The effect of microbiological properties of membrane bioreactor sludge on its filterability

Degree project in Biotechnology, second level
Hammarby Sjöstadsverk, Stockholm, 9 June 2015

SAMUEL KÄÄRIÄ

SUPERVISORS

Klara Westling
(IVL Swedish Environmental Institute)

Niklas Dahlén
(Stockholm Vatten AB)

EXAMINER

Gen Larsson
(School of Biotechnology at KTH Royal Institute of Technology)
Abstract

In preparation for a retrofit of Henriksdal waste water treatment plant to membrane bioreactors (MBR) this study on the effects of the microbiological properties of MBR sludge on its filterability was carried out. Filterability is closely related to MBR permeability (performance) and fouling (degradation of performance) and a better knowledge of the factors affecting filterability can lead to better understanding of the causes of fouling. Some of the microbiological factors known to have fouling propensity are mixed liquor suspended solid (MLSS) levels, relative hydrophobicity (RH) of the microbes, floc structure, extracellular polymeric substances (EPS) and soluble microbial products (SMP). These factors were analyzed during 12 weeks and correlations between the factors and filterability were calculated and evaluated to find the effect of microbiology on sludge filterability. Filterability was monitored using the sludge filtration index (SFI) method and its correlation with permeability was evaluated. Results show that RH had a negative effect on filterability. This can be explained by increased microbe-membrane/filter interactions and more deposition of particles as an effect of increased RH. The floc structure was studied by analyzing floc size, floc circularity and ratio of floc max and min diameters. Floc size was shown to have a positive effect on filterability and this is attributed the theory that large robust flocs form a secondary layer protecting the membrane from fouling caused by small particles. This can also be the cause of the lack of correlation between EPS and SMP with filterability despite rising levels. RH and EPS protein fraction were shown to have a negative effect and a positive effect on floc size, respectively. No effect on filterability by MLSS, EPS carbohydrate fraction or SMP protein fraction was found. SFI showed a positive correlation with permeability which means a high filterability correlates with low permeability. SFI was expected to have a negative correlation with permeability and the suitability of SFI as a filterability measurement to compare with permeability is therefore doubtful.
Sammanfattning

Som förberedelse för implementering av membranbioreaktorer (MBR) till Henriksdals avloppsreningsverk utfördes denna studie på hur filtrerbarheten hos slam i MBR påverkas av dess mikrobiologiska egenskaper. För MBR är filtrerbarhet nära relaterat till permeabilitet (prestanda) och fouling (försämring av prestanda), och en bättre förståelse om de faktorer som påverkar filtrerbarheten kan leda till en mer optimerad drift och användning av tidiga insatser för att motverka fouling. Mikrobiologiska faktorer som rapporterats orsaka fouling i MBR är bland annat graden av totalt suspenderat slam (MLSS), mikrobernas hydrofobicitet, flockstruktur, extracellulära polymera substanser (EPS) och lösliga mikrobiella produkter (SMP). Dessa faktorer studerades under våren 2015 och korrelationer mellan faktorerna och filtrerbarhet beräknades och analyserades.

Filtrerbarhet mättes med metoden sludge filtration index (SFI), vars korrelation till permeabiliteten också granskades. Resultaten visar att den relativa hydrofobiciteten (RH) hade en negativ effekt på filtrerbarheten. Detta kan förklaras av ökade interaktioner mellan mikroberna och membranet/filtret och ökad utfällning av små partiklar till följd av ökad RH. Flockstrukturen studeras genom att analysera flockstorlek, flockarnas cirkulärhet och förhållandet mellan flockarnas längsta och kortaste diameter. Flockstorleken visade sig ha en positiv effekt på filtrerabarheten, vilket kan förklaras med teorin att stora robusta flockar kan bilda ett sekundärt lager som skyddar membranet/filtret från fouling orsakad av små partiklar. Detta kan även vara orsaken till avsaknaden av korrelationer mellan filtrerbarhet och EPS och SMP trots höga nivåer. RH visade sig ha negativ effekt, och EPS proteinfraktion en positiv effekt, på flockstorleken. Inga korrelationer till filtrerbarhet kunde urskiljas bland faktorerna MLSS, EPS kolhydratfraktion eller SMP proteinfraktion. SFI visade en positiv korrelation till permeabilitet vilket betyder att hög filtrerbarhet korrelerar med låg permeabilitet. SFI förväntades ha negativ korrelation med permeabilitet och därför är SFIs lämplighet som filtrerbarhetsmetod för jämförelse med permeabilitet därför tveksam.
# Contents

1 Introduction.......................................................................................................................... 2
   1.1 Henriksdal waste water treatment plant ................................................................. 2
   1.2 Membrane bioreactor process .................................................................................. 2
   1.3 Membrane fouling ...................................................................................................... 3
   1.4 Sludge microbiology ................................................................................................. 4
   1.5 Factors with fouling propensity .................................................................................. 4
   1.6 Problem statement ...................................................................................................... 6
   1.7 Aim .............................................................................................................................. 6
   1.8 Strategy ...................................................................................................................... 6

2 Materials and methods ....................................................................................................... 7
   2.1 Hammarby Sjöstadsværk Line 1 MBR operating conditions ........................................ 7
   2.2 Sludge sample extraction ............................................................................................ 7
   2.3 Mixed liquor suspended solids .................................................................................. 7
   2.4 Filterability .................................................................................................................. 7
   2.5 Relative hydrophobicity ............................................................................................ 8
   2.6 Floc structure ............................................................................................................. 8
   2.7 Extracellular polymeric substances ............................................................................. 11
   2.8 Membrane permeability and resistance ..................................................................... 12
   2.9 Iron ............................................................................................................................. 13
   2.10 Statistical analysis .................................................................................................... 13

3 Results ................................................................................................................................. 14
   3.1 External events affecting MBR parameters ............................................................... 14
   3.2 Correlations ............................................................................................................... 15
   3.3 Correlations with filterability .................................................................................... 16
   3.4 Other correlations ..................................................................................................... 19

4 Discussion ............................................................................................................................ 24
   4.1 Overview ..................................................................................................................... 24
   4.2 Correlations ............................................................................................................... 25
   4.3 Filterability and RH ................................................................................................... 25
   4.4 Filterability and floc size .......................................................................................... 26
   4.5 RH, floc size and EPSp ............................................................................................. 26
   4.6 SFI and permeability ............................................................................................... 26
   4.7 Conclusion ............................................................................................................... 27

5 Acknowledgement ............................................................................................................... 28

6 Future work .......................................................................................................................... 28
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs</td>
<td>Absorbance</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>C</td>
<td>significance level critical value</td>
</tr>
<tr>
<td>CAS</td>
<td>Conventional activated sludge</td>
</tr>
<tr>
<td>df</td>
<td>degrees of freedom</td>
</tr>
<tr>
<td>DFCm</td>
<td>Delft Filtration Characterization Method</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular polymeric substances</td>
</tr>
<tr>
<td>EPSc</td>
<td>Extracellular polymeric substances carbohydrate fraction</td>
</tr>
<tr>
<td>EPSp</td>
<td>Extracellular polymeric substances protein fraction</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>IVL</td>
<td>IVL Swedish Environmental Institute</td>
</tr>
<tr>
<td>MBR</td>
<td>Membrane bioreactor</td>
</tr>
<tr>
<td>MLSS</td>
<td>Mixed liquor suspended solids</td>
</tr>
<tr>
<td>p</td>
<td>significance level</td>
</tr>
<tr>
<td>r</td>
<td>Pearson product-moment correlation coefficient</td>
</tr>
<tr>
<td>RH</td>
<td>Relative hydrophobicity</td>
</tr>
<tr>
<td>SFI</td>
<td>Sludge filtration index</td>
</tr>
<tr>
<td>SMP</td>
<td>Soluble microbial products</td>
</tr>
<tr>
<td>SMPc</td>
<td>Soluble microbial products carbohydrate fraction</td>
</tr>
<tr>
<td>SMPp</td>
<td>Soluble microbial products protein fraction</td>
</tr>
<tr>
<td>TMP</td>
<td>Transmembrane pressure</td>
</tr>
<tr>
<td>v/v</td>
<td>volume over volume</td>
</tr>
<tr>
<td>w/v</td>
<td>weight over volume</td>
</tr>
<tr>
<td>WWTP</td>
<td>Waste water treatment plant</td>
</tr>
</tbody>
</table>
1 Introduction

Henriksdal is a municipal waste water treatment plant (WWTP) in central Stockholm in the process of implementing membrane bioreactor (MBR) technology to meet the increasing demand for waste water treatment. In preparation for the change a downscaled copy of a future treatment line at Henriksdal has been set up at Hammarby Sjöstadsverk (www.sjostadsverket.se), a research and development WWTP on the premises of Henriksdal WWTP. This Line 1 at Hammarby Sjöstadsverk has been used to evaluate the performance of MBR and for various research projects (IVL and Stockholm Vatten, 2014).

