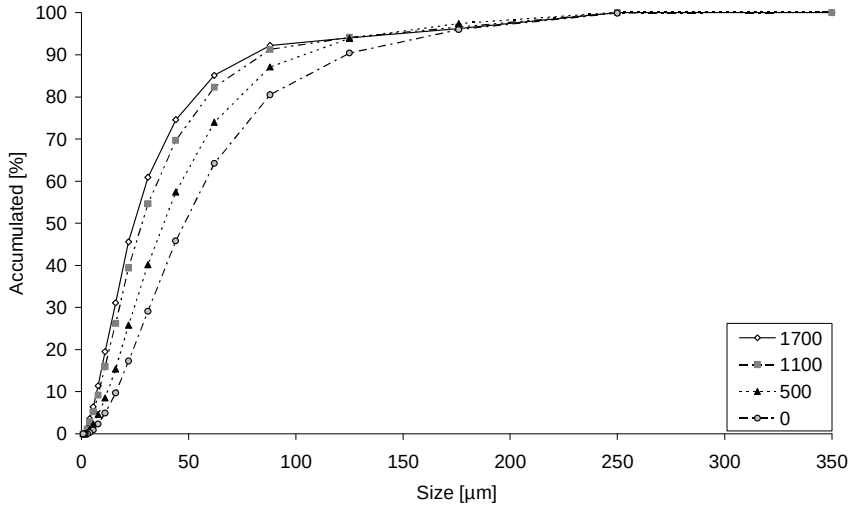

Fig. 15: The accumulated size distribution of sludge flocs after 5 h treatment at variable shear levels.

It is seen that a higher shear led to smaller sludge flocs in these short term experiments.

9.3.2 Sludge Samples from Pilot MBR and CAS at Lundtofte WWTP

The size distribution of sludge in the pilot MBR changed over time as shown in Fig. 16.

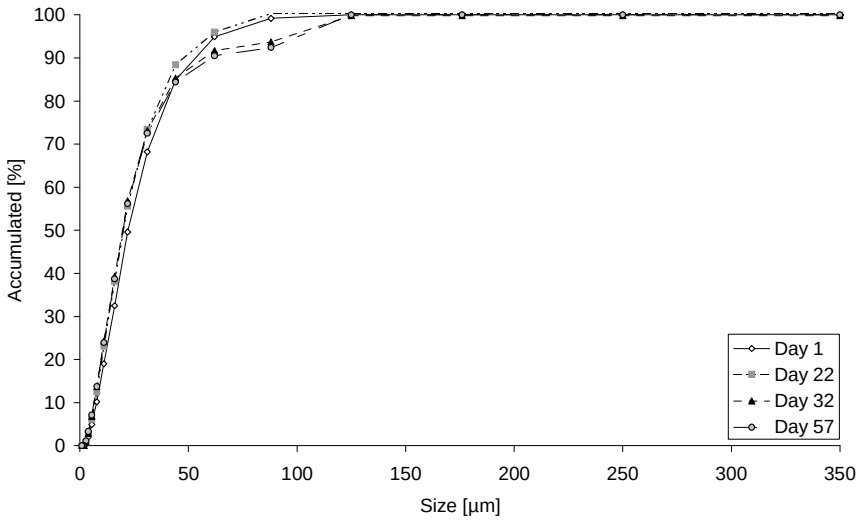

Fig. 16: Accumulated size distributions for samples from day 1 to day 57 of the pilot MBR.

The size distribution of the four samples shown in Fig. 16 changed towards a bimodal distribution over time with an increasing part of flocs within the range from 80 to 125 µm. The majority of flocs and particles were in the range from 4 to 65 µm.

The size distributions of the sludge from the pilot MBR and CAS are compared in Fig. 17.

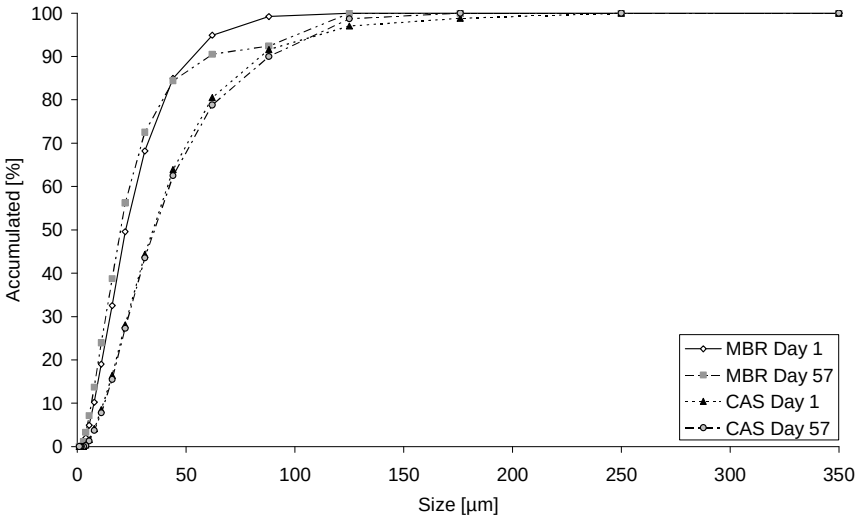

Fig. 17: Accumulated size distributions for samples from day 1 and 57 of the pilot MBR and CAS.

It is seen in Fig. 17 that the average floc size of the MBR sludge was lower than for CAS. The majority of flocs and particles in CAS were in the size range from 11 to 125 µm.

9.3.3 Sludge Samples from Labscale MBR

The floc size distributions for the labscale MBR sludge are shown in Fig. 18.

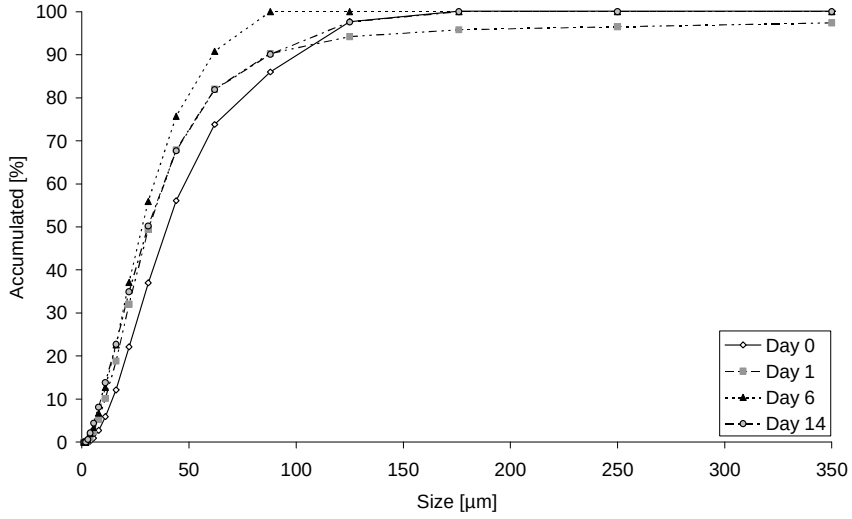

Fig. 18: Accumulated size distributions of samples from day 0 to day 14 of the labscale MBR. The sample from day 0 was taken out before the MBR system was started and hence not exposed to the shear levels of the MBR.

As seen in Fig. 18, the average floc size for samples from day 1 to 14 was significantly lower compared to the average size of the sludge sample from day 0 (i.e. CAS). For the samples from day 1 to 14, the majority of the sludge flocs were in the region from 4 to 125 µm, but the average size was increasing from day 6 to day 14.

9.4 Floc Strength of Sludge

9.4.1 Sludge Samples from Pilot MBR and CAS at Lundtofte WWTP

The results of floc strength measurements for samples from the pilot MBR and CAS are shown in Fig. 19.

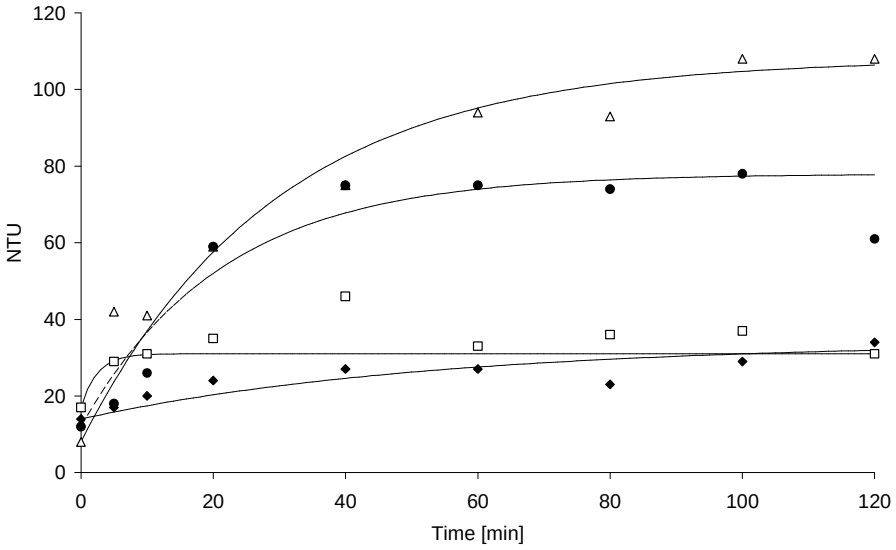

Fig. 19: The turbidity of sludge supernatant as a function time, indicating floc strength. Results for MBR sludge samples from day 4 (◆) and day 22 (□) and for CAS samples from day 4 (Δ) and day 22 (●) are presented. The lines are guides for the eyes. Note that the turbidity of the supernatant was measured in NTU units for the samples from the pilot MBR and CAS.

Fig. 19 shows that the measured turbidity (i.e. dispersed mass) for the samples from the pilot MBR were generally lower than for CAS. This indicates that the sludge flocs from the MBR were stronger than CAS flocs.

9.4.2 Sludge Samples from Labscale MBR

The development of floc strength of the sludge in the labscale MBR is seen in figure 20.

