Tampere University of Applied Sciences

# Impacts of temperature shock on membrane bioreactors

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BACHELOR'S THESIS
March 2020

Degree Programme in Energy and Environmental Engineering

## ABSTRACT

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Degree Programme in Energy and Environmental Engineering

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Bachelor's thesis 41 pages, appendices 5 pages
March 2020

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Operational parameters such as temperature can be a challenge for membrane bioreactors (MBRs) operated in regions with climate variations. This thesis aimed to evaluate the impacts of temperature shock on MBRs, focused on four aspects and their relationships: membrane fouling, extracellular polymeric substances (EPS), sludge characteristics and filamentous bacteria.

A laboratory-scale submerged MBR was used to demonstrate fouling tendencies in MBRs exposed to temperature shock. The MBR operated at 20°C for 18 days before the temperature was decreased to 10 ± 2°C, which was maintained for 10 days. Transmembrane pressure (TMP) was used to indicate fouling degree of the MBR. Extracellular polymeric substances (EPS) and SMP were extracted and analyzed for TOC, protein (PT) and polysaccharides (PS) concentration. Sludge characteristics and filamentous bacteria were assessed under the microscope.

The results of the experiment indicated that temperature shock had negative effects on fouling rate, regardless membrane replacement. Lower PS concentration of SMP was found after the shock, while PT concentration remained stable. Higher EPS concentration was measured in low temperature, in which majority were colloidal matters. The shock also weakened floc structure significantly, and both deflocculation and bulking were observed in the samples after the shock.

In conclusion, temperature shock is unfavorable for MBRs operation due to decreased filtration efficiency and changes in sludge characteristics. Standard methods should be developed for EPS and SMP extraction and analysis. Moreover, weakened floc structure may indicate decreased cell hydrophobicity in low temperature condition; thus, more in-depth study should be conducted concerning this hypothesis. From the fouling rate of the MBR, deflocculated sludge was concluded to have most impacts on fouling compared to bulking sludge. Conditioning strategies such as physical cleaning or dosing with coagulating reagents could be effective for fouling mitigation in MBRs operated under cold climate.

The materials for the thesis were supported by the Laboratory of Water Reclamation Engineering of Hokkaido University, under the guidance of Dr. Katsuki Kimura and Mr. Takayuki Kakuda.

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Key words: membrane bioreactors, temperature, membrane fouling, eps, smp, deflocculation, bulking

**CONTENTS**

## ABBREVIATION AND TERMS

| | |
|---|---|
| EPS | Extracellular polymeric substances |
| FI | Filament Index |
| HRT | Hydraulic retention time |
| MBRs | Membrane bioreactors |
| MLSS | Mixed liquor suspended solids |
| MLVSS | Mixed liquor volatile suspended solids |
| PS | Polysaccharides |
| PT | Protein |
| SMP | Soluble microbial products |
| SRT | Solid retention time |
| TMP | Transmembrane pressure |
| TOC | Total Organic Carbon |
| WWTP | Wastewater treatment plant |

# 1 INTRODUCTION

## 1.1 Developments of membrane bioreactors

Among different technologies for water reclamation, membrane bioreactors (MBRs) could be considered one of the best treatment methods that can produce excellent effluent quality to treat both industrial and domestic wastewater. An MBR, illustrated in Figure 1, consists of a membrane unit coupled with activated sludge, thus eliminates final sedimentation stage of conventional activated sludge. According to Krzeminski, Leverette, Malamis and Katsou (2017, 208), Henriksdal wastewater treatment plant (WWTP) of Sweden, capable of treating 864,000 $m^3$ of wastewater per day, is considered the world's largest MBR. Other countries such as United States and China also possess large MBR plants capable of treating hundreds of thousands cubic meter of wastewater per day. The growth of MBRs has increased rapidly in the last 15 years due to reduced membrane prices and optimized design and operation. MBRs have major advantages that common methods fail to meet: high treatment efficiency that can remove micropollutants, small footprint, fully automated operation system, aesthetic and odourless. These advantages enable MBR applications in decentralized WWTP and in facilities where stringent effluent limits are enforced, either for discharging into sensitive water bodies or for water reuse. (Krzeminski et al. 2017, 210.) Without final sedimentation, the technology is more resistance to bulking, which is a major problem in conventional activated sludge systems.

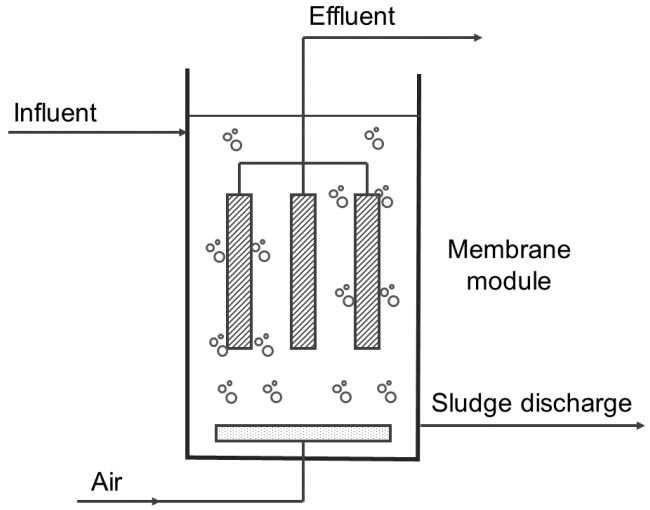



Figure 1. Diagram of submerged MBR set-up

## 1.2 Membrane fouling mechanisms

Over time, filtration efficiency of the membrane module reduces due to accumulation of various particles on the membrane. This phenomenon, also known as 'membrane fouling', obstructs wide-spread applications of MBRs and increases energy consumption, chemical usage, operational and maintenance costs (Krzeminski et al. 2017, 210). Hence, for the last 20 years, this has been the major topic for MBRs research, all devoted to the cause of membrane fouling and mitigation methods.

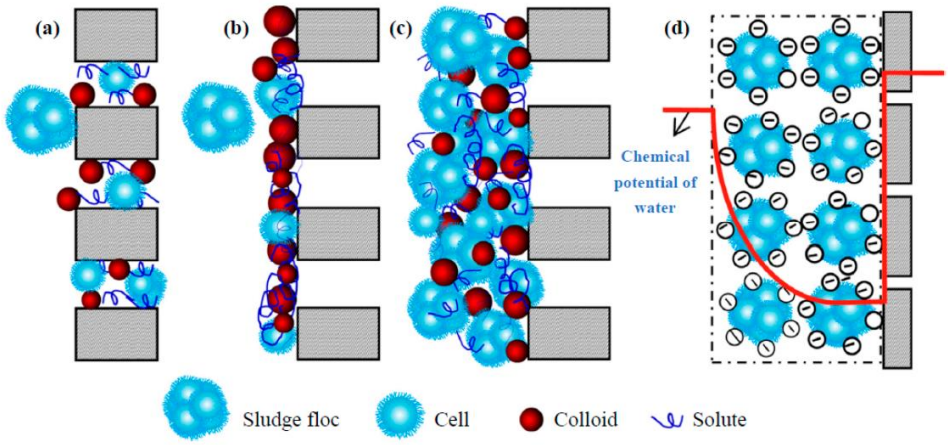



Figure 2. Modes of fouling: a) pore clogging, b) gel layer formation, c) cake layer formation, d) osmotic pressure effects (Figure by Gkotsis & Zouboulis licensed under CC BY 4.0)

Different fouling mechanisms displayed in Figure 2 are caused by different foulants. Pore clogging is caused by small particles such as solutes and colloids that enter membrane pores, reducing pore size. On the other hand, gel layer forms when colloids and suspended particles attach on membrane surface due to pore size rejection or pore clogging. Accumulation of these foulants over time results in a cake layer that covers membrane surface. However, if the cake layer has a significantly higher ion concentration, osmotic pressure effect will be formed due to different concentration between foulant side and permeate side, suppressing filtration efficiency of the module (Gkotsis & Zouboulis 2019).

Fouling is mostly categorized by removability, rather than fouling mechanisms. Commonly there are two types of fouling, physically reversible and irreversible

fouling. They both occur in the membrane module, in some cases one type can be more dominant than the other. Reversible fouling, which often occurs on membrane surface, can be removed by means of physical cleaning, such as air scouring and backwashing. Irreversible fouling on the other hand occurs in membrane pores and requires chemicals such as sodium hydroxide to extract the foulant.

## 1.3 Origins of fouling

Membrane performance was proven to be dependent on the relationship between operating condition, activated sludge and the membrane module. Membrane fouling can be influenced by the following factors (Rosenberger et al. 2006, 711):

- Membrane material and set up,
- Hydrodynamic condition: flux, transmembrane pressure difference (TMP), crossflow velocity,
- Operational condition: solid retention time (SRT), hydraulic retention time (HRT), temperature, influent and oxygen concentration,
- Properties of activated sludge: Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), extracellular polymeric substances (EPS), soluble microbial products (SMP), sludge's bio-composition, etc.

Temperature variation is one of the hardest operational parameters to control due to climate variations. This parameter was found to have significant impacts on membrane fouling, especially if SRT is short. Rosenberger et al. (2006) observed increased membrane resistance in lowered temperature, while Miyoshi et al. (2009) recorded impacts of both high and low temperature variations in an MBR with short SRT. Several factors can contribute to increased filtration resistance in MBRs in lowered temperature e.g. viscosity, declined sludge filterability. Moreover, at lowered temperature, declined microbial activity can have negative effects on pollutant removability of the MBR. Therefore, operation of MBRs in lowered temperature is unfavourable and optimization methods should be established for MBRs in locations with harsh climate.

Mixed liquor is a complex system consists of solid fraction and supernatant. The solid fraction includes flocs, suspended solids and fine particles, while the supernatant contains extracellular polymeric substances (EPS). EPS are products of cell excretions, cell material shredding, cell lysis and intake from the mixed liquor. They also play a role in protecting cells from environmental stress, maintain nutrients absorption and help creating flocs aggregates by binding cells togethers in a polymeric network. (Liu & Fang 2003, 237–238). EPS can be further categorized to colloidal and soluble fraction. Soluble EPS, also known as soluble microbial products (SMP), consists mostly of protein (PT), polysaccharides (PS), nucleic acids and humic substances, although impacts the latter two are often neglected (Liang, Liu & Song 2007, 95). SMP is popularly believed to be one of the key foulants in MBR, which can contribute up to 52% of membrane fouling at different testing condition (Liang et al. 2007, 96). However, multiple contradicts have been found between one study to another. This could be observed by Kimura, Naruse and Watanabe (2009, 1036) whose study show no correlation between membrane fouling and PS to PT concentration ratio of mixed liquor's SMP. On the other hand, Meng, Ladewig and Zhang (2011, 131) showed that higher PT to PS ratio of SMP reduces irreversible membrane fouling rate, which would be more beneficial in general. This raises questions on fouling potential of SMP, not only about the concentration but also the composition of it, in which PS is often regarded as the most damaging foulant.

