

The effect of pre-treatment with RO on biological stability in a drinking water treatment plant

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The effect of pre-treatment with Reverse Osmosis on the biological stability in a drinking water treatment plant

Research at ZS Lekkerkerk, Oasen

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Abstract

Drinking water companies in the Netherlands have not stood still when it comes to achieving a high level of drinking water quality. Optimizing conventional technologies, the use of more innovative techniques and tightening the drinking water quality norms are the main methods to improve drinking water quality at the customers' tap.

At drinking water company Oasen research is performed to the use of membrane filtration in future. Main goals of using reverse osmosis (RO) are too soften water to the desired level and to take into account future salination of the intake sources. At ZS Lekkerkerk a pilot RO installation is constructed. After anaerobic river bank filtrate is extracted, 50% of water is pre-treated with RO prior to conventional treatment steps of sand filtration, granular activated carbon (GAC) and UV-disinfection.

The key objective of this study was to determine the effect of pre-treatment with RO on the biological stability in a drinking water treatment plant. The effect of individual treatment steps on the biological stability of water was also investigated.

A high level of biological stability is aimed to achieve to limit regrowth of bacteria in the distribution network. Biological stability was monitored using direct measurements (ATP, TCC and nutrient sources) and by simulating biofilm formation (biofilm monitor and Modified Robbins Device). Using membrane fouling simulators (MFS), the (bio)fouling potential of water was monitored by pressure drop measurements, visual observations and analysis of accumulated material on spacers and membranes.

Due to small pore sizes ($<0.001 \mu\text{m}$) RO removes bacterial cells present in the water. From Figure 1 one can conclude that the physical removal of TCC remained significant in the treatment scheme. A TCC increase was observed after pre-filtration caused by the biological removal of ammonium, iron and manganese.

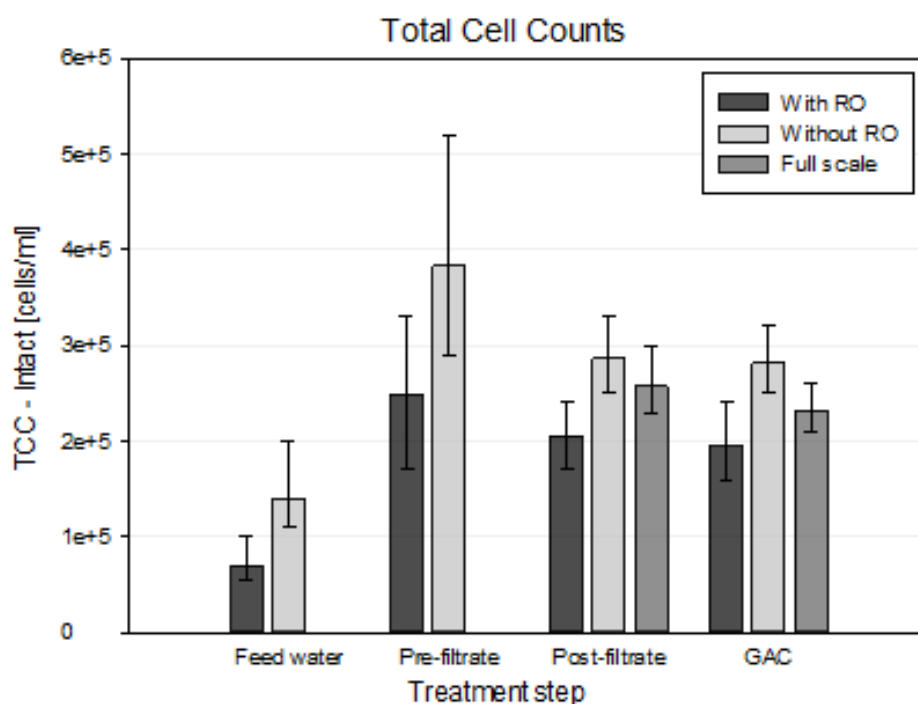


Figure 1 - Intact cell counts of flow with (50%) and without pre-treatment with RO. Full scale flow (reference) contains water from all eight filter sets that is mixed after post-filtration

The effect of pre-treatment with RO was not recognizable in ATP results found after investigated treatment steps. Dissolved organic carbon (DOC) was removed by RO and the removal remained significant in the course of the treatment scheme. Observed with the biofilm monitor, the biomass concentrations on glass rings at the beginning of the monitoring at ZS Lekkerkerk was very low. The effect of RO was visible, but it is doubtful if the observed difference is significant for concluding that biological stability of water improved. Extreme caution is needed in interpreting results as the run of the biofilm monitor was not finished in this thesis.

After post-filtration the increase in feed channel pressure drop obtained during MFS runs was smaller for water pre-treated with RO. This was likely caused by high effluent concentrations of iron and manganese from post-filters. No increase in feed channel pressure drop was observed after GAC experimental set-up. Increasing the linear flow velocity resulted in comparable results.

Biomass accumulation (ATP, TCC and TOC) found on spacers and membranes was lower for the flow consisting pre-treatment with RO. After GAC experimental set-up lower biomass accumulation was observed than after post-filtration, indicating that GAC treatment step had a positive effect on the biological stability.

One can conclude from this research that the biological stability of both flows investigated (with and without RO) was of a similar level and differences observed were small but visible for some methods used. With regard to the regrowth potential of water the effect of pre-treatment with RO was observed in biomass accumulation on spacers and membranes of MFS and biomass concentration on glass rings of the biofilm monitor (first 84 days)). But as absolute values observed were small it is doubtful if differences are significant. On the other hand, the physical removal of components (TCC and DOC) with RO was significantly recognizable throughout the course of the treatment scheme.

It can be said that RO, as pre-treatment, is a good step for achieving the main goals of softening water and the removal of salts. The effect on the biological stability of drinking water was limited, although some methods indicate a positive influence. The effect of pre-treatment with RO on the produced water is significant with regard to TCC and DOC. Although TCC and DOC are not direct indicators for bacterial regrowth it does tell that water that enters the distribution network is of a better initial quality.

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List of abbreviations

AOC	Assimilable Organic Carbon
ATP	Adenosine triphosphate
BDOC	Biodegradable Dissolved Organic Carbon
BFP	Biofilm Formation Potential
BFR	Biofilm Formation Rate
BFM	Biofilm Monitor
C	Carbon
DOC	Dissolved Organic Carbon
DNA	Desoxyribo Nucleic Acid
FCM	Flow Cytometry
GAC	Granular Activated Carbon
HNA	High Nucleic Acid
HPC	Heterotrophic Plate Count
LNA	Low Nucleic Acid
MFS	Membrane Fouling Simulator
MRD	Modified Robbins Device
N	Nitrogen
P	Phosphorus
PMMA	Polymethylmethacrylate
POC	Particulate Organic Carbon
PVC	Polyvinylchloride
RO	Reverse Osmosis
SW	Schuwacht (raw water intake source)
TCC	Total Cell Counts
TOC	Total Organic Carbon
TW	Tiendweg (raw water intake source)
UV	Ultraviolet
ZS	Treatment plant (Dutch: Zuiveringsstation)

Preface

The report in front of you is the result of eight months of internship at drinking water company Oasen. The research was performed within the framework of completing the study Civil Engineering at Delft University of Technology. By finishing this Master Thesis it means a period of studying is over.

There are some people I would like to thank for supporting me during this period. Special thanks go to Maarten Lut, who intensively supported me during the whole period at Oasen. Without his help, arranging materials for experiments at ZS Lekkerkerk would have been complicated. He was also willing to participate in the graduation board.

Thanks to the other members of the graduation board. Walter van der Meer for leading the graduation board as chairman and for taking the roll as supervisor with respect to Delft University of Technology. Jan Vreeburg for his useful comments during the graduation board meetings and his help in completing this report. Hans Vrouwenvelder for advising me on the use of several materials and methods in this study.

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Finally, I want to thank all people working at the Research Department at Oasen who made me a feel at home and for their interest in the subject. Special thanks to the operators of ZS Lekkerkerk, Patrick van Velden and Shyam Jagmohan, for their help at the treatment plant and for making my stay there comfortable.

The author

J. Dusseldorp

Gouda, January 2013

1 Introduction

1.1 Motive

Drinking water companies in the Netherlands have not stood still when it comes to achieving a high level of drinking water quality. Optimizing conventional technologies, the use of more innovative techniques and tightening the drinking water quality norms are the main methods to improve drinking water quality at the customers' tap.

One of the techniques to treat water is membrane filtration. Membrane filtration is increasingly being used at several drinking water companies replacing conventional sand filtration. Depending on the pore size of the membranes and the location in the treatment scheme other treatment steps (e.g. softening) can be replaced by membrane filtration too.

Membrane filtration with smallest pore size is Reverse Osmosis (RO). With pore sizes smaller than 0.001 μm it rejects all ions in the feed water. The produced water, permeate, is ultrapure with almost no components present.

In general, RO is mainly used for seawater desalination. In dry countries, where fresh water is scarce, RO is used to produce drinking water. Cruise ships are also provided with an RO installation to produce drinking water for passengers.

In the Netherlands the use of RO for producing drinking water is at an early phase. This is mainly due to the fact that fresh water is readily available. However, due to climate change more intake sources in the western part of the Netherlands detect increasing chloride concentrations (Jongerius, s.d.) caused by salt water intrusion from the North Sea.

At Oasen research is done regarding the use of RO at drinking water treatment plant ZS Lekkerkerk to decrease the hardness of water and to take into account future salination. A pilot RO installation treats anaerobic riverbank filtrate (groundwater). The permeate from the RO installation is equally (50/50) mixed with original intake water followed by conventional treatment of two steps of filtration. Pre-filtration (1st step) consists of a double layer filter material (sand and anthracite), whereas post-filtration (2nd step) consists of sand only. After filtration, treatment steps activated carbon filtration and UV disinfection are passed before the water is pumped into the distribution network.

Biological stability of water is still an unclear concept and hard to quantify. From literature one can conclude that biological stable water is water that does not support bacterial regrowth (Liu, 2010). Biological unstable drinking water can cause increases in (pathogenic) bacteria during distribution (Hammes *et al.*, 2008). Besides health risks an increase of bacteria in drinking water can cause taste and odor problems (Lee *et al.*, 1980). As RO permeate is ultrapure it is hypothesized that the permeate does not contain food sources for bacteria and therefore limits bacterial regrowth. Also bacteria are retained by the small pore sizes of the membrane. Questions arise if treating 50% of raw water by RO will significantly increase the biological stability in a drinking water treatment plant and of the produced water. In this thesis the biological stability in a drinking water treatment plant where a pilot RO installation is implemented was investigated.

1.2 Relevance of the project

At Oasen, Project 'Oasen-West' is in progress. The main goal of this project is to construct a new treatment configuration at the drinking water production location ZS Lekkerkerk.

Part of this project is to research the possibility of treating 50% of the extracted anaerobic groundwater with RO. This results in a decrease in hardness to the desired level. It also takes into account future salination of the intake sources.

Drinking water in the Netherlands is distributed without disinfectants (Van der Kooij *et al.*, 1999). Therefore it is required that regrowth of bacteria is limited as much as possible before reaching the customers' tap. Growth of (pathogenic) bacteria can lead to health risks and taste complaints (Hammes *et al.*, 2008). As residence time in the distribution network can be long (DiGiano *et al.*, 2000), it is required that the produced water at the production locations is of such a high level of biological stability that it does not support bacterial regrowth.

In theory RO will reject most of the food sources for bacteria and hence result in a higher level of biological stability. To extend the pilot RO installation at ZS Lekkerkerk to a full scale RO plant it is interesting to know what the effect of RO is on the biological stability in the drinking water treatment plant and of the produced water.

1.3 Research questions

In this research two separate feed water flows in a drinking water treatment plant were investigated. One flow consisting of 50% RO and 50% original intake water. The other flow (reference flow) consisting of original intake water only. Both flows were separately treated by pre-filtration and post-filtration. In the full scale drinking water treatment plant, after post-filtration both water flows are mixed together with six other post-filtrates and is treated further by granular activated carbon (GAC) and UV-disinfection.

In this research, for both flows two activated carbon simulation vessels were constructed (after post-filtration) to include the GAC treatment step for investigating the effect of pre-treatment with RO.

1.3.1 Primary research question

The primary research question is:

- *What is the effect of treating 50% of raw water intake with Reverse Osmosis on the biological stability of drinking water at treatment plant ZS Lekkerkerk?*

It should be noted that RO has only direct effects on the feed water quality (prior to filtration) at the beginning of the treatment at ZS Lekkerkerk.

To answer the primary research question sub-questions were investigated:

- *What is the difference in biological stability after pre-filter fed with 50% RO and 50% original intake water and pre-filter fed with 100% original intake water?*
- *What is the difference in biological stability after post-filter treated with 50% RO and 50% original intake water and post-filter treated with 100% original intake water?**
- *What is the difference in biological stability after granular activated carbon treated with 50% RO and 50% original intake water and granular activated carbon treated with 100% original intake water?****

- *To what extent can the influence of feed water quality on biological stability be measured in the full scale drinking water treatment plant ZS Lekkerkerk?*

* Post-filters were fed with pre-filtrate

** Two granular activated carbon simulation vessels were constructed and fed with post-filtrate

1.3.2 Secondary research questions

Besides the effect of two water flows (with and without RO) on the biological stability in a drinking water treatment, it was investigated how individual treatment steps influenced biological stability.

The secondary research question is:

- *What is the effect of individual (biological) treatment steps at ZS Lekkerkerk on the biological stability of drinking water?*

Resulting in the following sub-questions:

- *What is the effect of pre-filtration at ZS Lekkerkerk on the biological stability of drinking water?*
- *What is the effect of post-filtration at ZS Lekkerkerk on the biological stability of drinking water?*
- *What is the effect of granular activated carbon at ZS Lekkerkerk on the biological stability of drinking water?*

1.4 Structure of report

In Chapter 2 a subject analysis is given. General information about drinking water company Oasen is presented in Paragraph 2.1. At the moment of this MSc-Thesis a comprehensive project is in progress. This MSc-thesis is just a small research part of the 'Oasen-West' Project (Paragraph 2.2). Paragraph 2.3 discusses ZS Lekkerkerk, the location where the experiments were performed. This research was based on a pilot RO installation that was constructed at ZS Lekkerkerk. Paragraph 2.4 explains the concept of RO. In Paragraph 2.5 a discussion of literature about the concept of biological stability is given. A brief overview of the experimental set-up, research parameters and research tools is presented in Paragraph 2.6.

Chapter 3 gives a detailed description of the materials and methods used in this research. For research materials the properties and the way of operation is explained. For water sampling the principles of analysis methods are given.

In Chapter 4 the results and discussion of the experiments are listed. This chapter is divided into several parts to keep the overview of the extensive data set. First, in Paragraph 4.1.1 the regular water samples are discussed. Sub-paragraphs are introduced to discuss the differences between feed water quality (Paragraph 4.1.1.1), pre-filtrate (Paragraph 4.1.1.2), post-filtrate (Paragraph 4.1.1.3) and granular activated carbon filtrate (Paragraph 4.1.1.4). Successively, the results and discussion of the Membrane Fouling Simulator (Paragraph 4.1.2), biofilm monitor (Paragraph 4.1.3) and Modified Robbins Device (Paragraph 4.1.4) are presented. A short discussion on results obtained (at ZS Lekkerkerk) by KWR Watercycle research Institute is given in Paragraph 4.1.5. Finally, in Paragraph 4.2 an analysis is given of the performance of the several tools that were used in this study.

In Chapter 5 a conclusion of this MSc-Thesis is presented. Also recommendations towards this research, the 'Oasen-West' project and Oasen drinking water company are given.

2 Analysis

In the analysis background information of drinking water company Oasen, where research has been performed, is presented. It also discusses information needed to better understand the topic and the need for research on this topic. Some parts will be provided by information gained from Oasen whereas other parts of information were obtained from literature.

2.1 Drinking water company Oasen

Oasen is a Dutch drinking water company that supplies drinking water to the eastern part of the province of 'Zuid-Holland'. In total, 750 000 people and 7 200 business companies are provided with drinking water. Oasen manages the drinking water distribution network for their supply area of around 1 115 km². Around 43 million m³/year of drinking water is transported by 4 060 km of pipelines from the production locations to the customers' tap.

Compared to other drinking water companies Oasen is relatively small which is illustrated in Figure 2-1. The supply area of Oasen is presented in Figure 2-2 where ZS Lekkerkerk is located in 'Nederlek'.



Figure 2-1 - Drinking water companies in the Netherlands



Figure 2-2 - Supply area of Oasen

Like all drinking water companies in the Netherlands Oasen is an enterprise. In the Netherlands drinking water companies are non-profit organizations that do not operate in a free market. Each drinking water company is a monopolist within its own, by law, designated supply area. The main reason for this monopoly is that drinking water quality and availability of drinking water in the Netherlands are of such a social importance that it may not come under pressure because of economic considerations. The aim of Oasen is not to make profit, but to provide high quality drinking water and supply to their customers.

2.2 Project 'Oasen-West'

In November 2009 a new vision was formulated for the coming years to increase water quality and increase supply reliability in the western part of the supply area of Oasen. To realize this vision project 'Oasen-West' was created. In this plan investments on infrastructure (buildings and pipelines) for the years to 2020 are outlined.

To improve water quality and increase supply reliability it was decided in project 'Oasen-West' to construct a drinking water treatment plant north of the river Lek as customers in Krimpen aan den IJssel, Nederlek and Ouderkerk aan den IJssel do not receive soft water in the current situation. To realize this plan, rebuilding the existing treatment plant ZS Lekkerkerk in Krimpen aan de Lek is seen as most obvious. Also a pipeline will be constructed underneath the river Lek to transport water from the southern intake sources to the 'new' drinking water treatment plant.

With an increased implementation of membrane filtration in drinking water treatment plants in future, the decision was made to do a comprehensive research project to the possible use of RO. Using RO improves water quality and makes it possible to produce soft water. Supply reliability is increased due to building an extra pipeline under river Lek to connect the southern intake sources with the northern intake sources. Also, a new clear water reservoir with high pressure pumps will be constructed to increase the supply reliability.

2.3 ZS Lekkerkerk

Production location ZS Lekkerkerk is an important link in 'Oasen-West' project as this location will be reconfigured. A pilot RO installation was constructed at ZS Lekkerkerk to investigate the possibilities of using RO as full scale treatment to decrease hardness and to take into account future salination of the intake sources.

2.3.1 Intake sources

ZS Lekkerkerk uses undep anaerobic riverbank filtrate extracted from two locations: Tlendweg (TW) and Schuwacht (SW). In the plans of project 'Oasen-West' clear water from ZS De Put will be transported by a pipeline to ZS Lekkerkerk where it will be mixed prior to distribution. Intake sources are presented in Figure 2-3.



Figure 2-3 - Location of intake sources (Tlendweg and Schuwacht), ZS Lekkerkerk and ZS De Put (left) and a map of the western part of the Netherlands (right)

2.3.2 Current treatment scheme

At ZS Lekkerkerk extracted water is treated by a conventional treatment presented in Figure 2-4.

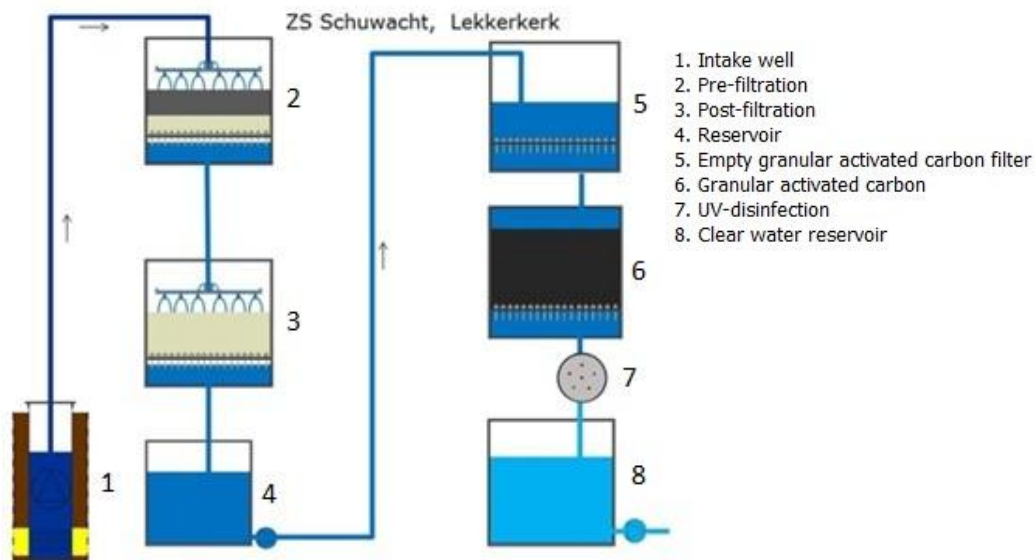


Figure 2-4 - Current treatment scheme ZS Lekkerkerk

After extraction of anaerobic river bank filtrate water is aerated using sprinklers. Oxygen is needed to oxidize Fe^{2+} to Fe^{3+} and to convert NH_4^+ to NO_3^- and Mn^{2+} to MnO_2 . After aeration the next step is pre-filtration containing sand and anthracite as filter material. The oxidized iron will form $Fe(OH)_3$ flocs that are physically removed by filtration, but part of the iron is also removed biologically. Manganese will be converted partly physically and partly biologically, while ammonium is converted fully biologically. The post-filtration step consists of filters containing sand only to take care of high effluent concentrations from pre-filtration. Both filtration steps are operated as dry filters because oxygen is stripping. Thus, methane removal is done fully physically.