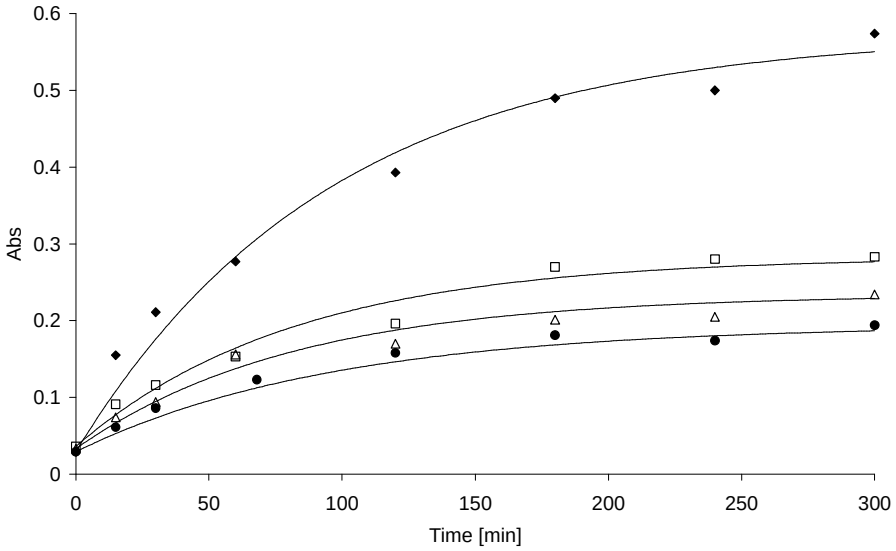

Fig. 20: The turbidity of sludge supernatant as a function of time, indicating floc strength of samples from the labscale MBR (◆ day 0, □ day 1, Δ day 6 and ● day 14). The lines are guides for the eyes.

It is seen that the sample from day 0 had a lower floc strength than the sludge from the following days (see Fig. 20). Besides, the floc strength is slightly increasing from day 1 to 14.

9.5 Determination of Carbohydrates, Proteins and Humus Amounts in EPS Samples

This section presents the determined amounts of carbohydrates, proteins and humus in samples of extracted and dissolved EPS (see Section 8.5).

9.5.1 Initial Experiments

The amounts of carbohydrates, proteins and humus in extracted EPS samples from sludge exposed to variable shear forces are presented in Fig. 21.

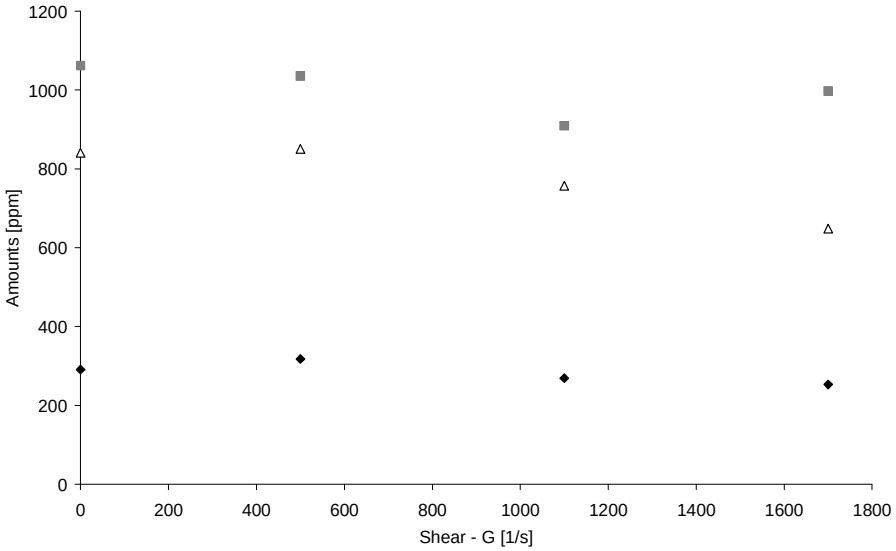

Fig. 21: Amounts of carbohydrates (◆), proteins (■) and humus (Δ) in extracted EPS from initial experiments with shear varying from 0 to 1700 s^{-1}.

It is seen that the extracted EPS contained higher amounts of proteins and humus compared to carbohydrates. No development of the amounts resulting from varied shear forces is seen.

Fig. 22 presents the determined amounts of carbohydrates, proteins and humus in dissolved EPS samples from the initial experiments.

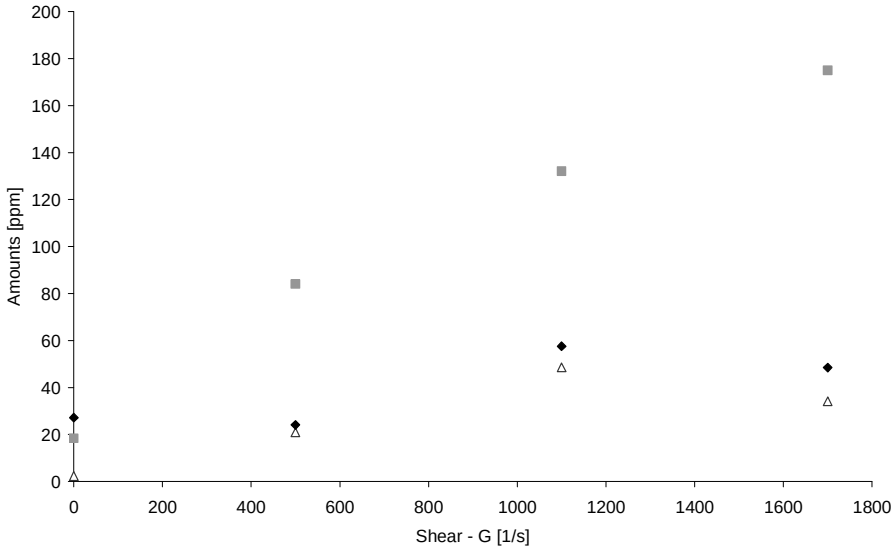

Fig. 22: Amounts of carbohydrates (◆), proteins (■) and humus (Δ) in dissolved EPS from initial experiments with shear varying from 0 to 1700 s^{-1}. Note the scale of the y-axis.

The amount of proteins increased with increasing shear whereas carbohydrates and humus amounts did not change significantly. As expected, the amounts were generally much lower than in the extracted EPS samples.

9.5.2 Samples from Pilot MBR and CAS at Lundtofte WWTP

To determine the deviation of the methods and storage, two of the extracted and two of the dissolved samples were measured three times with new standards every time (see Appendix F). The two samples were from day 4 and day 22 in the pilot MBR. These results led to the relative standard deviations seen in Table 7.

Table 7: The relative standard deviations in percent for the methods for determination of carbohydrate, protein and humus amounts. All calculated from 12 measurements.

Method	Standard deviation [%]
Carbohydrates	18
Protein	29
Humus	38

Other double determinations were made, but these are handled in Appendix F.

The development of carbohydrate, protein and humus amounts in extracted EPS samples from the pilot MBR is presented in Fig. 23.

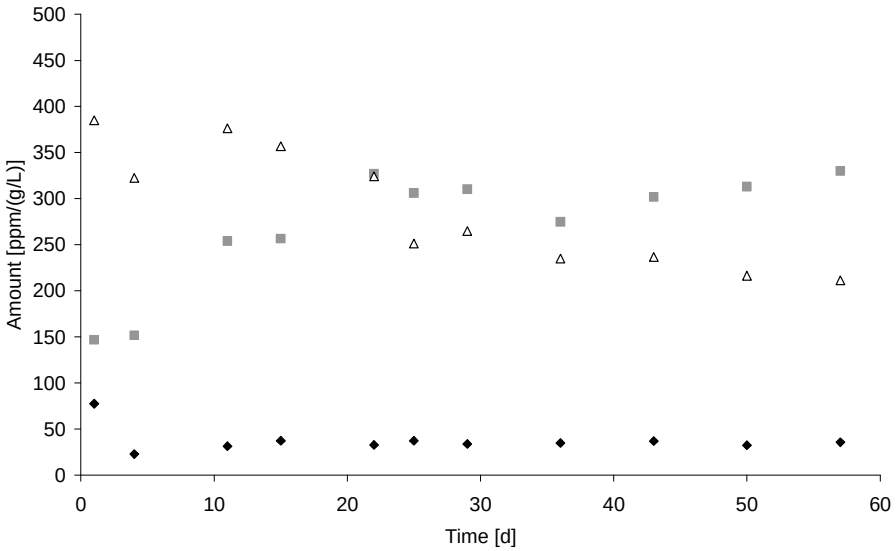

Fig. 23: The amounts (ppm) of carbohydrates (◆), proteins (■) and humus (Δ) in extracted EPS samples from day 1 to day 57 for the pilot MBR at Lundtofte WWTP. The presented amounts are relative to the dry matter contents (g/L) of the extracted EPS sample.

The amount of carbohydrates was significantly higher in the sample from day 1 compared to the following samples (see Fig. 23). The amount of proteins was generally increasing whereas the humus amounts slightly decrease in the amount of humus, but the changes for humus were lower than the standard deviation.

The corresponding measurements for extracted EPS samples from CAS at Lundtofte WWTP are seen in Fig. 24.

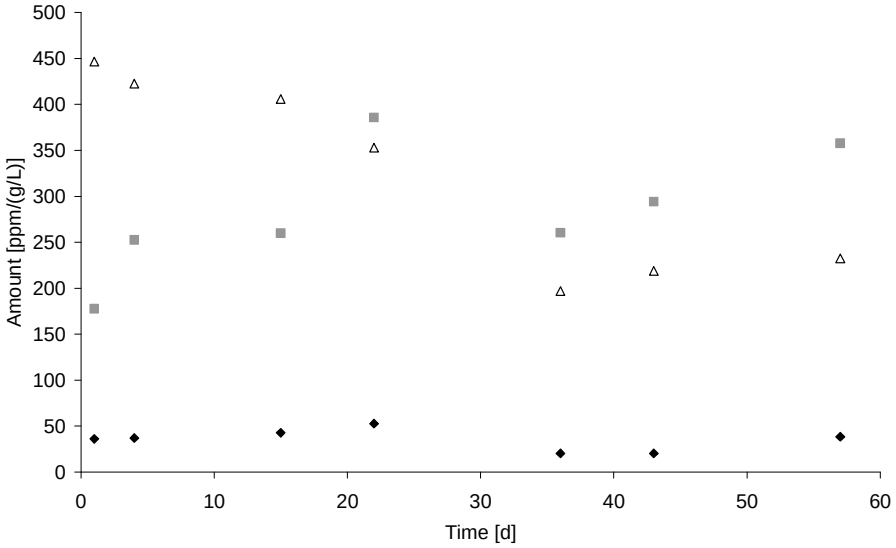

Fig. 24: The amounts of carbohydrates (◆), proteins (■) and humus (Δ) for extracted EPS from CAS at Lundtofte WWTP. The presented amounts are relative to the dry matter contents (g/L) of the extracted EPS sample.