1.1 Henriksdal waste water treatment plant

Figure 1 from Stockholm Vatten (2015) shows the process flow of Henriksdal waste water treatment and the ten steps. Incoming water (1) is first treated with chemicals to increase phosphorus precipitation (2) and then run through a bar screen (3) where large and medium sized objects are retained. A grit chamber (4) is used for sedimentation of sand and other heavy particles prior to the primary sedimentation (5) which facilitates sedimentation of phosphorus and primary sludge. Bioreactors (6) are then used to remove organic material and nitrogen with the help of microorganisms. Step number seven of Henriksdals current setup is a secondary sedimentation (7) step where the activated sludge from the bioreactors sediments. This is followed by chemical precipitation (8) and a sand filter (9) as polishing step before releasing the treated water (10). This configuration with bioreactors followed by secondary sedimentation and a polishing step is called a conventional activated sludge (CAS) process.

![Figure 1. Process flow chart of the waste water treatment at Henriksdal. Adapted from Stockholm Vatten (2015).](image)

1.2 Membrane bioreactor process

In an MBR plant the technologies of bioreactors and filtrating membranes are combined. MBR is similar to CAS in that it contains a biological step with anoxic and aerobic zones. Following the biological step CAS uses sedimentation to separate sludge from effluent whereas MBR uses a membrane which functions as a filter separating water from suspended solids and particles. With pore sizes ranging from 0.05 to 0.4 µm virtually no solids are able to pass which removes the need of a final polishing step that are commonly found in plants using CAS. This gives MBRs many advantages such as space reduction, high effluent quality and good disinfection ability. The disadvantages of MBR is a high investment cost, the need for high aeration with its accompanying energy requirements, and increased use of chemicals for cleaning of the membranes (Le-Clech et al., 2006). Although MBR has a higher efficiency it comes with a major drawback: membrane fouling. Membrane fouling occurs over time with operation and leads to a decrease of membrane performance and higher costs due to high aeration and cleaning chemicals. It is therefore desirable to understand the fouling process in order to minimize the costs. By finding factors that indicates fouling efforts can be taken in time to reduce fouling and optimize operation.
1.3 Membrane fouling

The performance of a membrane in an MBR is often measured as permeability (L m\(^{-2}\) bar\(^{-1}\) h\(^{-1}\)), which is the flux of water across the membrane (L m\(^{-2}\) h\(^{-1}\)) divided with the transmembrane pressure (TMP) (bar). Performance is influenced by many factors and decreases with time. This decrease of performance is called membrane fouling and occurs due to deposition or accumulation of microorganisms, solutes, colloids and cell debris on the membrane surface or within membrane pores (Hai et al., 2014). This increases resistance and thus the TMP required to preserve a specific flux. Fouling in MBRs is complex due to the varying composition of the suspended biomass. The two main types of fouling mechanisms are according to Hai et al. (2014):

a) **Cake fouling** – large particles such as suspended solids form a cake layer on the membrane which adds resistance (Figure 2, left)

b) **Pore blocking** – caused by smaller particles such as solutes and colloids (Figure 2, right)

![Figure 2](image.png)

**Figure 2.** Left: Formation of a cake fouling layer. Right: Pore blocking fouling. Images from Hai et al. (2014).

The different types of fouling are usually classified depending on the level of reversibility and cleaning treatment needed. Table 1 shows a common fouling type classification (Drews, 2010) and examples of fouling rate of the different types of fouling (Lousada-Ferreira et al., 2014). This shows the frequent need for mechanical cleaning and regular chemical cleaning. Reversible fouling is typically reduced by constant air scouring which is a major cost for MBR processes.

<table>
<thead>
<tr>
<th>Fouling type</th>
<th>Description</th>
<th>Fouling rate (\text{mbar/min})</th>
<th>Time interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible</td>
<td>Foulants accumulate on the membrane surface (cake formation), cleaning by mechanical treatment (filtration breaks or backflush cycles).</td>
<td>0.1–1</td>
<td>10 min</td>
</tr>
<tr>
<td>Irreversible</td>
<td>The foulants are strongly attached and need chemical treatment to be removed. Pore clogging is often irreversible.</td>
<td>0.001–0.1</td>
<td>Weeks, months</td>
</tr>
<tr>
<td>Irrecoverable</td>
<td>Fouling over a period of time causes permanent damage to the membrane.</td>
<td>0.0001–0.001</td>
<td>Several years</td>
</tr>
</tbody>
</table>

Although permeability is the most common parameter to monitor for MBR operators it is on its own a poor parameter to study fouling. Instead, sludge filterability is commonly used to study membrane fouling (Hai et al., 2014). Previous research at Hammarby Sjöstadsverk has indicated a positive correlation between sludge filterability and membrane permeability using the sludge filtration index (SFI) method (Apostolopoulou Kalkavoura, 2014). Different techniques have been developed for filterability assessment but there is yet no universal method.
1.4 Sludge microbiology
Apart from operating conditions, sludge microbiology is the main cause for membrane fouling. Activated sludge in both CAS and MBR is a mixture of microorganisms with very high diversity in organic compounds and particle sizes. The microorganisms use the waste as food source and most of the cell biomass grow in aggregated three-dimensional microbial communities called flocs. These flocs contain microflocs of bacteria and other organisms, various particles such as fibers, inorganic particles and extracellular polymeric substances (EPS) (Figure 3). Flocs are held together by EPS and filamentous bacteria, and contains channels which enable fluid to pass through (Seviour and Blackall, 1999).

Figure 3. Schematic view of a bacterial floc with examples of components. Image from Meeting Report 17 Jan 2014, Stockholm Vatten, Käppalaförbundet.

1.5 Factors with fouling propensity
All parts of MBR process design and operation affects membrane fouling which makes the phenomena complex and difficult to understand. Research to understand and find foulants have been going on for many years. Aeration rate and mixed liquor suspended solid (MLSS) were in the beginning thought to be the main factors but this was proven to be an incorrect statement (Le-Clech et al., 2006). Later research have been focused mostly on EPS and soluble microbial products (SMP) as they seem to influence the fouling rate in various ways (Le-Clech et al., 2006). EPS and SMP consists mainly of proteins and carbohydrates and some of the latest research have been focused on investigating the composition of these fractions (Drews, 2010). In this study, factors pertaining to the microbiology of the sludge was the focus of interest. Some of the major biomass related factors with fouling propensity are: MLSS, viscosity, floc structure, particle size, temperature, dissolved oxygen, relative hydrophobicity (RH), EPS and SMP (Hai et al., 2014; Le-Clech et al., 2006).