Activated carbon is the next step in the treatment scheme. Based on adsorption, components attach to the surface of the filter material. Activated carbon is used to remove odor-, taste- and color producing compounds. Furthermore, activated carbon is used to remove organic micro-pollutants, such as pesticides and pharmaceuticals.

Final step is UV disinfection which destroys the DNA of micro-organisms. This destruction blocks further multiplications of bacterial cells during distribution. Due to negative side effects (e.g. health risks and taste complaints) chlorine addition as disinfectant is not used.

2.3.3 Configuration

In Figure 2-5 an overview of the configuration is given to clarify the research location. As mentioned, water at ZS Lekkerkerk is extracted from two intake sources: Tiendweg and Schuwacht. The RO pilot installation is running to investigate if RO can be applied at full scale in the future. Tiendweg raw water is treated by RO. The permeate is mixed (50/50) with original Tiendweg raw water prior to pre-filtration. In total the treatment plant consists of eight pre-filters and eight post-filters that are running parallel. After two steps of filtration, water is mixed before entering the reservoirs prior to activated carbon filtration and UV-disinfection.

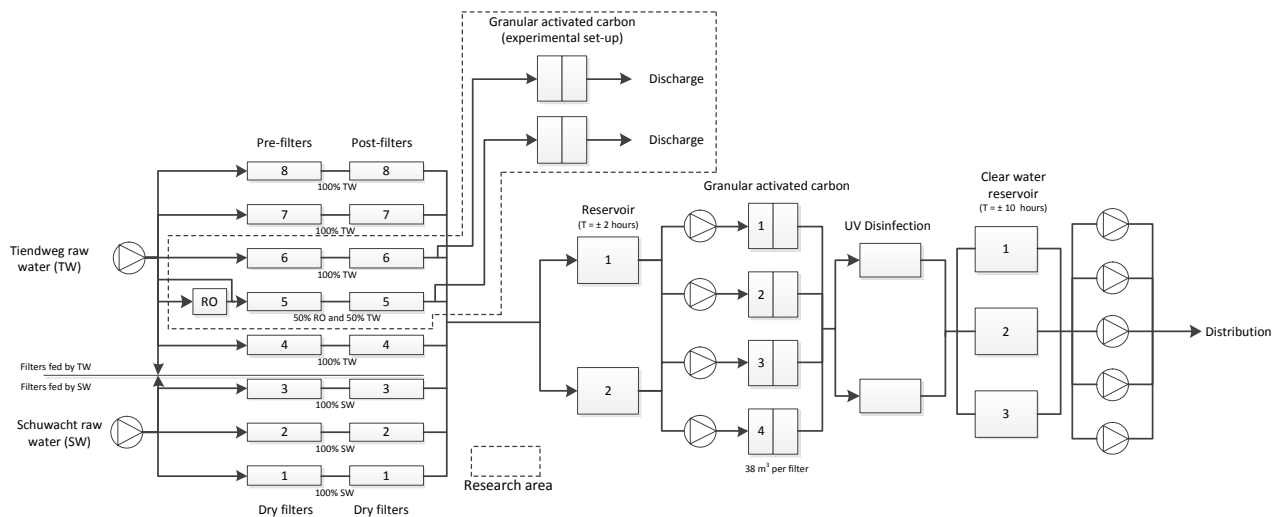


Figure 2-5 - Configuration of ZS Lekkerkerk where filter set 5 is fed for 50% with water that is pre-treated with RO

2.4 Reverse osmosis

Osmosis is a natural process of flow through a semi-permeable membrane. With a membrane pore size smaller than $0.001 \mu\text{m}$ suspended solids, bacteria, viruses and even monovalent ions (e.g. salts) will be captured. Based on osmotic pressure nature tries to equalize concentration differences. This means that water flows from low concentration of salts to higher concentrations as osmotic pressure is larger than the pressure difference between two water bodies, illustrated in the left diagram of Figure 2-6. If the pressure difference between the two water bodies is equal to osmotic pressure there is equilibrium. This occurs when the concentration of salts on both sides of the membrane is equal or if applied pressure is equal to the difference in osmotic pressure of the two water bodies.

RO is a treatment method where concentrated water passes the membrane to purify water. This conflicts with nature as concentration differences between two water bodies increases. To prevent water flowing from low concentration to high concentration the applied pressure on the concentrated water body should be higher than the difference in osmotic pressure between the two water bodies, illustrated in the right diagram of Figure 2-6. Thus, the driving force for RO is the applied pressure minus the osmotic pressure.

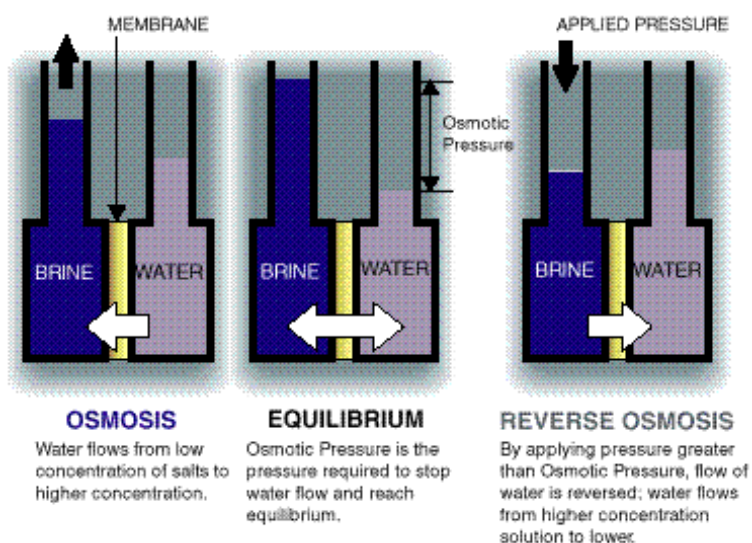


Figure 2-6 - Principle of reverse osmosis (Source: Lenntech)

Almost all RO membranes are spiral-wound, where water enters from one side into a module. Spiral-wound membranes have a large specific area (up to 1 000 m²/m³). RO is operated in cross-flow mode, which means only a small portion of the feed flow is produced as permeate. To increase the recovery, elements are placed in series. Applying multiple stages recovery is further increased.

RO permeate is ultrapure and not directly suitable for drinking water distribution. Post-treatment is needed to e.g. increase the hardness of the water to avoid corrosion of pipelines. At ZS Lekkerkerk RO permeate is mixed with original extracted water. By changing the proportion of permeate with original extracted water the desired level of hardness can be obtained.

2.5 Biological stability

Bacteria are autochthonous in drinking water. High concentrations of planktonic bacterial cells (>1e04 cells mL⁻¹) of broadly diverse populations are commonly found in tap water on consumption (Rinta-Kanto *et al.*, 2004; Berry *et al.*, 2006). These free-living bacterial communities found in water comprise of microorganisms that have proliferated predominantly in biofilms, either during the drinking water treatment process itself or in pipes during distribution of the water. The primary objective of drinking water treatment (from a microbiological perspective) is to ensure the absence of any pathogenic bacteria in the finished product and to limit any uncontrolled regrowth during distribution of the water (Hammes *et al.*, 2008).

Uncontrolled and excessive growth can lead to a deterioration of aesthetic water quality such as undesirable tastes, odors and visual turbidity and can also lead to process malfunctioning such as clogging of filters, bio-fouling and bio-corrosion (Lee *et al.*, 1980). In the Netherlands drinking water is distributed without a disinfectant residual in most supplies. Water treatment aims to achieve a level of biological stability that limits regrowth in distribution systems (Van der Kooij and Vrouwenvelder, 2001).

In literature the concept biological stability is interpreted in various ways. In general, there is no clear definition of the concept biological stability of drinking water used by authors. Concluded from literature can be that biological stable water is water that does not support bacterial regrowth, supported by a literature study of Liu (2010) on biological stability of drinking water:

"Biostability is a concept that addresses the overall tendency of the water to promote or suppress microbial proliferation."

Biological stability can be subdivided in two groups:

- Microbiological parameters in water phase and attached to surface material (biofilm growth) (Paragraph 2.5.1)
- Nutrient sources in water phase subdivided in organic carbon, nitrate-N and orthophosphate-P (indicators for biological stability) (Paragraph 2.5.2)

2.5.1 Microbiological parameters

Analysis of microbiological parameters is used to define the biological characteristics of drinking water produced by a drinking water treatment plant. In most current practices microbiological analysis is performed to secure water quality by satisfying norms set in legislation. In this context *Aeromonas*, *E-coli*, *Enterococci* and *Legionella* are important parameters. When researching the overall biological stability of drinking water general parameters, like total cell counts (TCC) and adenosine triphosphate (ATP) are more convenient.

Heterotrophic plate counts (HPC) and Total cell counts (TCC)

In the past, not too much attention was paid to total bacterial cell concentrations during production and distribution, as described by Hammes *et al.* (2008). This was caused by the fact that analysis was complicated and time consuming. Total bacterial cell concentrations are normally not considered during drinking water treatment as a design, operative or legislative parameter.

To properly understand microbial survival and growth during drinking water treatment the cultivation-dependent method of heterotrophic plate counts (HPCs) was introduced more than 100 years ago (reviewed in, e.g. Bartram *et al.*, 2003). In the recent past, HPC was still seen as one of the primary parameters for assessment of general microbiological quality of drinking water (Hammes *et al.*, 2008). More questions arise about what the method actually measures, how the method should be performed and how the results should be interpreted. Most critically, HPC detects only a small fraction of the total bacterial cells in aquatic environments (Allen *et al.*, 2004).

The need for rapid detection methods for planktonic bacteria in drinking water has been highlighted by Rinta-Kanto *et al.* (2004). Flow cytometry (FCM) has tremendous potential as an alternative tool for the analysis of bacteria in drinking water. FCM is a sensitive method that can be used to enumerate the bacteria in a water sample within 20 minutes and it provides a high degree of reproducibility (Hammes and Egli, 2005). Another advantage of FCM is that it can observe two defined clusters; the so-called low nucleic acid (LNA) and high nucleic acid (HNA). These represent two classes of bacteria typically observed in water. Gasol *et al.* (1999) have demonstrated the existence of LNA and HNA detected with SYBR Green I staining and FCM. These two classes are distinguished in size and fluorescence intensity, even though the composition and function of the two groups need to be clarified (Lebaron *et al.*, 2001).

Hammes *et al.* (2008) showed that HPC results had a standard error of >30% compared with FCM results with a standard error of <5%. The HPC method detects between 30 and 300 events in order to obtain a result, while FCM analysis detects between 40 and 20 000 events to obtain a result without any dilution required (Hammes and Egli, 2005). HPC takes 3-7 days to obtain results, while FCM requires 20 minutes.

Adenosine triphosphate (ATP)

ATP is another rapid method that can be used to assess the microbial quality of water. ATP is seen as the primary energy currency for all bacteria and therefore it is a parameter suited for the quantification of the active biomass (Velten *et al.*, 2007). An ATP measurement requires 5 minutes per sample and has the additional advantage that the equipment is simple to use and relatively affordable.

Correlations between different viability parameters

Berney *et al.* (2008) showed that cultivation independent viability indicators combined with total cell counts are useful parameters for the rapid detection of treatment process failure, overall water quality monitoring and for the assessment of regrowth potential. A high correlation between ATP data and HNA data from FCM has been observed. This is in line with the hypothesis that the bacterial population appearing as HNA contributes more to the overall activity of the bacterial biomass than LNA bacteria (Lebaron *et al.*, 2001). However, a better understanding of average ATP-per-cell values for environmental bacteria would contribute significantly to the use and interpretation of rapid ATP measurements for drinking water analysis.

Berney *et al.* (2008) show that no significant correlation was observed between HPC and other viability parameters, which confirms that the conventional HPC method is not a representative parameter for the general microbial quality of a water sample. This is mainly caused by the nutrient concentration on conventional HPC agar plates which is up to 1 000 times higher than the concentrations typically found in drinking water (Hammes *et al.*, 2008).

An overview of results of the correlations found between different viability parameters by Berney *et al.* (2008) is given in Figure 2-7.

Table 2 – Comparison between activity parameters measured for nine different water samples representing three types of drinking water

Parameter	R2A	PCA	Total ATP	Esterase	Polarized	Intact	HNA
Total counts	0.02	0.26	0.31	0.22	0.85	0.84	0.61
HNA	0.1	0.11	0.86	0.78	0.59	0.78	
Intact	0.003	0.11	0.64	0.52	0.88		
Polarized	0.005	0.18	0.44	0.35			
Esterase	0.35	0.04	0.95				
Total ATP	0.26	0.03					
PCA	0.07						

R²-values of the correlation between two parameters are listed.

Figure 2-7 - Overview of correlations found between different viability parameters (Berney *et al.*, 2008)

Biofilm formation rate (BFR) and biofilm formation potential (BFP)

Van der Kooij and Vrouwenvelder (2001) did research on the biofilm formation processes. Growth of micro-organisms on a surface exposed to flowing water leads to the formation of a biofilm, which is composed of bacteria, other micro-organisms (fungi, protozoa), extracellular products and inorganic compounds such as (hydr)oxides of iron, manganese and calcium. The main cause of biofilm formation is the availability of biodegradable compounds but temperature, flow velocity and surface characteristics also play important roles (Van der Kooij and Vrouwenvelder, 2001).

A tool used by Van der Kooij and Vrouwenvelder (2001) is the biofilm monitor which enables the determination of the BFR and BFP value of water. The accumulation of biomass on surfaces placed in the biofilm monitor is assessed using ATP analysis. BFR values of treated water are below 100 pg ATP/cm².d, but in slow sand filtrate values below 1 pg ATP/cm².d are observed. For treated ground water Van der Kooij *et al.* (1995) found BFR values ranging from 4.8 to 11.0 pg ATP/cm².d. Hence, assessment of the BFR values enables the detection of very low concentrations of growth promoting compounds in water. BFP values are obtained after long run times and are a measure of the maximum level of biomass accumulation.

2.5.2 Indicators for biological stability

Most authors in literature agree that nutrient sources, in particular organic compounds, are the key factor for bacterial regrowth (growth of biomass).

Hammes *et al.* (2009) supports this theory by mentioning that the presence of bacteria is usually not a result of exogenous contamination but rather due to micro-organisms that proliferate in these systems under the prevailing low nutrient conditions. The removal of biologically available organic carbon is the key process in the production of biologically stable drinking water.

Escobar *et al.* (2000) also explains what stimulates bacterial growth: for bacterial growth to occur, various nutrient sources must be present. In particular, organic compounds, either dissolved or particulate, provide energy and carbon sources for heterotrophic bacteria to produce new cellular materials.

Organic matter

As mentioned by Hammes *et al.* (2009) and Escobar *et al.* (2000) organic compounds are seen as the main nutrient for bacterial growth. Most studies focus on gross organic carbon parameters: total organic carbon (TOC), dissolved organic carbon (DOC), biodegradable organic carbon (BDOC)

and assimilable organic carbon (AOC). TOC represents the total organic carbon present in the water whereas DOC represents only the dissolved fraction of organic carbon (Van der Kooij *et al.*, 1982). BDOC represents the biodegradable fraction of organic carbon.

AOC was firstly proposed as a measurement for the organic content available for bacterial growth by Van der Kooij *et al.* (1982). AOC concentrations ranged 115-540 µg/l for ground and river waterworks with TOC concentrations of 0.6-4.0 mg/l (Miettinen *et al.*, 1997). Acetate has been mostly used as a reference for AOC as 97-100% acetate-C could be converted into biomass (Lu and Chu, 2005).

From results obtained by Van der Kooij (1992) it can be concluded that the concentration of AOC can be used to predict and control re-growth of heterotrophic bacteria. This conclusion is supported by the fact that a significant correlation was found between the AOC concentration and the density of heterotrophic bacteria in distribution of both drinking water prepared from surface water and drinking water prepared from groundwater (Van der Kooij, 1992).

For drinking water prepared from groundwater the lowest counts of heterotrophic bacteria were found in the drinking water with the lowest DOC concentration. Statistically significant linear relationships were found between the DOC value and the counts of heterotrophic bacteria (Van der Kooij, 1992) and between DOC concentration and the average ATP concentration (van der Kooij, 1992).

Inorganic nutrients – Nitrogen (N)

Nitrogen in drinking water is mostly present in the form of either nitrate (NO_3^- -N) under aerobic condition or ammonium (NH_4^+ -N) under anaerobic condition. However, N is usually not the limiting factor for microbial growth in drinking water. Lu and Chu (2005) and Frias *et al.* (1994) showed that adding NH_4^+ -N (up to 1 mg/l) or NO_3^- -N (up to 0.5 mg/l) did not stimulate further growth of bacteria.

Inorganic nutrients – Phosphorus (P)

Phosphorus in drinking water is usually present in the form of phosphate (PO_4^{3-} -P). Miettinen *et al.* (1997) found that microbial growth in drinking water is highly regulated not only by organic carbon, but also by the availability of phosphorus. Results show that addition of phosphorus (50 µg of PO_4^{3-} -P liter⁻¹) increased microbial growth in fresh drinking water produced from surface water or ground water. Even the addition of 1 µg/L of phosphate-P increased the microbial growth.

In many regions in Europe natural water has a higher content of mineral nutrients relative to AOC than that which exists in the boreal region where Miettinen *et al.* (1997) executed his study. There, organic carbon, not phosphorus or nitrogen, can be the most important factor to limit microbial growth in drinking water (Noble *et al.*, 1996).

2.6 Monitoring water quality

2.6.1 Feed water

Research at ZS Lekkerkerk was based on two water flows with a different feed water quality. Filter set 5 treats water that is pre-treated with RO (50%), while filter set 6 is treated directly by abstracted groundwater. Filter sets 4, 7 and 8 are treated with water from the same intake source. Filter sets 1, 2 and 3 are also treated directly by abstracted groundwater but another intake source is used resulting in a different feed water quality.

After filtration all water flows are mixed followed by treatment steps of activated carbon and UV-disinfection prior to distribution. As activated carbon is an important step regarding biological

activity a pilot installation was constructed after filter set 5 and filter set 6 (reference) to monitor the differences between the two water flows with different feed water qualities.

Figure 2-8 presents an overview of the locations at ZS Lekkerkerk that are investigated. To examine the direct effect of pre-treatment, RO research locations were chosen that follow flow 5 and flow 6 (left in Figure 2-8). To compare results with full scale and to investigate the effect of pre-treatment RO on produced water some research locations in the full scale treatment plant were chosen (right in Figure 2-8). Apart from the activated carbon experimental set-up (Paragraph 2.6.2), all other treatment steps of flow 5 and flow 6 are part of the full scale treatment plant. In Chapter 3 an extensive overview is given of the research locations, including materials and/or methods used at specific locations.