It is seen that the amount of carbohydrates did not change significantly over time for the extracted EPS samples from CAS. The amounts of proteins had an increasing tendency whereas amounts of humus had a decreasing, but again this was not larger than the standard deviation.

The amounts of carbohydrates, proteins and humus in dissolved EPS samples from the pilot MBR are presented in Fig. 25.

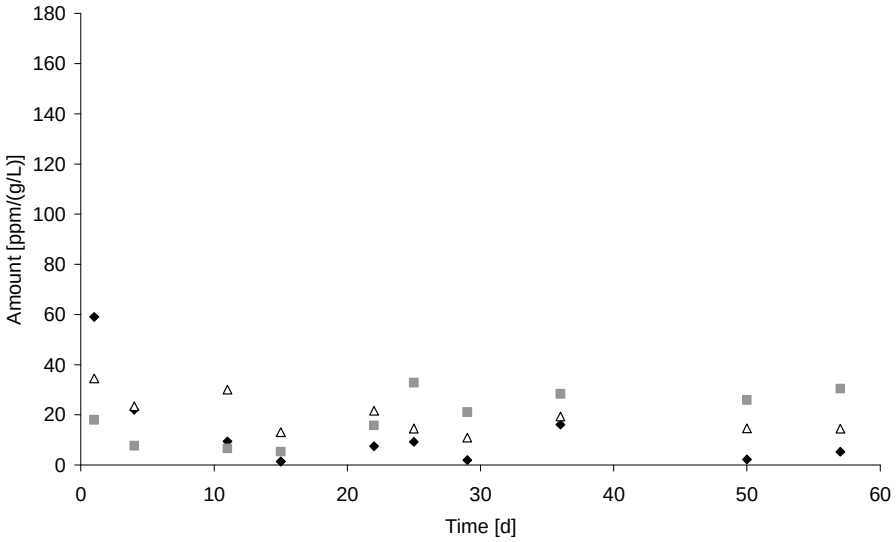

Fig. 25: The amount of carbohydrates (◆), proteins (■) and humus (Δ) in dissolved EPS samples from day 1 to 57 for the pilot MBR at Lundtofte WWTP. The presented amounts are relative to the dry matter contents (g/L) of the dissolved EPS sample.

It is seen that the amount of proteins and humus in the dissolved EPS samples did not change significantly. However, the amount of carbohydrates in the sample from day 1 had a significantly higher level compared to the following samples as for the extracted sample from same day.

The amounts of carbohydrates, proteins and humus in dissolved EPS samples of CAS from Lundtofte WWTP are shown in Fig. 26.

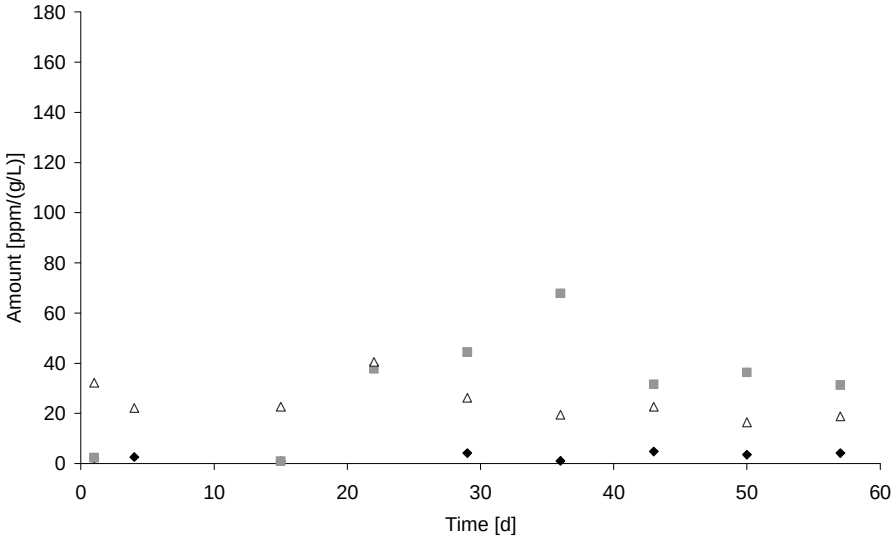

Fig. 26: The amounts of carbohydrates (◆), proteins (■) and humus (Δ) in dissolved EPS samples from day 1 to 57 for CAS at Lundtofte WWTP. The presented amounts are relative to the dry matter contents (g/L) of the dissolved EPS sample.

No significant changes of the contents in the dissolved EPS samples from CAS were observed. The amounts are similar to the levels seen for the MBR sludge (see Fig. 25).

9.5.3 Samples from Labscale MBR

The amounts of carbohydrates, proteins and humus in extracted EPS samples from the labscale MBR are presented in Fig. 27.

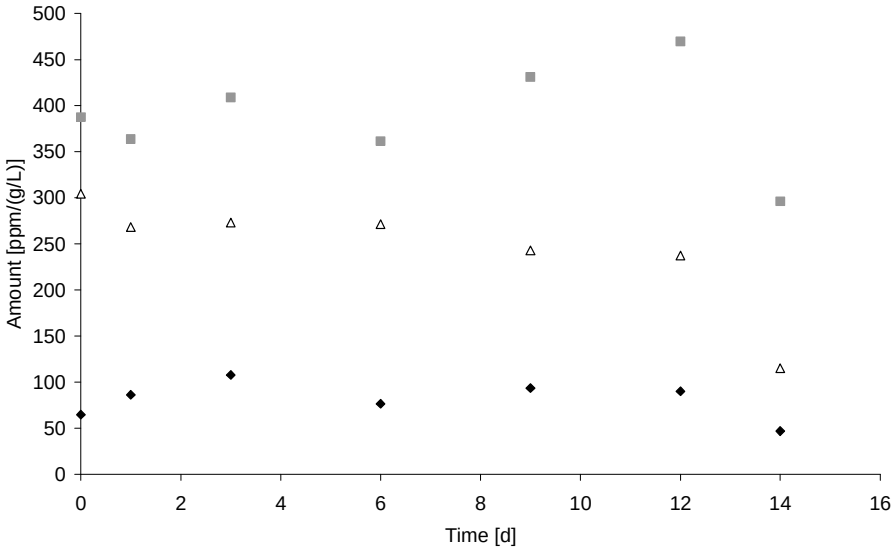

Fig. 27: The amount of carbohydrates (◆), proteins (■) and humus (Δ) in extracted EPS samples from the labscale MBR. The presented amounts are relative to the dry matter contents (g/L) of the extracted EPS sample.

No changes in amounts of carbohydrates, protein or humus are seen. The levels are similar to the levels determined for the pilot MBR and CAS (see Fig. 23 and 24).

Fig. 28 presents the determined amounts of carbohydrates, proteins and humus for the dissolved EPS samples from the labscale MBR.

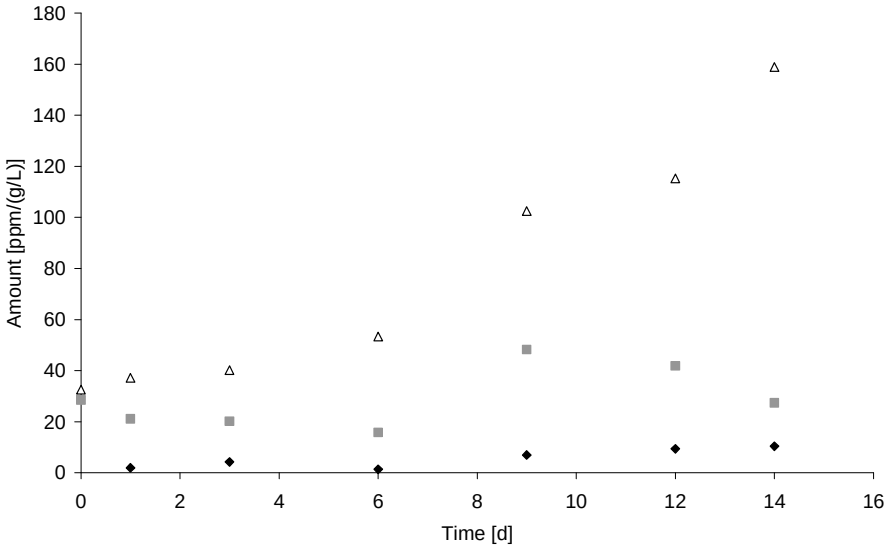

Fig. 28: The amounts of carbohydrates (◆), proteins (■) and humus (Δ) in the dissolved EPS samples from the labscale MBR. The presented amounts are relative to the dry matter contents (g/L) of the dissolved EPS sample.

It is seen that only the amounts of humus in the dissolved EPS changed significantly, with increased amounts throughout the operation time.

9.6 Hydrophobic Interaction Chromatography of Extracted EPS

This section presents results of HIC of extracted EPS samples. Only chromatograms obtained with the UV detector (at 225 nm) are included in this report. Other wavelengths (250 and 275 nm) and an ELSD detector were used in some of the measurements, but to limit the amount of data handling, these chromatograms have not been included. Hence, polysaccharides were not detected.

For samples of dissolved EPS and also of BSA and humic acid, no peaks other than the dead volume were detected. These results are not presented, but can be found on the CD-ROM.

9.6.1 Samples from Initial Experiments

The results of the hydrophobic interaction chromatography for extracted EPS are shown in Fig. 29.