Sludge characteristics and microbial composition are indicators of activated sludge's well-being. Floc structure contributes to dewatering capacity of sludge, while a balance and diverse microbial composition is crucial for effective pollutant removal. Filamentous bacteria play an important role in the system, act as the backbone of the system and connect small flocs together. However, their association with foaming and bulking in conventional WWTP requires strict monitoring with other parameters such as sludge volume index and MLSS. Although the impacts of bulking in MBRs are less significant in terms of effluent quality, filamentous bacteria should also be monitored to ensure efficient filtration process. Meng and Yang (2007, 55) observed a thick and dense cake layer on membrane surface caused by large flocs of bulking sludge. The phenomenon should receive more attentions in MBRs due to their impacts on membrane fouling and general wellness of sludge.

# 2 SCOPE OF THE THESIS

Climate change was anticipated to be one of the main challenges for wastewater treatment facilities in the next few decades. Heavy rainfall, which is a burden for wastewater treatment, has the potential to flood the facility and release untreated water to the environment. Extreme temperature changes also have impacts on biological treatments, decreasing treatment efficiency (Tolkou & Zouboulis n.d.). MBRs located in areas with temperature variance are also exposed to this risk. Thus, this thesis aims to assess the impacts of temperature shock on MBRs, focused on four aspects and their relationships: membrane fouling, EPS, sludge characteristics and filamentous bacteria. The results of the experiment will enable deeper understanding of fouling tendencies in MBRs exposed to temperature shock and could contribute in future applications of the technology in regions with climate variations. Moreover, as mentioned in section 1.3, there are multiple contradictions between the impacts of PT and PS concentration of SMP on membrane fouling (Kimura et al. 2009; Meng et al. 2011). The thesis will also seek solution to this debate based on the experiment set-up.

# 3 MATERIALS AND METHODS

## 3.1 Experiment set up

To successfully address the impacts of temperature shock on membrane fouling, a laboratory-scale MBR fed with synthetic wastewater was employed to simplify the temperature shock scenario and allowed overall control of the condition. The MBR used for the experiment was designed and set up by Laboratory of Water Reclamation Engineering of Hokkaido University (Sapporo, Japan).

### 3.1.1 Configuration of the MBR

The membrane module consists of three polyvinylidene difluoride (PVDF) flat-sheet membranes (Toray, Japan) with 0,1 μm nominal pore size, installed parallelly and submerged in an activated sludge tank. The sludge used to inoculate the MBR was acquired from a pilot scale MBR treating municipal wastewater in Sapporo city wastewater treatment plant. To control the temperature condition of the MBR, a low temperature circulation pump (EYELA, Japan) was employed to create a water jacket around the tank, allowing temperature adjustments.

A rolling pump (Masterflex L/S, USA) was used to create suction pressure and allowed liquid to pass through the membrane. The transmembrane pressure asserted by the pump was recorded with a digital pressure meter (Nagano Keiki, Japan) that recorded TMP value once every 10 minutes. This data can be extracted as an Excel file for analysis. To feed influent to the reactor, two rolling pumps (EYELA, Japan) were employed; one for concentrated synthetic wastewater, the other for tap water. These pumps were wired with sensors, which monitored the fluid volume and maintained constant HRT. As tank volume decreased during sludge withdrawal, the sensors would signal influent pumps to feed influent to the MBR. The full configuration of the MBR can be seen in Picture 1, in which the low temperature circulation pump and influent tanks were not shown as they were installed on the ground surface.


Picture 1. MBR set up

Annotation: 1. Activated sludge tank, surrounded by water jacket
2. Sensors
3. Effluent pump
4. Tap water pump
5. Concentrated feed water pump
6. Pressure meter
7. Sludge withdrawal valve

### 3.1.2 Operational parameters

The MBR operated under several constant parameters that are shown in the table below. Flux was maintained by increasing the flow the effluent pump which increases pressure asserted on membrane surface. To maintain constant SRT, a fixed volume of sludge was withdrawn every day. On the other hand, the sensors preserved HRT and effective volume of the tank. To optimize filtration process,

relaxation intervals were interweaved between filtration period, in this case the relaxation period happened once every 9 minutes of filtration.

TABLE 1. Operation condition on MBR

| Parameters | Unit | Value |
|---|---|---|
| Effective volume | L | 7,5 |
| Flux | $L/m^2.h$ or LMH | 18,2 |
| HRT | h | 7,5 |
| SRT | d | 15 |
| Membrane area | $m^2$ | 0,06 |
| Air | L/min | 15 |
| Filtration/Relaxation | min : min | 9 : 1 |

As mentioned in previous section, synthetic wastewater was used for influent. To avoid natural degradation of the influent, a fresh batch of concentrated influent was prepared on every day of the experiment. The concentrated influent was mixed with tap water with a ratio of 1:20 directly into the reactor tank. This produced a synthetic wastewater feed with COD approximately 500 mg/L. The content of the feed is given in the table below

TABLE 2. Feed content and concentration

| Components | Concentration (mg/L) |
|---|---|
| Glucose | 341 |
| Meat extract | 34,25 |
| Peptone | 102,25 |
| $NH_4Cl$ | 57,5 |
| $K_2HPO_4$ | 10,24 |
| $CaCl_2.2H_2O$ | 65 |
| $Mg_2SO_4.7H_2O$ | 25 |
| $MnCl_2.4H_2O$ | 0,28 |
| $FeCl_3.6H_2O$ | 2,42 |
| $NaHCO_3$ | 241 |

### 3.1.3 Reactor maintenance

During the operation of MBR, cleaning of the membrane was a necessary task as membrane fouling is inevitable. In this experiment, when TMP reached 25kPa, physical cleaning with a sponge was required to remove reversible fouling and maintain optimal operating condition. If fouling was still observed immediately after physical cleaning of the membrane, it can be concluded that irreversible fouling occurred. Thus, the membrane should be replaced and cleaned with sodium hydroxide solution for 24 hours in room temperature.

## 3.2 Experiment method

Prior to the experiment start date, the MBR remained in acclimatization period for three months to stabilize activated sludge's ecosystem. During acclimatization period, the MBR was maintained at 20$^{o}$C and received similar operational conditions as the experimental period. The following operational parameters of the MBR were monitored daily: pH, ORP, TMP, temperature, MLSS, MLVSS. These parameters must be stable for the experiment to start to avoid unwanted variations in the results.

The experiment started on day 0 with MBR temperature at 20$^{o}$C. To assess the impacts of temperature shock on the MBR, on day 18 the MBR was exposed to temperature shock of 10 ± 2$^{o}$C, which was continued for another 10 days. During the experiment period of 28 days, aside introducing temperature shock and adjusting flow rate to maintain constant flux, no other parameters were disturbed.

## 3.3 Analyses

### 3.3.1 Fouling monitoring

Membrane resistance is often used to characterize fouling in MBR. Due to accumulation of foulants on membrane surface, membrane resistance increases over

time at different rates i.e. fouling rate. Total resistance can be calculated using the following equation, which is commonly used by many researchers (Miyoshi et al. 2009, 5111; Wang, Wu & Tang 2009, 2507).

$$R = \frac{TMP}{\mu J} \quad (1)$$

where R is membrane resistance ($m^{-1}$), TMP is transmembrane pressure (Pa), μ is viscosity of the permeate (Pa.s) and J is permeate flux across the membrane ($L/m^2.h$ or LMH).

Proven by equation (1), if TMP and viscosity of the permeate remain unchanged during operation, higher membrane resistance will cause flux to drop in time. However, the MBR used in this thesis was operated under constant flux condition. In constant flux and viscosity, TMP has corresponding relationship with membrane resistance, shown in equation (1). Higher pressure should be asserted on membrane surface by increasing flow rate of permeate pump to maintain permeate flux. Thus, to simplify the analysis of the thesis, TMP was used to reflect fouling rate of the MBR. This data was extracted from the digital pressure meter of the MBR into an Excel file which allows analysis of the results.

### 3.3.2 Extraction and analysis of supernatant EPS and SMP

Supernatant EPS was extracted during sludge withdrawal by centrifuging the sludge at 5000 rpm in 10 minutes at similar temperature as the sludge itself. After the centrifugation, the liquid fraction separated from the sludge was extracted into a tube, hereby considered supernatant EPS. This supernatant was immediately filtered through a filter unit (Advantec, Japan) with 0,45 μm pore size. The filtered solution is regarded as the soluble EPS i.e. SMP, whereas the fraction retained by the filter is considered colloidal fraction. Supernatant EPS and SMP were frozen to preserve for analyses.

Protein (PT) and polysaccharides (PS) content of SMP were analysed using Lowry method (Lowry, Rosebrough, Farr & Randall 1951) and phenol-sulfuric

method (Dubois et al. 1956), respectively. Bovine serum albumin (BSA 98% Sigma, USA) was used as standard reagent for PT measurement, while glucose (Dextrose 98% Wako, Japan) was the standard reagent for phenol-sulphuric method. Since these analyses are colorimetric methods, the sample's absorbances were read using a spectrophotometer (Shimadzu, Japan), with PT measured at wavelength 750nm, and PS at 490nm. The results will be displayed equivalent to reagents' concentration, either mgBSA/L or mgGlucose/L.

Total organic carbon (TOC) was measured for supernatant and SMP samples to quantify these parameters using a TOC analyser (Shimadzu, Japan). The colloidal fraction, retained by the 0,45 µm filter unit, will be calculated by subtracting the TOC value of supernatant by TOC of SMP. Prior to measurement of TOC, the supernatant was homogenized using ultrasonic homogenizer for three minutes to mix the sample and break any remaining solids that might clog the TOC analyser.

### 3.3.3 Assessment of activated sludge properties

The methods for activated sludge properties analysis were done in accordance with the microscopic investigation manual by Eikelboom (2000). The sludge was analysed under three magnification objectives: 20x, 100x and 200x. The first magnification enables general observation of sludge structures and distribution of flocs under the slide. The second magnification was used most intensively, as it gives the most information on sludge morphology, floc size and filaments population, which is later used to establish the Filament Index (FI). This magnification was also used to count the flocs on the slide. The largest magnification, 200x, was used to indicate the filaments' shapes and identify some organisms.

The analysis was done entirely using a microscope (OLYMPUS, Japan). A camera (OLYMPUS CAMEDIA, Japan) was attached to the microscope and captured images from it. These images enabled further evaluations of the sample in the future. The set up of the microscope is indicated in the picture below.


Picture 2. Set up of the microscope

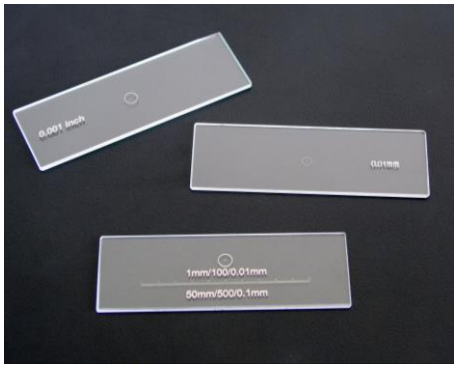
Annotation: 1. Microscope; 2. Camera; 3. Computer for previewing the image

Sampled was picked using a plastic pipette at the same spot of the reactor, which was a few centimetres below the water surface of the tank. One drop of sample was then immediately transferred to a microscope slide (MATSUNAMI, Japan). A cover slip was placed on top of the sludge drop to secure the sample. For each sampling date, two samples were collected to verify the results.