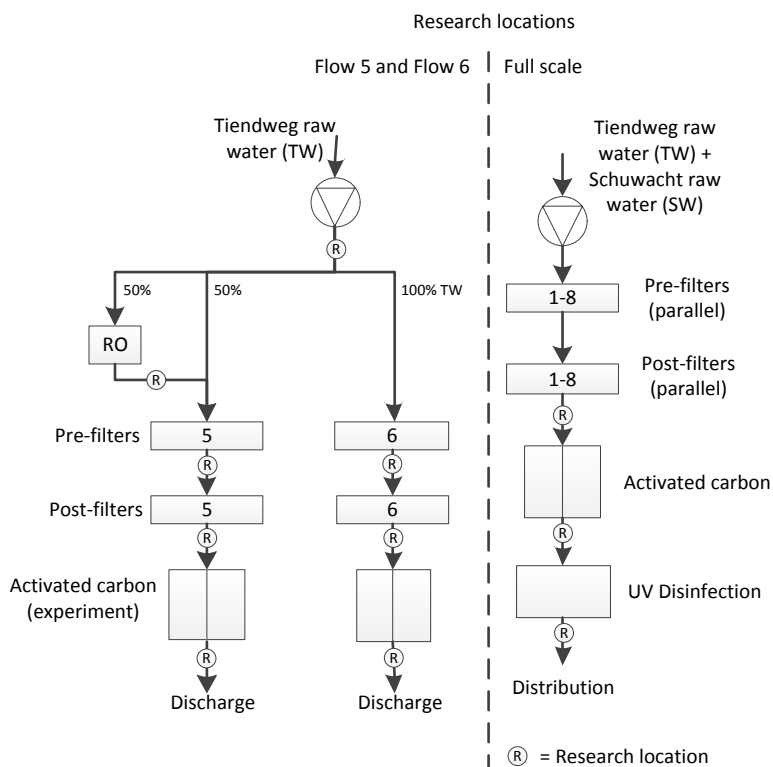


Figure 2-8 - Overview of research locations at ZS Lekkerkerk

2.6.2 Experimental set-up

After post-filter 5 (with RO) and post-filter 6 (without RO) an experimental set-up was constructed containing pilot activated carbon pressure vessels (Figure 2-9), storage for safety reasons (preventing pumps running dry) and dosage pumps (Figure 2-10) to control flow to measuring devices.



Figure 2-9 - Activated carbon pressure vessels



Figure 2-10 - Dosage pumps used for flow control to measuring devices

After filtration, water was collected in a small reservoir directly after the post-filters. Because water pressure was low (0.5 meters of water column) only a limited amount of flow could be transported by a regular sample point to the storage tanks constructed in the experimental set-up. Storage tanks were required for one specific reason. A sample point was out of use for the experimental set-up when regular samples were taken. Storing water in small buffer tanks provided enough time to prevent pumps running dry.

After storage, water was connected to the dosage pumps that control flow to measuring devices. In this way, filtrate from post-filter 5 and post-filter 6 was monitored. From storage, water was also pumped to the activated carbon pressure vessels (available height of 0.9 m and a diameter of 0.4 m) to include this treatment step. Contact time of water was equal to the full scale treatment plant (calculation in Appendix A). Afterwards water from activated carbon vessels was transported directly to measuring devices and to the dosage pumps to control flow. Figure 2-11 shows a schematic overview of the experimental set-up.

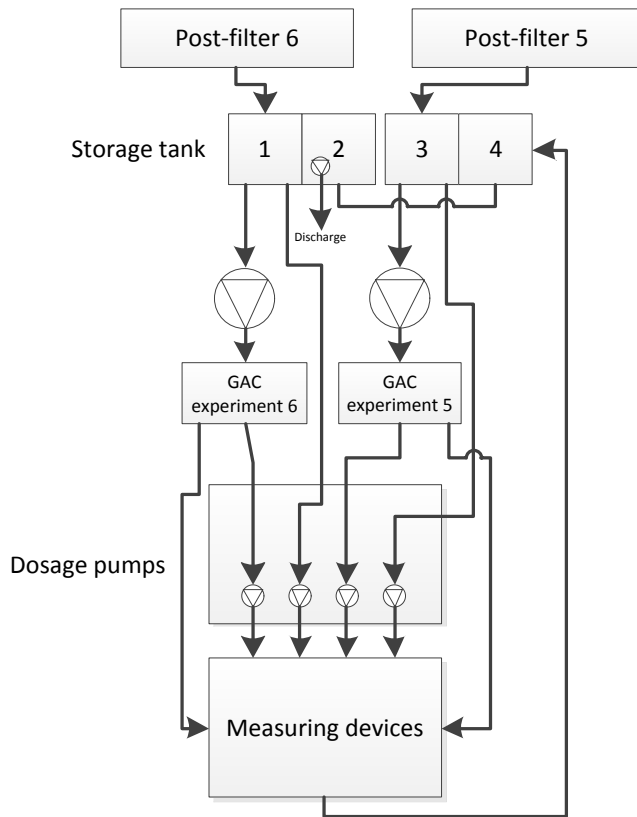


Figure 2-11 - Schematic overview of experimental set-up and the connection to measuring devices

3 Materials and methods

Biological stability was measured using parameters in the bulk water phase and by measuring the biofilm formation process. This chapter describes the materials and methods used in the research to the biological stability of water at ZS Lekkerkerk.

In Figure 3-1 an overview is given of all measurements performed in this research.

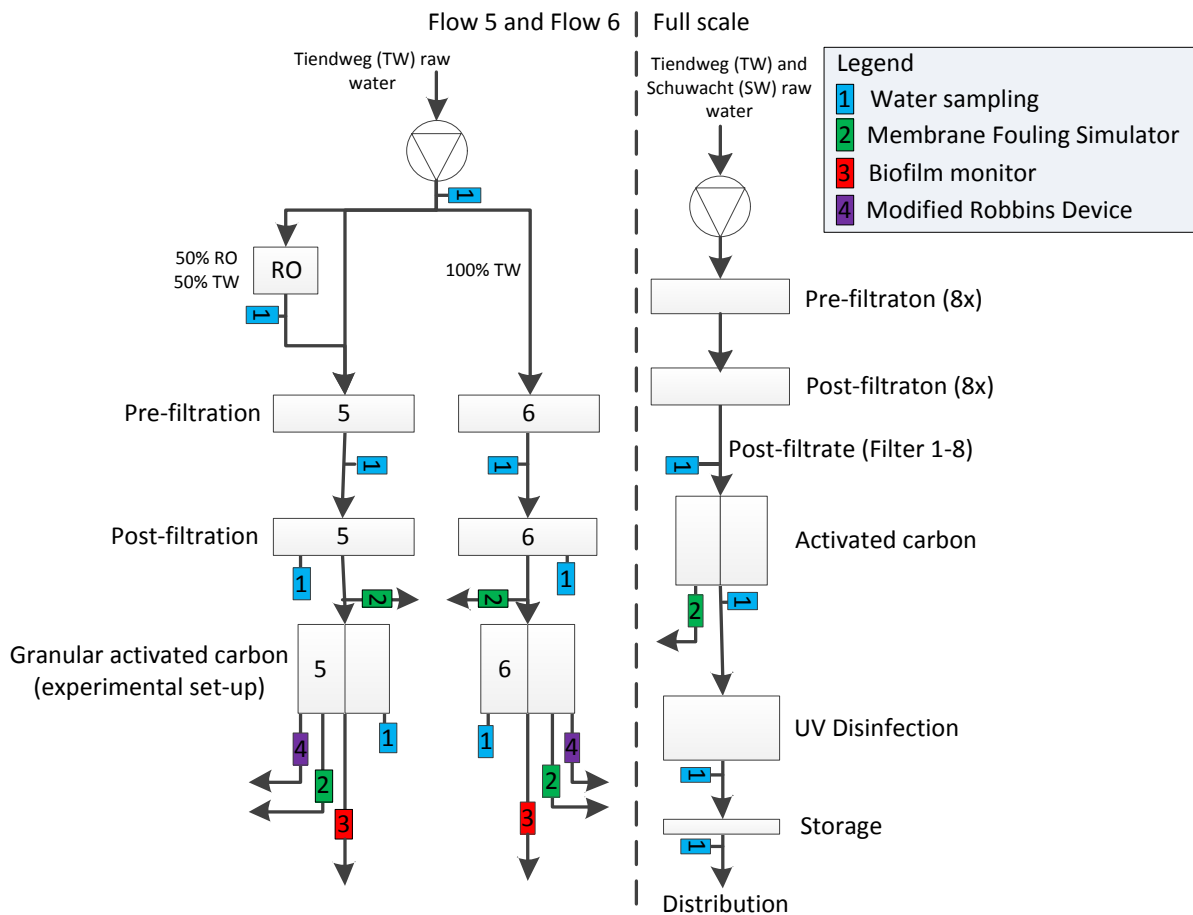


Figure 3-1 - Schematic overview of measurement locations and measuring devices used

Filter 5 and filter 6 are part of the full scale treatment where pre-filters and post-filters (8x) are running parallel.

3.1 Measuring devices

At ZS Lekkerkerk several measuring devices were used to investigate biological stability of different water bodies. This paragraph presents an overview of the measuring devices used and in what way they are operated.

3.1.1 Membrane Fouling Simulator (MFS)

MFS units were used to compare different biofouling potential promoted by different water qualities in the treatment scheme. MFS has been developed and applied for biofouling prediction in membrane filtration study (Vrouwenvelder *et al.*, 2006).

In spiral wound membrane elements, which are used in RO, two types of pressure drops can be distinguished: the trans-membrane pressure drop and the feed spacer channel drop. Trans-membrane pressure drop is the difference in pressure between the feed and the permeate lines, describing the resistance over the membrane. Flux decreases by an increase in trans-membrane pressure. The feed channel pressure drop is the pressure drop between the feed and concentrate lines (Flemming *et al.*, 1994). In practice, the feed channel pressure drop is the most serious problem related to biofouling (Vrouwenvelder *et al.*, 2009). Biofouling potential is measured using a differential pressure transmitter that measures pressure drop differences over a flow channel.



Figure 3-2 - Membrane Fouling Simulator for investigating (bio)fouling potential of water



Figure 3-3 - Differential pressure transmitter measuring the feed channel pressure drop

MFS units with sight window were used (Figure 3-2). These MFSs are made of PVC (bottom) and PMMA (top). A sight window makes it possible to recognize (bio)fouling immediately. The external dimensions of the MFS units are 260 mm x 85 mm x 55 mm making the measuring device compact and easy to install in a drinking water treatment plant. The dimensions of TRISEP membranes and 31 mil feed spacer sheets are 40 x 200 mm. MFSs were placed horizontally and a feed flow of 16 L/h was used in the first run resulting in an initial pressure drop of 35-40 mbar which is similar to previous studies (Araújo *et al.*, 2012). In the second run a feed flow of 41 L/h was used to fasten the process of (bio)fouling. Dosage pumps were used to control flow as this is not implemented in the device. Dosage pumps should be adjusted manually and on regular basis to correct flow. Feed channel pressure drop was measured about 2 or 3 times per week manually.

To measure the biofouling potential of water the MFS is an interesting device to use. However, one negative side effect is that feed channel pressure drop measured is a cumulative pressure drop caused by all components that will attach to the spacer and membrane implemented in the device. So bacteria and e.g. iron and manganese will accumulate on spacer and membrane, both causing an increase in feed channel pressure drop. When biofouling of water is high this will definitely be recognized in an increase in the feed channel pressure drop.

Fouling was monitored in various ways:

- Operational parameter: feed channel pressure drop.
- Visual, non-destructive observations using the sight window.
- Visual, non-destructive observations using a microscope and sight window (during run).
- Visual, destructive observations using a microscope (after run).
- Autopsy of coupons from the membrane and spacer sheets of the MFS.

Feed channel pressure drop was measured using a differential pressure transmitter (Figure 3-3). The pressure drop connections on the top of the MFS are made such that the inlet is connected to the high pressure port of the pressure transducer and the outlet to the low pressure port.

$$\Delta P = P_1 - P_2, \text{ where } P_1 = \text{inlet and } P_2 = \text{outlet}$$

Autopsy of coupons from MFSs was performed to measure the accumulation of ATP, TCC, TOC, iron, manganese and calcium on membranes and spacers. MFS units were transported on ice to a laboratory. Coupons were cut and the sections were placed in a capped bottle containing 100 ml of Milli-Q water. To determine the amount of attached materials, the bottles with membrane or spacer sections were placed in an ultrasonic cleaning bath. The low energy sonic treatment was repeated three times to remove all accumulated components. Volumes of water collected after sonic treatment were used to determine ATP, TCC, TOC, iron, manganese and calcium.

3.1.2 Modified Robbins Device (MRD)

MRD devices in Figure 3-4, developed by Tyler Research Corporation, are used to study biofilm formation promoted by different water qualities. It consists of a hollow structure to let water flow through. Press-fit plug holders are implemented in the device where biofilm is formed on stainless steel surfaces (Figure 3-5).

Two stainless steel surfaces per device were analyzed once per week on ATP. This results in a BFR of the investigated water. Because of time limitation BFP was not reached in this research.



Figure 3-4 - Modified Robbins Device to simulate biofilm formation



Figure 3-5 - Plug holding and metal biostud where bacteria adhere to

A low pressure MRD (LPMR-25) consists of 25 individual ports in a linear array along a channel of rectangular cross-section. The area of cross-section is 10 mm x 3 mm. External dimensions of MRD are 457 mm x 48 mm x 28 mm making it a compact device. A flow of 0.9 L/h was used.

Each individual port accepts a press-fit plug holding a sample coupon with a surface area of 50 mm² (Figure 3-5). These metal biostuds upon which the biofilm grows are made from stainless steel (LPMR-CTSS). The design of the plug is such that the surface of the coupon essentially becomes part of the channel wall. Bacteria introduced into the fluid flow adhere to the coupon and ultimately establish a biofilm, which can then be removed for analysis.

Samples were taken once per week in duplicates and were analyzed on ATP.

Before being installed the synthetic MRD devices were thoroughly cleaned with sodium hypochlorite solution (5%) and 70% ethanol and dried at 50°C. The press-fit plug including sample coupons were sterilized at 121°C for 15 minutes.

3.1.3 Biofilm monitor

The biofilm monitor, developed by Van der Kooij *et al.* (1995) was used in this research to evaluate biofilm formation. It consists of a vertically-placed glass column containing 20 glass cylinders. Water flows downward through the column, coming in contact with the surface of the cylinders.

Every two weeks two cylinders were removed and ATP analyses were performed. This results in the BFR of the investigated water. Because of time limitation BFP was not reached in this research.

As shown in Figure 3-6 the biofilm monitor consists of one vertically-placed glass column (F) of 600 mm in length and an internal diameter of 25 mm. This column contains 20 glass cylinders (G) on top of each other. These cylinders each have a total surface area of $17.4 \pm 0.2 \text{ cm}^2$ that is exposed to water. Water flows downward through the column with a flow of 175 L/h, coming in contact with the inner and the outer surface of the cylinders. The flow was checked regularly using a rotameter (H) and manually adjusted with a valve (J) when necessary. A pressure reduction valve (D) was used to minimize sudden fluctuations in the pressure drop. A Teflon tube (internal diameter 1 cm, length of 2.0 to 2.5 m) was used for connection with the water supply (A). The system was placed in the dark using shielding bags to prevent growth of phototrophic organisms.

Prior to their use, the cylinders were cleaned by boiling in a 1 N solution of sodium hydroxide in demineralized water, followed by rinsing with autoclaved water.

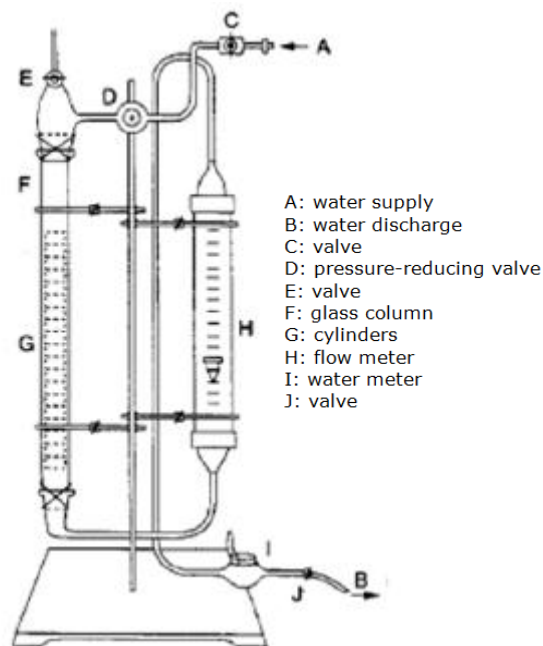


Figure 3-6 - Experimental set-up (not to scale) used for determining biomass accumulation on surfaces exposed to treated water

Run time of the biofilm monitor is about 150 days. A flow of 275 L/h is recommended for this device. Due to water availability at the research location the run was performed with a flow of 175 L/h. Less flow can cause some air bubbles in the device which are hard to remove. Those air bubbles create turbulence which can remove biofilm attached to the glass rings. Also results cannot be directly compared with previous studies. However, the effect of pre-treatment RO can be investigated as both BFM conditions were equal for both flows.

Although the complete run time could not be reached in this research biofilm monitors were installed. The first results can give an indication of the biofilm formation rate in the beginning of the experiment. Extreme caution should be taken about drawing conclusions from the first results as run time was not completed. After this thesis Oasen will finish the complete run to obtain the biofilm formation rate and biofilm formation potential.

A point of attention is the robustness of installing the biofilm monitor at a drinking water treatment plant. Due to operational problems pumps ran dry and feed water flow to biofilm monitor was stopped. Restarting feed water flow can have resulted in turbulence removing biofilm attached to the glass rings. This might result in a run that becomes useless. Because of long time period for a complete run it was inefficient to restart a run.

3.2 Water sample analysis

Water samples were taken for bacterial and non-bacterial analysis. Sampling frequency was dependent on the location in the treatment scheme. Water from pre-filters, post-filters and granular activated carbon experimental set-up were sampled most often.

Bacterial analysis consisted of total cell counts (TCC) by FCM, ATP and colony number (at 22 °C). Nutrient analysis mainly focused on DOC but also NO_3^- -N en PO_4^{3-} -P were taken into account. Ammonium, iron and manganese were also monitored as these are relevant parameters for anaerobic groundwater conditions.

3.2.1 Cells

ATP is considered to be the energy currency of life. It is the high-energy molecule that stores the energy. It consists of adenosine (composed of an adenine ring and a ribose sugar) and three phosphate groups. ATP is a molecule associated only with living cells. In drinking water ATP measurements are seen as a rapid and easy method for the detection of viable bacteria. (Hammes *et al.*, 2009)

The combination of TCC and total ATP can be used as a cultivation-independent approach to viability assessment in drinking water.

Flow cytometry (FCM)

FCM (BD Accuri C6® flow cytometer) was used to enumerate the total cell counts in water samples. In this research, distinction is made between total cell counts (TCC-Total) and intact cell counts (TCC-Intact). Intact cells are cells of which the cell wall is not damaged.

Prior to light scattering all cells are labeled with a fluorochrome. Fluorochrome SYBR Green I will pass through cell walls and attaches to the DNA material. Propidium iodide will only enter cells that are damaged and attaches to the DNA material. Using different wavelengths the total and intact cell counts can be determined. Figure 3-7 shows a picture of a single cell. As noticed SYBR Green I can enter undamaged cells while propidium iodide only enters damaged cells.

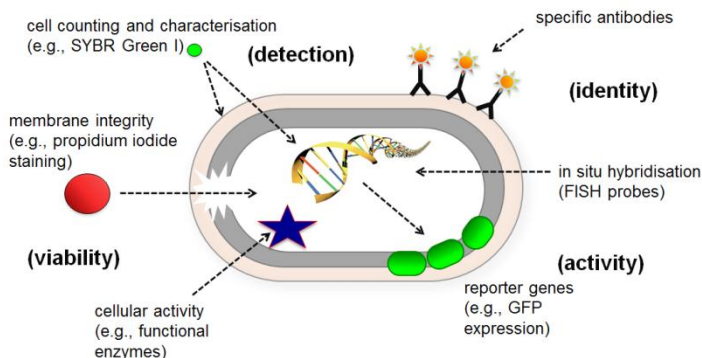


Figure 3-7 - Fluorochrome enters cells and attaches to DNA material

After 'labeling' cells with fluorochromes the water passes a capillary creating laminar flow which passes a laser beam of a specific wavelength. The passage is constructed in such a way that a flow of liquid droplets is obtained that each contain only one cell. Water flows undisturbed and no blocking occurs.

With the help of optical bandpass filters and dichroic mirrors SYBR Green I (light emission wavelength 475-700 nm) and propidium iodide (light emission wavelength 550-700 nm) are separated, after which the rays are detected by photometers. TCC-Total and TCC-intact are obtained.

By light scattering a cell will bounce back the light from the laser beam. The bounced light has the same wavelength and energy-content as the sent light from the laser beam but reflection occurs on a different angle. The intensity of forward scatter (FSC) is proportional to the size and shape of the cells. In this way distinction is made between HNA and LNA. Side scatter (SSC) can be used to research cell complexity and granularity.

The principle of FCM is shown in Figure 3-8.