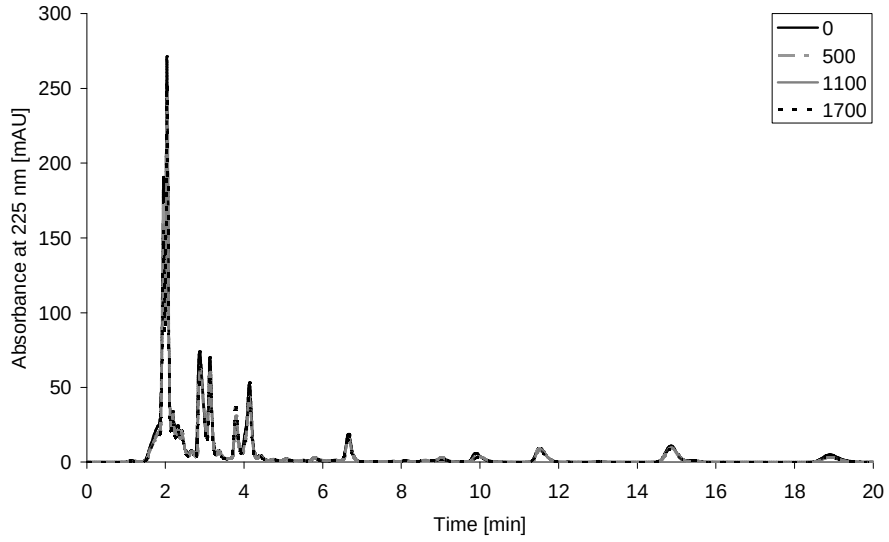

Fig. 29: Hydrophobic interaction chromatograms for the four samples of extracted EPS after exposed to different levels turbulent shear.

The first peak after approximately two minutes is the dead volume (see Fig. 29), that is not retained by the column. After the dead volume, the peaks are clearly separated. However, there is no sign of developments due to shear forces.

9.6.2 Samples from Pilot MBR and CAS at Lundtofte WWTP

In general, only the retention time from 1 to 5 minutes is included in the chromatograms presented in this section. As in Fig. 29, small peaks occurred in the retention time interval from 5 to 20 minutes. No significant changes were though observed from sample to sample and therefore this interval was skipped to clarify the interval from 1 to 5 minutes. Chromatograms for the full retention time can be found on the CD-ROM.

Fig. 30 and 31 present the chromatograms for four samples of extracted EPS from the MBR Pilot and CAS, respectively.

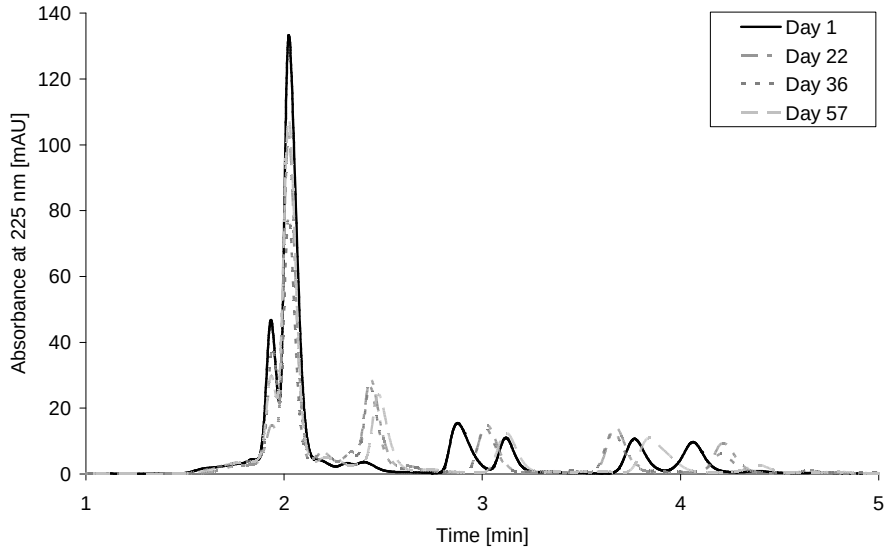

Fig. 30: Chromatogram of extracted EPS samples of MBR Pilot sludge from day 1, 22, 36 and 50. Absorbance has been normalized by dry matter contents of sample.

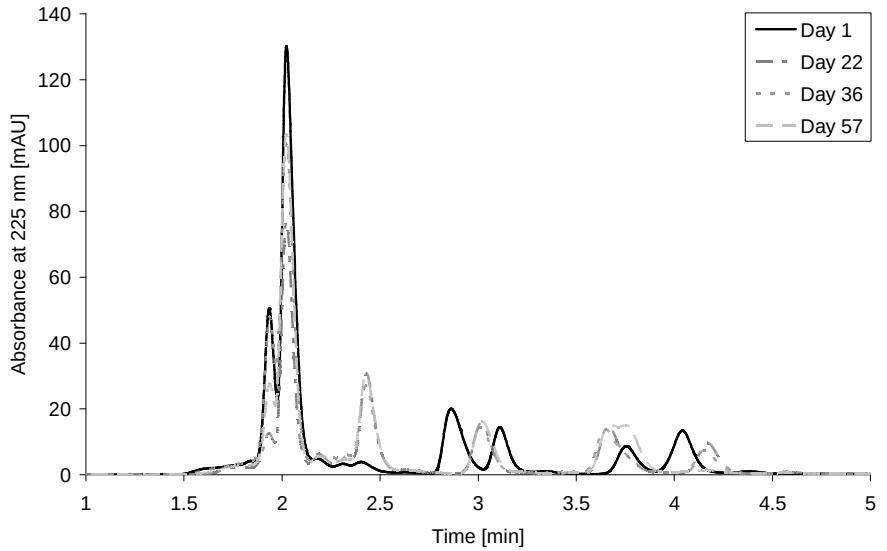

Fig. 31: Extracted EPS sample from CAS sludge from day 1, 22, 36 and 50. Absorbance has been normalized by dry matter contents of the sample.

In general, four distinct peaks are observed after the dead volume peak at 2 minutes. However, the retention time of the four peaks was different for day 1 compared to the other three samples which had comparable peak patterns. The sample from day 1 was not measured in the same measurement series as the other three samples.

Fig. 32 presents two chromatograms of the same sample of extracted EPS (Pilot MBR sludge Day 4) measured after different storage time (15 and 65 days).

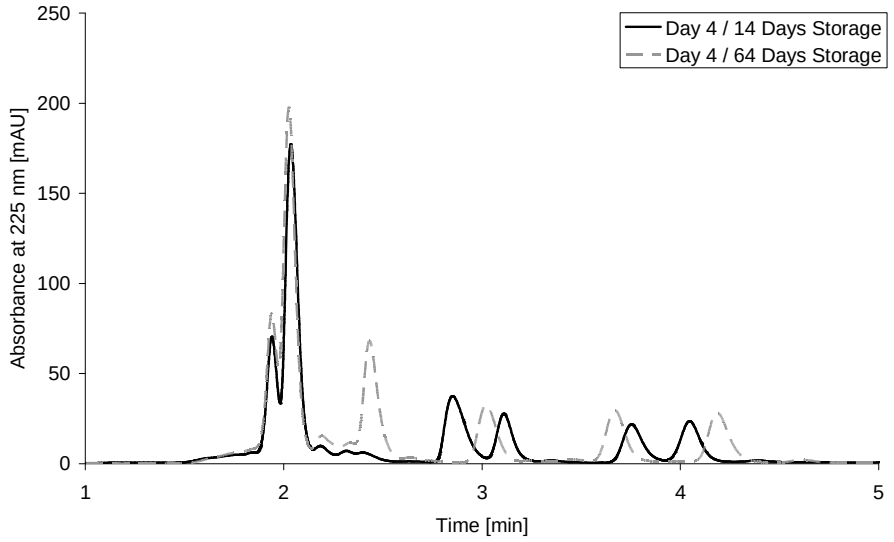

Fig. 32: Comparison of chromatograms of same sample (CAS Day 4) conducted after different storage time (14 and 64 days).

As in Fig. 31 and 32, four peaks occurred after the dead volume peak but with varying retention time. The changes in retention time were similar to the changes between different samples observed in Fig. 31 and 32.

9.6.3 Samples from Labscale MBR

Fig. 33 shows the chromatograms for four samples of extracted EPS from the labscale MBR.

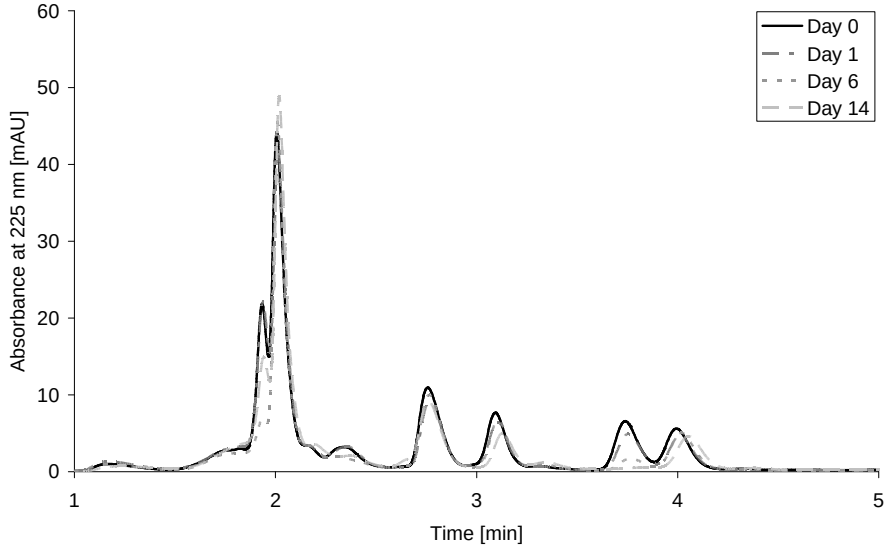

Fig. 33: EPS sample from Labscale MBR sludge from day 0, 1, 6 and 14. Absorbance has been normalized by dry matter contents of sample.

All the chromatograms had the same pattern, but the amounts of the polymers that resulted in the peak at ~3.7 minutes gradually decreased from day 1 to day 14, where it is no longer detectable.

9.7 Size Exclusion Chromatography of Extracted EPS

This section presents size exclusion chromatograms (UV detector at 225nm) of chosen extracted EPS samples. In general, only the retention time from 4 to 16 minutes is included since peaks only occurred in this interval. Samples of dissolved EPS were also separated using SEC yielding chromatograms with a more narrow range of separation but no significant changes of the chromatograms were observed over the analysis period and therefore these data are not presented here. The chromatograms for the full retention time as well as for dissolved EPS can be found on the CD-ROM.

9.7.1 Samples from Initial Experiments

The size exclusion chromatography for the extracted EPS exposed to different levels of turbulent shear is seen in Fig. 34.