Following is a short review on MLSS, RH, floc structure, particle size, EPS and SMP. These are easy to examine in simple lab environment and are all reported to have fouling propensity and an effect on sludge filterability (Hai et al., 2014; Le-Clech et al., 2006).

1.5.1 Mixed liquor suspended solids
MLSS was in the early days of MBRs considered as a major fouling factor but subsequent studies showed that there is no linear correlation between MLSS and membrane fouling (Hai et al., 2014). Lousada-Ferreira et al. (2010) suggests that there is a range for optimal MLSS concentration, and Gil et al. (2011) indicates that higher MLSS (up to 20 g/L) may give better filterability. Le-Clech et al. (2006) concludes a literature review on MLSS that due to contradictory results, MLSS is a poor indicating factor for biomass fouling propensity.
1.5.2 Relative hydrophobicity
The hydrophobicity of the membrane and microbial flocs are parameters that can help indicate membrane fouling. Membranes in MBR are often made hydrophilic to improve water permeability, whereas microbial flocs are usually hydrophobic for better microbe-microbe interactions. A lower RH of the flocs causes deflocculation and possibly increased microbe-membrane interactions which can lead to membrane fouling (Van den Broeck et al., 2011). Some have found that a higher hydrophobicity leads to a lower cake resistance (Le-Clech et al., 2006). There are however also reports of a positive correlation between RH and fouling (Hai et al., 2014).

1.5.3 Floc structure and particle size
Some authors have found a clear correlation between cell morphology (specifically the mean ratio of max and min diameters) and microbial filter cake resistance and compressibility (Le-Clech et al., 2006). It is suggested that large robust flocs form a secondary membrane which functions as a physically reversible dynamic membrane, protecting the actual membrane from fouling (Van den Broeck et al., 2010).

Particles of submicron-sizes also seem to have an effect on sludge filterability (Hai et al., 2014). These are believed to be released during deflocculation of larger flocs and/or derived from microbial activities. The particles would cause irreversible fouling by plugging or narrowing the pores of the membrane. They would also deposit on the membrane surface due to low back-transport velocity, which leads to a cake layer with much less filterability than those formed by stable large-sized flocs (Hai et al., 2014).

Van den Broeck et al. (2011) states that no parameter can on its own make a reliable prediction of sludge filterability and fouling effect. However, the authors showed that the combination of floc structure and RH can help categorize the sludge into two filterability categories: bad and poor to good.

1.5.4 Extracellular polymeric substances and soluble microbial products
EPS includes proteins, polysaccharides, lipids, nucleic acids and other polymeric substances produced by microorganisms. They are often reported as a main foulant in MBRs and can be categorized into EPS bound on bacterial flocs and EPS freely present in the bulk solution (also called SMP). Bound EPS are usually responsible for cake layer fouling while SMP is often accredited for pore blocking. Because of heavy flux and air scouring the cake layer tends to be of less significance which means that SMP are often the main foulant. Although including a variety of molecules the majority of EPS molecules are either proteins (EPSp) or polysaccharides/carbohydrates (EPSc) (Le-Clech et al., 2006).

Another explanation for the lesser impact of bound EPS is provided by reports stating that it has little or no significance in very low or very high levels, but a significant effect in-between (Le-Clech et al., 2006). This can be explained by the importance of EPS in the interactions between microbial cells in flocculation. Low levels of EPS can cause floc deterioration and thus membrane fouling, while too high levels will also cause fouling by increased microbe-membrane interactions. A theory of an optimum level of bound EPS for maintaining floc structure with minimum fouling effect has therefore been proposed (Le-Clech et al., 2006). A report by Nagaoka & Nemoto (2005) suggests that among the different bound EPS, those larger than 1000 kDa are the main responsible for membrane fouling.

As stated before, the SMP are considered the main foulants in pore blocking. SMP consists mainly of carbohydrates (SMPc) and proteins (SMPp). SMPc and SMPp are reported to have different impact on membrane fouling, where SMPc is considered a major foulant indicator whereas SMPp has less but not insignificant contribution. Although SMP is believed to mainly contribute to fouling through
pore blocking, they also contribute to the cake layer formation on the membrane. In the cake layer they provide a possible nutrient source for biofilm formation that adds to the hydraulic resistance. This is verified by a study showing a higher percentage of SMPc in the cake layer than in the activated sludge (Le-Clech et al., 2006).

Operating conditions, such as temperature and to some extent the solids retention time, are reported to alter the compositions of EPS. This is due to the change in microbiology caused by the change in conditions (Gao et al., 2013; Lesjean et al., 2005; Ma et al., 2013).

Drews (2010) made a compilation of the latest research and came to the conclusion that although SMP have been considered the main foulant, the diverse experimental results show that SMP concentrations in the supernatant alone might not be a reliable indicator of membrane fouling. The author suggests however, that SMP supernatant concentrations can correlate to membrane fouling in specific conditions, such as large pore sizes and low sludge age (Drews, 2010).

1.6 Problem statement
When studying the literature pertaining MBR fouling it quickly becomes clear that there are no universal methods or parameters that single-handedly can indicate fouling or bad filterability. Parameters are even shown to have different effect in different studies. This is mostly contributed to the differing composition of waste water and running operations of WWTPs, as well as the lack of standardized analytical methods (Drews, 2010; Hai et al., 2014; Le-Clech et al., 2006). Thus, it is difficult to predict which factors with fouling propensity might have the highest, if any, impact on sludge filterability in Hammarby Sjöstadsverk. By finding the factors that have the highest impact and monitoring them, mitigating measures can be taken in time to avoid severe fouling. To this end, this study was executed as a master thesis project supervised by IVL Swedish Environmental Institute and Stockholm Vatten AB.

1.7 Aim
The aim of this study is to identify and quantify the effect of microbiological factors on sludge filterability and to evaluate the correlation of SFI and permeability.

1.8 Strategy
A few selected factors will be monitored during 12 weeks to identify patterns in the variation of the factors and cross examine these with regard to sludge filterability. These factors are:

- MLSS
- RH
- Floc structure parameters:
  - Size
  - Ratio of max and min diameters
  - Circularity
- EPSc
- EPSp
- SMPc
- SMPp

Filterability, permeability and resistance will also be monitored. Filterability will be compared with permeability to assess correlation. Pattern identification will be conducted by correlation statistics and examination of plots.
2 Materials and methods
The aforementioned factors were studied using the methods described in this chapter three times a week during the period of February 25th – May 18th 2015.

2.1 Hammarby Sjöstadsverk Line 1 MBR operating conditions
The Line 1 at Hammarby Sjöstadsverk is equipped with a membrane filtration module from Alfa Laval, containing two filter membranes. The membranes are made of polyvinylidene fluoride and have a pore size of 0.2 µm. No information on their hydrophobic properties were available. They have continuous air scouring and use alternating relaxation periods to limit fouling (Alfa Laval, 2015). Sjöstadsverket Line 1 have several treatment steps as seen in Figure 4, they are: 1) incoming water from Henriksdal, 2) primary sedimentation, 3) bioreactors with anoxic and aerobic zones, 4) chemical precipitation (Fe-ions) for denitrification, 5) addition of carbon source for denitrification, 6) MBR, 7) treated water is returned to Henriksdal. The incoming water to Sjöstadsverket Line 1 comes from Henriksdal WWTP with dynamic flows, based on the incoming flow to Henriksdal (IVL and Stockholm Vatten, 2014).

![Figure 4. Simplified schematic view of Hammarby Sjöstadsverk line 1, adapted from IVL and Stockholm Vatten (2014).](image)

2.2 Sludge sample extraction
At the start of every day of analysis a volume of 3 L was gathered from the MBR in Hammarby Sjöstadsverk. The sample was taken from the top of the bulk mixture, close to the online MLSS sensor, and brought straight to the laboratory.