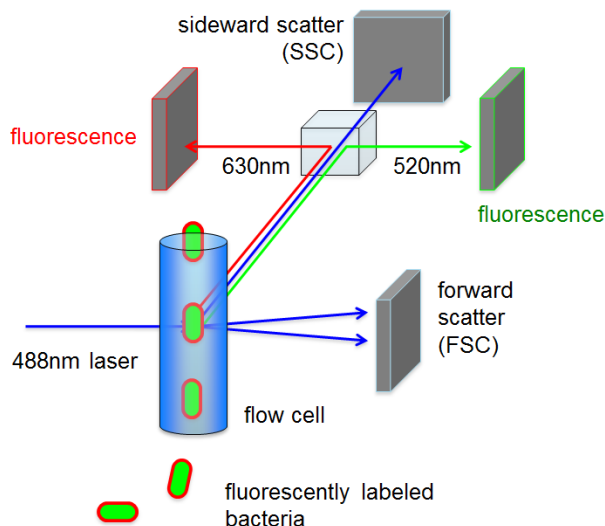
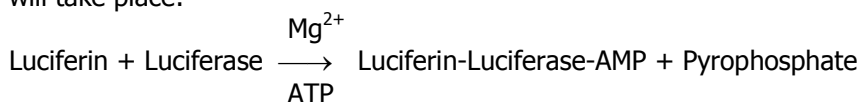


Figure 3-8 - Principle of flow cytometry using two stains with a different light emission wavelength to determine total and intact cell counts

Adenosine triphosphate (ATP)

ATP analysis is based on the reaction between two proteins derived from fireflies, luciferin (substrate) and luciferase (enzyme), which occurs in the presence of ATP. The light that is produced is measured and displayed in Relative Light Units (RLU).

A water sample is placed in a cuvette which is installed in a luminometer (biocounter). ATP may be present in the cells or freely in water samples. Next, reagents are added automatically. To lyse the cells LuminEX is added followed by adding substrate with enzyme LuminATE. Following reactions will take place:



(AMP = adenosinemonophosphate)

The emitted light is measured with a sensitive photometer and expressed as Relative Light Units (RLU). Under optimal conditions, 1 light photon per molecule of ATP is produced. The assay is performed in duplicate. Then, using a calibration method, the ATP-content of the sample is determined.

Biomass attached to metal biostuds of MRD and glass rings of biofilm monitor is removed before ATP analysis. Biomass removal is performed by ultrasonic vibrations (2 minutes) of the materials in sterile tap water. This treatment is repeated until all biomass is removed. The obtained suspension is used for ATP analysis.

3.2.2 Organic matter

Organic carbon is present as particulate organic carbon or as dissolved organic carbon (DOC). DOC is considered most important in drinking water because DOC provides the most easily consumable food source for aquatic bacteria (van der Kooij, 1992).

Dissolved organic carbon (DOC)

DOC is measured as the sum of organic bounded carbon present in water. After filtration by a membrane with pore size of 0.45 μm , organic carbon is catalytically combusted at 680 $^{\circ}\text{C}$ to carbon dioxide. Carbon dioxide that is released is measured using an infrared radiation detection device and then DOC is determined using a multi-point calibration.

3.2.3 Inorganic nutrients

Besides carbon the nutrients N and P are considered to be important with regard to bacterial growth. However, N is usually not the limiting factor for bacterial growth in drinking water. Some studies showed that adding $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$ did not stimulate further growth of bacteria (Frias *et al.*, 1994).

Orthophosphate ($\text{PO}_4^{3-}\text{-P}$) is considered to be the most important form of P, as it is the most readily available for biological consumption. When Phosphate-P has concentrations below 2 $\mu\text{g/l}$ it becomes the limiting factor for the growth of biofilm. Adding phosphates to these waters resulted in increased biofilm growth (Miettinen *et al.*, 1997).

Nitrate (NO_3^- -N)

AquaKem600, a discrete analyzer is used for spectrophotometric determination of nitrate in a water sample. Nitrate is reduced to nitrite using hydrazinesulphate. The gained nitrite together with nitrite present in sample reacts with sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride to a red colored diazo compound. Copper- and zincsulphate are catalysts in this reaction. The intensity of the color is measured at a wavelength of 540 nm and is a measure of the total concentration of oxidized nitrogen.

Orthophosphate (PO_4^{3-} -P)

AquaKem600, a discrete analyzer is used for spectrophotometric determination of orthophosphate in a water sample. In an acid environment, Molybdate forms together with ortho-phosphate ions and potassium antimony tartrate, by reduction of ascorbine acid a blue colored compound. The intensity of the color is measured at a wavelength of 880 nm and is a measure of the concentration of orthophosphate.

3.2.4 Ammonium, iron and manganese

Ammonium

AquaKem600, a discrete analyzer is used for spectrophotometric determination of ammonium in a water sample. Ammonium ions react with hypochlorite ions, formed by alkaline hydrolysis of sodium dichloro-isocyanurate and with salicylate at a pH of about 12.6, to a blue colored compound. The intensity of the color is measured at a wavelength of 660 nm and is a measure of the concentration of ammonium.

Iron and manganese

Iron and manganese analysis are determined by means of an inductively coupled plasma with mass spectroscopy (ICP-MS) in aqueous acidic solutions having a pH between pH 1 and pH 2. To reach this pH samples are acidified with HNO_3 . The sample is atomized with a nebulizer and enters as aerosol in the plasma. The plasma is a small cloud of very hot (6 000 – 10 000 K) and partially ionized argon gas. The plasma is generated and maintained in a radio frequency field. In the plasma water evaporates and present compounds disintegrate to atoms (dissociation and atomization). The atoms are almost completely ionized. In the mass spectrometer, the ionized atoms are separated and identified, depending on their mass and charge using a quadrupole.

3.3 Sampling locations

Measuring devices were put at specific locations to investigate the primary and secondary research questions. Also water samples were taken and analyzed on TCC, ATP, nutrient sources and other relevant parameters.

MFS

In total 5 MFS units were used in this experiment to investigate the biofouling potential of the two water flows (with and without RO). Therefore, a MFS unit was placed after post-filter 5 (with RO) and post-filter 6 (without RO). Also MFS units were placed after the two activated carbon vessels (experimental set-up). An additional unit was placed after the full scale treatment step of activated carbon to compare results.

MRD

To investigate the growth of biofilm of the two separate flows two MRD units are placed after activated carbon experiment flow 5 (with RO) and flow 6 (without RO).

Biofilm monitor

Parallel to MRD units, two biofilm monitors were placed to research the growth of biofilm. Also a comparison can be made between the performance of MRD and the biofilm monitor.

Water sampling

At all relevant locations in the treatment scheme, from raw water to clear water prior to distribution, water samples were taken and analyzed. Results can be used to answer primary and secondary research question.

4 Results + discussion

This chapter is an overview of results obtained in this research. Together with the discussion it will answer primary and secondary research questions. The chapter is split up into two parts. Paragraph 4.1 will focus on the results together with a discussion related to the research questions. In Paragraph 4.2 an analysis is given of the materials and methods used to investigate biological stability.

4.1 Results

To investigate biological stability several methods were used in this research. Distinction was made between direct measurements (Paragraph 4.1.1), (bio)fouling potential of water measured with the Membrane Fouling Simulator (Paragraph 4.1.2) and biofilm formation obtained by a biofilm monitor (Paragraph 4.1.3) and Modified Robbins Device (Paragraph 4.1.4). At ZS Lekkerkerk KWR Watercycle Research Institute also did research to the biological stability with regard to the effect of pre-treatment with RO. In Paragraph 4.1.5 a short comparison with this research is given.

4.1.1 Water sampling

Water samples were analyzed on TCC with flow cytometry and on ATP with a Luminometer. For TCC, the effect of pre-treatment with RO was visible throughout the treatment process. This was mainly caused by the physical removal of bacterial cells with RO. An increase in TCC was observed in water after pre-filtration caused by the biological removal of ammonium, iron and manganese in pre-filters. This was also recognized by ATP in water after pre-filtration. High ATP values up to 120 ng/l were observed indicating a low level of biological stability. After second filtration step ATP values dropped below 10 ng/l. This was also observed in water after GAC experimental set-up. With regard to ATP the effect of pre-treatment with RO was not recognizable.

Organic carbon is seen as an important food source for bacteria. DOC is almost completely removed by RO. The effect of pre-treatment with RO was visible and significant for DOC throughout the treatment scheme.

TCC and ATP in water observed at different locations in the treatment process are given in Figure 4-1 and Figure 4-2. Feed water is water that is fed to pre-filter 5 (with RO) and to pre-filter 6 (without RO). Feed water to pre-filter 5 is a theoretical value based on values observed in raw water that is extracted from the intake source and RO permeate with the assumption that it was mixed 50/50.

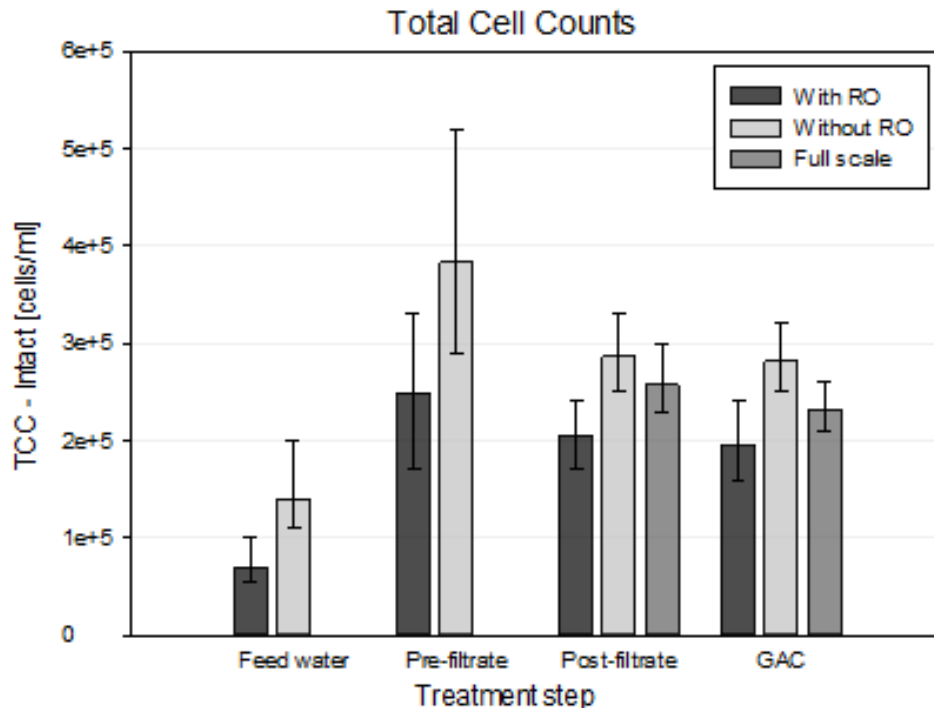


Figure 4-1 - Intact cell counts of flow with (50%) and without pre-treatment with RO. Full scale flow (reference) contains water from all filter sets that is mixed after post-filtration

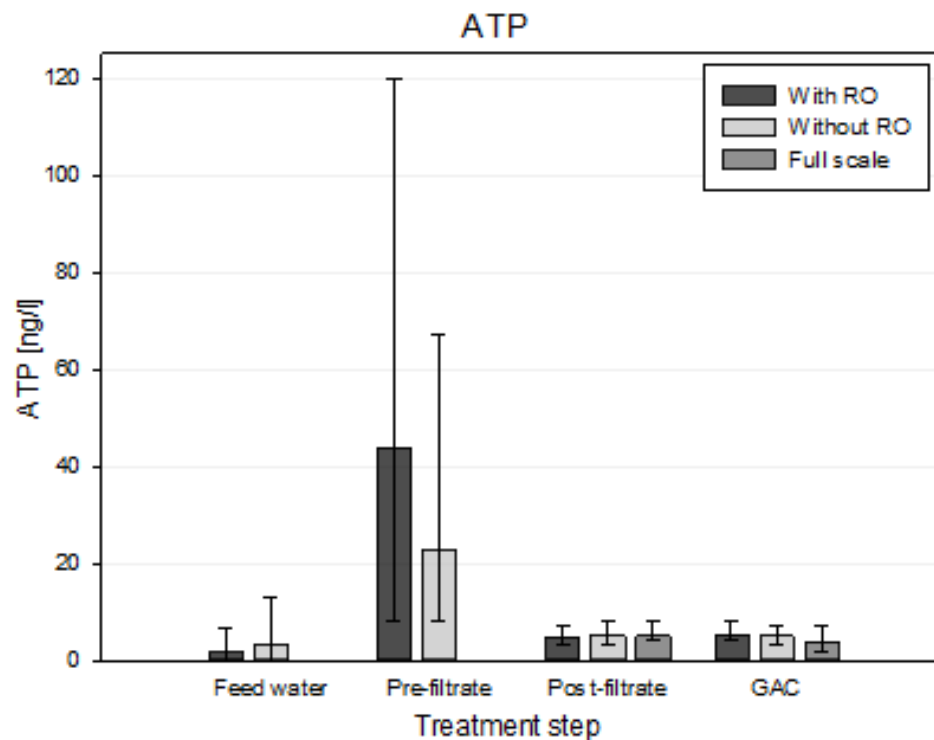


Figure 4-2 - ATP of flow with (50%) and without pre-treatment with RO. Full scale flow (reference) contains water from all filter sets that is mixed after post-filtration

One can conclude from Figure 4-1 that the effect of pre-treatment with RO on TCC was caused by the physical removal of bacterial cells. TCC does not indicate if regrowth potential of water is high. But TCC is an indicator for telling what the effect of several treatment step is on the biological growth. From this perspective it can be said that the growth of bacterial cells was caused by the biological removal of ammonium, iron and manganese in the pre-filters. 100% removal of methane was achieved by stripping.

TCC in water that was mixed after post-filtration is presented as the full scale flow. As expected, the TCC is in between the flows with and without RO. But as the contribution of RO to the full scale flow is very small (6-10%) it was expected that TCC observed in full scale flow was very close to TCC observed in flow without RO pre-treatment. An explanation for this significant difference is given in Appendix B1.

With regard to biological stability ATP is seen as a better indicator (Velten *et al.*, 2007) as ATP does tell about the level of activity of biomass present in water samples. Caution is needed in stating that ATP is the leading parameter for biological stability.

ATP peak values after pre-filtration were observed. A higher average ATP value after pre-filter fed with water that was pre-treated with RO cannot be explained. Substrate conversion in pre-filter was higher for pre-filter fed with water not pre-treated with RO. This is shown in Paragraph 4.1.1.2. Therefore a lower level of activity of biomass in filter fed with water pre-treated with RO was expected.

After post-filtration and after GAC experimental set-up the effect of pre-treatment with RO was not recognized with regard to ATP. For both flows (with and without RO) ATP was lower than 10 ng/l, which is seen as a reference value for biological stable water (Van der Kooij *et al.*, 1999). Compared to pre-filters the biological activity in post-filters and GAC pressure vessels was lower and was also recognized in a drop in TCC.

4.1.1.1 Feed water (Raw + RO Permeate)

The effect of pre-treatment with RO was directly visible when comparing the feed water qualities to pre-filter 5 (with RO) and pre-filter 6 (without RO). Almost all bacterial cells were removed physically with RO as observed in RO permeate. As expected, for all RO permeate samples taken the ATP was below detection limit (1 ng/l). The removal of iron, manganese, DOC and orthophosphate-P was also very high. Ammonium and methane in gas phase partly passed the RO membranes.

ATP in feed water flows to pre-filtration is presented in Figure 4-3 (left) and TCC in feed water flows to pre-filtration in Figure 4-3 (right).

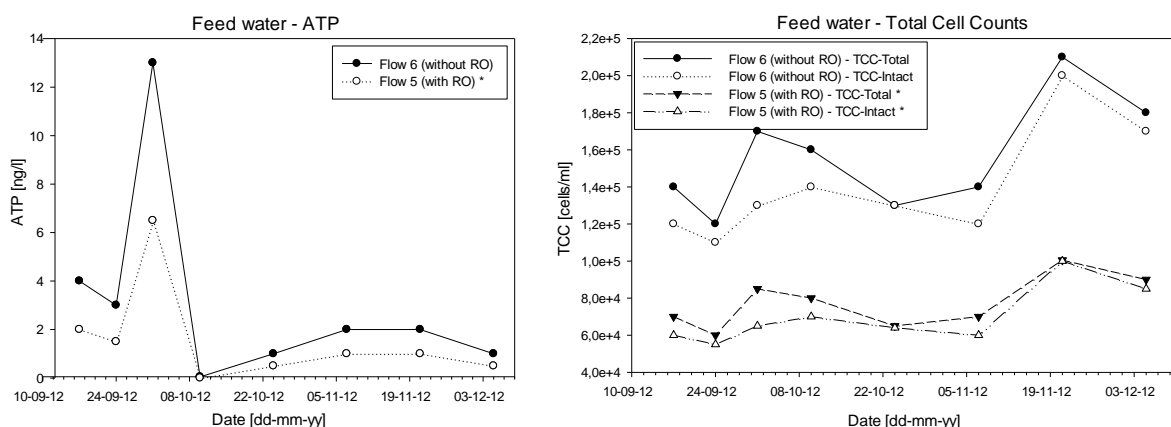


Figure 4-3 - ATP (left) and TCC (right) of feed water flows (with and without pre-treatment RO) to pre-filters
*Flow 5 is theoretical value based on 50% RO permeate and 50% TW raw water

As the actual feed water flow (in m³/h) to both pre-filters differed, the load of ammonium, iron and manganese to both filter beds was also different. This influenced the amount of substrate conversion and therefore may have influenced the biological activity and/or growth in pre- and post-filters. In Appendix B2 an estimation of the feed water load to both pre-filters is presented.

For all RO permeate samples, ATP was below the detection limit (1 ng/l). As expected also TCC values were extremely low with no samples exceeding 1 000 cells/ml. Theoretically it can be

assumed that more than 99.9% of cells should be removed by RO. However, two samples observed TCC values slightly higher than the detection limit (600 cells/ml). This can be caused by the fact that cells may be present in sampling pipes where residence time of water was high and thus cells present have time to attach on walls and multiply. The time between sampling and analysis could also have caused some slight increases in TCC. In addition FCM measuring technique is not 100% accurate in distinguishing background from actual cells.

Except for one peak value, raw water observed low ATP values up to 4 ng/l. Peak value may be caused by an error during analysis. If a flock of cells is present in the water sample this can result in a high error of the ATP measurement. TCC values varied between 1.1×10^5 and 2.1×10^5 cells/ml for raw water. Some variation may be caused due to changing the combination of intake wells over time.

TCC was obtained using FCM. Analysis of the results was done using CFlow software. For raw water and RO permeate an analysis is given in Appendix B3.

4.1.1.2 Pre-filtration

The first step in the treatment scheme was dual media filtration. Filter material used was sand and anthracite. Main processes that occurred in the pre-filters were the removal of ammonium, iron and manganese. As these processes are mainly biological an increase in biological activity was expected after pre-filtration. This is confirmed by high ATP observed in water for both flows (with and without pre-treatment RO). An absolute increase of TCC (compared to feed water) for both flows was also observed. 100% methane removal was achieved physically by stripping.

Again, the physical effect of water pre-treated with RO was visible in TCC as presented in Figure 4-4 (right). Observed ATP was high and variable for both flows (Figure 4-4 (left)).

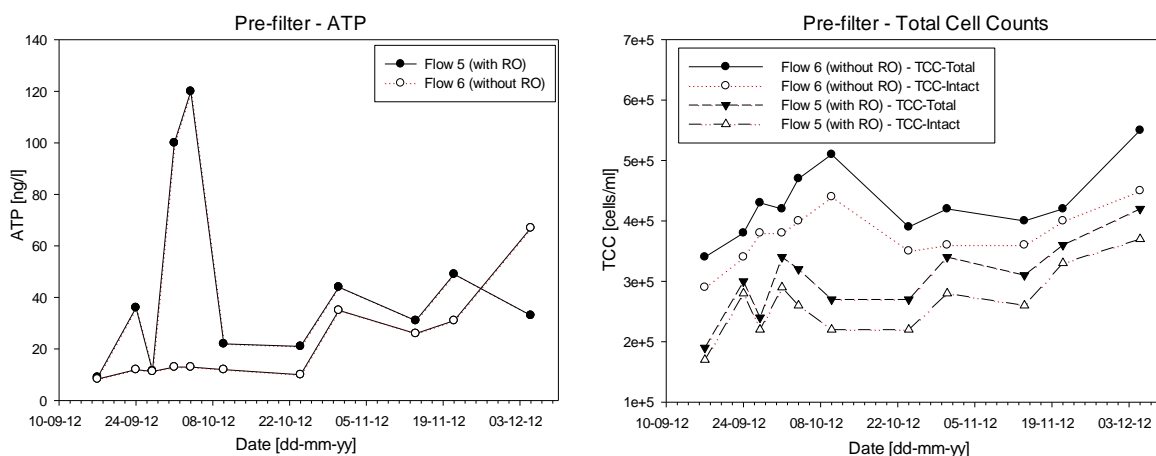


Figure 4-4 - ATP (left) and TCC (right) of water after pre-filtration (with and without pre-treatment RO)

Because of RO pre-treatment the load of filter set 5 was about 65% of the load of filter set 6 as estimated in Appendix B2. TCC-Intact values in pre-filtrate without RO varied between 2.9×10^5 and 4.4×10^5 cells/ml, while TCC-Intact in pre-filtrate with RO varied between 1.7×10^5 and 2.9×10^5 cells/ml. For all samples taken TCC in water that was pre-treated with RO observed lower values as a result of the physical removal of bacterial cells with RO.

