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NOM REMOVAL THROUGH COAGULATION, SEDIMENTATION AND FILTRATION AS A REMEDIAL ACTION TO PREVENT ADVERSE EFFECTS OF RE-GROWTH IN NETWORKS

Fjerning av NOM ved koagulering, sedimentering og filtrering som tiltak mot biofilmdannelse i ledningsnett for drikkevann

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Abstract

A qualitative and quantitative monitoring of biofilm formation was performed in four categories of water: raw water, filtered water (after removal of natural organic matter (NOM)), disinfected water and distributed water. The biofilm formation was reduced by 95–99 % as a result of the NOM removal, a reduction considerably more significant than indicated by the change in biodegradable organic carbon in the water. The biofilm formation was almost constant in the distribution system regardless of residence time. The mould in the biofilm changed from excess growth in biofilm grown in raw water to marginal or no growth in treated water or distributed water. The amount of bacteria in the biofilm was considerably reduced during the treatment, and particularly low in biofilm grown in water taken shortly after disinfection. Presumptive *Pseudomonas*, presumptive *Aeromonas*, coliform bacteria and *E. coli* were identified in biofilm grown in raw water, but not in biofilm grown in treated water or distributed water. Presumptive *Legionella* was identified in biofilm grown in raw water, in water after NOM removal but before disinfection and in distributed water. Confirmed *Legionella* was identified in biofilm grown in water after NOM removal and during distribution.

Key words – NOM, coagulation, regrowth, biofilm

Sammendrag

En kvalitativ og kvantitativ kartlegging av biofilmdannelsen ble gjennomført for råvann, filtrert vann etter fjerning av naturlig organisk materiale (NOM), rentvann fra behandlingsanlegget og distribuert vann. Fjerning av NOM reduserte biofilmdannelsen med 95–99 %, noe som var en vesentlig større reduksjon enn reduksjonen i biologisk nedbrytbart organisk karbon (BDOC) skulle tilsi. Biofilmdannelsen var i liten grad påvirket av oppholdstid i ledningsnettet. Mengden muggsopp i biofilmen ble redusert fra overvekst i råvann til tilnærmet ingen vekst i behandlet og distribuert vann. Totalantall bakterier i biofilm ble betydelig redusert som følge av vannbehandlingen og var spesielt lavt etter desinfeksjon. Presumptivt *Pseudomonas*, presumptivt *Aeromonas*, koliforme bacterier og *E. coli* ble påvist i biofilm i råvann, men ikke i biofilm i behandlet eller distribuert vann. Presumptivt *Legionella* ble påvist i biofilm i råvann, filtrert vann og distribuert vann. *Legionella* ble påvist i filtrert og distribuert vann.

Introduction

Increased heavy rain as associated to climate change is expected to increase the discharge of natural organic matter (NOM) from the catchment area to the surface water sources in Norway. Further, increased temperature will enhance biomass production in forests and fields in the catchment areas. This will increase the NOM load to the raw water and is expected to increase the load of biodegradable organic matter as well. For drinking water production this is an issue, as biodegradable carbon is the major source for microbial growth in the distribution system and elevated concentrations may affect the microbial safety of the distributed water.

The focus on micro-biological growth in the distribution system is mainly related to biofilm formation on the pipe wall and in deposits. Biofilm formation in the distribution system may create taste and odour problems, increase the turbidity and increase the chlorine decay (Norton, 1999, Franzmann et al., 2001). A high biofilm formation potential may also increase the bacterial number as plate count in the distribution system (Huck and Gagnon, 2004) and increase the possibility for survival of pathogenic micro-organisms. It is shown that biofilm in pipes increases survival of spores and cysts even at high chlorine doses (LeChevallier et al., 1987). The potential for growth of coliform bacteria increases with increasing biofilm formation potential measured as assimilable organic carbon (AOC) (Rice et al., 1991, LeChevallier et al., 1996).