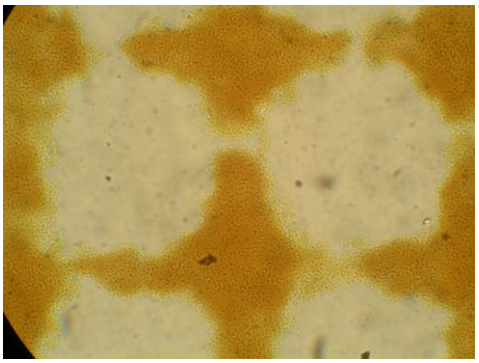
The sample was first observed at 20x and some photos were taken. The sludge's general properties were recorded to the datasheet (Appendix 1) modified from the analysis form by Eikelboom (2000), and the largest floc's size was identified. Next, the sample was observed under 100x magnification, which contains the most information needed for the analysis of sludge characteristics, defined by sludge morphology, filamentous bacteria, free-living cells and protozoa/metazoa population of the sludge. The latter parameter was assessed on the scale from 0 – 3, which is equivalent to none to numerous cells per slide. For the free-living cells, the scale is narrowed to field of view scale, where 3 is equivalent to hundreds of cells per field of view. And last, at 200x magnification, some organisms were assessed closely to identify their species.

The images acquired from the camera were processed with ImageJ software to convert the coloured images to grayscale images, which reduce bias of colour and enhance the contrast between sludge and surrounding liquids. The ImageJ software also allows setting a length scale for the image. This can be done using a stage micrometre (Picture 3), while in this experiment, a slide with of 0,5mm

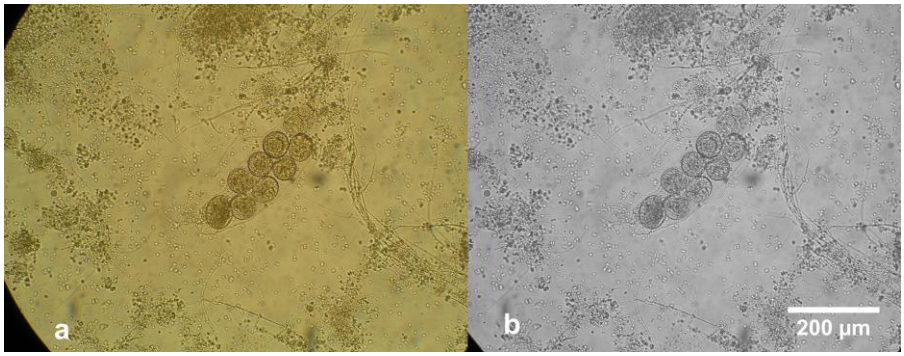
grids was used to calibrate the images (Picture 4). After the scale was established, a scale bar can be placed on the image which indicates the size of the object in the photo. The post-processed result can be seen in Picture 5b.




Picture 3. Stage micrometre (WPI n.d.)



Picture 4. Gridded slide for calibration




Picture 5a & 5b. Original image at 100x magnification (a) and processed (b)

### 3.3.4 Assessment of filamentous bacteria

Filamentous bacteria were assessed by the Filament Index (FI) onsite and using the images acquired from the microscope. FI was used as a tool to estimate the number of filamentous bacteria outside of the floc, weighted by scale 0-5, where 5 is equivalent to numerous filaments per floc. To assess FI, the sample's flocs were compared with a reference image (Appendix 2) by Eikelboom (2000). By comparing the number of filaments surrounding the floc with the reference images, the FI value could be determined for the sample. FI is commonly assessed by visually averaging FI value of flocs on the slide (Eikelboom 2000). However, in this thesis, each floc's FI were recorded to a datasheet and logged to Excel for analysis and enabled better statistical comparison between samples.

# 4 RESULTS AND DISCUSSIONS

## 4.1 Fouling rate

Fouling rate of the MBR weighted by TMP increment rate is displayed in the figure below. The vertical axis indicates both TMP and temperature, while the horizontal axis shows the operational days starting from day 0 to end of day 27. The temperature shock is represented by the red vertical line that clearly separates two different periods of the MBR: normal and lowered temperature stage. The thin arrows mark physical cleaning, while the thicker arrows mark that the membrane was changed.

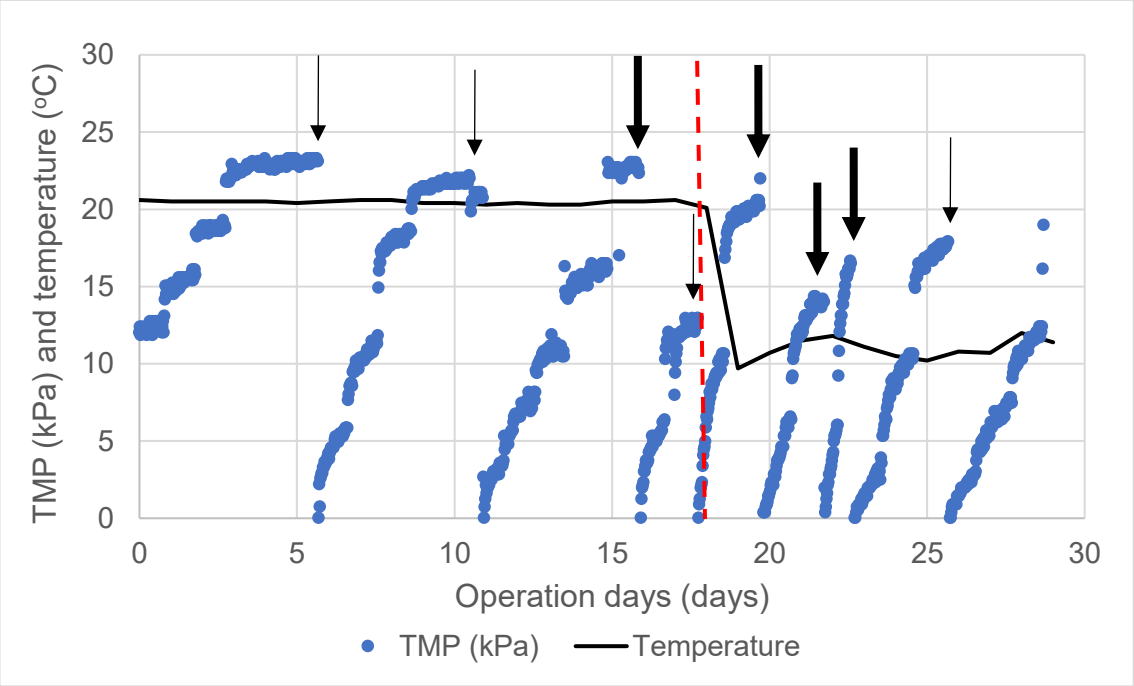



Figure 3. Membrane resistance indicated by TMP increment rate

In the first period, when the temperature of the MBR was maintained at 20°C, the membrane received several physical cleanings whenever TMP reaches 25kPa. It took 3 to 4 days for TMP to reach 20 kPa and 5 days to reach 25 kPa. This rate was considered reasonable and after physical cleaning, membrane resistance was seen to have similar increment trend. Temperature of the MBR was stable during this period at around 20,4°C.

After physical cleaning of the membrane of the 18th day, temperature was decreased to 10°C. Immediately, the increment rate of TMP increases rapidly, from 0 to 22kPa in 2 days thus changing of the membrane was required to sustain the MBR. On the following days, the similar trend was observed even with membrane replacement. A membrane breakage incident happened consecutively on 22nd and 23rd operation day that caused the influent come out cloudy, thus changing of membrane was needed two days in a row. After the incident, it was advised by the research supervisor that the optimal TMP range should be under 20kPa to avoid damaging effects of fouling. Thus, on the 25th day, physical cleaning of membrane was needed before TMP reach 20kPa.

It was clearly seen that the increment rate after temperature shock was gradually higher, as it took only 2 days for TMP to hit 20 kPa. On the other hand, in normal temperature operation, it was 3 to 4 days. The raise was most visible on the 18th day when temperature shock occurred. Nevertheless, on the last few days of the experiment, TMP increased with a stable rate and reached 20kPa limit after 3 days, similar to normal temperature operation. This suggests that the MBR might recover from the shock if it is operated for a longer period after the shock. This thesis however failed to conclude on this matter due to short-term experiment period.

Higher membrane resistance in lowered temperature was also observed in other publications (Chiemchaisri & Yamamoto 1994; Rosenberger et al. 2006). This can be concluded by several factors: higher viscosity of the liquid fraction at lowered temperature, and considerably lower sludge filterability, which enhances fouling. Even if viscosity is taken into account and membrane resistance was adjusted accordingly, fouling tendencies was still observed in lowered temperature (Rosenberger et al. 2006). Therefore, low temperature has more detrimental effects on MBRs operation than just increasing the viscosity of permeate, for instance on filtration and permeate quality (Chiemchaisri & Yamamoto 1994). It can also lead to membrane breakage observed in the experiment. Thus, from the results of this thesis and other publications, it can be concluded that temperature shock has negative impacts on membrane resistance, and long-term operation of MBR in lowered temperature is undesirable in terms of membrane life span.

## 4.2 Supernatant and SMP characteristics

Six samples were collected throughout the experiments, the first three samples were retrieved on the first stage of the experiment on day 4, 14 and day 18 prior to lowering the temperature, while the remaining were collected in the low temperature period, on day 20, 24 and 27. These samples were used for both PT and PS analysis, as well as TOC measurements.

### 4.2.1 Protein and polysaccharides concentration of SMP

The concentration of SMP, characterized by its PT and PS content is displayed in the figure below, displayed as mgBSA/L or mgGlucose/L.