When looking at the ATP an interesting conclusion can be made. For both pre-filtrates the ATP values were high. Unexpectedly, the ATP values for pre-filtrate with RO seemed to be higher than for pre-filtrate without RO indicating a higher level of activity of the bacteria. From both ATP and TCC it can be concluded that ATP per cell was higher for pre-filtrate 5. This was unexpected and unexplainable as substrate conversion in pre-filtrate was believed to be lower. E.g. ammonium load of feed water was estimated lower (Table B-5) and ammonium concentration in water after pre-filtration was equal or higher for flow with RO for many samples over time.

From ATP results one can conclude that water after pre-filtration was not of a high level of biological stability caused by the high biological removal of substrate and therefore high level of activity of bacteria.

From literature (Figure 2-7) a good relationship ($R^2 = 0.86$) was found between total ATP and HNA fraction of TCC (Berney *et al*, 2008). Interesting to see is that for filter 5 (with RO) the percentage of HNA on TCC increased from 12% to around 27% and for filter 6 (without RO) from 12% to around 20%. This indicates that the relative growth of the larger cells in the water was higher.

Research was done to the variety of TCC over time. Focus was on the difference in pattern of both flows presented in Appendix B4.

Ammonium removal

When looking at the ammonium effluent concentrations of the pre-filters (Figure 4-5) one can conclude that the performance of especially pre-filter 6 was not constant over time. Another interesting property from Figure 4-5 is that ammonium concentration of pre-filtrate 5 was equal or higher for many samples taken over time. This was not expected as influent ammonium concentration of pre-filter 5 was lower than for pre-filter 6.

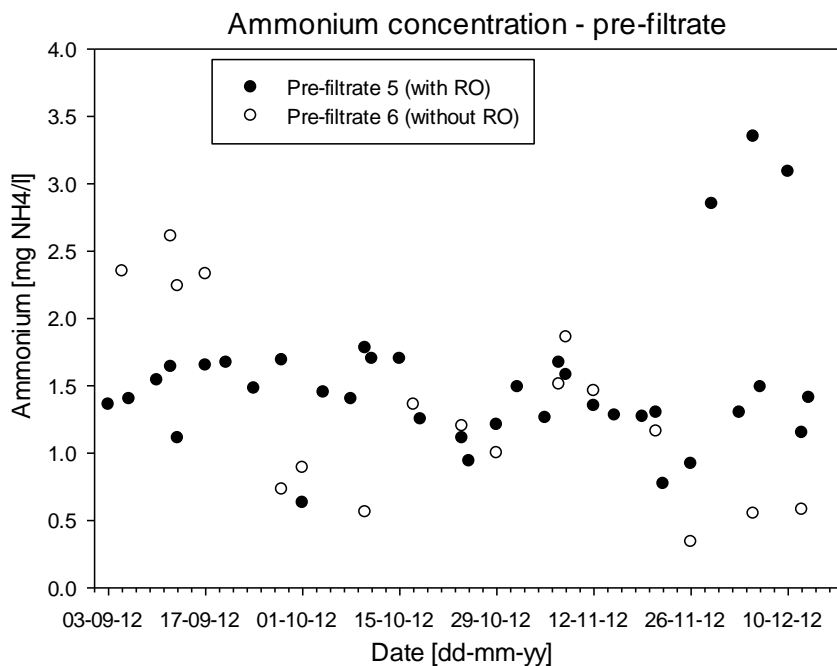


Figure 4-5 - Ammonium concentration in water after pre-filtration (with and without pre-treatment RO)

Ammonium peak values in pre-filtrate 5 at the end of the research period were caused by the fact that due to operational problems RO permeate water was not fed to filter 5, but the bypass was used consisting of 100% raw water without pre-treatment with RO.

Nutrient sources – DOC, Nitrate-N and Orthophosphate-P

Besides the growth of nitrifying bacteria, growth of bacteria can also be caused by the availability of nutrient sources. Table 4-1 shows the concentration of nutrients sources in water after pre-filtration.

Table 4-1 - Nutrient sources (DOC, Nitrate-N and Orthophosphate-P) in water after pre-filters

Type of water	DOC [mg/l]	Nitrate-N [mg/l]	Orthophosphate-P [mg/l]
Pre-filtrate 5 (with RO)	1.08	1.29	0.03
Pre-filtrate 6 (without RO)	2.15	3.57	0.03

Comparing both pre-filters it can be concluded that organic carbon, seen as the most important food source, was significantly lower in water after filter fed with water pre-treated with RO. Only a slight decrease in DOC was observed between feed water and water after pre-filtration. Treating 50% of water with RO, almost 50% of DOC was removed in feed water to pre-filter 5. For both feed waters the nitrate-N concentrations were very low (<0.1 mg/l). Due to the conversion of ammonium, nitrate in water increased. As mentioned before pre-filter 6 had a higher ammonium concentration in the influent but for many samples an equal or lower ammonium concentration in the effluent compared to pre-filter 5. This is confirmed by the amount of nitrate in water from pre-filters.

4.1.1.3 Post-filtration

After post-filtration, consisting of sand as filter material, clear observations (based on direct measurements) can be made regarding the effect of pre-treatment with RO on the biological stability of water. No significant difference in ATP between flow with and without pre-treatment RO was recognized. For TCC the physical removal of TCC with RO was still observed after post-filtration. Same accounts for DOC.

In Figure 4-6, ATP (left) and TCC (right) of water after post-filtration is presented.

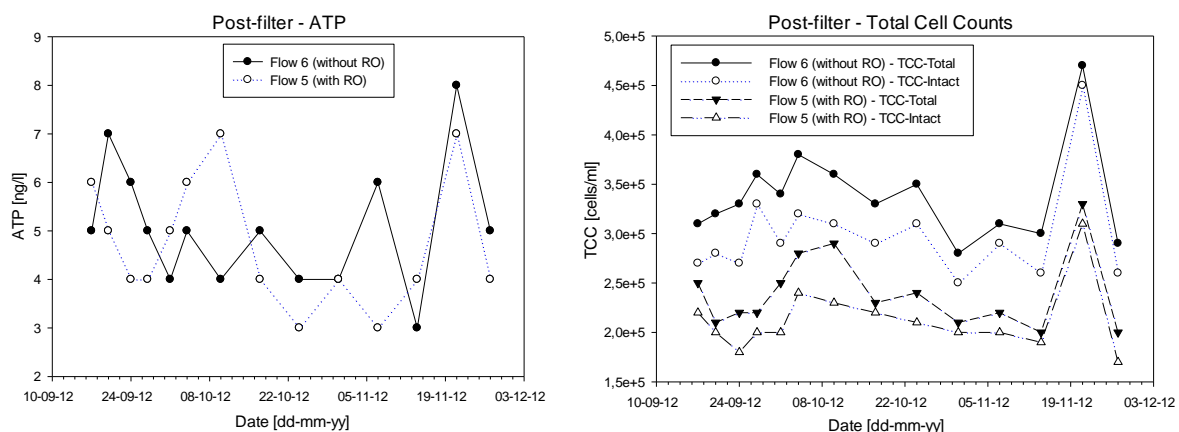


Figure 4-6 - ATP (left) and TCC (right) of water after post-filtration (with and without pre-treatment RO)

Compared to pre-filtrate, ATP results for both flows were significant lower. This was caused by less biological activity than during pre-filtration. It is expected that substrate load over the filter bed was decreasing; thus more substrate was available in the top compared to the bottom of the filter bed. This results in lower activity of bacteria present in the bottom part of the filter. This is confirmed by the high removal rates given in Appendix B5.

For TCC observed in water after both post-filters same conclusions can be drawn again. TCC in water that was pre-treated with RO was structural and significantly lower than water that was not pre-treated with RO likely caused by the physical removal of TCC with RO.

One peak value was observed for both post-filtrate 5 and post-filtrate 6 (Figure 4-6). Compared to the other results it seems significant, but an increase of about 1.0e+05 cells/ml is not that high. In most studies bacterial parameters are presented on a logarithmic scale. In this thesis this has not

been chosen as the effect of pre-treatment with RO on TCC will be hardly visible when presented on a logarithmic scale. As for both post-filtrates the same peak value occurred it can be assumed that it was caused by a change in the feed water quality. When looking at the TCC of raw water and after GAC experiments an increase in TCC was also observed. Surprisingly this peak was not observed in the pre-filtrate.

4.1.1.4 Granular activated carbon (experimental set-up)

For direct measurements same conclusions can be drawn for water after granular activated carbon pressure vessels as for water after post-filtration. The effect of pre-treatment with RO was not visible regarding ATP. The physical removal of bacterial cells obtained by pre-treatment with RO is still significant after the GAC pressure vessels. Compared to post-filtration no significant changes in DOC, nitrate-N and orthophosphate-P was observed. ATP and TCC results in water after GAC experimental set-up are given in Figure 4-7.

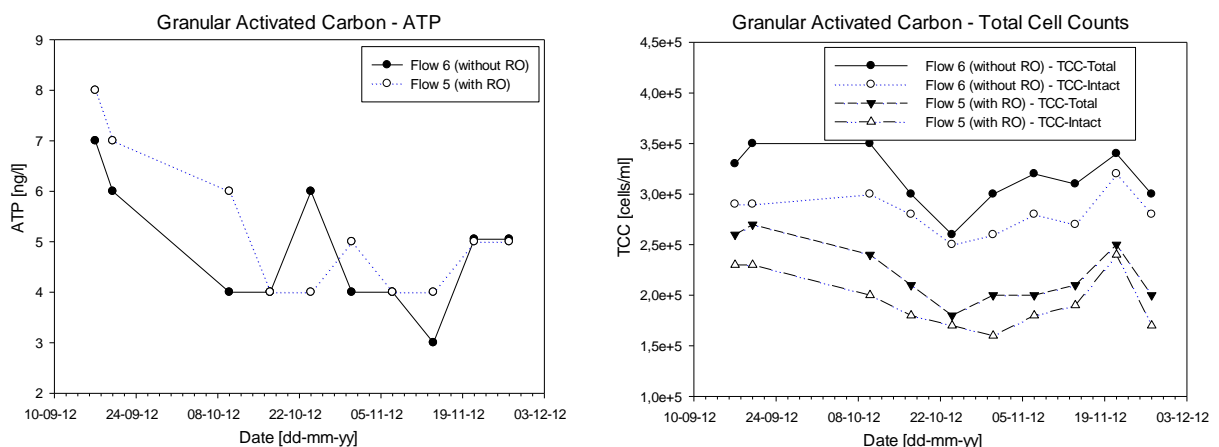


Figure 4-7 - ATP (left) and TCC (right) of water after post-filtration (with and without pre-treatment RO)

ATP values were low (3-8 ng/l) and no structural difference was observed between both flows. As expected, the TCC values were similar to results of post-filtrate where TCC values for GAC flow 5 is significantly lower than for GAC flow 6.

With regard to ATP results water seemed of a high level of biological stability. Again, TCC results are not a direct indicator for the regrowth potential. However, it does show the effect of pre-treatment with RO on cells that enter the distribution network.

4.1.2 Membrane fouling simulator

The effect of pre-treatment with RO on the (bio)fouling potential of water was investigated using MFS units with a flow of 16 L/h. Five monitors were operated at five locations in the treatment scheme: after post-filter 5 (with RO), after post-filter 6 (without RO), after GAC experiment 5 (with RO), after GAC experiment 6 (without RO) and after GAC full scale (reference).

The highest increase in feed channel pressure drop over time was observed at MFS unit placed after post-filter 6 (without RO). An increase was also recognized for MFS unit placed after post-filter 5 (with RO). As no increase in feed channel pressure drop was observed at MFSs placed after GAC experimental set-up (both with and without RO) it is assumed that feed channel pressure drop was likely caused by iron and manganese concentrations from post-filtration. This is confirmed by visual observations obtained using the sight window.

Although biofouling potential of water could not be recognized in an increase in feed channel pressure drop, the effect of pre-treatment with RO was observed with regard to biomass accumulation on spacers and membranes in MFS units. The biomass accumulation (ATP, TCC, TOC) on spacers and membranes was lower for MFSs placed at the flow that was pre-treated with

RO. On spacers and membranes of MFSs placed after granular activated carbon less biomass accumulation was observed than after post-filtration.

To fasten the biofouling process a second run was performed with a higher flow (41 L/h) at the five locations. Comparable results were obtained than during the first run.

4.1.2.1 First run

For the first run the results consist of:

- Feed channel pressure drop (during run)
- Visual observations using the sight window
- Visual observations using a microscope (after run)
- Autopsy of membrane and spacer (after run)

The first run lasted from 24 September 2012 to 27 November 2012. Due to practical reasons some units were installed some days after the initial start date.

Feed channel pressure drop

The feed channel pressure drop is the difference in pressure between the feed and the concentrate lines. It was measured using a differential pressure transmitter. Figure 4-8 shows the results of the first run. Because of instant fouling of the spacer in MFS unit after post-filter 5 the run was stopped (1) and restarted (2) using a new membrane and spacer.

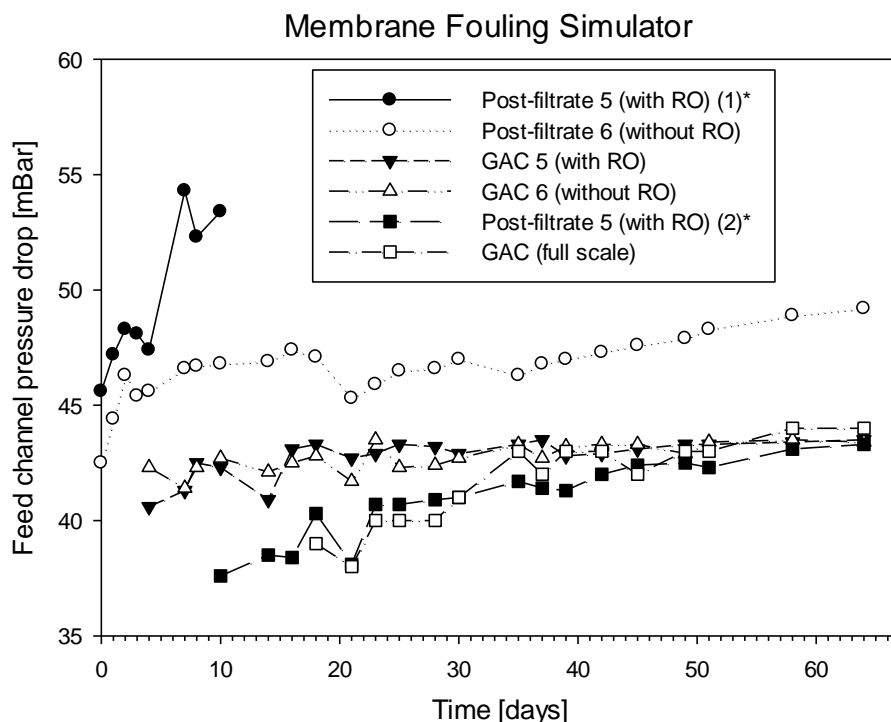


Figure 4-8 - Feed channel pressure drop in time of MFS's placed after post-filtration (with and without pre-treatment RO) and GAC experiments (with and without pre-treatment RO)

*Due to instant fouling of the MFS after post-filter 5 (with RO) run (1) was stopped and a new run (2) was started

A sudden increase in feed channel pressure drop was observed at the monitor placed after post-filter 5. Due to operational problems filter set ran dry. Most likely, deposits in the storage at the bottom of the post-filters were transported into the experimental set-up causing fouling. A second reason of fouling can be that higher iron effluent concentrations occurred after the filter was used again. Visual observation with a microscope of membrane and spacer used with regard to instant fouling is given in Appendix C1.

From Figure 4-8 it can be concluded that overall feed channel pressure drops over time were low. This is consistent with results from Vrouwenvelder (2010) where only small pressure drops were observed for water to which no substrate was added (acetate dosage).

At MFSs placed after post-filtration an increasing trend in feed channel pressure drop was recognized. This was caused by components in the post-filtrate that were retained in the GAC treatment step (see visual observation in Appendix C1). Although differences between MFSs placed after post-filtration were small it can be seen that the gradient of feed channel pressure drop is slightly higher for flow 6 (without RO) than for flow 5 (with RO). This may indicate that fouling potential of flow 6 is slightly higher.

(Bio)fouling potential after the GAC experimental set-up was for both flows very low as only a negligible increase in feed channel drop was observed. Differences with GAC full scale could be lead back to the way of operation. No dosage pump was used for controlling the flow, but a valve under natural pressure was constricted.

Visual observations using a microscope

After a run time of 2 months all spacers and membranes were placed under a microscope. Figure 4-9 shows the microscopic results of the spacers. Visual observations of membranes show less or equal information.

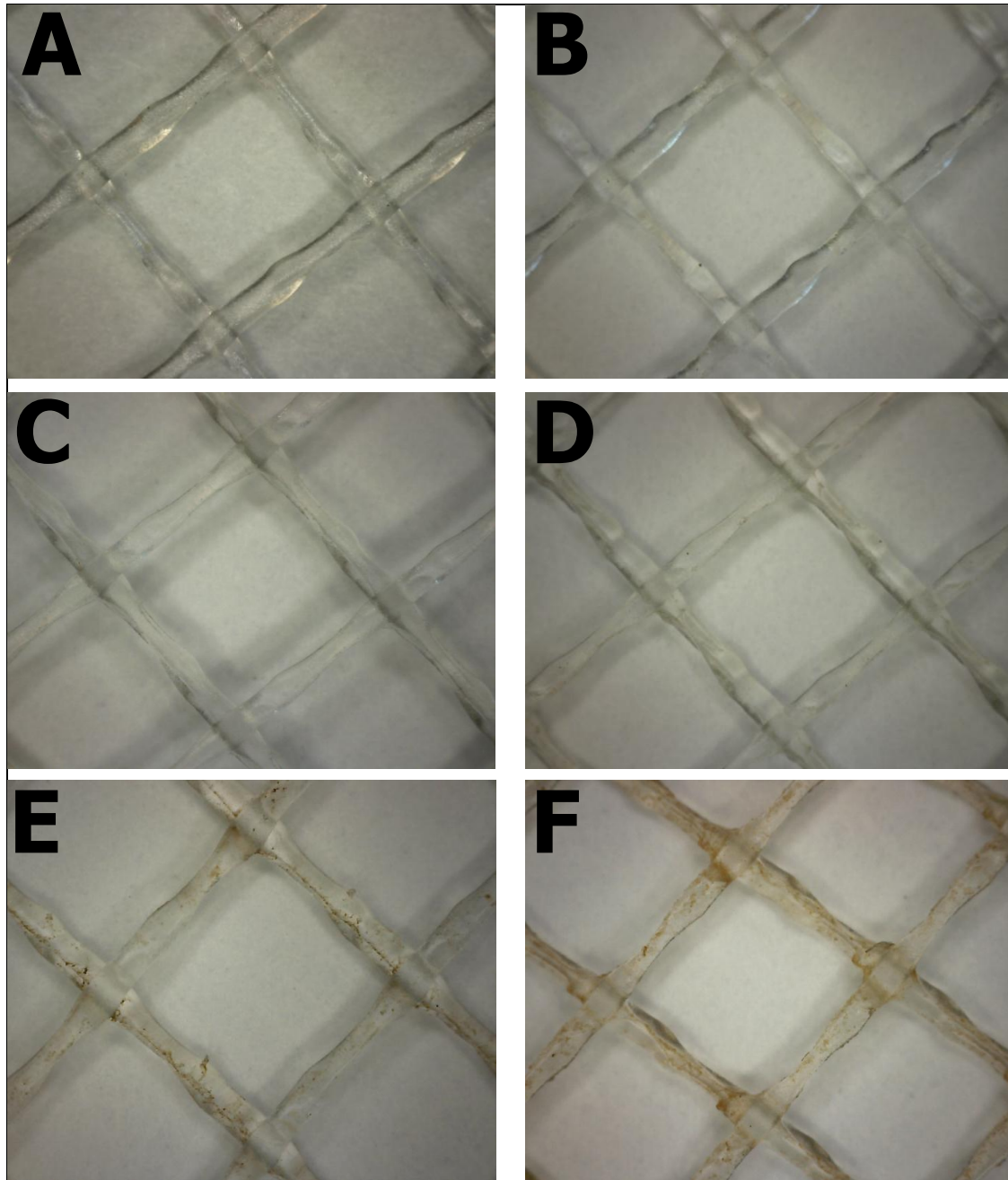


Figure 4-9 - Microscopic observations of feed spacers (placed on a blank paper) after the first MFS run (flow from left to right)

(A: Clean, B: Activated carbon full scale, C: Activated carbon experiment 5 (with RO), D: Activated carbon experiment 6 (without RO), E: Post-filtrate 5 (with RO), F: Post-filtrate 6 (without RO))

With regard to visual observations the effect of pre-treatment RO was not visible after the GAC experimental set-up. After post-filtration the MFS unit placed in flow with pre-treatment RO did show less fouling of the spacer and membrane. This indicates that fouling potential of water pre-treated with RO is lower. However, after GAC treatment the effect of RO (with regard to fouling) was not recognizable.