The biofilm may contain opportunistic pathogenic micro-organisms like *Aeromonas spp., Flavobacterium spp., Legionella pneumophila* and *Pseudomonas aeruginosa* (Gibbs and Hayes, 1989, Holmes and Nicholls, 1995, Juhna et al., 2007) and several pathogenic and toxigenic moulds and yeasts (Ramage et al., 2013, Siqueira and Lima, 2013, Sardi et al., 2014). Further, several pilot and full-scale studies have shown that also highly pathogenic micro-organisms may occur in biofilms in distribution systems, like *Legionella pneumophilia* (Armon et al., 1997, Berry et al., 2006), *Helicobacter pylori* (Mackay et al., 1998, Moreno et al., 2007), *Salmonella typhimurium* (Armon et al., 1997) and *Mycobacterium xenopi* (Berry et al., 2006). In most cases this is believed to be due to the increased survival in biofilms compared to in the flowing water. Some pathogenic and opportunistic pathogenic micro-organisms may, however, not only survive but also grow in the biofilm, as shown for *Vibrio cholerae* (Hall-Stodley og Stodley, 2005), *Pseudomonas aeruginosa* (Hall-Stodley og Stodley, 2005), *Escherichia coli, Klebsiella oxytoca* and *Klebsiella pneumonia* (LeChevallier, 1987, Mackerness et al., 1993, Keevil, 1994, Camper at al., 1996), *Aeromonas* (Långmark et al., 2007) and *Mycobacteria* (Norton et al., 2004, Emtiazi et al., 2004, Vaerewijck et al., 2005). Fass et al (1996) showed that *E.coli* could grow in the biofilm at 20°C. Available biodegradable organic matter (BOM) in the water is among the factors deciding whether such growth will occur. However, Wingender and Flemming (2004) concluded that neither pathogenic micro-organisms nor the indicator organisms were common in such biofilms.

In Norway, the biofilm formation potential has been measured in a number of raw and treated waters (Hem og Charnock, 1999). The measurements showed that the potential exceeds the 10 µg AOC/L suggested by van der Kooij et al. (1999) as a limit for biofilm formation. NOM removal by coagulation and filtration may, based on previous studies (Hem and Charnock, 1999), reduce the biofilm formation potential while chlorination increased this formation. UV-disinfection had minor influence on the potential. High NOM content may increase the biofilm formation potential measured as AOC in raw water (Hem and Efraimsen, 2001).

The raw water source for Oset WTP is Maridalsvannet, a small to medium sized lake. The NOM content and colour are medium high for Norwegian conditions, with approximately 4 mg TOC/L and 25–30 mg Pt/L. The raw water quality is rather stable throughout the year with regard to organic matter and particulate material. In 2009 a new water treatment plant supplying 90 % of Oslo's drinking water was set in operation by the Oslo municipality. This new plant includes NOM removal through coagulation, sedimentation and filtration. UV disinfection is used for disinfection. The previous plant had micro-straining and chlorination only. In 2008 and 2009 the microbiological composition of treated water from Oset water treatment plant was studied, and the previous treatment without NOM removal was compared with the new treatment with NOM removal (Hem et al., 2012). The amount of moulds was considerably reduced as a result of the introduction of NOM removal. The change in the bacterial composition was, however, more marginal. The genera *Sphingomonas* and *Legionella* and the order *Burkholderiales* were detected in all samples, regardless of sampling point and the water treatment at the time of sampling.

The aim for the present study was to examine the effect of the new water treatment/NOM removal on reduction of biofilm formation and the presence of moulds and opportunistic pathogenic bacteria in biofilm in the distribution system.

Materials and methods

Biodegradable dissolved organic carbon (BDOC) analysis

Sampling for analyses of BDOC was performed in March 2012. Analyses of BDOC were performed according to Eikebrokk et al. (2007).

Biofilm sampling

Sampling points

Altogether 165 samples of biofilms were collected from the following sampling points from August to December 2012:

SP1. Raw water from Maridalsvannet (33 samples)
SP2. Filtered water, i.e. before disinfection (Oset WTP) (33 samples)
SP3. Treated water from Oset WTP (33 samples)
SP4. Water from Furulund basin, representing approximately 2.5 hours residence time in the distribution system (33 samples)
SP5. Water from Havnabakken, representing approximately 24 hours residence time in the distribution system (33 samples)

Sampling procedure

Glass pipes in series filled with glass coupons were installed at each sampling point (SP1-5). The same setup was previously used by Hem (2003, 2009). The procedure for biofilm monitoring is a modified version of the procedure described by van der Kooij and Veenendaal (1993). The glass coupons, formed as cylinders with length 10 mm, outer diameter 15 mm and a wall thickness of 1.2 mm, were placed inside the glass pipes as a stack. Both the inside and outside of the coupons was then exposed to the water. The up flow water velocity was approximately 0.2 m/s and this was controlled at each sampling. Between the samplings, the glass pipes were covered with black plastic sheets to prevent algae growth.

During the160 days the experiment lasted, three coupons were sampled from each sampling point for biofilm formation analyses every 14th day, representing from 14–160 days of micro-biological growth. Each coupon was placed in a small glass filled with 10 mL distilled or ultrapure water. At the end of the experiment, three coupons were also sampled for analyses of moulds and another three for analyses of selected opportunistic pathogenic bacteria.