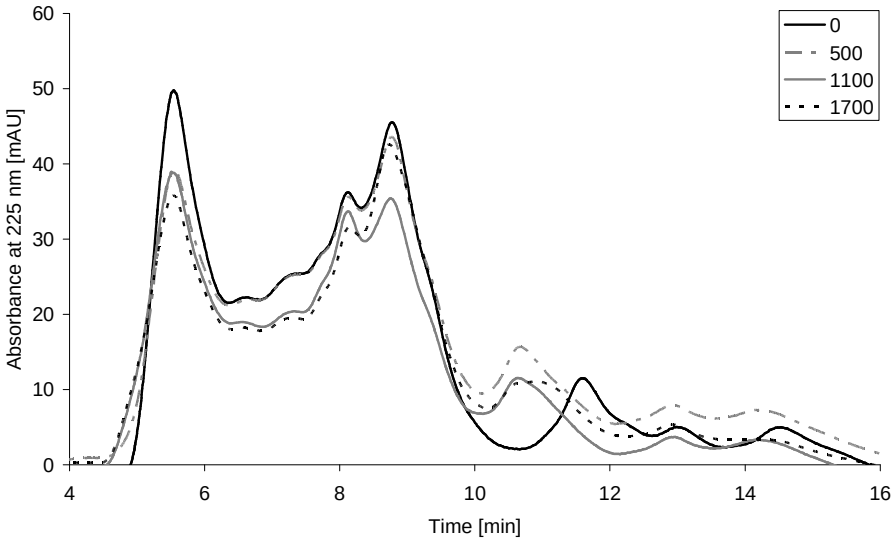

Fig. 34: Size Exclusion Chromatogram for the four samples of extracted EPS exposed to different levels of shear.

The dead volume was detected at approximately 9 minutes. There were small deviations within the chromatograms but the patterns of the chromatograms were similar.
BSA and humic acid separation yielded the chromatograms seen in Fig. 35.

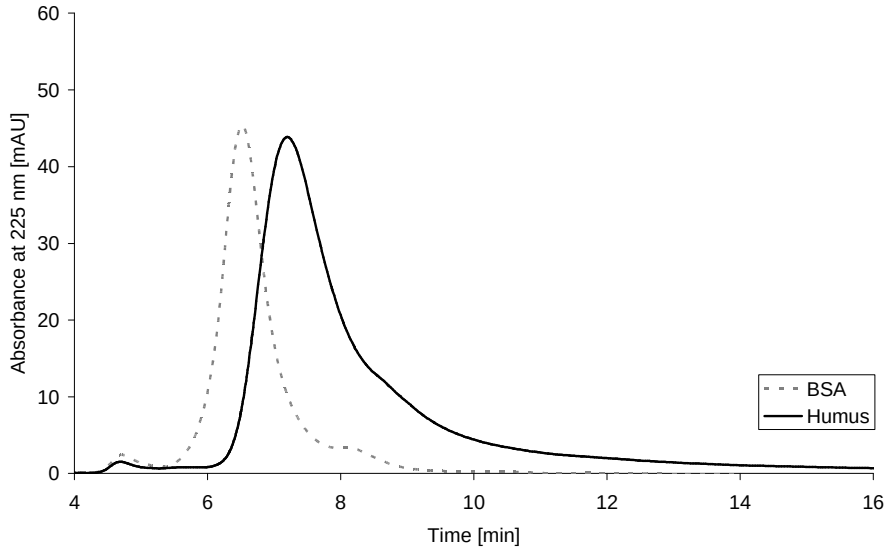

Fig. 35: Size Exclusion Chromatograms of BSA and Humic Acid.

It is seen that BSA had the lowest retention time and that both had lower retention times than the dead volume.

9.7.2 Samples from Pilot MBR and CAS at Lundtofte WWTP

Fig. 36 shows chromatograms of samples from the MBR pilot.

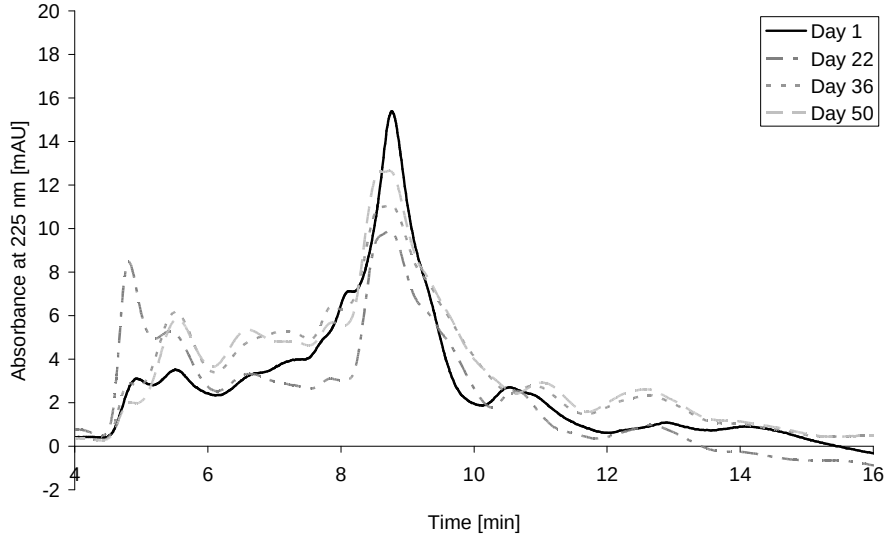

Fig. 36: Chromatograms of extracted EPS samples of pilot MBR sludge from day 1, 22, 36 and 50. Only the retention interval from 4 to 16 minutes is included. Absorbance has been normalized by dry matter contents of sample.

The peak of the dead volume was more significant for the sample from day 1, which was conducted in another measurement series than the other samples. Variations of the peak patterns occurred especially in the retention time interval from 4 to 6 minutes with more significant peaks of the samples from day 22, 36 and 50 compared to day 1. In the retention interval after the dead volume, the absorbance baseline for day 50 shifted so that the measured values were below 0 as seen in Fig. 36. However, the absorbance pattern was similar to the other samples.
Chromatograms for extracted EPS samples from CAS are shown in Fig. 37.

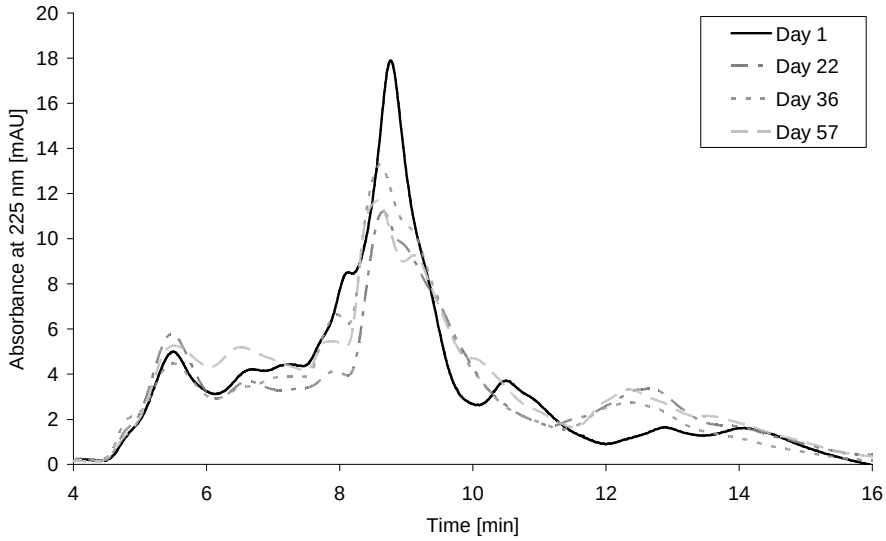

Fig. 37: Chromatogram of extracted EPS sample from CAS sludge from day 1, 22, 36 and 57. Only the retention interval from 4 to 16 minutes is included. Absorbance has been normalized by dry matter contents of sample.

The pattern of peaks were similar for all samples except for day 1 for which the dead volume and peaks after deviated slightly. This sample was measured in another series than the other samples.
Fig. 38 compares chromatograms of the same sample with varied storage time.

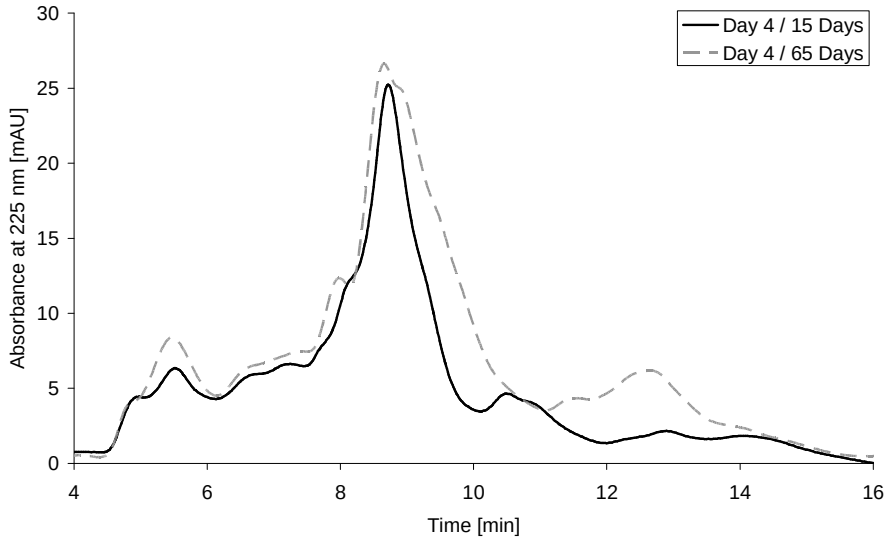

Fig. 38: Comparison of chromatograms of same sample (pilot MBR day 4) conducted after different storage time (15 and 65 days). Only the retention interval from 4 to 16 minutes is included.

The patterns of the chromatograms are similar before the dead volume whereas they deviate after the dead volume. This deviation is comparable to the deviations seen after the dead volume in Fig. 37 and 38.

9.7.3 Samples from Labscale MBR

Chromatograms of extracted EPS samples from the labscale MBR are shown in Fig. 39.