Autopsy of membrane and spacer (after run)

After the first run MFS units were transported to a laboratory for membrane and spacer analysis. Analysis was done on ATP, TCC, TOC, iron, manganese and calcium. An overview of the results is given in Table 4-2 (bacterial) and Table 4-3 (non-bacterial).

Table 4-2 - Biomass concentration found on membranes and spacers in monitors placed at several locations after a run time of 54 to 64 days

Type of water	Run time [days]	Membrane (M) or Spacer (S)	ATP [pg/cm ²]	TCC-Total [cell/cm ²]	TCC-Intact [cell/cm ²]
Post-filtrate 5 (with RO)	54	M	620	44E+05	16E+05
		S	430	14E+05	4.5E+05
Post-filtrate 6 (without RO)	64	M	1400	120E+05	42E+05
		S	2100	130E+05	43E+05
GAC (experiment) 5 (with RO)	60	M	370	34E+05	12E+05
		S	150	4.6E+05	1.5E+05
GAC (experiment) 6 (without RO)	60	M	650	38E+05	15E+05
		S	570	14E+05	4.1E+05
GAC (full scale)	60	M*	1700	38E+05	17E+05
		S	280	3.8E+05	0.78E+05

* Possible contamination during analysis

Table 4-3 - Relevant parameters found on membranes and spacers in monitors placed at several locations after a run time of 54 to 64 days

Type of water	Run time [days]	Membrane (M) or Spacer (S)	TOC [pg/cm ²]	Iron [mg/cm ²]	Manganese [mg/cm ²]	Calcium [mg/cm ²]
Post-filtrate 5 (with RO)	54	M	2.0	0.38	<0.02	<0.50
		S	1.3	0.52	0.09	<0.50
Post-filtrate 6 (without RO)	64	M	2.1	1.00	0.10	<0.50
		S	3.4	2.10	0.32	<0.50
GAC (experiment) 5 (with RO)	60	M	2.0	0.20	<0.02	<0.50
		S	0.4	0.09	<0.02	<0.50
GAC (experiment) 6 (without RO)	60	M	2.2	0.19	<0.02	<0.50
		S	3.1	0.18	<0.02	<0.50
GAC (full scale)	60	M	4.8	0.22	<0.02	<0.50
		S	2.3	0.15	<0.02	<0.50

From bacterial analysis of membrane and spacers one can conclude that biomass accumulation (ATP in pg/cm²) on all spacers and membranes was low. After a run time of 54 to 64 days biomass accumulation on spacers ranged from 150 to 2 100 pg/cm². Araújo *et al.* (2012) found ATP values of around 300 pg/cm² after a run time of 6 days. The biomass accumulation per day (ATP of 50 pg/cm².d) in the study of Araújo *et al.* (2012) is higher than found during highest biomass accumulation in this research (ATP post-filtrate 6 of 32 pg/cm².d). Taken into account should be the different qualities of feed water. However, Araújo *et al.* (2012) also used drinking water prior to distribution prepared from anaerobic groundwater.

Although microbiological values were low the effect of pre-treatment with RO was visible. For MFSs placed in the flow that was pre-treated with RO, the biomass accumulation (ATP and TCC) was significantly lower than in the flow not pre-treated with RO. This accounted for both MFSs placed after post-filtration as MFSs placed after GAC experimental set-up.

GAC treatment step had also a positive effect on the biomass accumulation. MFSs placed after GAC experimental set-up observed lower ATP and TCC accumulation on spacers and membranes than MFSs placed after post-filters. These results indicate that biofouling potential of water decreases after GAC treatment.

In Appendix C2 an answer is given of what components in the water investigated have largest impact on the increase of the feed channel pressure drop.

4.1.2.2 Second run

To fasten the process of biofouling a second run was performed with a flow of 41 L/h. This resulted in a higher linear velocity over the spacers and membranes. A higher flow means that more substrate passed the spacers and membranes in MFS units. Again, MFS units were placed at same locations as in the first run to investigate the effect of pre-treatment with RO.

For the second run the results consist of:

- Feed channel pressure drop (during run)
- Visual observations using the sight window
- Visual observations using a microscope (during run)

The relative increase in feed channel pressure drop observed in the second run was comparable with the first run. Due to a higher flow the initial pressure drop was higher. The absolute values of the increase in feed channel pressure drop was also higher caused by higher linear flow velocities resulting in more resistance over the spacer. Observed trends were similar to the first run. Visual observations obtained during the second run were comparable with the first run.

Results of the second run are presented in Appendix C3.

4.1.3 Biofilm monitor

Two biofilm monitors were placed after the GAC pressure vessels of the experimental set-up. Run time of both biofilm monitors for this thesis was limited from 25-10-12 to 17-1-2013. After this thesis biofilm monitors will continue to run. Extreme caution in interpreting results is needed as monitoring was not finished. From the first 84 days the effect of pre-treatment with RO seemed to be visible. For most samples taken the biomass concentration was lower on glass rings for biofilm monitor installed at the flow containing pre-treatment with RO. However, biomass concentrations obtained were low compared to Van der Kooij (1995). It is doubtful if the effect of pre-treatment with RO is significant.

Figure 4-10 shows the growth of biofilm expressed in biomass concentrations on the glass rings of the biofilm monitor that were removed for analysis every two weeks.

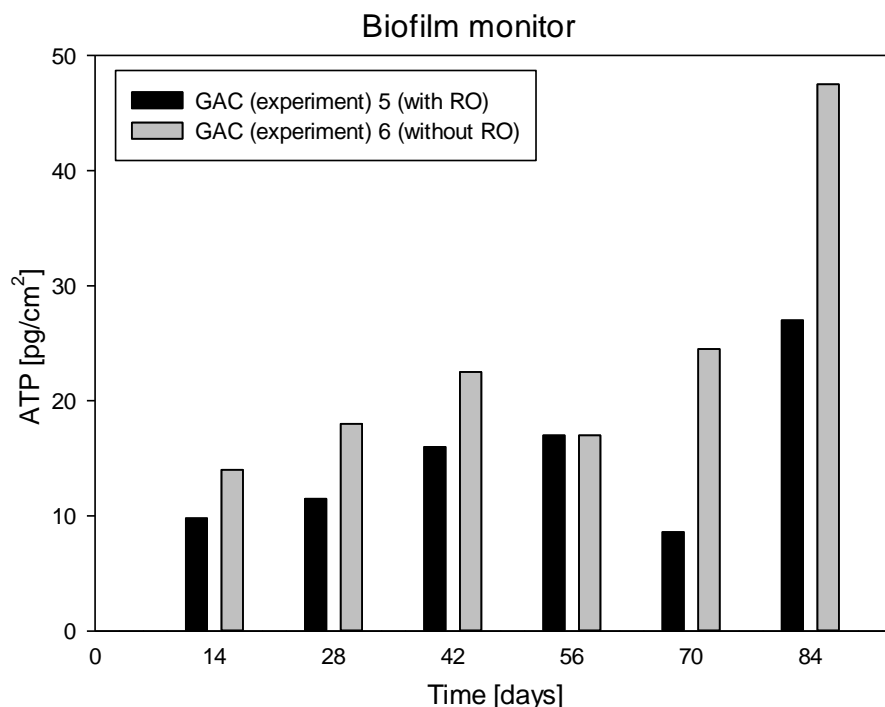


Figure 4-10 - Biomass concentration in time found on glass rings of the biofilm monitor
*all samples were taken in duplicates

A difference was observed between the two investigated flows. The flow consisting of water pre-treated with RO observed less biomass concentrations on surfaces for many samples. Initially, biomass concentrations increased slowly but an exponential trend is expected at the end of the monitoring. Probably this exponential trend started after 70 days of running after a sharp increase was observed.

Although the biomass concentrations observed were lower for the flow with RO, the BFR at the start of the monitoring of both flows seems to be comparable indicating that the effect of pre-treatment with RO is limited with regard to biofilm growth.

In agreement with previous results discussed in this report the level of biological stability of water was high for both flows. From day 14 to day 42 of the experiment the biomass formation rate was 0.29 pg ATP/cm².d for water after GAC pressure vessel 6 (without RO). For treated groundwater Van der Kooij (1995) found BFR values ranging from 4.8 to 11.0 pg ATP/cm².d. Van der Kooij and Vrouwenvelder (2001) observed values below 1 pg ATC/cm².d for slow sand filtrate. Caution is needed in comparing results as feed water quality is different in the various studies. Again, BFR found in literature is a result of a complete run. Previous research at ZS Lekkerkerk of clear water

observed a BFR of 6.4 pg/cm².d indicating that the correct BFR values were not reached in this research.

BFP was not reached in this research due to time limitation. However, based on the small BFR in the beginning of the monitoring the expectation is that the run time (140 days) of the biofilm monitor in this research will be too short to reach the maximum level of biomass formation.

4.1.4 Modified Robbins Device

MRD was used to simulate biofilm growth. The principle that biomass attaches to surfaces is the same as the proven biofilm monitor. Stainless steel bolts were implemented in the device where biofilm is formed on stainless steel surfaces. A flow of only 0.8 L/h was used as recommended by the developers of the system. Results obtained were highly variable and no trends were observed making the method inadequate for measuring biofilm formation.

In Figure 4-11 the results obtained after analysis of steel bolts in a laboratory are presented. Every week two stainless steel bolts were removed per device.

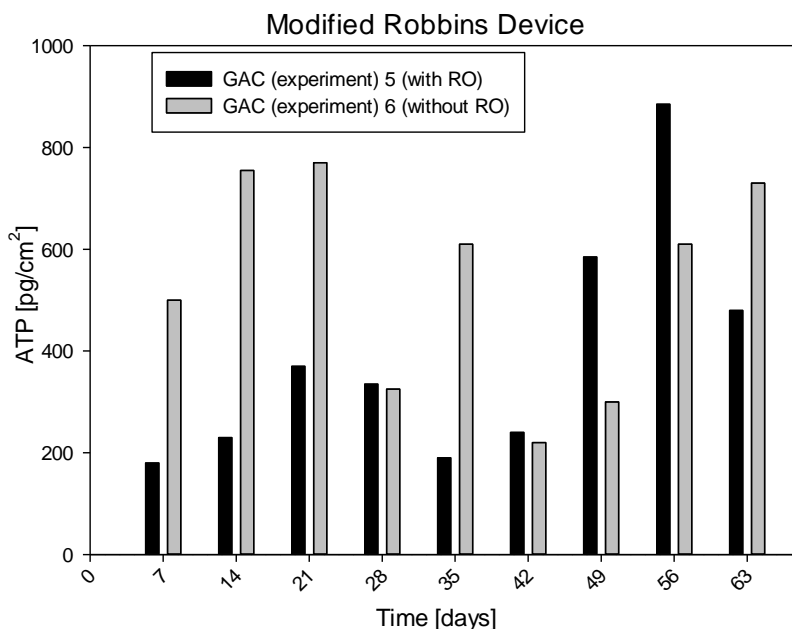


Figure 4-11 - Biomass concentration in time found on metal studs of Modified Robbins Device

A growth in biomass concentration was expected over time. For both MRDs results were highly variable over time. There was no trend observed in the difference between both flows (with and without RO) investigated. Compared to the biofilm monitor the biomass concentrations varied from 190 to 850 pg ATP/cm² compared to maximum values of the biofilm monitor of less than 50 pg ATP/cm² in the same time span.

One can conclude that results of MRD were inconsistent for investigating biofilm growth of drinking water. Possible cause could be the used flow of only 0.8 L/h. Previous research performed by Liu (2012) also showed inconsistent results with a flow of 16 L/h. During operation of MRDs air entered the flow channel due to the fact that some biostuds could not be fit good enough into the system. During the removal of the metal biostuds the flow channel was exposed to open air and thus contamination might occur. For the removal of some samples force was needed as biostuds were fit too tight in the device. This caused movement of the MRD and inadequate sampling as a result. After the biostuds were removed the stainless steel bolt had to be removed using a screwdriver. With a tweezers the stainless steel bolts were removed for transportation to a laboratory.

All reasons above led to inadequate sampling and inconsistent results.

4.1.5 Research by KWR Watercycle Research Institute

As part of a cooperation with Oasen, KWR Watercycle Research Institute also investigated the biological stability of water at ZS Lekkerkerk with regard to the effect of pre-treatment with RO.

KWR used three methods to investigate the biological stability:

- Continuous Biofilm Monitor (CBM)
- Hemoflow concentration technique
- Biomass Production Potential (BPP)

A description of the methods and the results including discussion are described in Hijnen *et al.* (2013).

Continuous Biofilm Monitor

The effect of pre-treatment with RO was visible after pre-filtration where biomass accumulation rate (BAR) of water pre-treated with RO was lower than the BAR for water without pre-treatment RO. This was not recognized for water after the GAC pressure vessels in the experimental set-up as differences in BAR between both flows (with and without RO) was small.

Interesting observation is that the BAR after pre-filters is significantly higher than the BAR after GAC pressure vessels. This confirms the statement that after pre-filtration, due to biological activity in the pre-filters, water was of a lower level of biological stability.

Hemoflow concentration technique

The effect of pre-treatment with RO was visible with regard to carbohydrates and particulate organic carbon observed in the concentrate. Water pre-treated with RO observed lower concentrations confirming that the physical removal of components with RO (like DOC in the research of this thesis) remained visible during the treatment process.

The effect of GAC treatment step as seen with e.g. MFS was not recognized in the concentration of carbohydrates and particulate organic carbon observed.

Biomass Production Potential

After 14 days of incubation the cumulative ATP value of water after post-filtration pre-treated with RO was slightly lower than for water not pre-treated with RO. After GAC pressure vessels of the experimental set-up no difference in the cumulative ATP values between both flows (with and without RO) was observed. This can confirm the statement that the effect of pre-treatment with RO after GAC treatment is not significant.

4.2 Performance of measuring devices

During this research methods have been used to measure the biological stability of drinking water in a treatment plant. For some methods the effect of pre-treatment RO was visible, but likely not always significant. Also the effect of the several treatment steps in the treatment scheme of ZS Lekkerkerk was recognizable. However, comparing the results of several methods some methods show clear differences while other methods did not show a significant effect of pre-treatment RO on biological stability.

The performance of measuring devices is based on several criteria that are important for investigating research questions. Results obtained from measuring devices should be reproducible, accurate and relevant within a specific time scale to answer research questions. Important for researching the effect of pre-treatment RO is that measuring devices can measure the differences between two water flows. Regarding primary research question (effect of pre-treatment with RO) a relative analysis is more important than the absolute obtained values.

Based on these criteria an analysis will be given in this paragraph about the devices used to measure biological stability in a drinking water treatment plant:

- I. Water sample analysis
 - a. Cells – ATP and TCC
 - b. Nutrient sources – DOC, NO_3^- -N and PO_4^{3-} -P
- II. Membrane Fouling Simulator
- III. Biofilm Monitor
- IV. Modified Robbins Device

4.2.1 Methodology

Ad I. Water sampling (spot measurements)

Water sampling is an easy and fast method to investigate (microbial) water quality. From TCC results the effect of pre-treatment RO was directly visible. The effect of several treatment steps was also observed using TCC.

Water containing ATP lower than 10 ng/l is seen as the upper limit for biological stable water (Van der Kooij *et al.*, 1999). Caution is needed for stating that water is stable or unstable based on ATP only. It is not proven that water containing low amounts of ATP automatically implies that regrowth during distribution is limited. Also it is not proven that if water observe high ATP values it automatically implies that water is biological unstable.

Physical removal of nutrient sources by RO was directly visible in water sample analyses (DOC, NO_3^- -N and PO_4^{3-} -P) and was recognized in further treatment scheme.

Obtained ATP and TCC results were accurate and relevant with regard to the research questions.

Ad II. Membrane Fouling Simulator

MFS units were used to investigate the (bio)fouling potential of water on spacers and membranes. MFS units are compact and easy to install. Sight window is effective for direct recognition of fouling of the spacers and membranes.

Results obtained are accurate and reproducible as both runs performed (16 L/h and 41 L/h) showed same trends.

Method can be relevant for researching the effect of pre-treatment RO on the biological stability. In this research the potential for biofouling of both water flows (with RO and without RO) was extremely low. Only after post-filtration a minute increase in feed channel pressure drop was

observed. Biomass accumulation observed on spacers and membranes were low, but the effect of RO was visible.

Regarding feed channel pressure drop it would be more interesting if biofouling potential of water was high. Likely, the effect of pre-treatment with RO would be more visible than in this research. In this research an increase in feed channel pressure drop was mainly caused by the accumulation of iron and manganese rather than by biomass accumulation. This is concluded from analyses performed on spacers and membranes after the MFS run.

Operational problems in the treatment scheme were directly recognized by the feed channel pressure drop as a sudden increase was observed caused by high effluent concentrations after filtration.

Ad III. Biofilm monitor

The biofilm monitor is a proven method to obtain the biofilm formation rate of water. During distribution biofilm formation on pipe walls is formed. Results obtained by the biofilm monitor are relevant as it simulates real practice.

As run time of the biofilm monitor is long the run was not completed in this research. Interpreting the first results should be done with extreme caution. After this research Oasen will continue the run until it is finished.

For both flows biofilm formation was formed very slowly. Although this minute growth of biofilm the effect of pre-treatment RO seemed to be visible in the biomass concentrations observed on the glass rings. If these differences are significant to say that the effect of pre-treatment RO on biological stability regarding biofilm growth is better is doubtful. After a complete run better conclusions from BFR and BFP can be made.

Ad IV. Modified Robbins Device

Results obtained by MRD are not reproducible and thus not MRD is not a good method for investigation biofilm formation of drinking water. Biomass concentrations on metal biostuds were highly variable for both flows. As two metal biostuds were removed every week for analysis an increasing trend was expected in biomass concentrations observed as biofilm is expected to grow in time.

4.2.2 Applicability to use in a drinking water treatment plant

Methods used can be relevant, accurate and reproducible for investigating the research questions. However, when doing research at a drinking water treatment plant other parameters can be important for the decision to choose what method to use.

Criteria for the applicability to use a method in a drinking water treatment plant:

- Ability to measure biological stability: direct measurements (physical effect) and/or indirect measurements (regrowth potential)
- Time period to obtain valuable results
- Applicability to install at a drinking water treatment plant
- Robustness
- Water use
- Costs

All four methods used in this research are analyzed on these criteria. Table 4-4 presents an overview of the analyses performed. It should be mentioned that criteria like relevancy and reproducibility are more important for the decision to choose what method. However, practical criteria can play an influence in the decision process. If methods are cheap and easy to use in a

full scale drinking water treatment they may be preferable over another method although results are less relevant.

Table 4-4 - Multi-criteria analysis materials and methods

	Ability to measure biological stability	Time period to obtain valuable results	Applicability to install at a drinking water treatment plant	Robust -ness	Water use	Costs
Water sampling	+/-	++	++	+	++	-
Membrane Fouling Simulator	+	+	+	-	+/-	+
Biofilm Monitor	++	--	+/-	-	--	-
Modified Robbins Device	--	--	+	+/-	+	+

(--) very negative; (-) negative; (+/-) moderate; (+) positive; (++) very positive

Ad I. Water sampling (spot measurements)

Water sampling is an easy and fast method to investigate (microbial) water quality. With regard to TCC and DOC, the effect of pre-treatment with RO was recognizable throughout the whole treatment scheme. The time period to obtain these results was short.

With regard to regrowth potential water sampling is not that effective. From literature (van der Kooij, 1999) ATP is seen as an indicator. But caution is needed for interpreting results as ATP cannot be used as a leading parameter for indicating bacterial regrowth during distribution.

Ad II. Membrane Fouling Simulator

MFS units are compact and quite easy to install. One disadvantage is that no flow control was implemented in the device. So dosage pumps were needed to use the MFS units. Using dosage pumps automatically implied that storage tanks were needed for preventing pumps running dry.