2.3 Mixed liquor suspended solids
Online MLSS readings were noted each time a sample was taken for use in dilutions during the experiments. For more accurate data, MLSS was analyzed according to routine practice at Sjöstadsverket with triplicate samples. A filter paper (1.6 µm pore size) was weighed and used to filter 10 ml of activated sludge sample using tap water suction for faster filtration. The filter paper and dry sample was then heated in an oven at 105°C for 3 hours, and then cooled down in a desiccator before weighing. MLSS was then calculated according to Equation 1.

$$\text{MLSS (g/L)} = \frac{m_{\text{filter paper+heated sample}} - m_{\text{filter paper}}}{\text{sample volume}} \quad \text{Equation 1}$$

2.4 Filterability
The filterability of the sludge was assessed using the SFI method by Thiemig (2012). This method aims at measuring the reversible fouling. Triplicate tests were performed during the first two weeks of experiments and then raised to five tests from March 6th until the end of the experimental period. The analysis was performed by first placing the sludge sample in a water bath to increase its temperature to 20°C. The equipment was assembled as seen in Figure 5. A Büchner funnel was
placed above a 500 ml measuring cylinder with clamps, and a 0.6 µm filter paper with 150 mm diameter was placed inside the funnel. A blade agitator was fixated about 10 mm above the filter paper and run during filtration at an approximated rotating speed of 200 revolutions per minute. A sludge sample of 500 ml was poured into the funnel and the time for permeate to increase from 100 to 150 ml was measured. SFI was then normalized against % MLSS (w/v) according to Equation 2. Good filterability yields low SFI values and poor filterability high.

\[
SFI\left(\frac{s}{\%\text{ MLSS (w/v)}}\right) = \frac{\Delta t (s)}{\left(\frac{gL_{\text{MLSS}}}{1000 g/L}\right)} \times 100\%
\]

Equation 2

![Figure 5. Experimental setup for SFI measurements.](image)

2.5 Relative hydrophobicity
RH was measured by the microbial adherence to hydrocarbons method (Rosenberg et al., 1980) modified by Van den Broeck et al., (2011). Triplicate samples were tested each time. A sludge sample was washed twice by centrifuging at 5000x g for 10 min and resuspending by mixing to a concentration of 2.5 gMLSS/L with PBS buffer to a total volume of 40 ml. The starting sample volume was usually around 10 ml, varying with the MLSS concentration. After washing, a part of the sample was filtrated with 0.45 µm filter and used as a zero measurement for spectrophotometric absorbance measurement at 650 nm. Absorbance was measured using a photoLab® 6600 UV-VIS spectrophotometer and polystyrene cuvettes. The absorbance of the sample (Abs₁) was measured and then 3 ml sample was mixed with 3 ml n-hexadecane by vigorous shaking for two minutes. The sample was then given 5 minutes to settle after which the aqueous solution was extracted and its absorbance (Abs₂) measured. The RH was then calculated by Equation 3.

\[
RH (\%) = \left(1 - \frac{\text{Abs}_2}{\text{Abs}_1}\right) \times 100\%
\]

Equation 3

2.6 Floc structure
The floc structure was analyzed with a digital image analysis method similar to Van den Broeck et al. (2011).

2.6.1 Image capture
The sample was diluted to 1 gMLSS/L and two separate droplets of diluted sample were applied unto a glass slide with a cover glass each and then examined in an optical microscope. If the microscopic
content of the droplets were not similar two new droplets were used. With a total magnification of 100x, a minimum of 90 images were taken in a structured manner across both droplets with about half of the images from each of the droplets. In order to achieve an objective result the pictures were taken on random with no regard to the objects in the image. The images were stored as JPG images with resolution 1280x1024 pixels. The microscope used was a Nikon Optiphot-2 and the images were captured by a CCD camera connected to a computer with DinoLite Digital Microscope software (version 2.9.0.0) by AnMo Electronics Corporation.

For scale determination of the digital images a cover glass with a 1/100 mm scale was used, see Figure 6. A conversion from pixel size to µm size of the digital images was performed by measuring the pixels between the µm marks in the microscopic image (Figure 7).

![Figure 6. Cover glass with µm scale (1/100).](image)

![Figure 7. Microscopic image of the µm scale.](image)

### 2.6.2 Image analysis

The images were analyzed using the microbial ecology image analysis software DAIME version 2.1 (Daims et al., 2006). To load the images into DAIME a conversion to TIFF image format was first required. This was accomplished with the freeware XnConvert version 1.66 for Windows.

Once a batch of pictures was loaded (as color images) and the µm scale set, a segmentation of the images was required in order to identify the objects in the image. This was accomplished by using DAIMEs own automatic segmentation tool for 2D segmentation using edge detection, which algorithms detect objects by searching for edges in the image, i.e. borders of objects. During segmentation, a black and white object layer is created where white pixels indicate image object and black pixels indicate no object. The settings used are seen in Figure 8. The program was set to exclude objects smaller than 2000 pixels or approximately 650 µm² to sort out noise.
Figure 8. DAIME was used to segment the images in order to identify the objects.

After segmentation the images are checked to validate the definition of objects and reject any objects that are not bacterial flocs, to avoid incorrect data. The objects from the object layer is viewed on top of the normal image to easily distinguish between flocs and other objects. Figure 9 shows an image where four non-floc objects (marked with red circles) have been rejected. Internal dark regions are included for all objects. This procedure was repeated for every image in the batch.

Once rejection of non-floc objects is completed an image analysis is performed to measure features of selected objects in the object layers. DAIME calculates features such as total area (µm²), perimeter (µm), perimeter/total area, circularity, min and max diameter (µm) and ratio of diameters (see Figure 10). The perimeter is an approximation due to the adjustment of the algorithms to fit round objects better than straight lines.
The data is then exported for evaluation. Mean and median values of total area (µm$^2$), circularity and ratio of diameters were saved for data analysis. The total area is the amount of pixels in the object times the size of one pixel in µm$^2$. The ratio of diameters is the ratio of the largest caliper diameter to the smallest (1 for spherical objects). The circularity is calculated according to Equation 4:

$$\text{circularity} = 4\pi \times \frac{A}{P^2}$$  \hspace{1cm} \text{Equation 4}

Where:  
$A = \text{area of the object (µm}^2\text{)}$  
$P = \text{perimeter of the outer edge of the object (µm)}$

2.7 Extracellular polymeric substances

2.7.1 Extraction of SMP and EPS

The extraction of SMP and EPS from activated sludge was performed using a modification of the heating method described by Le-Clech et al. (2006) (Figure 11), with triplicate samples. 40 ml MLSS sample was centrifuged in a 50 ml falcon tube for 5 min at 5000x g after which the supernatant, which contained the SMP fraction, was filtrated through a 0.45 µm filter. The falcon tube was refilled to 40 ml with deionized water and the pellet resuspended by mixing. A volume of 15 ml was then transferred to a glass vial for heating at 80°C for 10 min, after which it was centrifuged in a falcon tube for 10 min at 7000x g. The supernatant, containing the EPS fraction, was then filtered through a 0.45 µm filter.

Figure 11. Heating method for extraction of SMP and EPS from activated sludge, adapted from Le-Clech et al. (2006).
2.7.2 Protein quantification
A modified version of the Lowry method (Lowry et al., 1951) by Frølund et al. (1996) was used to quantify the protein fraction of the SMP and EPS samples. The following reagents were used:

- Reagent 1 143 mM NaOH, 270 mM Na$_2$CO$_3$
- Reagent 2 57 mM CuSO$_4$
- Reagent 3 124 mM Na-tartrate
- Reagent 4 Mix of reagents 1, 2 and 3 in the proportion 100:1:1
- Reagent 5 Folin reagent 1N

Bovine serum albumin (BSA) was used for the standard curve. Reagents 4 and 5 were prepared the same day as analysis.