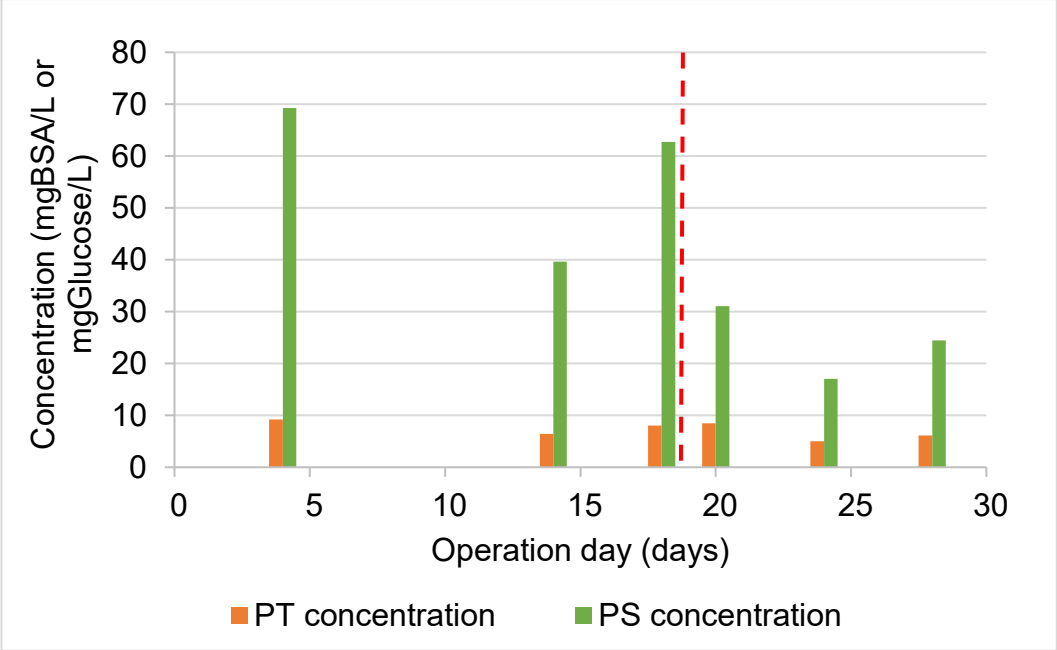



Figure 4. PT and PS concentration of SMP with operation day

The figure clearly defined two different stages of the experiment by the red line. Generally, PT fraction of SMP remained stable through all 6 samples, indicating that temperature shock did not disturb the generation of PT in SMP. The average concentration of PT was 7,2 mgBSA/L, with the lowest value of 5 mgBSA/L on day 24. On the other hand, the concentration of polysaccharides after the shock was considerably smaller than in normal temperature. Again, on day 24, the lowest concentration of PS was found, which was only 17 mgGlucose/L. This value

was significantly lower than the samples collected on the 4$^{th}$ day of the experiment, which was 69 mgGlucose/L.

The findings of the experiment were contradicting with multiple researches which has proven that higher concentration of polysaccharides is related to higher fouling rate (Rosenberger et al. 2006, 715; Brink et al. 2011, 4496–4497). The similar analysis method for PS was used by Brink et al. (2011, 4494), who has found that low temperature was responsible for the increment of polysaccharides in the soluble fraction of supernatant. From this, the research concluded that polysaccharides could be linked to higher fouling rate. However, it was also found that standard deviation of the measurements was high, thus more reliable analysis method should be developed (Brink et al. 2011, 4496). Therefore, using only polysaccharides concentration to conclude on the fouling situation of the MBR is insufficient, and further analysis is needed. Based on similar conclusions, Kimura et al. (2009, 1034) had employed advanced analysis methods to characterize fouling which returned positive results. The research concluded that excitation - emission matrix (EEM) fluorescence spectroscopy was an effective investigation tool to study fouling in MBRs (Kimura et al. 2009, 1038).

Overall, it could be concluded that the concentration of different fractions of SMP was insufficient to characterize fouling in MBRs. First, MBR is a complex and dynamic system, and results of concentration could be considered temporary and inadequate to characterize fouling. Second, there is no standard method to extract and analyse SMP (Kunacheva & Stuckey 2014, 14). Some extraction methods can yield higher content than others; therefore, results may vary between researches. Last, due to different operation parameter and sludge condition, it can not be concluded that one result is correct, and others are false. Similar to activated sludge, MBRs is dependent on operational parameters such as HRT, SRT, etc. Different operation parameters and configurations could greatly affect the content of supernatant and SMP (Gkotsis & Zouboulis 2019). Therefore, the results of this experiments were insufficient to conclude that PT and PS concentration have strong relationship with fouling in MBRs.

### 4.2.2 TOC analysis of SMP and supernatant

TOC analysis can be an efficient tool to quantify the concentration of SMP and supernatant. Due to loss of sample, the samples drawn from day 4 and day 27 were not available for TOC analysis. Therefore, only four samples were assessed using this method. The results of TOC analysis are displayed in the figure below.

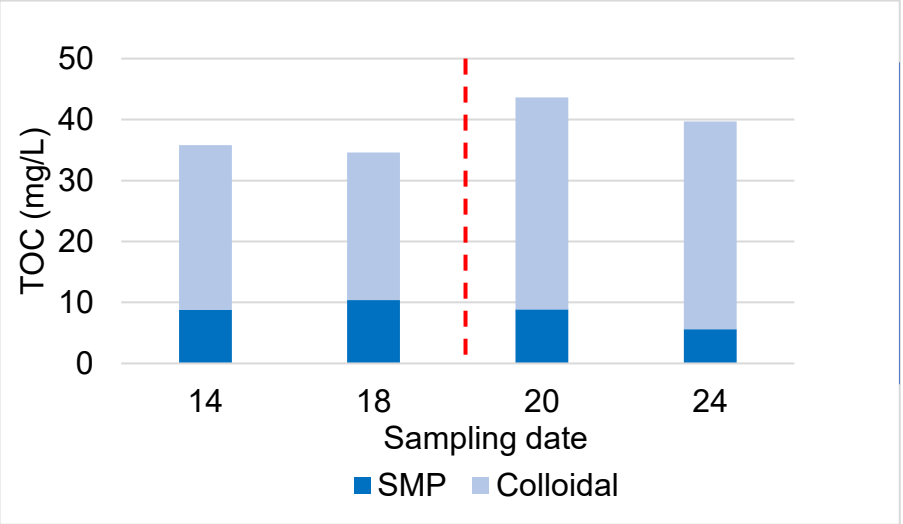



Figure 5. Quantifying SMP and colloidal fraction of supernatant by TOC analysis

Similar TOC values approximately 35 mg/L were found for supernatant in normal temperature, while an SMP content of 10 mg/L was measured for these samples. After the shock, the supernatant content was 15% higher compared to normal condition, while SMP concentration reduced slightly. These results do not align with the theory that more SMP is produced in stressed condition, such as extreme temperature stress (Azami, Sarrafzadeh & Mehrnia 2012, 3). On the other hand, the colloidal fraction had increased about 30% after the shock was introduced. This result indicated that rather than soluble fraction, colloidal fraction was released more intensively. Similar results were found by Remy, Temmink, Brink and Rulkens (2011, 405) in which higher colloidal fraction of supernatant was measured at 14°C, compared to sludge at 20°C.

It can be concluded that more colloidal particles are released in MBR exposed to temperature shock. It is possible that the colloidal matters were released as a coping mechanism to protect the cells from the shock. Therefore, from this result and the fouling rate observed in Figure 3, the colloidal fraction is expected to be associated with membrane fouling, rather than soluble fraction of supernatant.

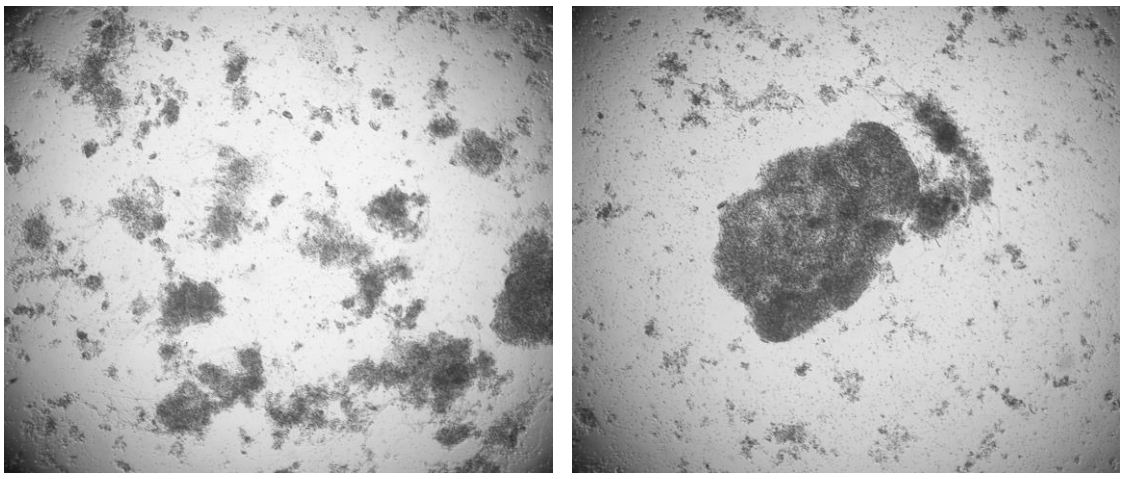
However, due to different fouling mechanisms of soluble and colloidal particles, more specific analyses should be conducted to determine which fraction has more influences on membrane fouling.

## 4.3 Sludge structure and characteristics

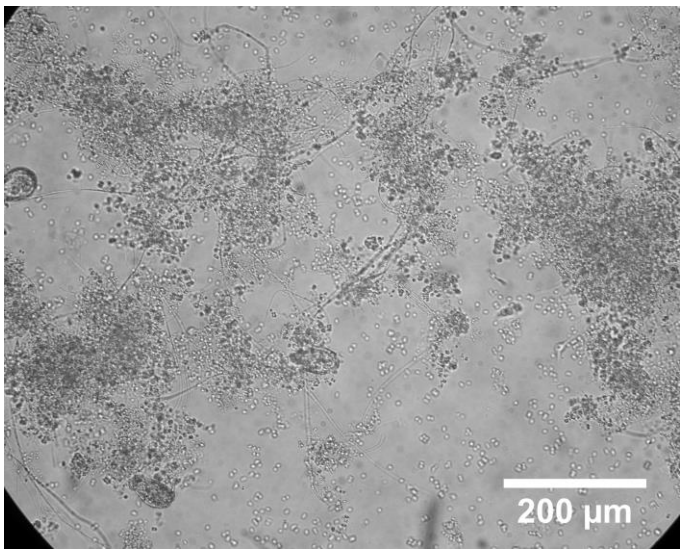
The sludge was analysed on day 7, 13, 19, 24 and 27 of the experiment. The first two were used as reference of normal sludge, while the remaining samples should provide how the sludge reacts to sudden temperature changes.

### 4.3.1 Changes in floc structure

In the first period of the experiment, although samples taken on day 13 has slightly better structure, both samples had similar characteristics and similar floc morphology. Regarding the size, more than 80% of the flocs are 0,2mm or above, with the biggest floc diameter of 1,5mm to 1,8mm (Picture 7). The flocs were rounded, with a few exceptions of irregularly shaped flocs that were surrounded by filaments. Clear difference between flocs and liquid fraction was seen (Picture 6 and 8), and the bacteria that formed the floc were dense and closely bounded. Only a few small flocs were weak and loosely bound together. Thus, it can be concluded that the flocs were compact and robust, and the sludge was in good condition.