Results obtained with MFS can be fast if biofouling potential of water is high. This can be observed by the feed channel pressure drop which should be checked regularly. A disadvantage for investigating biological stability (biofouling) is that fouling due to high effluent concentrations disturbs measurements. Due to operational problems in a drinking water treatment plant this method was less suitable to place after post-filters.

An advantage is that the costs of materials used are low. Only the differential pressure transmitter used is costly. Water use is also quite low, although MFS units run continuously.

Ad III. Biofilm monitor

Biofilm monitor is a proven monitor and easy to install at drinking water treatment plant. One problem can be the size of the monitor and the space (height) needed for sampling taking. The biofilm monitors used in this research used a water flow of 175 L/h where 275 L/h is recommended. This was because not enough water was available after post-filters due to natural pressure.

Time period to obtain valuable results is long. In the first 84 days of this research differences were visible, but as the run was not finished conclusions are hard to draw. Therefore, when fast recognition of biological stability is needed this method is not preferable. On the other hand, biofilm growth is definitely a slow process and the biofilm monitor directly simulates this process. So although run times are long the biofilm monitor does produce valuable results with regard to the biological stability.

Disadvantage of the biofilm monitor is the robustness of the method. When flow to monitor is suddenly stopped (e.g. caused by operational problems in a drinking water treatment plant) the complete run can be useless as bacteria will not have food sources to survive. Turbulence can remove biofilm when feed water flow is restarted.

Ad IV. Modified Robbins Device

The biofilm monitor is a compact device with low water use. In theory, biofilm formation will be formed on the metal biostuds and therefore should be a good simulator for investigating biofilm growth. However, results were variable and inconsistent making the method inadequate to use in a drinking water treatment plant.

5 Conclusions and recommendations

During the research a lot of data was obtained from measurements performed at ZS Lekkerkerk to investigate primary and secondary research questions. In Chapter 4 an extensive overview is given of all the results. A discussion is performed to give answers on primary and secondary questions. In this Chapter conclusions (Paragraph 5.1) and recommendations (Paragraph 5.2) are given.

5.1 Conclusions

Before answering primary research about the effect of pre-treatment with RO on the biological stability in a drinking water plant it should be noticed that both flows investigated (with and without RO) seemed of a high level of biological stability after post-filtration and GAC experimental set-up.

Primary research question:

What is the effect of treating 50% of raw water intake with Reverse Osmosis on the biological stability of drinking water at treatment plant ZS Lekkerkerk?

The physical removal of bacterial cells and nutrient sources in water by RO was visible throughout the course of the treatment scheme. TCC in water flow that was pre-treated with RO was significantly lower than for water not pre-treated with RO. This accounts for water sample analyses performed after pre-filtration, post-filtration and GAC experimental set-up. Same trends were observed for DOC.

The effect of pre-treatment RO on regrowth potential was hardly visible. Some methods observed differences that were small and it is doubtful if the effect of pre-treatment with RO was significant. In the first 84 days of the biofilm monitor the biomass concentrations found on glass rings was smaller for water pre-treated with RO. Extreme caution is needed about drawing conclusions as monitoring run was not finished (not possible to compare BFR and BFP with literature).

Biofouling potential of water was not visible by the increase in feed channel pressure drop of MFS units placed. Biomass accumulation on spacers and membranes was small, but the effect of pre-treatment with RO was recognized both after post-filtration and after GAC experimental set-up.

The effect of pre-treatment with RO was not visible with regard to ATP in water samples. After post-filtration and GAC experimental set-up ATP of both flows were similar. Thus, the active biomass in both flows (with and without RO) was comparable.

To what extend can the influence of feed water quality on biological stability be measured in the full scale drinking water treatment plant ZS Lekkerkerk?

The physical effect of RO was visible throughout the treatment scheme with regard to TCC and DOC. The regrowth potential of both flows (with and without RO) measured with indirect methods after post-filtration and GAC experimental set-up was small or not recognized. It is doubtful if the differences observed are significant for concluding that pre-treatment with RO in this situation has a direct effect on the regrowth potential of bacteria in water.

What is the effect of individual (biological) treatment steps at ZS Lekkerkerk on the biological stability of drinking water?

Due to the biological removal of ammonium, iron and manganese in pre-filters the water is of a low level of biological stability with regard to ATP. This was confirmed by results found by Hijnen *et al.* (2013).

After post-filtration the biological stability improved significantly with regard to ATP. This is confirmed by the low biofouling potential observed with MFS.

Results after GAC experimental set-up showed that GAC treatment step improved the level of biological stability further. Biomass accumulation on spacers and membranes was lower after GAC than after post-filtration. Visual observations of MFS units also showed that fouling (iron) potential of water was lower after GAC experimental set-up. Hijnen *et al.* (2013) observed this effect of GAC with the CBM and BPP test.

5.2 Recommendations

From results obtained the biological stability of drinking water at ZS Lekkerkerk seemed of a high level of biological stability. From this research some recommendations can be made related to the results and the materials used. Most important recommendation is not to use pre-treatment RO solely for improving the biological stability of drinking water as this was not clearly shown in this research.

Further research to the effect of pre-treatment with RO on biological stability:

- As the level of biological stability at ZS Lekkerkerk was high for both flows (with and without RO) the difference of the regrowth potential of water was not always visible. Results of the complete run of the biofilm monitor can possibly show the effect of pre-treatment with RO better. If not, it would be interesting to do research at a location (consisting a RO installation) where level of biological stability is known to be very low.

Question arises why the effect of pre-treatment with RO on biological stability is hardly visible. This may be caused by the high biological activity during pre-filtration that eliminates the effect of RO:

- Further research to what type of micro-organisms are present in both filter sets.
- What type of micro-organisms contribute most to bacterial regrowth.
- Further research to HNA/LNA of cells obtained by FCM. A sharp increase in HNA bacteria (compared to LNA) was observed during pre-filtration where biological growth was high due to the biological removal of ammonium, iron and manganese.

Discussion about switching RO to another location in the treatment scheme. Recommendations related to biofouling of membranes:

- After pre-filtration: biological unstable water, so strongly not recommended.
- After post-filtration: water of high level of biological stability. Take into account high effluent concentrations of iron and manganese as observed with MFS units.
- After GAC experimental set-up: water of a higher level of biological stability than after post-filtration. No fouling was observed with MFS units

Use of monitors:

- Biofilm monitors are recommended to run until mid-March. Interesting to see if expected exponential growth will occur. As initial biofilm formation is low, biofilm formation potential is possibly not reached in this study.
- Membrane Fouling Simulators are recommended to use again when known biofouling potential of water is expected to be very high. MFS can also be used to recognize high effluent concentration of e.g. iron, as coloring of spacers was observed with the sight window. A different way of operation (e.g. changing membrane and spacer thickness) might

- help investigating water with low biofouling potential as resistance over unit increases when membrane thickness increases.
- Modified Robbins Device is not recommended to use anymore. Results are highly variable and inconsistent for the detection of biofilm.

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Annexes

Annex A – Calculation activated carbon

To include granular activated carbon in the research a pilot installation was constructed. A pilot installation was built as the contribution of reverse osmosis on the full scale activated carbon filters is too low to draw conclusions.

The amount of activated carbon needed is based on the required flow and the contact time of water with the filter material. The required flow is the sum of flows required for the measuring materials. After activated carbon a biofilm monitor (175 L/h), a MFS unit (16 L/h) and a MRD unit (1 L/h) are placed.

Required discharge:

$$Q = 0.175 \frac{m^3}{h} + 0.016 \frac{m^3}{h} + 0.001 \frac{m^3}{h} = 0.192 \frac{m^3}{h}$$

The pilot installation consists of two pressure vessels with available height of 0.9 meters and a diameter of 0.4 meters:

$$H_{available} = 0.9 \text{ m}$$
$$D = 0.4 \text{ m}$$

The surface area of the pressure vessel:

$$A = \frac{1}{4} \cdot \pi \cdot D^2 = 0.126 \text{ m}^2$$

With the required flow the filtration velocity becomes:

$$v = \frac{Q}{A} = 1.53 \text{ m/h}$$

The contact time of water with the filter material is about 20 minutes in the full scale treatment plant. To compare the full scale treatment with the pilot activated carbon installation a similar contact time is required:

$$T = \frac{1}{3} \text{ h}$$

The height of the filter material becomes:

$$H_{needed} = v \cdot T = 0.51 \text{ m}$$

Annex B1 – Water sampling: different feed water qualities

The TCC results obtained for the full scale flow, consisting of the mixed filtrate from all eight filter sets, was lower than expected (Figure 4-1). As contribution of RO to the full scale flow was low (6-10%) TCC in full scale flow was expected to be slightly lower than for water that was not pre-treated with RO. However a significant difference was observed. This was likely caused by the difference in feed water quality fed to the eight filter sets. The difference in performance of filter set may also be a reason. Performance of filters is influenced by several parameters e.g. flow, velocity, time after backwashing, filter age and feed water quality.

As mentioned the difference between full scale flow and flow without pre-treatment with RO was likely caused by the difference in feed water qualities. In Table B-1 an overview of all filter sets including remarks is given. TCC results should be interpreted carefully as data set is limited. The TCC-Intact value for both Schuwacht raw water (single value) and Tiendweg raw water (average of 8 values) was 1.4e+05 cells/ml.

Table B-1 - TCC-Intact for comparison filter sets at ZS Lekkerkerk

Filter set	Remark	n	TCC-Intact pre-filtrate (cells/ml)	n	TCC-Intact post-filtrate (cells/ml)
1	Intake source Schuwacht	1	3.0e+05	1	2.4e+05
2	Intake source Schuwacht	1	3.4e+05	1	2.6e+05
3	Intake source Schuwacht	1	2.9e+05	1	2.5e+05
4	Separate biological iron removal	0	-	0	-
5	Pre-treatment with RO (50%)	10	2.5e+05	13	2.1e+05
6	Recirculation	10	3.7e+05	13	3.0e+05
7	Dosage of phosphate	1	3.9e+05	0	-
8	-	2	3.0e+05	1	3.3e+05

Ammonium and iron concentrations of raw water from intake source Schuwacht was lower than from intake source Tiendweg. This resulted in less substrate available for bacteria that were present in pre- and post-filters. Lower substrate concentrations resulted in less biological growth which was recognized in TCC values as TCC for filter sets 1-3 was slightly lower than for filter sets 6-8. This explains that TCC of mixed post-filtrate was not similar to post-filtrate 6 (without RO) as was hypothesized at the start of the research.

Annex B2 – Water sampling: feed water load

Raw water contains substrate for bacteria. An overview of the main parameters is given in Table B-2. Important to state is that ammonium was removed biologically whereas iron and manganese are partly physically and partly biologically removed. Organic carbon is considered to be the main food source for bacteria. As recirculation was part of flow 6 (without RO) the effluent concentrations of post-filter 6 are also presented.

Table B-2 - Concentration of most relevant parameters in raw water, RO permeate and post-filtrate 6 for determining feed water quality to pre-filters

Type of water	Ammonium concentration (mg NH ₄ /l)*	Iron concentration (mg Fe/l)*	Manganese concentration (mg Mn/l)*	Dissolved organic carbon (mg/l)	Orthophosphate-P (mg/l)	Nitrate-N (mg/l)
Raw water (TW)	5.57	5.25	0.48	2.27	0.74	0.06
RO Permeate	0.29	0.01	0.001	0.06	0.001	0.06
Post-filtrate 6	0.02	0.005	0	2.11	0.03	4.48

*Representative data value is determined using data from mid-September 2012 to mid-December 2012

Iron and manganese were almost completely removed by RO, while ammonium concentration was still significant. This is because ammonium in gas phase partly passes the RO membranes.

From Figure 2-8 it seems that filter set 5 (with RO) and filter set 6 (without RO) received same quantities of water with the difference that filter set 5 had an additional pre-treatment of RO. Unfortunately it cannot be concluded that feed water to filter set 5 consisted of exactly half of the components than feed water to filter 6. This is due to following reasons:

- Ammonium and methane were not fully removed with RO
- Flow difference in feed water
- Recirculation at filter set 6 for better performance pre-filter

To compare both water flows the load of feed water to filter set 6 was estimated.

In Table B-5 the load of two feed water flows is presented. This is a result of a calculation based on actual flows and on the ammonium-, iron- and manganese concentrations. Table B-2 shows concentrations of ammonium, iron and manganese in different types of water. Table B-3 (filter 5) and Table B-4 (filter 6) show the actual (estimated) flows in the treatment plant. It should be noted that feed water quality was slightly variable over time due to the fact that investigated filter sets were part of a full scale treatment plant where water quality and flows varied over time.

Table B-3 - Feed water flow to filter 5 (with RO)

Type of water	Flow (m ³ /h)*
Raw water (TW)	25
RO Permeate	25

*Flow meters

Table B-4 - Feed water flow to filter 6 (without RO)

Type of water	Flow (m ³ /h)
Raw water (TW)	40*
Recirculation	7-8*

*Estimation and variable (no flow meter)

Appendix D shows electrical conductivity measurements which confirm that water from feed water to filter set 5 was equally mixed (50% RO permeate and 50% Tiendweg raw water).

Table B-5 - Load of ammonium, iron and manganese to pre-filtration

Type of water	Ammonium load (kg NH₄/h)*	Iron load (kg Fe/h)*	Manganese load (kg Mn/h)*	Total load (kg/h)
Feed water filter 5 (with RO)	0.147	0.131	0.012	0.290
Feed water filter 6 (without RO)	0.223	0.210	0.019	0.452

* Estimation as flows are uncertain and variable over time

From Table B-5 one can conclude that (based on estimation) the feed water flow to pre-filter 5 consisted of about 65% of the components compared to feed water flow to pre-filter 6.

Annex B3 – Water sampling: flow cytometry

CFlow software was used to have a closer look at the TCC results obtained with FCM. Figure B-1 shows graphs of raw water (left) and RO permeate (right) obtained by CFlow. The red gate was placed manually to distinguish between bacteria and background. So all dots outside the gate is considered to be background and everything inside is bacteria. The positioning of the gate was done manually and based on experience, causing some uncertainty in measurements.

When staining the cells with SYBR Green I fluorescence only, all cells are stained and appear in the red gate. So both damaged and undamaged cells are shown in the red gate resulting in TCC-Total. From the x-axis the DNA content (size) of the cells can be recognized, where large cells are shown to the right of the red gate. Based on experience the size of the cells can be divided into LNA and HNA. For raw water most dots appear in the left of the red box representing a large LNA fraction of the total cells.

When staining cells with propidium iodide and SYBR Green I the intact cells are counted. As propidium iodide only enters damaged cells it means that all the damaged cells will have high red fluorescence and will appear outside the gate. The intact cells will have a normal green fluorescence and remain in the gate.

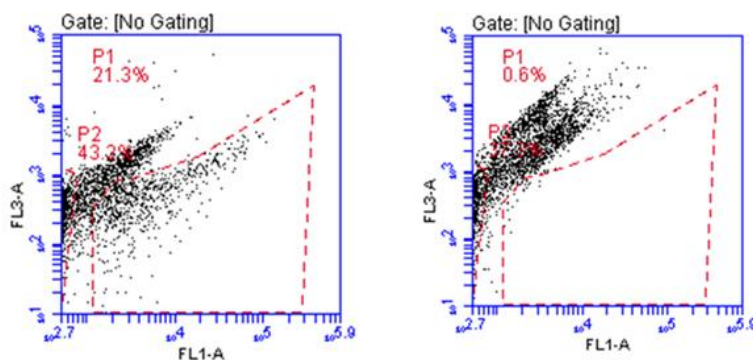


Figure B-1 - Flow cytometry results where dots inside the red gate represent bacteria and dots outside the red gate is background noise (left: raw water, right: RO permeate)

A clear difference was observed between raw water and RO permeate. For RO permeate it could be seen that almost no black dots, and therefore no bacteria appeared inside the red gate.

Annex B4 – Water sampling: TCC varieties after pre-filtration

Another interesting property of Figure 4-4 is the difference in pattern observed between pre-filtrate 5 and pre-filtrate 6. Table B-6 shows this difference by presenting the ratio of TCC-Intact of pre-filtrate 5 with pre-filtrate 6.

Table B-6 - TCC-Intact pre-filtrate 5 (with RO) and pre-filtrate 6 (without RO)

Date	TCC-Intact pre-filtrate 5 (with RO) [cells/ml]	TCC-Intact pre-filtrate 6 (without RO) [cells/ml]	Ratio TCC pre-filtrate 5 / pre-filtrate 6 [-]
17-9-2012	1.7e+05	2.9e+05	0.59
24-9-2012	2.8e+05	3.4e+05	0.82
27-9-2012	2.2e+05	3.8e+05	0.58
1-10-2012	2.9e+05	3.8e+05	0.76
4-10-2012	2.6e+05	4.0e+05	0.65
10-10-2012	2.2e+05	4.4e+05	0.50
24-10-2012	2.2e+05	3.5e+05	0.63
31-10-2012	2.8e+05	3.6e+05	0.78
14-11-2012	2.6e+05	3.6e+05	0.72
21-11-2012	3.3e+05	4.0e+05	0.83

TCC-Intact ratios were variable which indicates that processes in the pre-filters were not constant. Variability can also be caused by the fact that feed water quality was not constant over time. But, a better feed water quality to pre-filter 6 automatically implies a better feed water quality to pre-filter 5 as they both used same raw water intake source.

Another possible reason for variability could be the time between sampling and backflushing of the pre-filters. To flush out materials attached to sand and anthracite filters were backflushed every 3 days to prevent clogging of the filters. Clogging of filters increases the resistance and can cause higher effluent concentrations which should be prevented. Research was done at the time of flushing and the TCC values of the pre-filtrate. From the results one can conclude that there was no relationship between the time of flushing and the time that samples were taken (data not shown).

The most likely reason for different patterns between pre-filter 5 and pre-filter 6 was the performance of both filters under specific conditions. Both filter sets had different properties and thus were hard to compare. Variability in feed water load, flow, velocity, time of flushing, recirculation at filter set 6 and filter age are parameters that can influence the performance of filters. Higher velocities can result in more outflow of biomass.

Although the reason for varieties was not 100% clear, a trend is observed that TCC values of filter set 6 are significantly higher caused by both a higher TCC of the feed water and a higher absolute growth within the filter set. The ratios given in Table B-6 are understandable as the ratio in load between two filters was estimated at about 0.65.

Annex B5 – Water sampling: removal rates filtration

Table B-7 shows concentrations of most important substrates in water after post-filtration. The removal rate compared with feed water quality is also presented. Regarding the high removal rate of these components it may be concluded that substrate in the lower part of the post-filter bed was limited for bacterial growth.

Table B-7 - Ammonium-, iron- and manganese concentrations in water after post-filtration and removal efficiency with regard to feed water

Type of water	Ammonium		Iron		Manganese	
	mg/l	log removal	mg/l	log removal	mg/l	log removal
Post-filtrate 5 (with RO)	0.001 - 0.047	1.8 - 3.5	0.004 - 0.008	2.5 - 2.8	<0.001 - 0.001	2.4 - >2.4
Post-filtrate 6 (without RO)	<0.001 - 0.082	±1.7 - >±3.6	0.004 - 0.008	±2.7 - ±3.0	<0.001 - 0.001	±2.7 - >±2.7

Annex C1 – Membrane Fouling Simulator: visual observations

The top parts of the MFS units are transparent which made it is possible to look onto the membrane and spacer while it was in operation. As mentioned, fouling over time was low resulting in small or no differences in what was observed.

The left MFS unit in Figure C-1 shows the MFS unit placed after GAC experiment 6 (without RO) where it can be seen that the membrane and spacer were not discolored. No visual information from the sight window of GAC experiment 5 (with RO) and GAC full scale is given in this report as membrane and spacer were also not discolored. The right MFS unit shows the MFS unit located after post-filter 6. No significant difference was observed in post-filter 6 and post-filter 5 using the sight window. In Figure C-1 the difference in discoloration is noticeable showing that the fouling potential of water after post-filtration was higher than the fouling potential after GAC treatment step. The same observation was made of MFS units placed after post-filtrate 5 and GAC experiment 5.



Figure C-1 - Visual observation of MFS placed after GAC experiment 6 (without RO) (left) and MFS placed after post-filter 6 (with RO) (right)

From Figure C-1 the difference in discoloration is noticeable showing that the fouling potential of water after post-filtration was higher than the fouling potential after GAC treatment step. The same observation is made of MFS units placed after post-filtrate 5 and GAC experiment 5.

Visual observations using a microscope to show instant fouling

Figure C-2 shows deposits attached on the spacer and membrane in MFS used after post-filtrate. The attached deposits resulted in a sudden feed channel pressure drop at post-filtrate 5. Fouling occurred homogeneous over the feed channel. A new spacer and membrane was installed in the MFS unit and the run was restarted.