The procedure was as follows: 1000 µl Reagent 4 and 750 µl sample was added to a glass vial and mixed by shaking for 1 min. 150 µl Reagent 5 was added and the solution mixed for 1 min after which it was left to react for 45 min in room temperature. The absorbance was then measured at 750 nm. The cuvettes used were poly(methyl methacrylate) and the spectrophotometer same as for RH measurements. Deionized water was used as blank, deionized water instead of sample was used as control and a standard curve was made with BSA.

2.7.3 Carbohydrate quantification
A modified version of the Antrhone method (Frølund et al., 1996) was used to quantify the carbohydrate fraction of the SMP and EPS samples, with triplicate samples. A reagent containing 0.5% anthrone (w/v) in 75% H$_2$SO$_4$ (v/v) was prepared the same day as use and cooled down with cold tap water to room temperature. 400 µl sample and 2000 µl reagent was mixed in a glass vial and placed in a heater at 120°C for 10 min. The sample was then cooled down with running tap water for 5 min, and the absorbance measured at 625 nm with the same instrument and cuvettes as for protein quantification. Deionized water was used as blank, deionized water instead of sample was used as control and a standard curve was made with glucose.

2.8 Membrane permeability and resistance
2.8.1 Membrane permeability
The permeability for the two membranes in the MBR module was gathered from online sensors and later normalized against temperature. Normalization against temperature is common while studying MBR permeability and is used to compensate for the temperature effect on water viscosity, which affects the flux of water. The viscosity of water can be calculated with the following empirical formula (United States Environmental Protection Agency, 2005):

$$\mu_T = 1.784 - (0.0575 * T) + (0.0011 * T^2) - (10^{-5} * T^3)$$  \hspace{1cm} \text{Equation 5}

Where:
- $\mu_T$ = water viscosity at temperature $T$ (cP)
- $T$ = water temperature (°C)

For MBR processes a reference temperature of 20°C is commonly used, for which the viscosity is approximately 1 cp. The relationship of observed and normalized flux is according to Equation 6, the observed permeability as Equation 7 and the normalized permeability is obtained by multiplying the observed permeability with the ratio of $\mu_T/\mu_{20}$ according to Equation 8 (United States Environmental Protection Agency, 2005).

$$J_{20} * \mu_{20} = J_T * \mu_T$$  \hspace{1cm} \text{Equation 6}
Permeability was also tested with a normalization against hypothetical long term fouling with the same method used in the work by Apostolopoulou Kalkavoura (2014). By looking at the trend of permeability decrease over a two month period with stable operation a trend was observed and the trend line was used to “add” permeability and thus compensate for the permeability lost by fouling. The equation used was

\[ P_{\text{temp. and fouling normalized}} = P_{\text{temp. normalized}} + 2.3454 \times \text{number of days} \]  

\[ \text{Equation 9} \]

This value was however used only to see if it correlates better with SFI than permeability only normalized against temperature.

2.8.2 Membrane resistance

The total membrane resistance \( R_t \) was also monitored. \( R_t \) is extracted from the permeability according to Equation 10 (Meng et al., 2006):

\[ R_t = \frac{1}{\mu_T} \left( \frac{\text{TMP}}{J_T} \right) = \mu_T^{-1} \times P^{-1} \]  

\[ \text{Equation 10} \]

2.9 Iron

Sludge samples were taken by Hammarby Sjöstadsverk staff weekly and iron concentrations were determined by an accredited laboratory run by Eurofins.

2.10 Statistical analysis

2.10.1 Correlation

To identify the effect of a factor on SFI and between the factors, the Pearson product-moment correlation coefficient \( r \) was calculated and a correlation matrix formed. The correlation coefficient between two sets of parameters is calculated according to Equation 11 and gives the linear relationship of the sets (Miller and Miller, 2005).

\[ r(X, Y) = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}} \]  

\[ \text{Equation 11} \]

Where

\( X = \) Array of values
\( Y = \) Array of values
\( \bar{x} = \) Average of X values
\( \bar{y} = \) Average of Y values
The correlation coefficient is always between -1 and 1 where -1 is a perfect negative linear correlation, and 1 a perfect positive linear correlation. In a scatter plot with X and Y a perfect correlation would be seen as all the data points on a straight line with either positive or negative slope. If the coefficient is zero or close to zero there is no correlation (Miller and Miller, 2005). To sort out very weak relationships an arbitrary limit has to be set to 0.4, meaning that correlations between -0.4 and 0.4 were disregarded. The limit 0.4 was also chosen for easier comparison of the results with a similar study performed by Meng et al. (2006).

2.10.2 Significance test
The probability that the correlations occurred by chance was tested using a two tailed significance test with 95% significance level (p). The degrees of freedom (df) was N-2 where N is the number of pairs of data. With \( r, p, df \) and \( N \) the critical value (C) for Pearson correlation was obtained from statistical tables (Fort Lewis College, 2015; University of Connecticut, 2015). Any correlations where \(-C < r < C\) were regarded as not significantly different from zero. The more pairs of data, the lesser the critical value will be.

3 Results
MLSS, RH, floc size, ratio of max and min floc diameters, floc circularity, EPSc, EPSp, SMPp and filterability was successfully measured and the following sections will present the variations over time and the correlations. Due to experimental failure the data sets for the following factors had a later start than planned: SFI (February 27th) and RH (March 4th). SMP and EPS measurements were started on March 30th. SMPc measurement failed due to method limitations. Permeability, resistance and iron levels were collected. An overview of the MBR operation during the experimental period and the correlations between the factors will follow.

3.1 External events affecting MBR parameters
During the experimental periods some external events had a significant impact on MBR parameters. Figure 12 shows the online data for MLSS, inflow, permeability and resistance together with eight external events impacting the operation of the MBR.

![Figure 12. Permeability, MLSS and water inflow to MBR line over time. Eight major external events affecting the operation occurred during the test period, marked in blue for heavy rain and red for MBR stop.](image-url)
Event 1: Heavy rain caused a peak in inflowing water with an accompanying drop of permeability, and a small increase of MLSS.

Event 2: Heavy rain during several days caused high inflow of water which caused an increase of MLSS and a rapid loss of permeability and raise of resistance. Since iron ion dosage is proportional to inflow this caused an accumulation of iron ions in the system.

Event 3: Due to rapid increase in TMP the MBR membranes shut down automatically on Saturday 11th April and was restarted on Monday 13th (seen in Figure 12 as no resistance data for a few days). This was due to high iron dosage during period of high inflow. The abnormal peaks are due to operational stop. Flow was stopped but the recirculating pump from MBR to bioreactors was still functioning and some MLSS was lost.

Event 4: The MBR was run with lower average inflow until cleaning.

Event 5: Chemical cleaning of the membranes was performed on 22-23 April by back flushing with oxalic acid (pH 1.6) and sodium hypochlorite (pH 11), resulting in decreased TMP and increasing permeability. The average inflow was increased after cleaning.

Event 6: Rain caused a peak of inflowing water and a small decrease of the increasing permeability.

Event 7: All operational functions preceding the MBR was out of order for two days. This stop together with heavy rain caused peaks in MLSS and resistance and drop of permeability.

Event 8: Substantial amounts of rain water caused a drop of permeability and an unusually high and fast increase of MLSS.

3.2 Correlations
Correlations between all factors were calculated and a significance test evaluation performed. These correlations were put into a matrix seen in Table 2. The green cells indicate positive correlations and orange cells indicate negative correlations. White cells are coefficients that are either below the 0.4 limit, or above the limit but failed the significance test.