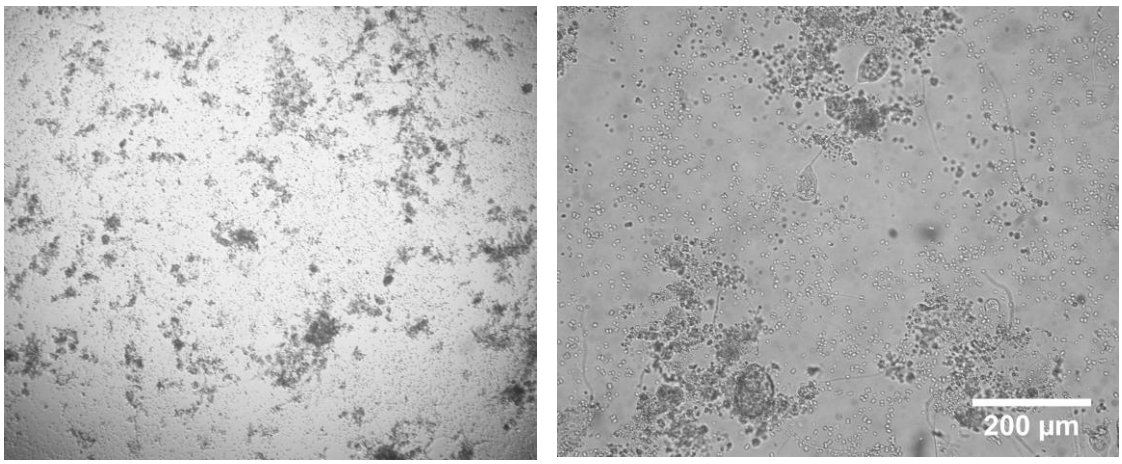


Picture 6 & 7. Sludge structure on day 7 (left) and biggest floc of day 13 (right)



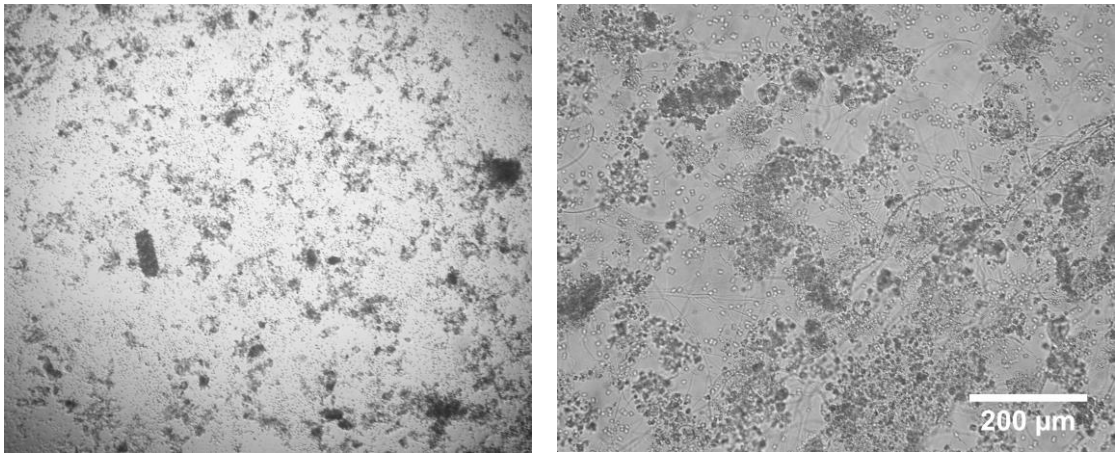

Picture 8. Compact flocs on day 13

In the following period, dramatic changes in structure were observed. On day 19, the size of floc drastically reduced to the point where 60% of the flocs are smaller than 0,2mm. The biggest floc observed from both samples has a diameter of 0,8mm, which is only 40% the diameter of the biggest floc in day 13. It was recognized that the floc has broken down due to the shock and became weaker, less compacted (Picture 9). Around the edges of the flocs, many bacteria were weakly bonded to each other, thus the flocs were open and weak (Picture 10). The increased number of flocs due to broken down of bigger flocs hindered the feasibilitiy to count and assess FI for each floc due to limited scanning time, which should be done in less than 30 minutes before the slide dry out.



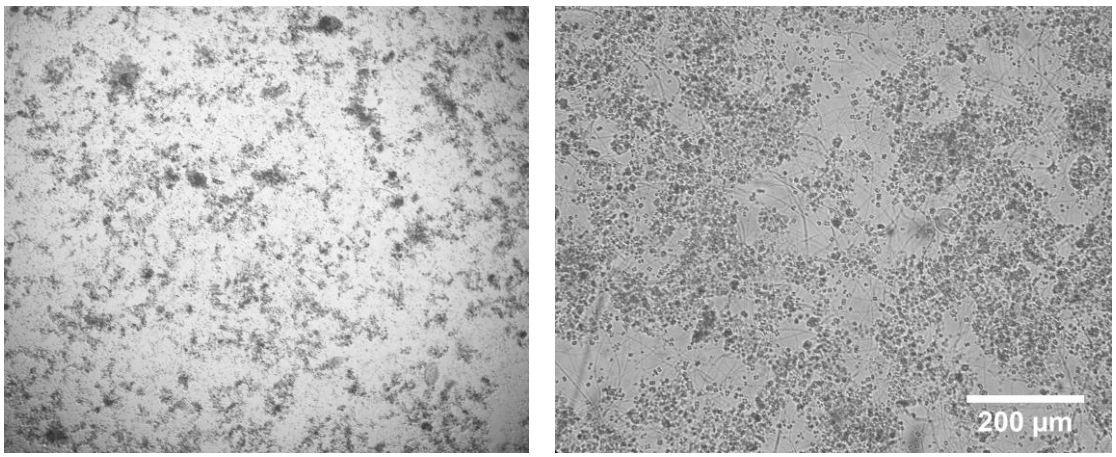

Picture 9 & 10. Loose floc structure on day 19 (left) and open flocs (right)

On day 24, dense floc distribution was found in the sample observed at 20x (Picture 11). The biggest floc observed had a diameter of 0,7mm, suggesting that the size of the floc remained as small as on day 19. Most of the flocs were around 200µm to 250µm and were bonded together by filaments to form a matrix. Smaller flocs had irregular shape to due to their open, weak structure (Picture 12).

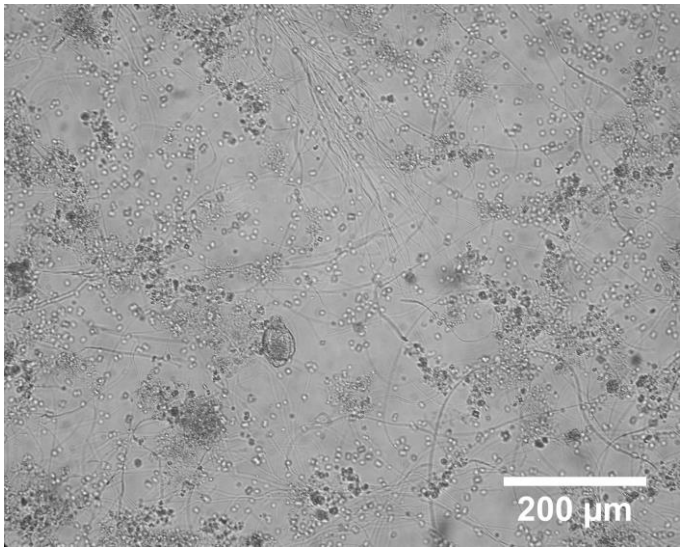



Picture 11 & 12 . Floc distribution (left) and loose floc structure (right) on day 24

On day 27, filamentous bulking situation was observed in the samples. The details on the bulking will be discussed in proceeding sections regarding filamentous bacteria, this phenomenon however had significantly influenced sludge structure. The biggest floc has a diameter of 1,6mm that consisted of medium-sized flocs and filaments bound together in a tight-knitted web. The flocs typically ranged from 100 µm to 200µm while with some in between 200-400 µm. The microbes inside the flocs held together very weakly (Picture 14 and 15). The structure can be observed in 20x magnification, which clearly show its dispersed structure (Picture 13).




Picture 13 & 14. Filament matrix structure (left) and filament web (right) of day 27



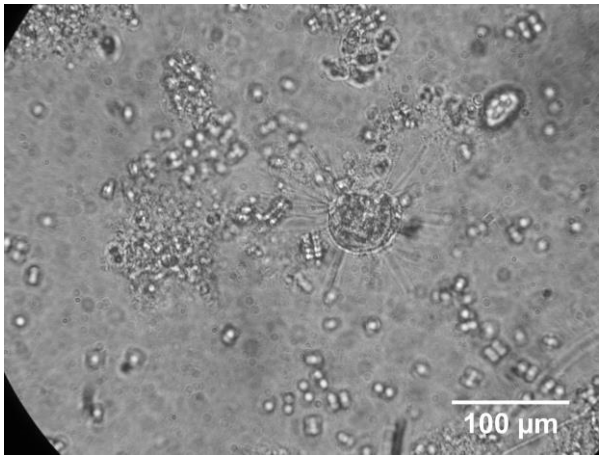

Picture 15. Deflocculated, small flocs observed on day 27

In conclusion, interesting changes in floc structure was observed: initially, a very stable sludge structure was established, the flocs were rounded, compacted and robust. After the shock, the flocs first disintegrated, then bulking occurred, thus created a filament structure that bind the broken flocs into a complex matrix of flocs. Regarding the floc's weak structure after the shock, it can be anticipated that hydrophobicity of cells' wall decreases at lowered temperature, since the bacteria inside the flocs are more likely to attach to liquid rather than binding together, causing flocs to have weak, open structure. This theory is yet confirmed based on available literatures, and detailed analyses on relative hydrophobicity of flocs could be done to confirm the hypothesis.

The experiment clearly shown that deflocculation occurred at low temperature, which drastically reduced floc size and probably stimulated the release of colloidal fraction of supernatant observed in TOC analysis. Similar deflocculation phenomenon was observed by Brink et al. (2011, 4495, 4497), whose experiment confirmed the reduction of particle size in when the temperature was decreased to 7$^{o}$C. Brink et al. (2011, 4494) used a particle size analyser to reach this conclusion, while the images acquired from the microscope were also sufficient to confirm the results.

### 4.3.2 Changes in sludge diversity

The ecosystem within the sludge was diverse in normal temperature period. Numerous active free-swimming rotifers and a few inchworming species were seen. Several ciliates species including *Colpidium*, *Carchesium* and *Aspidisca* were found. Aside from ciliates and rotifers that are frequently seen in activated sludge, several amoeba and heliozoa (Picture 16) were observed. Nematodes and worms that are known to consume flocs were also found at low number, around 10-20 individuals per slide. High diversity in the sludge indicates healthy sludge, as there are different organism species to break down organic matters from the influent.




Picture 16. Heliozoa observed on day 13

After the shock was introduced on day 18, the sample on day 19 indicated that protozoa and metazoan were the first organisms to respond to the shock. No worm was observed in both samples, and only a few nematodes were seen. The diversity from previous weeks was reduced greatly, the sample did not contain any heliozoan or tardigrade. The most resilience organisms were ciliates and rotifers, which had existed in the sludge since acclimatization period. Few free-moving ciliates were seen, but the number of sessile ciliates remained unchanged. Rotifers remained active and numerous. The table below concluded the assessment of protozoa/metazoa population of the sludge throughout the experiment.