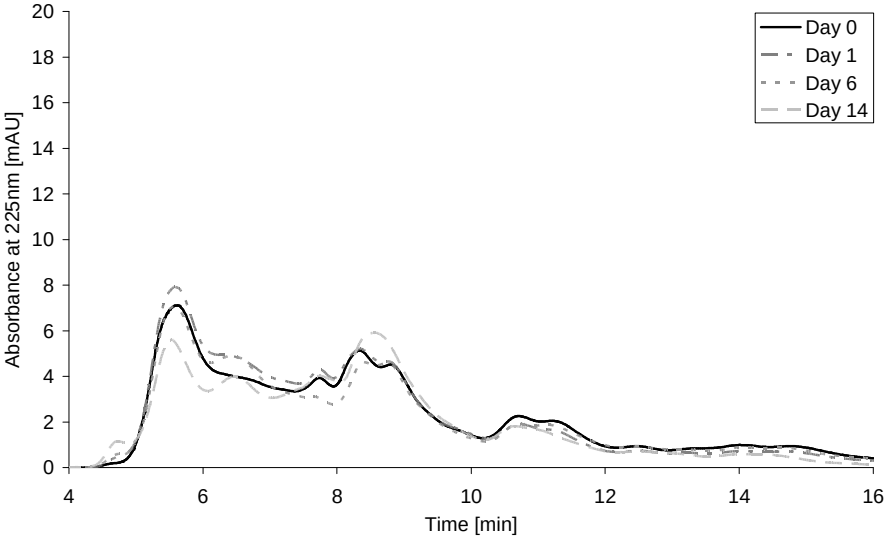

Fig. 39: Chromatograms of extracted EPS samples from labscale MBR from day 0, 1, 6 and 14. Absorbance has been normalized by dry matter contents of sample. Only the retention interval from 4 to 16 minutes is included.

The chromatograms were similar in most regions, but deviations in absorbance peak patterns were especially observed in the retention time interval from 6 to 8 minutes. Specifically, at ~6.5 minutes a peak increased regularly from day 0, where it was not detectable, to day 14.
The chromatogram patterns were similar to the samples from the pilot MBR, except for the dead volume peak which was generally lower for the samples from the labscale MBR.

10 Discussion

10.1 Operation of the Pilot and Labscale MBR

The flux development for the whole operation period of the labscale MBR operated at constant TMP was in accordance with the stages described in the theory (See Section 4.3). In the initial phase, the flux declined significantly (see Fig. 11) resulting from formation of irremovable and irreversible fouling. The flux then reached a more stable level with a constant fouling resistance. This level is determined by the balance between the scrubbing forces removing fouling and the attraction forces of foulants either towards other foulants or the membrane surface (see section 4.2). The decrease in the end of the operation period e.g. results from changes in the fouling layer structure or accumulation of irreversible and irremovable fouling. For the pilot MBR, the flux was more varying due to setup adjustments and stops in the followed operation period.

In Fig. 12, it is seen that the flux rapidly declined to a stable level after relaxation for the labscale MBR whereas it was steadily decreasing throughout the 10 minutes for the pilot MBR (see Fig. 10). The latter can though partly result from the low logging rate (1 min^{-1}). The decrease results from the formation of removable fouling, which was removed by the scrubbing during relaxation. For the labscale MBR, it was formed very fast whereas the process is slower for the pilot MBR. However, since the aeration setup flow, the applied TMP and the dimensions of the membrane units were different in the two systems, the scrubbing effect was not necessarily comparable in the two systems.

Due to a high pressure loss over the flowmeter, the applied TMP in the labscale MBR was 80 mbar which was the maximal TMP possible to apply in the build system. The actual TMP working over the membrane was estimated to be 25 mbar assuming that the flux was below critical flux. This assumption is supported by the fact that the flux reached a steady state with the applied TMP of 80 mbar, and steady state only occurs below critical flux (see section 4.2). The pilot MBR was operated at approximately 7.5 $L/(h \cdot m^2)$ in the operation period included in this project, but the final aim of flux was 15 $L/(h \cdot m^2)$ with a TMP of ~30 mbar. Hence, the actual TMP of the labscale MBR was slightly lower but still comparable to aimed flux for the pilot MBR. In further work with the labscale MBR, the setup should though be adjusted so that the flowmeter is not a limiting factor.

10.2 Floc Size and Floc Strength

10.2.1 Sludge Samples from Initial Experiments

In the initial experiments, the absorbance of sludge supernatant was used as a measure of changes of the sludge flocs under the applied turbulent shear level. After 5 hours of applied shear, the supernatant absorbance only increased slightly. Hence, 5 hours of varying turbulent shear was applied to test changes of sludge characteristics resulting from varying shear.

The supernatant absorbance measurements also showed that the final amounts of dispersed material increased with the applied turbulent shear in these short term experiments (see Fig. 14). The average size of the sludge flocs decreased with increasing shear level (see fig. 15). This is in accordance with the theory of sludge floc size and strength presented in section 3.1. The average floc size depends on the equilibrium between flocculation and deflocculation, which is determined by the intermolecular forces within the sludge flocs assuring flocculation and the applied shear forces resulting in deflocculation. Hence, assuming that the intermolecular forces did not change in such a short term experiment, more deflocculation must occur with increasing shear forces resulting in more dispersed materials and a lower average size of sludge flocs. Hence, the expected changes were observed with these methods, and therefore they were also applied in MBR sludge analysis.

10.2.2 Slugde Samples from Pilot MBR, CAS and Labscale MBR

The mesurements of size distributions showed that the majority of the sludge flocs were in the size range from 5 to 125 µm (see Fig. 15, 16 and 17), which is considered reasonable compared to size distributions for sludge flocs presented in the literature, e.g. Le-Clech *et al.* [2006] and Mikkelsen and Keiding [1999]. Generally, the average size of sludge flocs from the pilot MBR was lower compared to CAS (see fig. 16) and the same was seen by comparison of sludge from day 0 and the following samples from the labscale MBR (see fig. 17). Doing the same comparison in terms of floc strength, shows a corresponding trend as MBR sludge had a higher floc strength. The changes occurred within 24 hours of operation of the MBRs and are similar to the changes seen during the short term shear exposure of the initial experiments. Therefore, it is assumed that this general difference results from higher shear in the MBR system.

The average floc size of sludge both from the pilot and labscale MBR increased slightly in the end of the analyzed periods. Over longer terms, operation parameters such as temperature did however also change slightly for the pilot MBR, and the MLSS was increasing throughout the period. For the

labscale MBR, the feed was changed since it was not possible to feed with real wastewater. Hence, the observed long-term changes could result from these changes. Another possibility is that the microorganisms over time gradually adapted to the changed environment in the MBR.

10.3 Determination of Carbohydrates, Protein and Humus Amounts in EPS Samples

10.3.1 Samples from Initial Experiments

The contents of proteins were highest and carbohydrates lowest for extracted EPS samples (see Fig. 21, results), and this corresponds to EPS compositions presented in the literature, e.g. [Wilen *et al.* 2008]. No significant composition changes were observed with increasing shear for the extracted EPS, but in the dissolved EPS samples, the contents of proteins increased significantly with increasing shear (see Fig. 22). This corresponds with the increased turbidity of the sludge supernatant measured after the 5 hours of shear exposure (see Fig. 14). One might expect that the increasing amounts of proteins dispersed from the sludge flocs also imply corresponding lower amounts of same in the extracted EPS samples, but the increase is low compared to the amounts determined for the extracted EPS samples.

10.3.2 Samples from Pilot MBR, CAS and Labscale MBR

With regards to amounts of proteins and humus, extracted EPS samples from the pilot MBR and CAS sludge had similar developments during the operation time. Hence, the decrease in humus and increase in protein amounts (see Fig. 23 and 24) must result from changed in external changes in operation parameters such as temperature or feed composition. The amounts of proteins and humus in the dissolved EPS samples did not change significantly.

The amounts of carbohydrates were significantly higher both for the dissolved and extracted EPS sample from day 1 of the pilot MBR compared to the samples for the rest of the period and to the stable level seen for CAS samples (see fig. 24). These higher amounts might be an initial effect of the increased shear forces in the MBR system.

For the EPS samples from the labscale MBR, amounts similar to the pilot MBR and CAS were also found and the amounts were stable throughout the operation period, except for the humus amounts in the dissolved samples which was increasing throughout the operation period but since the changes were gradual and not immediate as the changes in floc size and strength, the changes might result e.g. from the changed feed.

The determined standard deviations included both variations resulting from the method and from storage of the samples (see section 8.5), since the EPS samples were analyzed in series and therefore were stored for varying time before analysis. The standard deviations were generally high, especially for the modified Lowry method in which humus determination accumulates the error for both humus and protein amounts and therefore yields a higher standard deviation. Besides, these methods measure the overall amounts of the three components but even though the total amounts e.g. of proteins do not change, changes of the types of proteins might change.

10.4 Hydrophobic Interaction Chromatography of Extracted EPS

10.4.1 Samples from Initial Experiments

In the presented chromatograms, many distinct peaks were detected after the dead volume peak (see Fig. 29). Hence, a variety of polymers which interact with hydrophobic column material were present in the extracted EPS samples, and the higher the retention time, the stronger is the interaction between the polymers and the column material. However, the chromatograms were completely similar for all samples so with the used method there were no detectable composition changes of the polymers depending on the applied shear levels.

BSA and humic acid were not separated by the column, so other standards should be applied if the results using this method should be fully verified. However, it was still considered interesting to se if any changes occur for MBR sludge compared to CAS.

10.4.2 Samples from Pilot MBR, CAS and Labscale MBR

The hydrophobic interaction chromatograms did not show any detectable changes of EPS composition for the pilot MBR and CAS sludge. The only changes observed resulted from the fact that not all samples were measured in the same measurement series (see Fig. 32). For the labscale MBR, one of observed peaks changed significantly from the sample from day 0 to day 14. However, the changes occured gradually over the whole operation period and hence might result from the feed. It is though remarkable that the chromatograms for the labscale MBR were similar to MBR pilot even though the origin of the sludge was not the same and the operation parameters were not completely similar. This indicates that the presence of the polymers separated by this chromatography technique is less sensitive to changes in environment, both physical and biological.

In further studies it would be interesting to characterize the separated fractions and to apply the technique in analysis of different fouling layers to see if the same polymers are present there.

10.5 Size Exclusion Cromatography of Extracted EPS

10.5.1 Samples from Initial Experiments

The SEC of extracted EPS samples yielded fingerprints (see Fig. 34), which were comparable to the results presented in the literature, e.g. [Garnier *et al.* 2005; Lyko *et al.* 2007]. The fingerprints were similar for all samples of these initial experiments, except for slight changes after the dead volume. In this region, other mechanisms than size exclusion affect the retention time and the same similar peaks occurred but with different retention time for one of the samples. Hence, the extracted EPS composition did not change detectably within the applied variations of shear in these experiments. BSA and humic acid were separated within the range of separated polymers from the sludge samples. However, a wider range of standards should be applied in further studies.