Floc structure parameters are given in both mean and median values, except for the correlations with permeability and $R_t$ where the median was chosen due to its higher correlation. The median of floc structure parameters gave in general better correlations. No correlations with SMPc could be made because the measured values were on or below the detectable range of the method used. This was also the case for some of the EPSc measurements. The degrees of freedom and critical values are found in Appendix 1 and Appendix 2, respectively.
Table 2. Correlation matrix between all variables measured. Fe concentration, permeability and membrane resistance with 95% significance. Green cells indicate positive correlation and orange cells indicate negative. Non-colored cell indicate either no or very weak correlation (-0.4<r>0.4) or a correlation that did not pass the 95% significance test. For correlations between floc structure parameters and permeability or resistance the median value was used. Internal correlations of floc structure parameters are presented as mean-mean and median-median.

<table>
<thead>
<tr>
<th></th>
<th>SFI</th>
<th>MLSS</th>
<th>RH</th>
<th>Floc size</th>
<th>Floc circ.</th>
<th>Floc ratio</th>
<th>SMP prot.</th>
<th>EPSP</th>
<th>EPSc</th>
<th>EPS tot.</th>
<th>Fe</th>
<th>Perm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSS</td>
<td>0.106</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH</td>
<td>0.609</td>
<td>0.302</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floc size Mean</td>
<td>-0.459</td>
<td>0.020</td>
<td>-0.469</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med.</td>
<td>-0.532</td>
<td>0.066</td>
<td>-0.573</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floc circ. Mean</td>
<td>0.370</td>
<td>-0.316</td>
<td>0.393</td>
<td>-0.760</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med.</td>
<td>0.390</td>
<td>-0.273</td>
<td>0.390</td>
<td>-0.792</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floc ratio Mean</td>
<td>-0.334</td>
<td>0.144</td>
<td>-0.440</td>
<td>0.443</td>
<td></td>
<td>-0.744</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med.</td>
<td>-0.282</td>
<td>0.251</td>
<td>-0.394</td>
<td>0.714</td>
<td>-0.920</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMPp</td>
<td>-0.304</td>
<td>-0.445</td>
<td>0.173</td>
<td>0.458</td>
<td>0.245</td>
<td>0.290</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPSP</td>
<td>-0.299</td>
<td>-0.716</td>
<td>-0.570</td>
<td>0.778</td>
<td>0.670</td>
<td>-0.348</td>
<td>0.106</td>
<td></td>
<td></td>
<td>0.317</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.433</td>
<td>0.320</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPSc</td>
<td>0.143</td>
<td>-0.329</td>
<td>0.425</td>
<td>-0.330</td>
<td>0.482</td>
<td>-0.188</td>
<td></td>
<td></td>
<td></td>
<td>0.307</td>
<td>-0.141</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.526</td>
<td>0.404</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPS tot.</td>
<td>0.300</td>
<td>-0.232</td>
<td>-0.067</td>
<td>0.442</td>
<td>0.317</td>
<td>-0.197</td>
<td>-0.005</td>
<td>0.027</td>
<td>0.715</td>
<td>0.592</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.166</td>
<td>0.027</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.195</td>
<td>0.726</td>
<td>0.049</td>
<td>-0.074</td>
<td>-0.489</td>
<td>0.412</td>
<td>0.523</td>
<td>-0.780</td>
<td>0.515</td>
<td>-0.323</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.135</td>
<td>0.502</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perm. A</td>
<td>0.517</td>
<td>0.439</td>
<td>0.297</td>
<td>0.742</td>
<td>-0.580</td>
<td>0.446</td>
<td>-0.432</td>
<td>-0.055</td>
<td>-0.734</td>
<td>0.557</td>
<td>-0.095</td>
<td>-0.105</td>
</tr>
<tr>
<td>B</td>
<td>0.245</td>
<td>0.297</td>
<td>0.467</td>
<td>-0.359</td>
<td>0.426</td>
<td>-0.359</td>
<td>-0.119</td>
<td>-0.734</td>
<td>0.502</td>
<td>-0.116</td>
<td>0.028</td>
<td>1</td>
</tr>
<tr>
<td>Rb</td>
<td>-0.257</td>
<td>-0.278</td>
<td>-0.536</td>
<td>0.482</td>
<td>-0.320</td>
<td>0.297</td>
<td>-0.010</td>
<td>0.621</td>
<td>-0.348</td>
<td>0.178</td>
<td>-0.035</td>
<td>-0.881</td>
</tr>
<tr>
<td></td>
<td>-0.286</td>
<td>-0.330</td>
<td>-0.489</td>
<td>0.524</td>
<td>-0.358</td>
<td>0.302</td>
<td>0.078</td>
<td>0.698</td>
<td>-0.262</td>
<td>0.293</td>
<td>-0.050</td>
<td>-0.901</td>
</tr>
</tbody>
</table>

3.3 Correlations with filterability

Table 2 shows that SFI has positive correlation with RH and permeability and a negative correlation with floc size, i.e. a good filterability correlates with low RH, high floc size and decreasing permeability. Figure 13 shows the correlation plots of SFI with RH, floc size, permeability and resistance. The plots show that other than linear correlations sometimes fit the data better.
Figure 13. Correlations between SFI and RH, Floc Size, Permeability (membranes A and B) and $R_t$ (membranes A and B) with best fit curves shown. The $R^2$ values for the trend lines are RH: 0.37, Floc size: 0.39, Permeability: 0.33 (A) and 0.30 (B), $R_t$: 0.09 (A) and 0.12 (B).

Figure 14 shows SFI with RH and median floc size. The plot also indicates a positive correlation between SFI and RH, and a negative correlation between SFI and floc size.

Figure 14 confirms the positive correlation between SFI and permeability, i.e. a good filterability follows low permeability. SFI drops with increasing [Fe] and increases rapidly to normal conditions once [Fe] drops a little, only to drop again after the chemical cleaning on 22-23 April (Event 5).
Figure 15. Plot showing SFI, [Fe], permeability (membrane B) and $R_t$ (membrane B). Since the values of the two membranes are similar, membrane A is not shown in this plot to reduce noise and increase the simplicity and visibility of the plot.

Table 2 shows that among the floc structure parameters, only floc size have a correlation with SFI. Figure 16 shows the variation of the floc structure parameters together with SFI. The figure indicates that SFI have weak or no correlations with circularity and diameter ratio, and a weak negative correlation with floc size.

Figure 16. SFI plotted with floc structure parameters floc size, floc circularity and ratio of floc diameters.

The correlation between SFI and MLSS was also tested with SFI raw data i.e. without normalizing against MLSS. The correlation coefficient was 0.702 and the correlation plots are shown below in Figure 17.
3.4 Other correlations

Other correlations than those connected with SFI were found. Some of these include correlations between RH, floc size, floc ratio, EPSp, permeability and resistance.

3.4.1 RH

Apart from the correlation with SFI Table 2 shows that RH correlates negatively with floc size (-0.573), the ratio of floc diameters (-0.440), EPSp (-0.570) and resistance (-0.536), and positively with permeability (+0.742). Figure 18 shows RH plotted with floc structure parameters over time, where there is a positive correlation between floc size and ratio of diameters, and a negative correlation between these and RH.

Figure 17. Correlation plots of SFI filter time and MLSS.

Figure 18. RH plotted with floc structure parameters floc size, circularity and ratio of diameters.

Figure 19 shows correlation plots of RH with floc size, ratio of diameters, permeability and EPSp with examples of trend lines. These are the best fitting trend lines and have $R^2$ values of 0.50 (floc size), 0.56 (permeability membrane A), 0.38 (permeability membrane B), 0.16 (ratio) and 0.34 (EPSp).
RH and membrane resistance had negative correlation of -0.539 (membrane A) and -0.489 (membrane B) and is shown as a plot in Figure 20. The linear fits has a $R^2$ values of 0.30 (A) and 0.25 (B). Figure 20 shows that in April when the resistance increased, RH decreased.

**Figure 19.** Correlation plots of RH with floc size, ratio of diameters, permeability and EPSp.

**Figure 20.** Left: RH and resistance correlation plot. Right: RH (blue) and resistance (purple) with variation over time.