TABLE 3. Protozoa/Metazoa population evaluation of sludge

| Organism | Sample date | | | | |
|---|---|---|---|---|---|
| | **7** | **13** | **19** | **24** | **27** |
| Ciliates | 2 | 3 | 3 | 3 | 2 |
| Flagellates | 0 | 0 | 0 | 0 | 0 |
| Amoeba | 0 | 2 | 1 | 1 | 1 |
| Testate amoeba | 1 | 1 | 1 | 1 | 1 |
| Heliozoa | 0 | 1 | 0 | 0 | 0 |
| Rotifers | 3 | 2 | 2 | 2 | 2 |
| Nematodes | 1 | 1 | 1 | 1 | 0 |
| Worms | 2 | 1 | 0 | 0 | 0 |
| Free living cells | 2 | 2 | 2 | 2 | 3 |

From the evaluation of 24$^{th}$ and 27$^{th}$ day, clearly the diversity of the sludge did not seem to improve after the shock. The samples were still heavily populated by ciliates and rotifers, and 'higher' organisms such as tardigrade or worm were not observed. Interestingly, the number of free-living cells was extremely high on the 27$^{th}$ day. In Picture 15, the solution surrounding the flocs were clouded by the free-living cells. This condition could be explained due to the deflocculation condition of the sludge that causes the flocs to break down to cellular level, which is extremely undesirable for viability of activated sludge.

In conclusion, very low sludge diversity was observed after the shock, proving temperature's adverse effects on sludge wellness and probably MBR's effluent quality. This can be proven by comparing parameters such as $PO_4^{3-}$, $NO_3^-$, BOD, COD of the effluent and influent. In addition, the mass death of cells and sludge microfauna due to sudden temperature could explain the increment of colloidal fraction in supernatant. However, these matters were not discussed as it is out of the scope of the thesis. More in-depth analyses should be conducted to verify the hypothesis.

## 4.4 Filamentous bacteria

### 4.4.1 Filament Index (FI) and fouling

Each sludge sample was measured in duplicate to verify the results of FI analysis, which were counted and recorded. The outcomes of duplicated samples (Appendix 3) indicated similar trends and had small deviation; thus, the results of FI analysis, shown in the chart below, was averaged between two samples. The average FI of the samples on day 7, 13 and 19 belonged to grade 2, while day 24 had an average value of 3. The highest FI recorded was for day 27, with an average FI of 4. It could be seen that the number of flocs increases with the operation day, due to the deflocculation phenomenon described previously.

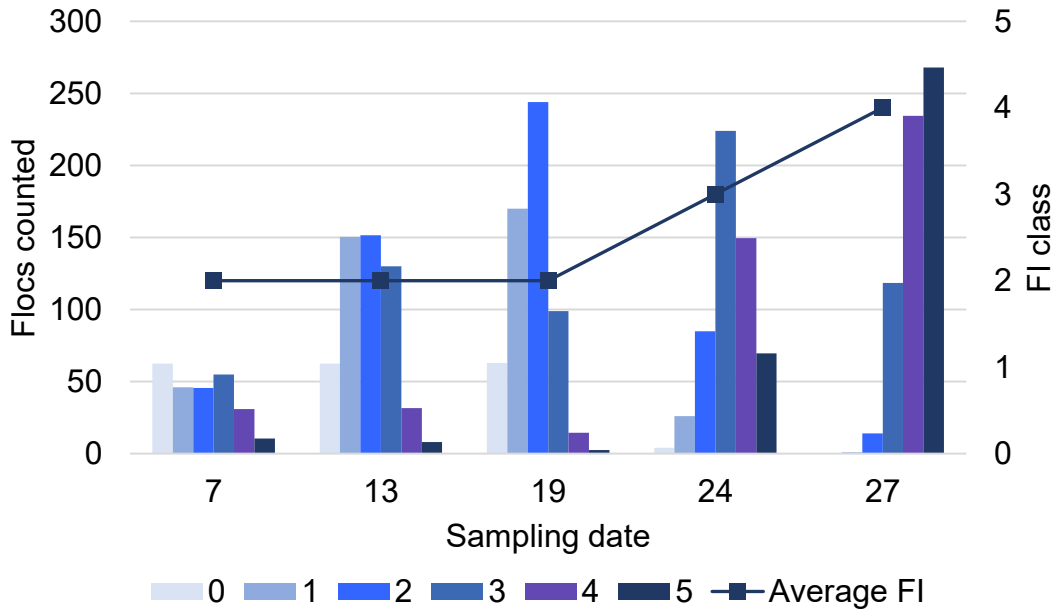
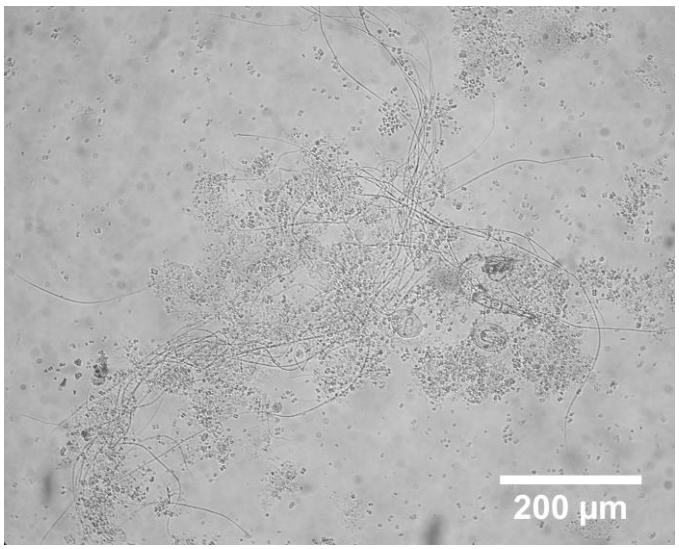


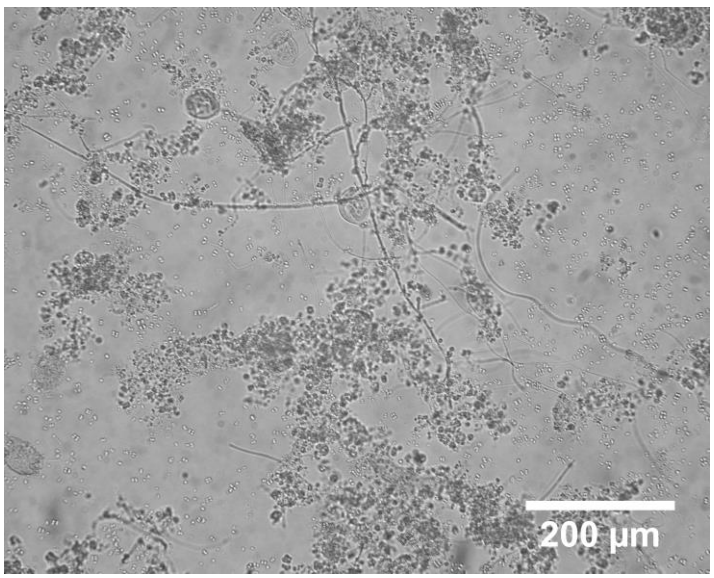

Figure 6. FI assessment of samples

In the normal temperature stage, the amount of class 5 flocs was very low comparing to other classes, with less than 20 flocs per slide. This indicates that filamentous bacteria were not the dominant organism in the ecosystem. They maintained their role as the backbone of activated sludge process: to maintain floc structure and form aggregates. The filaments found in the sludge had long, bent structure that ran through and around the flocs (Picture 17), affecting the shape of some flocs. Filaments were also observed to be free instead of being attached to flocs.




Picture 17. Filament structure found on day 9

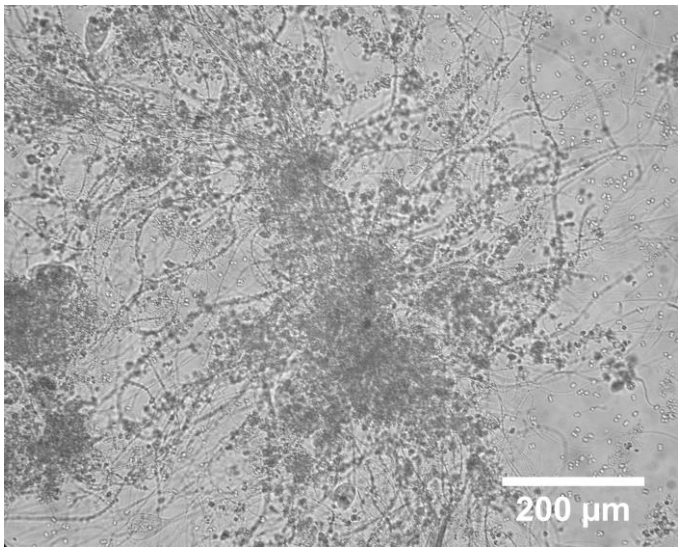
On day 19, a dramatic change in sludge structure was observed and filamentous bacteria were also affected. In this sample, many flocs had short filament surround them, with relative FI value of 2. The filaments became short and crumbled together instead of being long and slightly bent (Picture 18). Deflocculation might be associated with this phenomenon. Very few flocs had FI=5, while 50 to 60 percent of the flocs belonged to FI class 2 and 1. Therefore, it could be concluded that temperature shock had influenced the structure of flocs and caused deflocculation, which reduced the number of filaments in the flocs.




Picture 18. Irregular filament shape

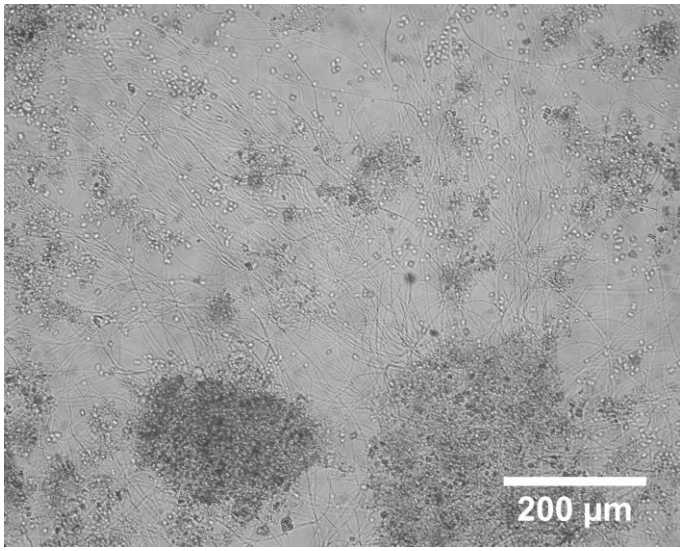
However, the sample on day 24 recorded a remarkable growth of filamentous bacteria, suggesting that they were not affected long-term by temperature shock.