10.5.2 Samples from Pilot MBR, CAS and Labscale MBR

In the retention time range before the dead volume, gradual changes over operation time were observed both for sludge from the MBR pilot and the labscale MBR but not to same extent for CAS sludge. This indicates that the composition of EPS in the sludge flocs actually change resulting from the changed operation parameters in the MBR compared to CAS.

For the MBR pilot, the changes were most significant from day 1 to day 36 whereas the composition was more stable in the remaining period (see Day 36 and Day 50). In the labscale MBR, the composition is slightly changing throughout the whole analysis period.

Thus, the changes occur over longer terms than the changes in floc size and strength and the chromatograms of pilot MBR and CAS are still comparable. Hence, these changes do not necessarily result directly from increased shear, but instead from slight changes of operation parameters as described in earlier sections. Still, the changes are interesting but the main object in this project was the comparison of the CAS and MBR sludge, further studies of the changing fractions were not carried out.

Changes of peaks were also observed in the retention range after the dead volume, but as for the samples from the initial experiments, the peaks had similar shape and therefore changes are expected to result from the fact the not all samples were measured in the series (see Fig. 38).

11 Conclusion

The objective of this project was to investigate changes in sludge floc characteristics resulting from higher shear forces in a MBR system compared to conventional activated sludge processes. Sludge flocs from both a pilot MBR and a labscale MBR with comparable setups were analyzed and compared with CAS sludge of same origin.

On the macroscopic level, the average floc size was lower and the floc strength higher for the sludge in the MBR system compared CAS. These changes occurred within 24 hours of operation for both MBRs.

On the microscopic level, long-term changes of EPS composition in terms of carbohydrate, protein and humus contents were observed for the MBR sludge, but these changes were not ascribed directly to changed shear forces. Hydrophobic interaction chromatography and size exclusion chromatography of EPS revealed slight changes of EPS composition, but again the changes were observed over longer terms and assumed not to result directly from the changed shear forces.

12 Further Work

This project has studied sludge characteristics in the bulk phase of the MBR systems. The next step in understanding the fouling problems in MBRs is to study the interactions between the bulk phase and the fouling layers at the membrane surface. This step includes a thorough study of composition of individual fouling layers/classes and their impact on the total fouling resistance. Here the methods used for determination of EPS composition within this project could be applied. The polymer fractions separated by HIC and SEC should though be further characterized. This could be done e.g. by applying IR spectroscopy, MS and/or NMR.

Furthermore, the labscale MBR should be placed at a municipal WWTP since the use of synthetic feed instead of wastewater will affect the MBR sludge in long-term operations.

13 References

ATKINSON, S., 2006. Research studies predict strong growth for MBR markets. *Membrane Technology 2006*, 8-10.

BRADSHAW, T. P., 2005. *Introduction to peptide and protein HPLC*. California: Phenomenex.

CHRISTENSEN, M. L., NIELSEN, T. B., ANDERSEN, M. B. O., KEIDING, K., 2009. Effect of water-swollen organic materials on crossflow filtration performance. *Journal of Membrane Science*, 333, 94-99.

ÇIÇEK, N., FRANCO, J. P., SUIDAN, M. T., URBAIN, V., MANEM, J. 1999. Characterization and comparison of a membrane bioreactor and a conventional activated-sludge system in the treatment wastewater containing high-molecular-weight compounds. *Water Environment Research*, 71, 64-70.

DANSK STANDARD 204, 1980. *Determination of total residue and total fixed residue in water, sludge and sediment.* Copenhagen: Dansk standardiseringsråd.

DANSK STANDARD 207, 1985. *Total non filterable residue and fixed matter in total non filterable residue.* Copenhagen: Dansk standardiseringsråd.

FRØLUND, B., PALMGREN, R., KEIDING, K., NIELSEN, P. H., 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research*, 30, 1749-1758.

GARNIER, C., GÖRNER, T., LARTIGES, B. S., ABDELOUHAB, S., DE DONATO, P., 2005. Characterization of activated sludge exopolymers from various origins: A combined size-exclusion chromatography and infrared microscopy study. *Water Research*, 39, 3044-3054.

GAUDY, A, F., 1962. Colorimetric determination of protein and carbohydrate. *Ind. Water Wastes*, 7, 17-22**.**

HOLLAND, M., 2004. *Citing References.* Poole: Bournemouth University.

ISRAELACHVILI, J., 1991. *Intermolecular & Surface Forces*. 2nd ed. London: Academic Press.

JARVIS, P., JEFFERSON, B., GREGORY, J., PARSONS, S.A., 2005. A review of floc strength and breakage. *Water Research*, 39, 3121-3137.

JUDD, S., 2006. *The MBR book: Principles and Applications of Membrane Bioreactors in Water and Wastewater Treatment.* Oxford: Elsevier.

LE-CLECH, P., CHEN, V., FANE, T. A. G., 2006. Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science*, 284, 17-53.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265-275.

LYKO, S., AL-HALBOUNI, D., WINTGENS, T., JANOT, A., HOLLENDER, J., DOTT, W., MELIN, T., 2007. Polymeric compounds in activated sludge supernatant – Characterisation and retention mechanisms at a full-scale municipal membrane bioreactor. *Water Research,* 41, 3894-3902.

MENG, F., CHAE, S., DREWS, A., KRAUME, M., SHIN, H., YANG, F., 2009. Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. *Water Research,* 43, 1489-1512.

MIKKELSEN, L. H., 1999. *A Physico-Chemical Approach to the Floc Strength Concept – with Dewatering Implications*. Thesis (PhD). Aalborg University.

MIKKELSEN, L. H., KEIDING, K., 1999. Equilibrium aspects of the effects of shear and solids content on aggregate deflocculation. *Advances in Colloid and Interface Science,* 80, 151-182.

MIKKELSEN, L, H., KEIDING, K., 2002. The shear sensitivity of activated sludge: An evaluation of the possibility for a standardised floc strength test. *Water Research,* 36, 2931-2940.

MULDER, M., 1996. *Basic Principles of Membrane Technology*. 2nd ed. Dordrecht: Kluwer Academic Publishers.

SHANNON, M. A., BOHN, P. W., ELIMELECH, M., GEORGIADIS, J. G., MARINAS, B. J., MAYES, A. M., 2008. Science and technology for water purification in the coming decades. *Nature*, 425, 301-310.

WILÉN, B. M., JIN, B., LANT, P., 2003. The influence of key chemical constituents in activated sludge on surface and flocculating properties. *Water Research,* 37 (9), 2127-2139.

WILÉN, B. M., LUMLEY, D., MATTSSON, A., TAKASHI, M., 2008. Relationship between floc composition and flocculation and settling properties studied at a full scale activated sludge plant. *Water Research,* 42 (16), 4404-4418.

14 Notation

BOD	Biological Oxygen Demand
BSA	Bovine Serum Albumin
CAS	Conventional Activated Sludge
CIL	Clean In Line
CIP	Clean In Place
COD	Chemical Oxygen Demand
E	Efficiency
ELSD	Evaporative Light Scattering Detector
EPS	Extracellular Polymeric Substances
F/M	Feed to Microorganism ratio
G	Turbulent shear level
HRT	Hydraulic Retention Time
J	Flux
k_e	Death rate constant
MBR	Membrane BioReactor
MLSS	Mixed Liquor Suspended Solids
OLR	Organic Load Rate
PE	PolyEthylene
PVDF	PolyVinyliDene Flouride
R_f	Fouling resistance
R_m	Membrane resistance
SRT	Solid Retention Time
TMP	TransMembrane Pressure
V	Volume
WWTP	WasteWater Treatment Plant
Y	Biomass yield
μ	Dynamic viscosity

Appendix A – Dimensions of Membrane Module

Fig. 40 shows dimensions of the flat sheet membrane module in the labscale MBR.

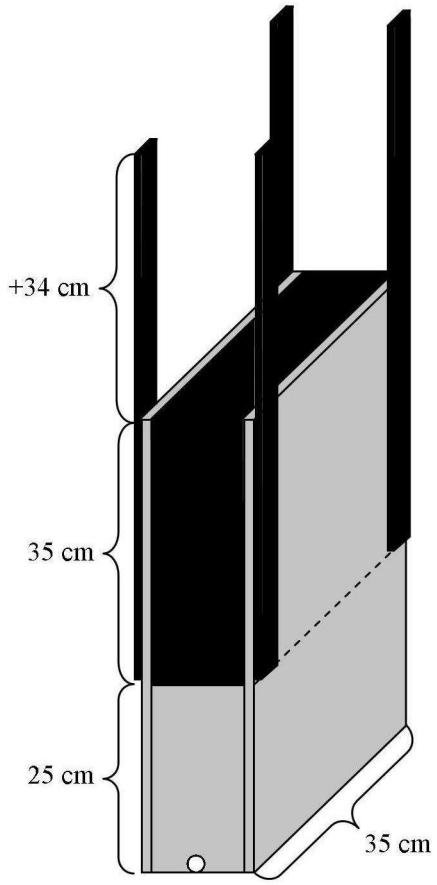

Fig. 40: The membrane module in the labscale MBR. The grey part of the figure is the plastic cage for the membrane and the black part in between the grey are the membrane. The four black plates are for attaching the membrane module to the reactor. The aeration was placed under the membrane in the plastic cage and this was symbolized by the white circle.

Appendix B – Lowry Method

This appendix describes the exact procedures of the modified Lowry method used for determination of protein and humus.

Three basic solutions of reagents called A, B and C was made to create the used reagents solutions D, E and F.

A: 5.71 g NaOH and 28.571 g Na_2CO_3 dissolved in 1000 mL demineralised water.

B: 0.7143 g $CuSO_4 \cdot 5H_2O$ dissolved in 50 mL demineralised water.

C: 1.4286 g Na-tartrate dissolved in 50 mL demineralised water.

D: A, B and C in the ratio 100:1:1 respectively.

E: A, C and demineralised water in the ratio 100:1:1 respectively.

F: 5 mL Folin-Ciocalteus and 6 mL demineralised water.

Every of the following samples were made twice.

500 µL standard or sample was put into reagent tubes four of each samples were diluted if necessary. Then half of the samples and standard were added 700 µL reagent D and the other half 700 µL reagent E. All samples were mixed on a vortex-mixer. Then all samples stand for at least 10 min. 100 µL reagent F was added and mixed on the vortex-mixer directly after this addition. After standing for 45 min. the absorbance was measured at 750 nm.