Biofilm analysis

Biofilm formation analyses

The analysis of the coupons was based on the methodology developed by van der Kooij and Veenendal (1993). The following analysis was performed for each coupon, meaning there were three parallel measurements per sampling. The median was used in the evaluation and calculations.

Biofilm was removed by placing the sampling glass with coupon and water in an ultrasonic bath for 4.75 min and analysed for adenosine tri-phosphate (ATP). The analysis for ATP is based on the transformation of ATP to adenosine mono-phosphate (AMP) with light emission at a wavelength 562 nm by bioluminescense. First, nuleotide releasing reagent for microbial ATP (NRM) that extracts ATP from the cells, is added. Then the enzyme luciferin-luciferase that reacts with ATP with light as one of the products is added.

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow{\text{(Luciferase)}} \text{Oxyluciferin} + \text{AMP} + \text{pyro-phosphate} + CO_2 + \text{light}$$

The analysis of ATP was performed in a luminescence monitor.

The first days after start-up of the biofilm monitoring there will be a lag-phase with almost no significant growth followed by a period when the biofilm will grow exponentially limited by the amount of biomass. Then the growth will be "stable" limited by the growth area and the available substrate until the biofilm detachment becomes significant, from when the biofilm may vary considerably. The biofilm formation rate is calculated from the stable growth phase.

Mycological analysis

Biofilm was removed from the coupon physically. Some of the sampling material was transferred to the surface of DG18 Hocking and Pitt, 1980) plates for qualitative analyses. Some material was also dissolved in sterile salt water 1:10 for 20 min before dilution from 10^{-2} to 10^{-6} and seeding on duplicate DG18 plates. The plates were incubated at 25 ±1°C in 7 days, placed upside down in ventilated bags. The moulds on the dishes were then classified and quantified. Representative colonies were identified to family or genus level.

Bacteriological analysis

Biofilm from the coupons were homogenised in 10 mL distilled water for 5 min in an ultrasonic bath. The biofilm samples were analysed for total heterotrophic plate count by use of two non-selective agars (R2A-agar and water plate count agar (ISO 6222)(WPCA)) in addition to total bacterial counts by microscope using a FISH probe for Eubacteria (EUB 338) and Dapi staining. Selective medium were used for presumptive analyse for *Aeromonas spp.*, *Pseudomonas spp. (*ISO 16266:2006), *Legionella spp.*(GVPC agar) and specific analysis of coliforms /*Escherichia coli* (Colilert-18). *Legionella spp.* was confirmed by testing for need for cysteine in media.

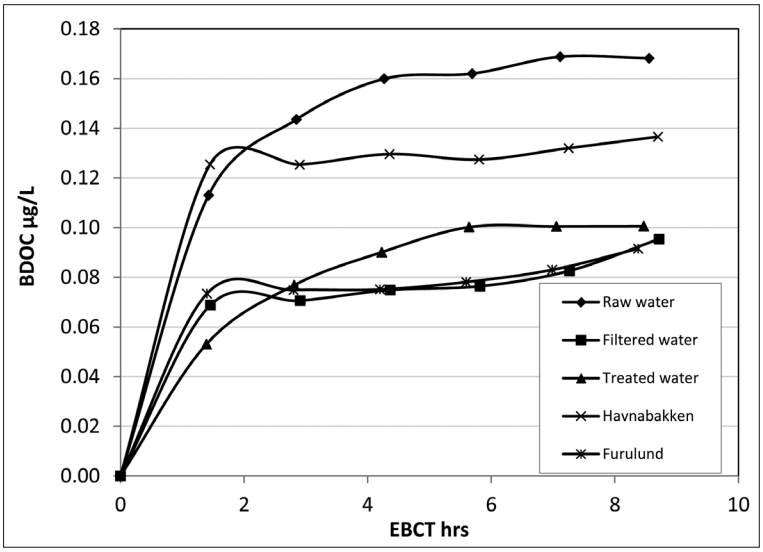

Figure 1. *Results from the BDOC analysis (EBCT=empty bed contact time).*

Results and discussion

Biodegradable dissolved organic carbon

The results of the BDOC analysis are shown in Figure 1 and Table 1.

The BDOC results show that the amount of the DOC being biodegradable was almost reduced by 50 % as a result from the new treatment including NOM removal. The effect from UV disinfection and the small amounts of chlorine added was marginal. When the water's residence time in the distribution system is rather long the BDOC content seems to show a slight increase, for which there is no obvious physical reason.