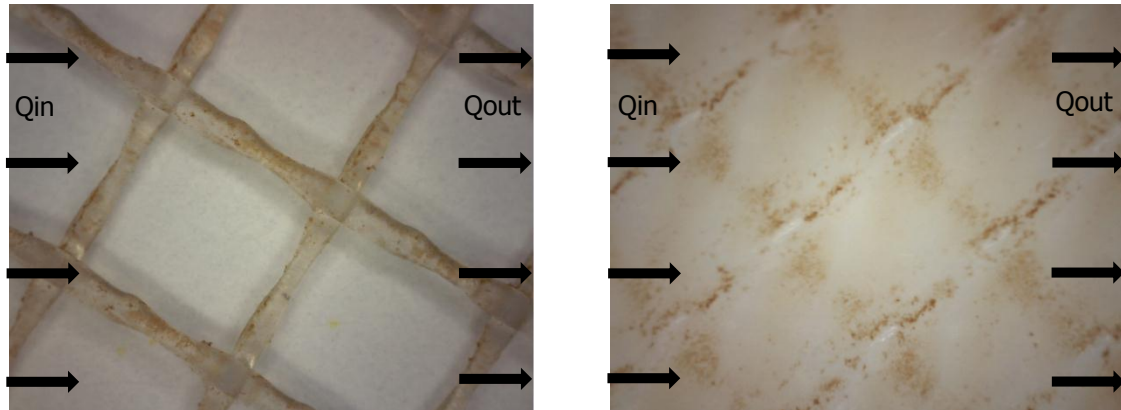


Figure C-2 - Deposits on feed spacer (left) and membrane (right) of MFS placed after post-filter 5 resulting in a sudden feed channel pressure drop

Annex C2 – Membrane Fouling Simulator: Components in water that influence feed channel pressure drop

Table C-1 and Table C-2 present concentrations (ATP, TCC, TOC, iron, manganese and calcium) found on spacers and membranes implemented in MFS units.

Table C-1 - Biomass concentration found on membranes and spacers in monitors placed at several locations after a run time of 54 to 64 days

Type of water	Run time [days]	Membrane (M) or Spacer (S)	ATP [pg/cm ²]	TCC-Total [cell/cm ²]	TCC-Intact [cell/cm ²]
Post-filtrate 5 (with RO)	54	M	620	44E+05	16E+05
		S	430	14E+05	4.5E+05
Post-filtrate 6 (without RO)	64	M	1400	120E+05	42E+05
		S	2100	130E+05	43E+05
GAC (experiment) 5 (with RO)	60	M	370	34E+05	12E+05
		S	150	4.6E+05	1.5E+05
GAC (experiment) 6 (without RO)	60	M	650	38E+05	15E+05
		S	570	14E+05	4.1E+05
GAC (full scale)	60	M*	1700	38E+05	17E+05
		S	280	3.8E+05	0.78E+05

* Possible contamination during analysis

Table C-2 - Relevant parameters found on membranes and spacers in monitors placed at several locations after a run time of 54 to 64 days

Type of water	Run time [days]	Membrane (M) or Spacer (S)	TOC [pg/cm ²]	Iron [mg/cm ²]	Manganese [mg/cm ²]	Calcium [mg/cm ²]
Post-filtrate 5 (with RO)	54	M	2.0	0.38	<0.02	<0.50
		S	1.3	0.52	0.09	<0.50
Post-filtrate 6 (without RO)	64	M	2.1	1.00	0.10	<0.50
		S	3.4	2.10	0.32	<0.50
GAC (experiment) 5 (with RO)	60	M	2.0	0.20	<0.02	<0.50
		S	0.4	0.09	<0.02	<0.50
GAC (experiment) 6 (without RO)	60	M	2.2	0.19	<0.02	<0.50
		S	3.1	0.18	<0.02	<0.50
GAC (full scale)	60	M	4.8	0.22	<0.02	<0.50
		S	2.3	0.15	<0.02	<0.50

An interesting question that arises is what components of the water investigated had the largest impact on the increase of the feed channel pressure drop? The iron and manganese concentrations found on the membranes and spacers after the GAC experiments were low and did not result in an increase in feed channel pressure drop.

A comparison can be made between MFS unit placed after post-filtrate 5 and MFS unit placed after GAC experiment 6. Although water was from a different flow and at a different location in the treatment scheme, similar bacterial (ATP and TCC) concentrations were found on the membranes and spacers. Feed channel pressure drop slightly increases for post-filtrate 5 and no increase was observed for the MFS unit placed after GAC experiments. This may indicate that the observed iron and manganese concentration on the membrane and spacer caused the slight increase in feed channel pressure drop.

This may indicate that biofouling of the water observed in the treatment plant was too low to be recognized in the feed channel pressure drop over time. Although increases of the feed channel pressure drop in post-filtrates were small it is likely to be caused by non-bacterial components like

iron and manganese. This is confirmed by iron found in the water phase. Iron concentrations were low, but higher in water after post-filters than in water after the GAC experimental set-up. This indicates that iron removal took place in the GAC filters. Be careful about concluding that GAC is a good treatment step for iron removal as iron concentrations in the post-filtrate were lower than 0.01 mg/l.

Annex C3: Membrane Fouling Simulator: Second run

The feed channel pressure drop was measured using a differential pressure transmitter. All MFS runs started at 30-11-2012. Due to the fact that pumps ran dry run MFS units placed at flow 6 were stopped after 19 days.

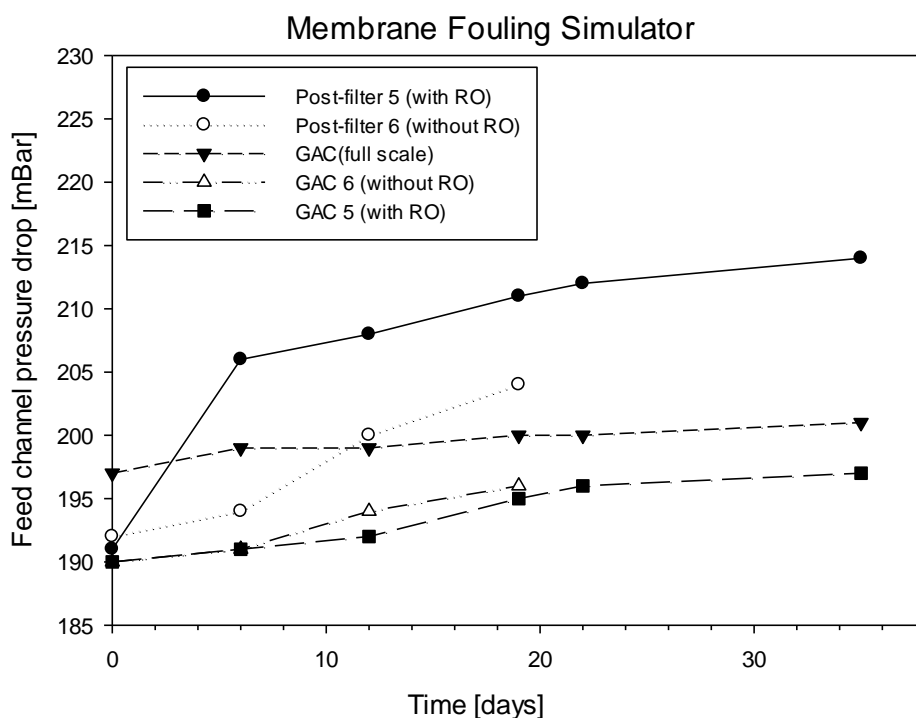


Figure C-3 - Feed channel pressure drop in time of MFS's placed after post-filtration (with and without pre-treatment RO) and GAC experiments (with and without pre-treatment RO)

At the start of the second run a sharp increase in pressure drop was observed at the MFS unit of post-filter 5. Like in the first run, due to a failure in the RO system the RO pilot plant was shut down twice in the period between two measurements (30-11-2012 and 5-12-2012). During the shutdown the bypass system was set in operation so filter sets did not run dry. But probably high effluent concentrations in the post-filtrate due to high influent concentrations (no pre-treatment RO) is the reason for some fouling of the MFS unit placed after post-filtrate 5. This was also recognized in visual observations presented in Figure C-4.

Hypothesis of using higher flows is to recognize fouling in an earlier stage as more water, and therefore more substrate is passing the MFS units in a shorter time. Again, differences between different water flows were hard to observe. However, with a close look the gradient of post-filtrate 6 seems to be steeper than post-filtrate 5 indicating the fouling potential of water not pre-treated with RO was slightly higher. The increase in feed channel pressure drop over time for the MFS units placed after GAC experiments and GAC full scale was small. This again confirms that biofouling potential after GAC treatment step was low.

Visual observations using microscope (in situ)

The microscope used was placed on the MFS units while running. Pictures were taken, in situ, at 4 different moments during the second MFS run to obtain visual observations during a run.

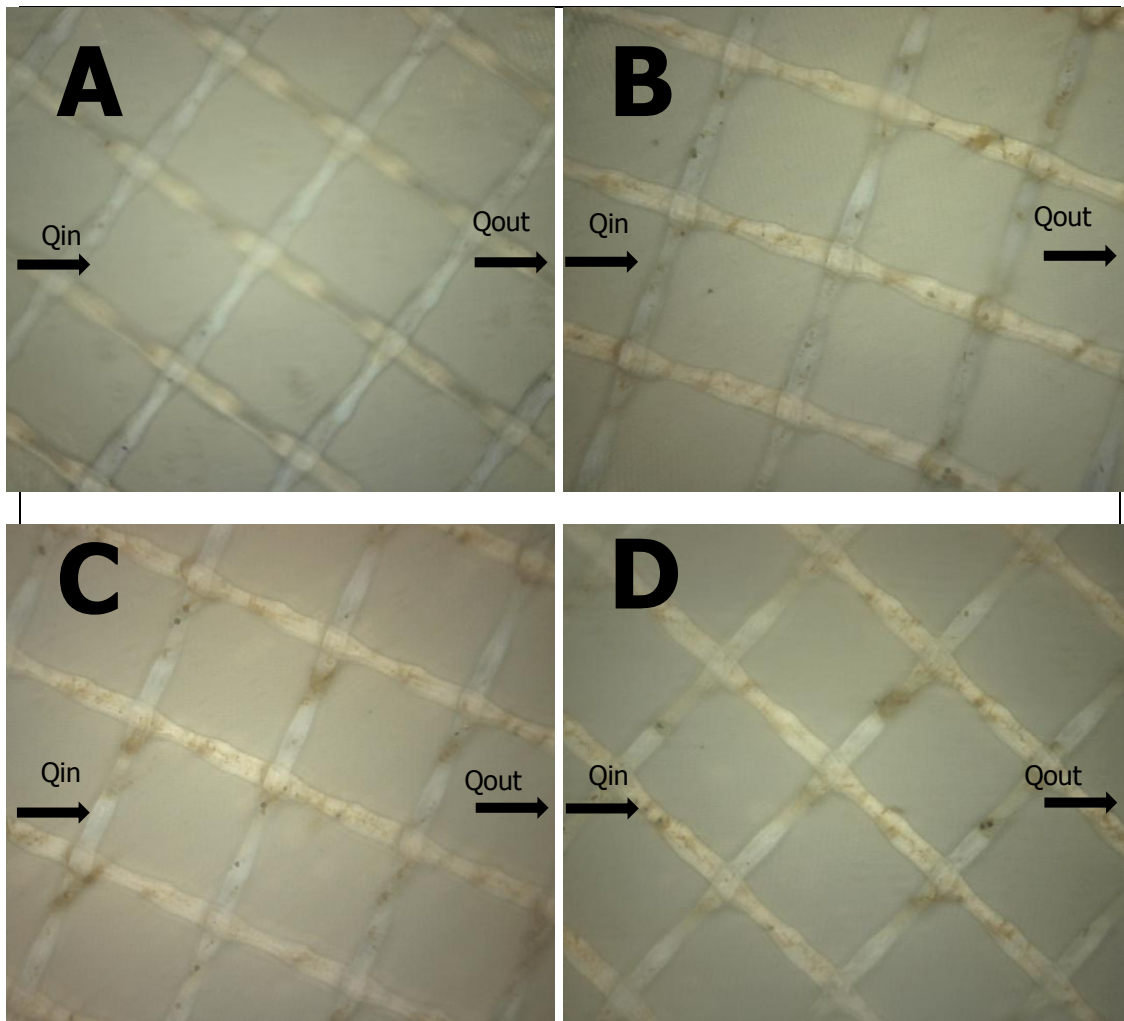


Figure C-4 - In-situ visual observations of spacer and membrane in MFS unit after post-filter 5 (with RO) using a microscope (A: at day 0; B: at day 11; C: at day 18; D: at day 35)

Between day 0 and day 11 instant fouling occurred due to failure in the RO pre-treatment pilot installation. Bypass was activated and higher influent concentration into filter set resulted in higher effluent concentrations. A small but sudden increase in feed channel pressure drop was recognized. During the remainder of the run fouling seemed to be limited.

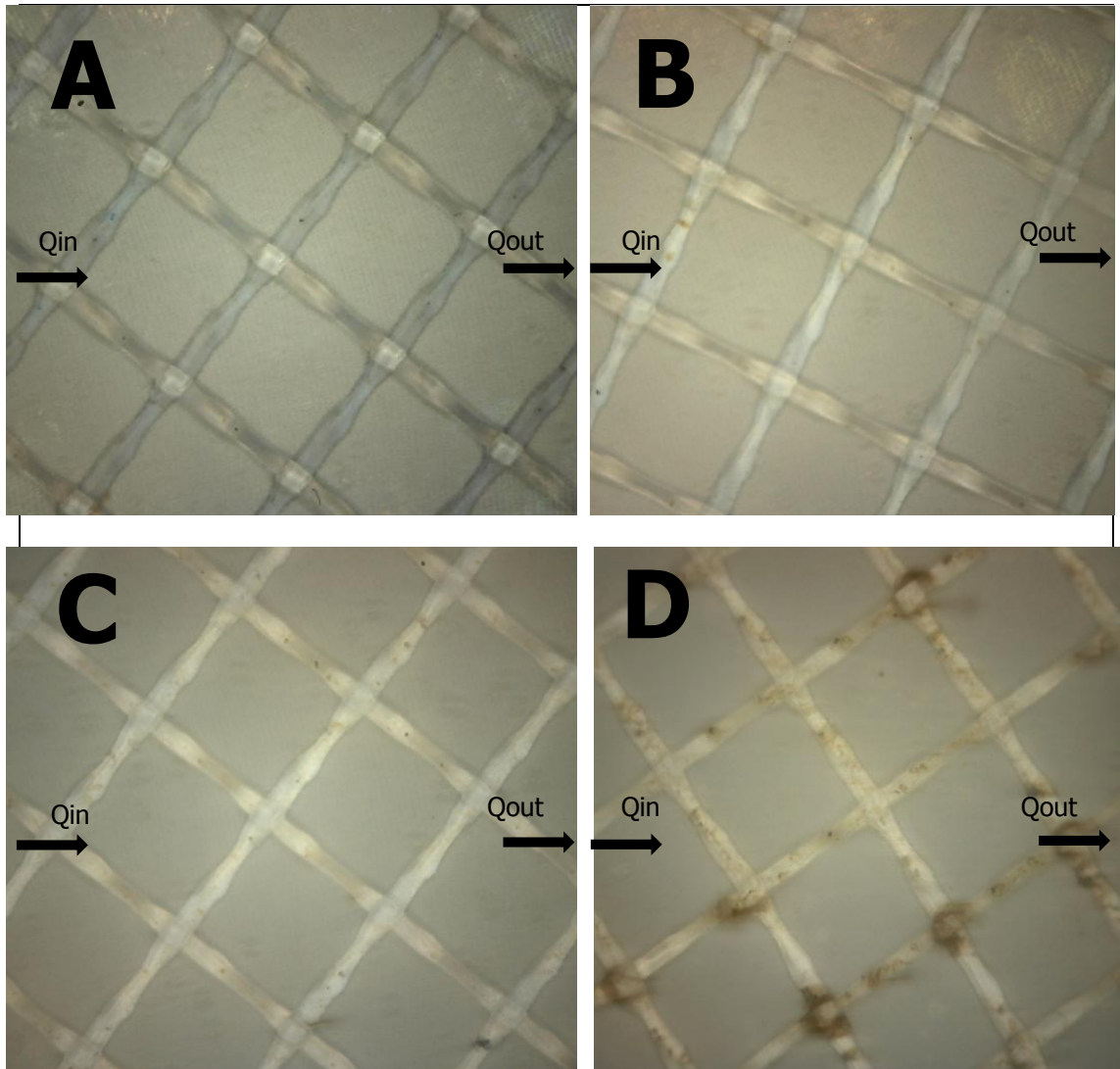


Figure C-5 - In-situ visual observations of spacer and membrane in MFS unit after post-filter 6 (without RO) using a microscope (A: at day 0; B: at day 11; C: at day 18; at day 35)

From the start of the run up to and including day 18 fouling was limited. Due to maintenance at the treatment plant filter set 6 ran dry. No water was supplied to the storage tanks and pumps ran dry. This resulted in fouling (Figure C-5-D) of the spacer and run was stopped.

Annex D – Electrical conductivity

Electrical conductivity is measured at several locations in the treatment scheme of ZS Lekkerkerk for two reasons:

- To check if feed water of filter 5 is equally mixed (with RO)
- To determine contribution of RO on produced water

Figure D-1 shows an overview of the electrical conductivity in the treatment train of ZS Lekkerkerk.

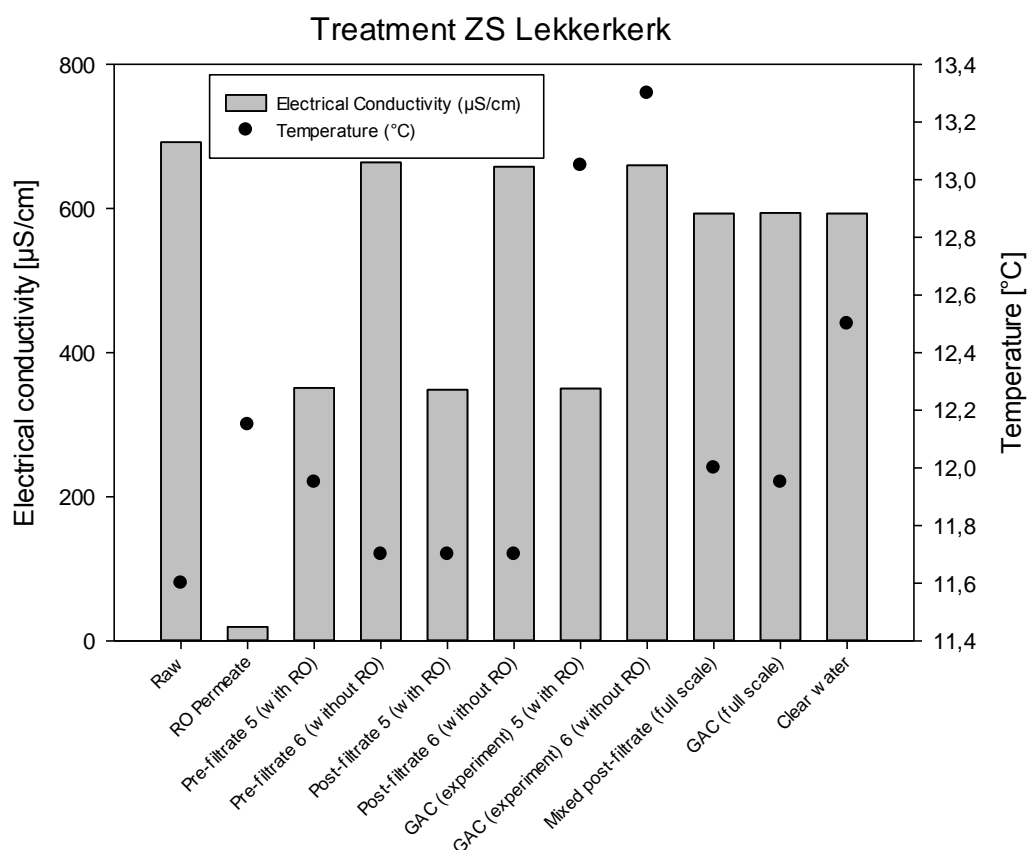


Figure D-1 - Temperature and electrical conductivity at several locations in the treatment scheme of ZS Lekkerkerk

Electrical conductivity slightly decreases between raw water, pre-filters and post-filters due to removal of metals in the filtration steps.

Concluded can be that feed water is mixed almost equally (49.5% RO and 50.5% TW) and that the contribution of RO at produced water is about 6-10%.