### 3.4.2 Floc size
Floc size has a positive correlation with EPSp and $R_t$ and negative correlations with SFI, RH and permeability. This is viewed in Figure 21 and Figure 22 where the correlation plots are shown.
Figure 21. Plot showing the correlation of floc size with SFI, RH and EPSp.

Figure 22. Floc size correlations with permeability and resistance. Dark green and purple represents membrane A, light green and purple represents membrane B. The $R^2$ values for the trend lines are: Permeability A 0.36, Permeability B 0.29, $R_A$ 0.26 and $R_B$ 0.30.

Figure 23 shows the positive correlation between floc size and EPSp concentrations, and the negative correlation between floc size and RH.
3.4.3 Ratio of floc max and min caliper diameters

The ratio of floc max and min diameters had negative correlations with RH and permeability. Figure 24 shows the correlation plots and ratio, RH and permeability over time. Ratio and RH has $R^2=0.20$. 

Figure 23. RH, median floc size and EPSp plotted together.

Figure 24. Up: RH, floc max and min diameter ratio and permeability. Down left: RH and ratio correlation with trend line. Down right: Permeability and ratio correlation.
3.4.4 EPSp
EPSp has negative correlations with MLSS, RH and permeability, and positive correlation with floc size and resistance. Figure 25 shows an increase of EPSp when permeability decreases and resistance increases.

![EPSp vs Permeability and R_t](image_url)

Figure 25. EPSp with permeability and R_t.

3.4.5 Permeability
Positive correlations were found between permeability and SFI, RH and floc circularity. Negative correlations were found between permeability and floc size, ratio of floc max and min diameters and EPSp.

Normalizing the permeability against fouling caused a linear raise of the permeability curve with increasing date and is an attempt in finding the true permeability since without fouling, the long term permeability would be higher. The plot is shown in Figure 26. The correlation coefficients were 0.494 and 0.383 for membrane A and B, respectively. This version of permeability was not used in other correlations.

![SFI and fouling normalized permeability](image_url)

Figure 26. SFI plotted against permeability normalized against fouling.
3.4.6 Resistance
Resistance had positive correlation with floc size and EPSp, and negative with RH.

4 Discussion
4.1 Overview
Figure 27 shows two plots with the variation of all factors over the whole experimental period. Some interesting trends in the correlations and some effects of external factors are seen and discussed here.

![Variation over time of all factors](image)

Figure 27. Up: Median floc size, EPSp, Fe and MLSS plotted over time. Down: SFI, RH, permeability, resistance and inflow plotted over time.

A closer look at Figure 27 shows that changes in most factors begin to happen in March. In the beginning of March, the chemical for iron dosage is changed and the initial dosage is low but subsequently raised (5-19 mg Fe/L). The dosage is based on the incoming water flow. The iron level is thus increased throughout the March and a week into April. During this increase of Fe concentration, floc size and EPSp increase and RH decrease. Filterability increases at the same time as permeability decreases.

The increase of Fe and drop of permeability can be explained by the heavy rainfall causing a high inflow of water. Since Fe is dosed proportionally with inflow but the inflowing water have lower concentration of organic matter due to the dilution caused by the rain, the Fe concentration increases, MLSS in the MBR increases and the permeability decreases. Fe has previously been shown to have a positive effect on permeability (Apostolopoulou Kalkavoura, 2014) but this decrease of permeability may be due to an excess of Fe which in turn affected other factors such as EPS, floc size and RH, more on this in section 4.6.
Permeability continues to drop during the first half of April, at the same time as EPSp and floc size increase and RH decreases. Some research states that EPSp is important for microbe interactions and are needed for flocculation (Le-Clech et al., 2006) and this study confirms that EPSp levels are correlates positively with floc size.

In mid-April, floc size and EPSp peak and SFI and RH reaches a bottom on April 17-19. As Fe drops, so does floc size and EPSp after peaking. SFI and RH both increase at the same time. SFI reaches a small peak on April 22th, which can be explained by a raise in RH and decrease of floc size, EPSp and Fe. SFI then drops with RH, and floc size and EPSp increase. After hitting a low on April 29th, SFI starts to increase with increasing RH and Fe, whereas floc size and EPSp seem unstable. Why SFI doesn’t drop with increasing Fe as earlier might be due to a delay in the change of sludge microbiology. Since the microbiology of activated sludge is a complex system with many different species changes in concentrations or operational settings may take weeks to be visible in the sludge microbiology. More on SFI and permeability in section 4.6.

4.2 Correlations
Some of the factors studied had a significant correlation with SFI and may as such have an effect on sludge filterability. The microbial factors with correlations with filterability were RH and floc size. All other factors had very weak or no correlation with SFI. When studying correlations it is important to remember that correlation does not imply causation, especially in complicated biological systems such as activated sludge in MBRs. It is also important to evaluate the correlations by plotting them since a high correlation coefficient does not always represent a good correlation, as seen in Figure 24 down right where ratio of diameters and permeability are plotted. The plot show poor correlation although the correlation coefficient is 0.4.

The correlation between SFI and permeability as well as some correlations between other studied factors will also be discussed here.

4.3 Filterability and RH
The correlation factor between SFI and RH was 0.609 which is well above the critical value (0.369), proving a relatively strong positive correlation. Since SFI is reversely proportional to filterability, this correlation means that as RH increases, filterability gets worse. Higher RH was thought to cause more interactions between microbes (which facilitates flocculation of microbes into larger flocs) and between microbes and membrane surface. Some reports state that microbes attached to the membrane are more hydrophobic than those in suspension, indicating that hydrophobic microbes attach easier to the membrane (Meng et al., 2006). This would not only cause blocking of the filter by formation of a biofilm and reversible fouling, but also increased deposition of particles causing irreversible fouling.

The result that higher RH decreases filterability is in line with the work of Meng et al. (2006) who found that an increase of RH had a strong correlation with an increase of membrane fouling resistance in MBRs (which should give worse filterability and higher SFI). It is however the opposite of the findings from Van den Broeck et al. (2011) who found that an increased RH also increased filterability while using the Delft Filtration Characterization Method (DFCm) to quantify reversible fouling. The DFCm is more similar to SFI than MBR resistance measuring but still gave different results. A plausible reason for this difference between results may be, among others, the differing hydrophobic properties of the membranes or filters used, and the microbiological composition of the sludge.
4.4 Filterability and floc size
The second factor to correlate with SFI is the floc size, which had a negative correlation. As for all the floc structure parameters this was measured in both mean and median values. The mean values proved to have weaker correlations than the median values, which is most likely due to the higher sensitivity for extreme values for the mean. Floc size had a correlation factor of -0.532 for the median which is far from the critical value -0.381. This means that larger flocs correlates with better filterability. One explanation is the theory that large flocs can form a secondary layer that protects the MBR membrane from irreversible fouling caused by small particles (Van den Broeck et al., 2010). This can also be the cause of the lack of correlation between EPS and SMP with filterability. This is however unexpected since SFI is an attempt to measure the reversible fouling, not irreversible. Since cake layer formation is the main cause of reversible fouling it is suggested that a decreased floc size facilitates cake layer formation. The result is in line with other studies confirming that deflocculation deteriorates sludge filterability (Alkmim et al., 2014; Meng et al., 2006; Van den Broeck et al., 2011).

4.5 RH, floc size and EPSp
A higher RH was thought to lead to flocculation and increased floc sizes due to increased microbe-microbe interactions. Instead, the negative correlation between RH and floc size indicates that higher RH leads to lower floc sizes. This correlation is not very strong but not insignificant. From Figure 17 it is clear that a linear relation is not the best correlation. It is seen that there might be a threshold RH percentage above which a linear negative relation might be found between RH and floc size, and below which deflocculation would occur rapidly – decreasing the median floc size. This threshold would be around 20% but needs more data to be confirmed.