After nearly a week following the shock, the filaments population increased dramatically. Small flocs were tied together by dense filament that results in aggregates with FI=5. These flocs however were distributed sparsely, but still connected by filaments. The filaments found in this sample were long and branched, suggesting they might be *M. parvicella* which is notorious for its *en masse* growth in cold temperature and associations with bulking and foaming (Eikelboom 2002, 64). In conclusion, instead of forming bigger flocs with low to medium FI, a web of small flocs bundled by long, dense filaments were created (Picture 19). An interesting point to note, is that whether this has any impacts on filterability or dewatering property of the sludge. Since the flocs were open, and held together by filaments in a web, they created space for water retention that could be responsible for decreased filterability of sludge (Seviour & Nielsen 2010, 201).




Picture 19. Bulking sludge on day 24

On day 27, the sample had FI grade 4, which was the highest FI recorded in this MBR. The filaments could be described as long, bent and twisted bundles with branches (Picture 20), although it was unclear whether they were real or false branching. The webs of flocs and filaments were large and distributed entirely on the slide. Nearly no flocs had FI=0 and 1, while up to 250 flocs belonged to FI class 5, which proved the overgrowth of filamentous bacteria in the system. Moreover, severe bulking situation obstructed the analysis due to unclear filaments distribution on flocs. In conclusion, it seems that elongated operation time of MBR under lower temperature condition had induced sludge bulking, which may lead to severe cake fouling.




Picture 20. Severe bulking observed on day 27

The results of microscopic analysis of activated sludge have shown that temperature shock can damage sludge structure and mircrofauna stability. After the shock, the sludge deflocculated, significantly reduced the number of filaments. Regardless of this, after 5 days, bulking occurred with the broken flocs, creating bundles of small flocs surrounded by filaments. The most dramatic bulking situation was observed on day 27, with the highest number of flocs with FI=5.

In MBRs, both deflocculation and bulking were observed to have detrimental effects on fouling in the membrane modules. According to Meng and Yang (2007, 55), deflocculated sludge induces irreversible fouling by adsorption and accumulation of colloids or solutes into the pores of membrane and the cake layer. For bulking sludge, fouling is enhanced by accumulation of flocs on membrane surface due to irregular floc shape and high viscosity. In this experiment, a sharp increase of TMP was observed in deflocculated stage, while for the bulking stage, the increment was less visible. Therefore, deflocculated sludge had more pronounced effects on membrane fouling than bulking sludge.

In conclusion, although bulking sludge and deflocculated sludge are associated with different fouling mechanisms, the results of this thesis indicated that deflocculated sludge has more detrimental impacts on fouling. Consequently, conditioning strategies should be developed for MBRs operated in lowered temperature. Cake fouling caused by bulking sludge can be mitigated with chemically

enhanced backwash, which prevents accumulation of foulants on membrane surface. In addition, Remy et al. (2011) found that a low dose of powdered activated carbon (PAC) can reduce floc disintegration in MBRs subjected to lowered temperature. Therefore, PAC could be used to combat deflocculation of sludge in autumn and winter, when temperature variations are most defined.

### 4.4.2 Filamentous bacteria and EPS

As mentioned in section 4.2.2, results indicated that the increment of EPS is mostly due to colloidal fractions. Similar findings were observed by Meng and Yang (2007, 55), who showed that bulking sludge has less soluble EPS and more colloidal-EPS concentration compared to normal sludge. Filamentous bacteria act as a bridging element that allow attachment of free EPS on filaments. They retain the soluble fraction within the flocs, lowering the amount of soluble EPS extracted in bulking scenario compared to sludge in other conditions (Meng & Yang 2007). On the other hand, it was found that deflocculated sludge can release more soluble EPS, due to reaction of microorganisms toward environmental stress. This finding is in opposition to this thesis' results with soluble fraction remains stable throughout the trial. This could be explained by different extraction methods, as Meng and Yang (2007, 50) used cation ion exchange resin to extract EPS, while only centrifugation and filtration was used in this thesis.

# 5 CONCLUSIONS

Impacts of temperature shock on MBRs were successfully characterized with the laboratory-scale MBR set up. After the shock, fouling rate was vastly higher than the rate of normal temperature condition even with membrane replacement. Operation of the MBR in this condition also induced membrane breakage. In the last few days of the experiment, TMP increased with a gradual and stable rate similar to normal condition, suggesting that the MBR might adapt to low temperature condition and recover from the shock. Longer experimental period is required to confirm this hypothesis.

Temperature shock had lowered PS concentration of SMP, while PT concentration was very stable throughout the experiment. On the other hand, TOC analysis had proven that EPS concentration in low temperature phase is higher than normal phase, in which more colloidal matters are released rather than soluble particles. Contradictions between these findings and other publications are associated with different extraction methods, thus standard methods should be developed to ensure equal substrate yield in different studies.

The shock had significantly decreased the diversity of sludge and induced sludge deflocculation, which weakened floc structure. However, after a very short period, filamentous bacteria thrived among others and lead to severe bulking. Small flocs were covered in thick filaments, thus increased the overall FI evaluation of samples collected in this period. Based on fouling rate of the MBR, it was also concluded that deflocculated sludge had more severe impacts on fouling compared to bulking sludge, due to its association with pore clogging.

In conclusion, temperature shock is unfavourable for MBRs operation due to decreased filtration efficiency and changes in sludge characteristic. Conditioning strategies such as backwash or dosing with coagulating reagent should be used to mitigate fouling. Moreover, no strong relationship was established between SMP composition and membrane resistance; thus, this parameter solely is insufficient to represent fouling in MBRs.

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# APPENDICES

Appendix 1. Datasheet for microscopic sludge investigation

Modified from Eikelboom (2002)

Sampling date:

| Characteristic | Parameter | Assessment | Remarks |
|---|---|---|---|
| Morphology | Shape | | |
| | Structure | | |
| | Strength | | |
| | Size | | |
| Filamentous bacteria [a)] | FI | | |
| Proto/Metazoa [b)] | Ciliates | | |
| | Flagellates | | |
| | Amoeba | | |
| | Testate amoeba | | |
| | Heliozoa | | |
| | Rotifers | | |
| | Nematodes | | |
| | Worms | | |
| Other characteristics | Free living cells [c)] | | |

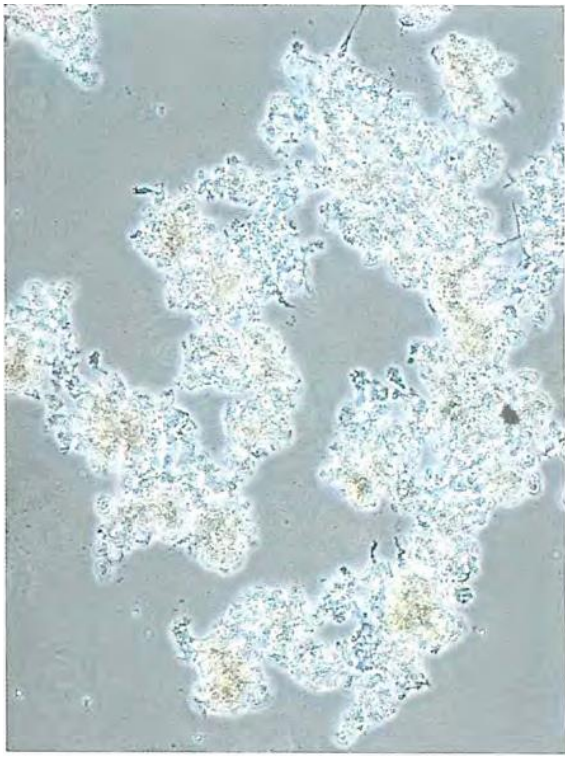
Assessment scales:

a) Scale 0 – 5: none – numerous filamentous organism

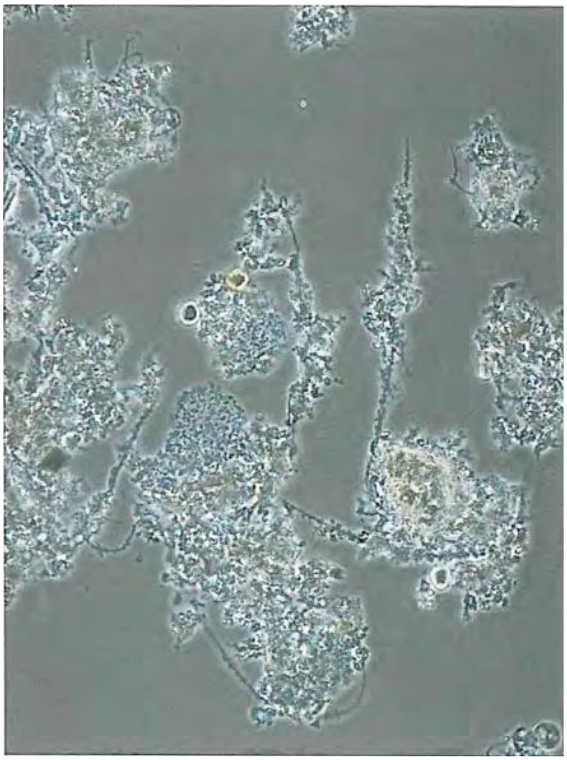
b) Scale 0 – 3: none – numerous cells/colonies per slide

c) Scale 0 – 3: none – hundreds of cells per field of view

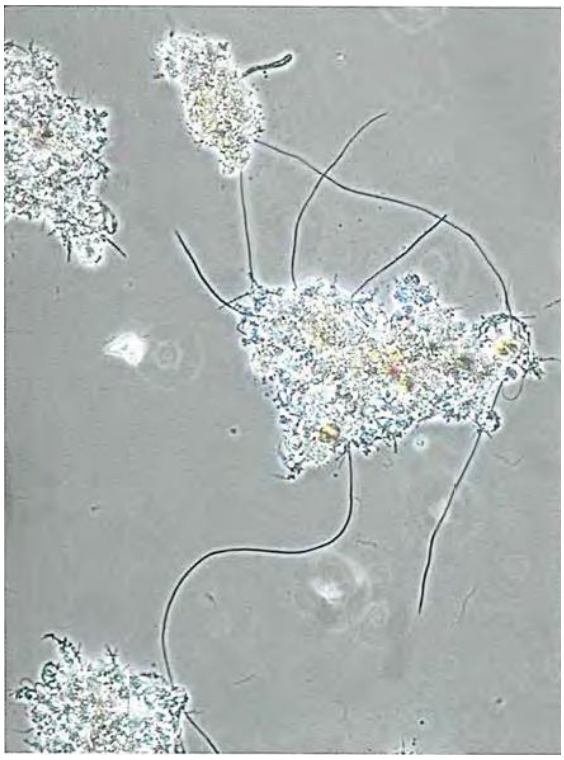
Appendix 2. Reference images for FI (Eikelboom 2002) 1 (3)