Standard curves were made from 0 to 100 ppm for both proteins and humus.

The total absorbance (ABS_{total}) was the samples mixed with reagent D. The samples mixed with reagent E was called blind (ABS_{blind}). The absorbance of protein ($ABS_{protein}$) and the absorbance of humus (ABS_{humus}) were calculated as seen below.

$$ABS_{total} = ABS_{protein} + ABS_{humus}$$

$$ABS_{blind} = 0.2 \cdot ABS_{protein} + ABS_{humus}$$

$$\Downarrow$$

$$ABS_{protein} = 1.25(ABS_{total} - ABS_{blind})$$

$$ABS_{humus} = ABS_{blind} - 0.2 \cdot ABS_{protein}$$

Appendix C – Anthrone Method (1)

This appendix describes the procedures of the anthrone method used for determination of carbohydrate contents.

Two reagents was used - A and B.

A: 27.5 mL demineralised water and 472.5 mL and 0.625 g anthrone.

B: The same as A without the anthrone.

Both A and B were cooled down to approximately 4 °C before use.

1.0 mL sample or standard was added to four tubes. 2.0 mL reagent A were added to two of the each sample or standard and the other two were added 2.0 mL reagent B. All samples and standards were mixed on a vortex-mixer.

Both samples and standards were boiled at 100 °C for 14 min and then cooled down in a ice bath. Afterwards every sample and standard was measured at 625 nm.

Standard curve was made from 0 to 100 ppm.

Appendix D – Operation Conditions of MBR systems (1)

pH Results

The results from pH measurements on both pilot MBR sludge, CAS and labscale MBR shows no sign of development. The pilot MBR has an average pH of 7.36, CAS is a little lower at 7.17 and the labscale is the highest at 7.43.

Conductivity Results

The measurements of the conductivity are changing over time as seen in Fig. 41.

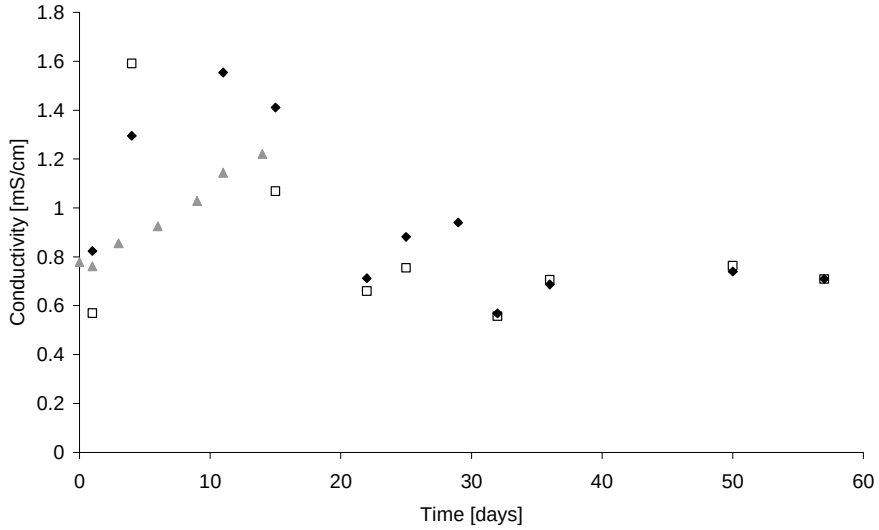

Fig. 41: Conductivity development over time. ◆ is the pilot MBR, □ is CAS and ▲ is the labscale MBR.

The pilot MBR and CAS changes seen in Fig. 41 are approximately the same and are therefore considered to be due to feed changes. The changes in the conductivity for labscale MBR are increasing which means that the something is influencing on the conductivity. This could be the dog food and the fact that the permeate is return in to the aerated tank. The return of the permeate means that small ions that are normally lead out through the permeate stays in the system.

MLSS Results

Fig. 42 shows the development in MLSS as a function of time in the two MBR system.

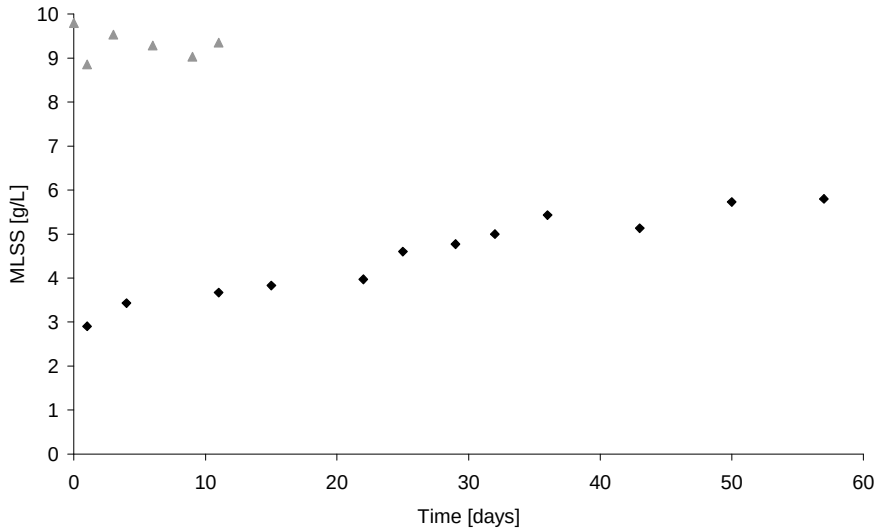

Fig. 42: MLSS development over time. ◆ is the pilot MBR and ▲ is the labscale MBR.

MLSS increases over time for pilot MBR as expected. However a small decrease in MLSS is detected for the labscale MBR.

Permeate MLSS and Turbidity

On the permeate from the labscale MBR MLSS and Turbidity at 650 nm was measured. Both values was so low that the measurements did not result in any usable values.

Appendix E – Permeate Flow from Pilot MBR

Fig. 43 shows the flow measurements from the 4th of February to the 6th of April from the pilot MBR at Lundtofte WWTP.

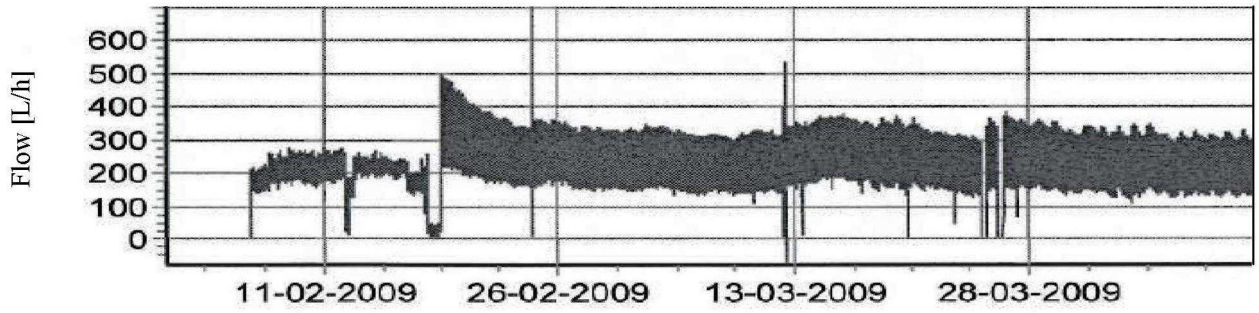

Fig. 43: The permeate flow (L/h) over time for the pilot MBR at Lundtofte WWTP.

Appendix F – Carbohydrate, Protein and Humus DeterminationS

Triple determination

Triple determination for standard deviation of carbohydrates, proteins and humus for day 4 and 22 from the pilot MBR is seen in Table 8.

Table 8: The three measurements on the same sample for day 4 and 22 in the pilot MBR at Lundtofte WWTP both dissolved and extracted EPS.

MBR dissolved day 4	Carbohydrates [ppm/(g/L)]	Protein [ppm/(g/L)]	Humus [ppm/(g/L)]
Measurement 1	22	8	23
Measurement 2	18	28	10
Measurement 3	12	18	13
MBR dissolved day 22	Carbohydrates [ppm/(g/L)]	Protein [ppm/(g/L)]	Humus [ppm/(g/L)]
Measurement 1	8	16	22
Measurement 2	6	22	11
Measurement 3	6	17	15
MBR extracted day 4	Carbohydrates [ppm/(g/L)]	Protein [ppm/(g/L)]	Humus [ppm/(g/L)]
Measurement 1	23	152	394
Measurement 2	21	207	166
Measurement 3	18	258	151
MBR extracted day 22	Carbohydrates [ppm/(g/L)]	Protein [ppm/(g/L)]	Humus [ppm/(g/L)]
Measurement 1	33	327	324
Measurement 2	26	248	206
Measurement 3	24	263	199

Double determination

To see the influence of storage time on the content of carbohydrates, protein and humus the sludge from day 57 from the pilot at Lundtofte WWTP was extracted twice with 24 h of storage between the two extractions. The results of this can be seen in Table 9.

Table 9: The difference in the dissolved and extracted amount of carbohydrates, protein and humus for the sludge from day 57 from the MBR at Lundtofte WWTP. + 24 h means that the sludge has been stored for 24 h at 5 °C before extraction.

	Carbohydrates [ppm/(g/L)]	Protein [ppm/(g/L)]	Humus [ppm/(g/L)]
MBR dissolved day 57	5	31	15
MBR dissolved day 57 + 24 h	3	28	21
MBR extracted day 57	33	330	211
MBR extracted day 57 + 24 h	36	328	218

The small difference between the two extractions (see Table 9) is of no significance since they are not larger than the standard deviation. Also the difference could be ascribed to the deviation in the extraction method. Therefore the sample from day 9 from the labscale MBR was extracted twice at the same time. These results are shown in Table 10.

Table 10: The difference in the dissolved and extracted amount of carbohydrates, protein and humus for the sludge from day 9 from the labscale MBR. The extraction was done twice at the same time hence I and II.

	Carbohydrates [ppm/(g/L)]	Protein [ppm/(g/L)]	Humus [ppm/(g/L)]
MBR dissolved day 9 I	7	64	164
MBR dissolved day 9 II	7	48	103
MBR extracted day 9 I	94	431	243
MBR extracted day 9 II	102	506	275

The differences in the results shown in Table 10 are also within the standard deviation and therefore no tendencies are to be determined.