RH was found to correlate negatively with EPSp. The correlation coefficient was -0.570, which is only slightly below the critical value of -0.531. The correlation is of average strength but close to the limit of being insignificant. Meng et al. (2006) found a positive correlation between RH and EPSp and states that most EPS proteins have mostly hydrophobic amino acids. This seems not to be the case at Hammarby Sjöstadsverk.

Floc size and EPSp have positive correlation coefficients of 0.778 (mean) and 0.670 (median), both well above the critical value of 0.532. This contribution of EPSp to flocculation and floc stabilization is also found in literature (Le-Clech et al., 2006) where lower EPSp concentrations tend to cause floc deterioration and membrane fouling. Tian and Su (2012) however found that high levels of EPSp might lead to a more loose sludge structure and poorer floc stability, together with higher RH.

4.6 SFI and permeability
One reason for choosing SFI as the method for filterability assessment was the result of previous research at Hammarby Sjöstadsverk stating that out of a few tested methods, SFI correlated the best with permeability (Apostolopoulou Kalkavoura, 2014). SFI was shown to have a negative correlation, i.e. good filterability (low SFI) correlated with good (high) permeability. The results of this study imply the opposite. SFI and permeability had correlation coefficients of 0.517 and 0.439 for membrane A and B respectively. These correlations are of moderate strength and above the critical value for significance (0.381). One major factor for this correlation is the decrease of both SFI and permeability following the events in late March and during April. However, as the permeability decreases SFI is expected to increase since bad permeability usually correlate with poor filterability (high SFI). One must however keep in mind that SFI only measures reversible fouling whereas the membrane permeability is also affected by longer-term fouling such as irreversible and irrecoverable.

The explanation to this may be that some factors may have a different effect on permeability than on filterability due to the different types of fouling affecting the filter and the membrane. As seen in
Figure 27 permeability seems more receptive to external factors such as inflowing water to the treatment line. The effect of inflowing water may be a push of sludge towards the end of the line, i.e. to the MBR. Also, as previous stated Fe levels correlated positively with permeability during an earlier study which indicates that higher Fe gives better permeability (Apostolopoulou Kalkavoura, 2014). The current study had however different operating conditions because the Fe dosing chemical was changed at the beginning of the experimental period, and the concentrations of Fe in the sludge was during this study twice those of the previous study (around 800-1400 mg L\(^{-1}\) compared to around 350-750 mg L\(^{-1}\) (Apostolopoulou Kalkavoura, 2014)).

SFI is a dead-end filtration method relying on gravity which is very different from the tangential flow filtration with transmembrane pressure configurations used in full scale MBRs. Lousada-Ferreira et al., (2014) therefore states that SFI might just be a measurement of sludge dewaterability instead of filterability.

To see the effect of long term fouling permeability was normalized against fouling as described in the work by Apostolopoulou Kalkavoura (2014) but this gave poorer correlation coefficients than without fouling normalization. A better estimation would have been to examine the permeability with pure water instead of sludge, but this was not possible in this study. Another way of connecting filterability and membrane performance is by looking at the membrane resistance. By subtracting the resistance caused by normal flux from the total resistance, the specific resistance caused by fouling can be measured (Meng et al., 2006).

As with all the factors, the correlation of SFI and permeability may be defined with more surety if measured for a longer period. Regardless, the conclusion is that the suitability of SFI as a filterability measurement for fouling monitoring is uncertain. Other common filterability methods include the DFCm (which is a more advanced setup quantifying the fouling potential), Capillary Suction Time, Time to Filter and Filter Test (Alkmim et al., 2014; Lousada-Ferreira et al., 2014). In a work to find the best filterability method to evaluate sludge quality Alkmim et al. (2014) found that Time to Filter best captures the variations in sludge quality. The same authors also concludes that filterability has the potential to forecast the fouling process and together with other parameters will allow for process optimization.

4.7 Conclusion

In literature discussing MBR foulants many parameters are shown to have different effects in different studies, making it difficult to find any scientific consensus on their effects. This is usually contributed to the differing composition of waste water and running operations of WWTPs, as well as the lack of standardized analytical methods (Drews, 2010; Hai et al., 2014; Le-Clech et al., 2006). It is consequently not surprising that the findings of this study are both consistent with and contradictory to other studies. The availability of previous works on the subject is also limited, especially on the effect of microbiological factors on sludge filterability.

The following important points were concluded in the discussion:

- RH has a negative effect on filterability
- Floc size has a positive effect on filterability
- RH has a negative effect on floc size
- EPSP\(e\) has a positive effect on floc size
- EPSP\(e\) has a negative effect on RH
- SFI might not be a suitable filterability measurement to monitor fouling
5 Acknowledgement

The author wish to thank Stockholm Vatten AB and IVL Swedish Environmental Institute for financing this study. Many thanks also to the following people for support, advice and help in various forms: Elin Ottosson (IVL), Hugo Royen (IVL), Jill Mattsson (Käppalaförbundet), Klara Westling (IVL), Niklas Dahlén (Stockholm Vatten), Peter Lindström (Stockholm Vatten) and Sofia Andersson (IVL).

6 Future work

Since the result of this study pertaining SFI and permeability conflicts with previous research it would be interesting to measure SFI for a longer period of time to see if it was caused by the abnormal stop of MBR operation in April. Other filterability methods could also be explored in order to find a correlation between filterability and permeability.

Some other factors than filterability could also benefit from a longer experimental period, especially EPSc, EPSp, SMPc and SMPp since a lot fewer data points were available for these than for other parameters. Generally, more data points give better assurance to the results and similar studies are often performed for a year or longer (Van den Broeck et al., 2011). Also, for carbohydrate quantification another method would be preferred as the detection limit was not low enough.
7 References


## Appendix

### Appendix 1. Degrees of freedom (number of data pairs - 2)

<table>
<thead>
<tr>
<th>SFI</th>
<th>RH</th>
<th>Mean Size</th>
<th>Mean Circ</th>
<th>Mean Ratio</th>
<th>Med Size</th>
<th>Med Circ</th>
<th>Med Ratio</th>
<th>SMIP</th>
<th>EIPS</th>
<th>EPSc</th>
<th>EPS Tot</th>
<th>Fe</th>
<th>MLSS</th>
<th>PermA</th>
<th>PermB</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH</td>
<td>23</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Size</td>
<td>25</td>
<td>23</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Circ</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Ratio</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med Size</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med Circ</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med Ratio</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMIP</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIPS</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPSc</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPS Tot</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLSS</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PermA</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>27</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>PermB</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>27</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Rt A</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>27</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Rt B</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

### Appendix 2. Correlation coefficient critical values for 95% significance

<table>
<thead>
<tr>
<th>SFI</th>
<th>RH</th>
<th>Mean Size</th>
<th>Mean Circ</th>
<th>Mean Ratio</th>
<th>Med Size</th>
<th>Med Circ</th>
<th>Med Ratio</th>
<th>SMIP</th>
<th>EIPS</th>
<th>EPSc</th>
<th>EPS Tot</th>
<th>Fe</th>
<th>MLSS</th>
<th>PermA</th>
<th>PermB</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH</td>
<td>0.396</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Size</td>
<td>0.381</td>
<td>0.396</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Circ</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Ratio</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med Size</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med Circ</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med Ratio</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMIP</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIPS</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>0.532</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPSc</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPS Tot</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>0.576</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.632</td>
<td>0.632</td>
<td>0.602</td>
<td>0.602</td>
<td>0.602</td>
<td>0.602</td>
<td>0.602</td>
<td>0.602</td>
<td>0.878</td>
<td>0.811</td>
<td>0.95</td>
<td>0.95</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLSS</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PermA</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PermB</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rt A</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rt B</td>
<td>0.381</td>
<td>0.396</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>0.374</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

32