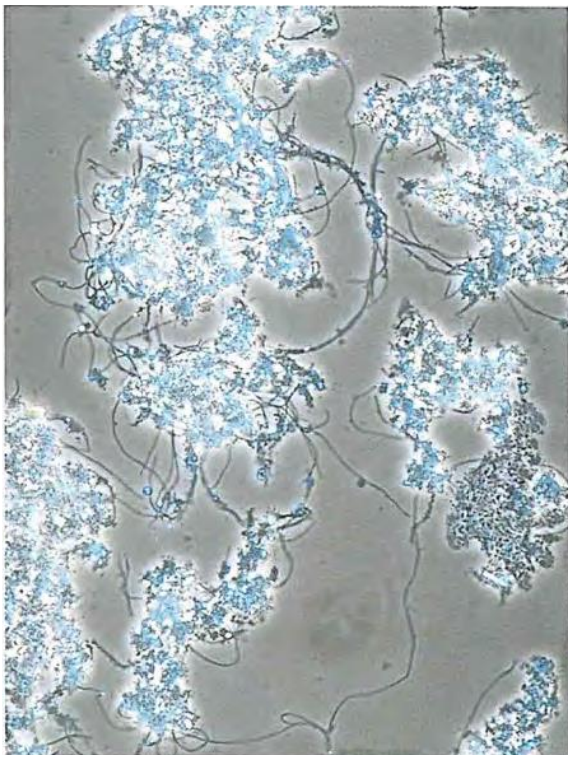
Picture 1. FI = 0 (150x)



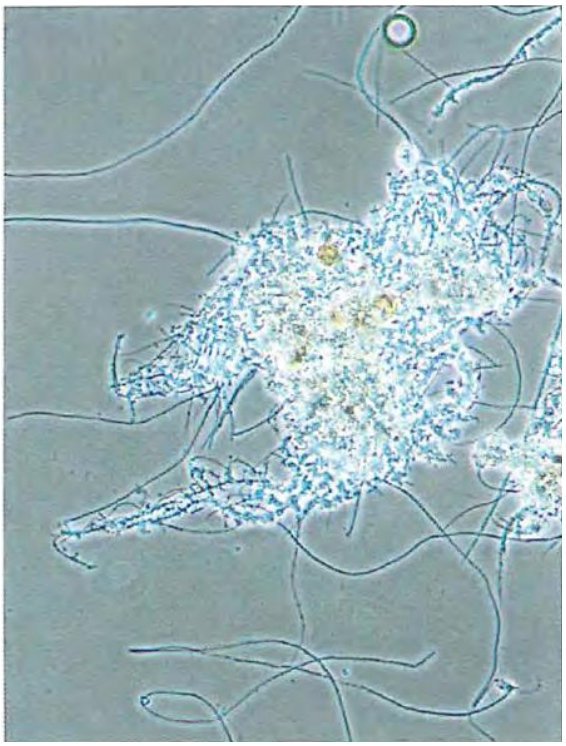
Picture 2. FI = 1 (150x)



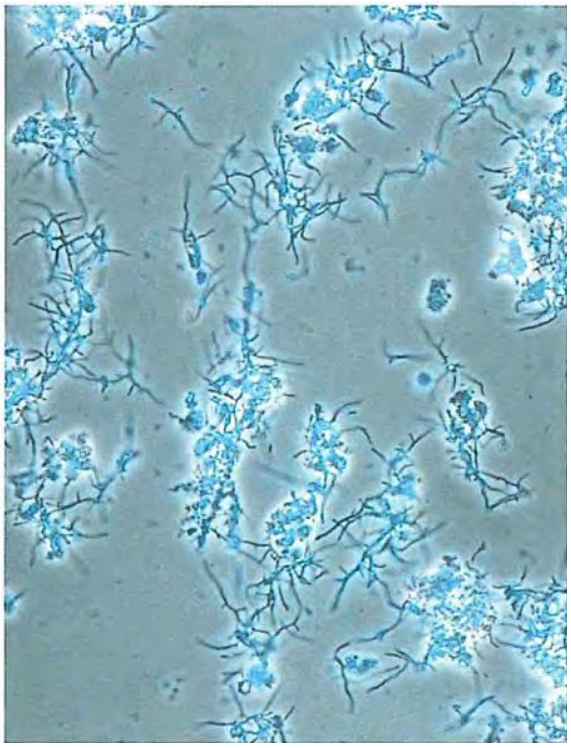
Picture 3. FI = 2; robust filaments (150x)



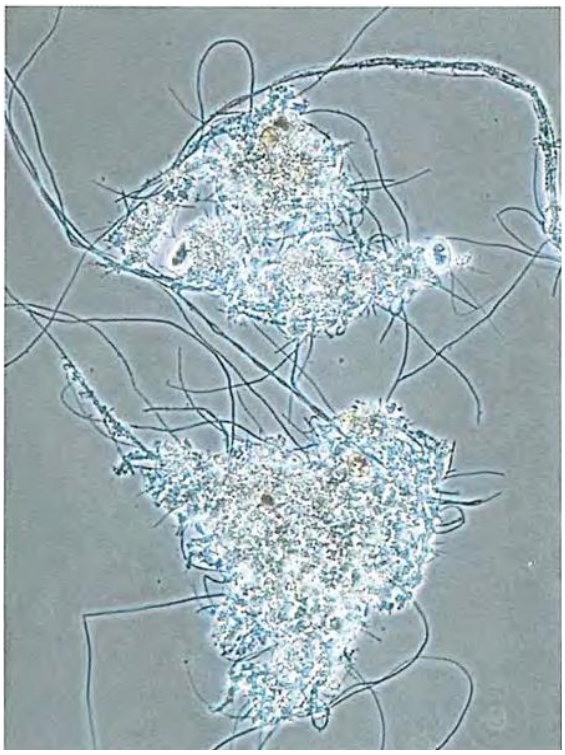
Picture 4. FI = 2; thin filaments (300x)



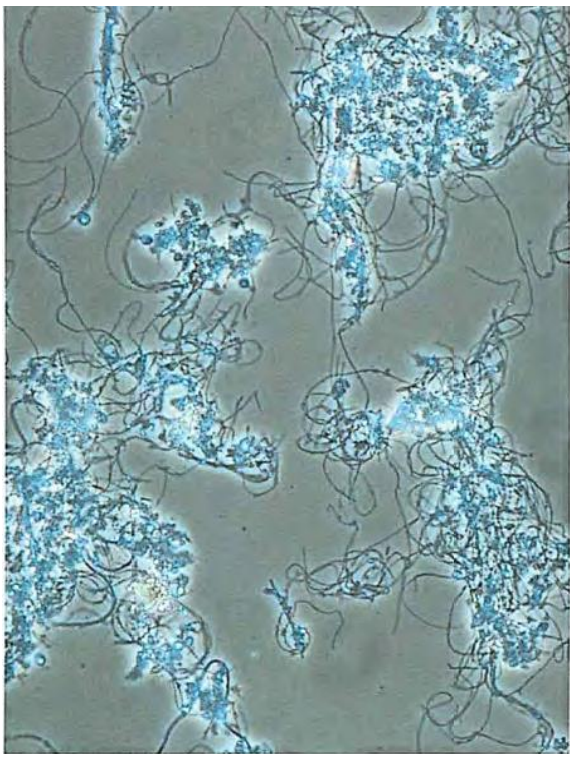
Picture 5. FI = 3; robust filaments (150x)



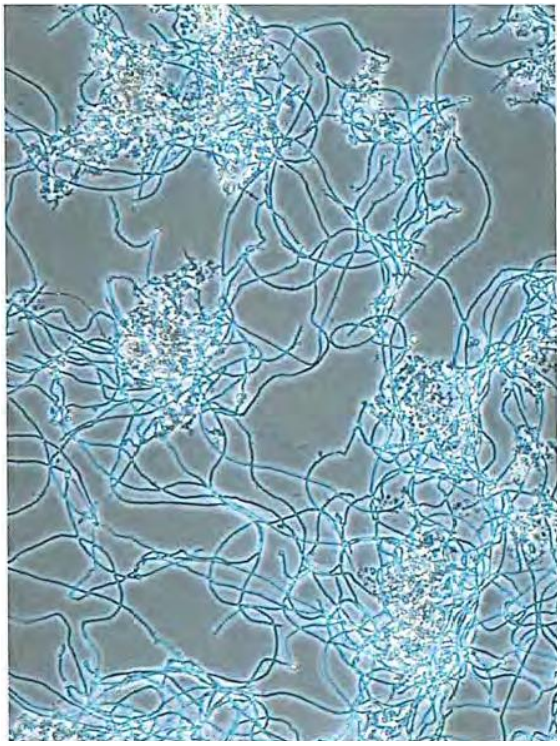
Picture 6. FI = 3; thin filaments (300x)



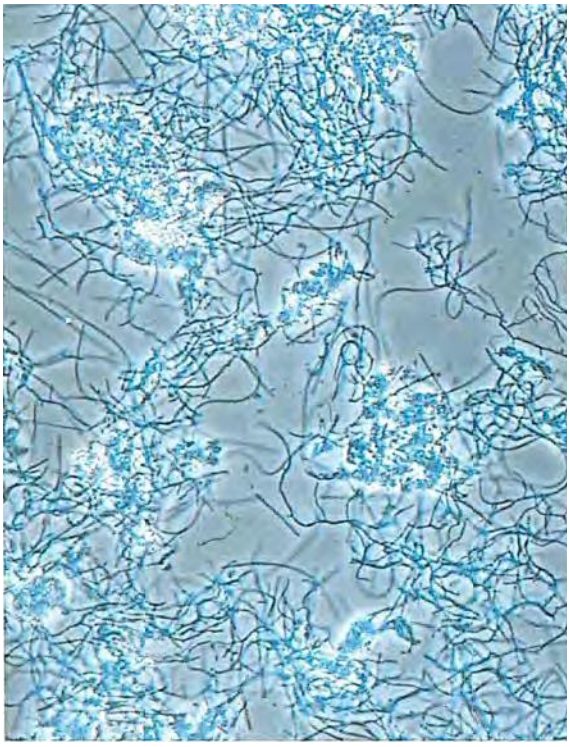
Picture 7. FI = 4; robust filaments (150x)



Picture 8. FI = 4; thin filaments (300x)



Picture 9. FI = 5; robust filaments (150x)



Picture 10. FI = 5; thin filaments (300x)

Appendix 3. Full results of FI analysis

| Sample date and number | | Filament Index count | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| 7 | Sample 1 | 47 | 42 | 39 | 67 | 39 | 14 |
| | Sample 2 | 78 | 50 | 52 | 43 | 23 | 7 |
| 13 | Sample 1 | 65 | 146 | 128 | 132 | 32 | 12 |
| | Sample 2 | 60 | 155 | 175 | 128 | 31 | 4 |
| 19 | Sample 1 | 78 | 173 | 227 | 103 | 12 | 2 |
| | Sample 2 | 48 | 167 | 261 | 95 | 17 | 3 |
| 24 | Sample 1 | 1 | 10 | 75 | 245 | 166 | 88 |
| | Sample 2 | 7 | 42 | 95 | 203 | 133 | 51 |
| 27 | Sample 1 | 0 | 0 | 15 | 72 | 214 | 251 |
| | Sample 2 | 0 | 2 | 13 | 165 | 255 | 285 |