Table 1. *Summary of the results from the BDOC analysis.*

Sampling point	BDOC (mg/L)
Raw water	0.17
Filtered water	0.10
Treated water	0.10
Furulund	0.09
Havnabakken	0.14

Biofilm formation

The results of the biofilm formation measurements are shown in Figure 2 and Table 2.

When raw water was supplied to the biofilm monitor a considerable biofilm formation was measured. For all other tested water types the biofilm formation was close to zero.

The biofilm formation potential was 30–140 times higher for raw water than for the other water types. The

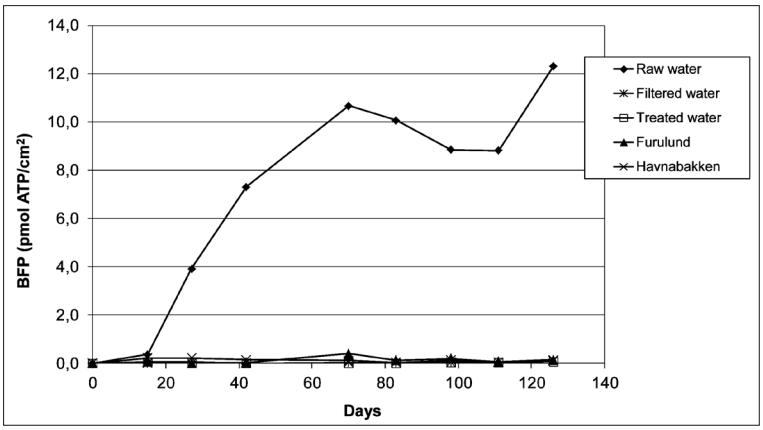

Figure 2. *Biofilm formation potential (BFP) during 125 days of biofilm build up in the biofilm monitor.*

Table 2. *The biofilm formation potential and rate.*

Sampling point	Maximum biofilm formation potential (pmol ATP/cm^2)	Maximum biofilm formation rate (pmol ATP/cm^2*d)
Raw water	12.3	0.30
Filtered water	0.14	0.006
Treated water	0.09	0.005
Furulund	0.40	0.013
Havnabakken	0.22	0.015

Table 3. *Moulds identified in the biofilm from the glass coupons.*

	No of colonies		
Parallel	A	B	C
Raw water	Excess growth *Trichoderm*a sp. + 1 Mucoraceae	Excess growth *Trichoderma* sp.	Excess growth *Trichoderma* sp.
Filtered water	1 *Cladosporium* sp.	1 *Trichoderma* sp.	No growth
Treated water	No growth	No growth	No growth
Furulund	No growth	No growth	No growth
Havnabakken	No growth	1 *Cladosporium* sp.	No growth

maximum biofilm formation rate was 20–60 higher for raw water compared to the other water categories. In treated water and during distribution the biofilm formation potential was 0.09–0.4 pmol ATP/cm^2 and the maximum biofilm formation rate was 0.005–0.015 pmol ATP/cm^2*d. These numbers harmonize with results from treated water from two waterworks in Vestfold, Norway, where they have a similar raw water quality and NOM removal by coagulation and filtration, but chlorination for disinfection (Hem, 2009).

The effect of the water treatment on the biofilm formation was far higher than the effect on BDOC. The water was analysed for possible limiting nutrients, but both the nitrogen and phosphorous contents were almost unchanged during water treatment.

The biofilm will partly consist of colonising microorganisms and partly of microbiological growth within the biofilm. In the biofilm monitoring the total growth is measured and the importance of either of the two contributions to the total growth is not defined.

Moulds

The results from the analysis of moulds in the biofilm are shown in Table 3.

From samples of biofilm grown in raw water there were excess growth of mould colonies, while none or only one colony was identified from biofilm samples grown in other water types. The reduction of the growth as a result of the water treatment was expected, since previous analysis of mould colonies in the water phase showed high numbers with the previous water treatment, meaning micro-sieving and chlorination, but close to none growth with the new treatment (Hem et al., 2012).

Bacterial composition of the biofilm

The results from analyses of total number of bacteria and plate count are shown in Table 4.

The reduction in cultivable bacteria was less than expected compared to raw water resulted in over grown plates for plate counts. However, the direct microscopic counts showed that there were higher bacteria counts in raw water, but also considerable numbers in biofilm grown in the other water types.

Table 4. *Total bacteria (CFU/cm^2 biofilm) on biofilm coupons by plate count (WPCA, R2A) and direct microscopic count (FISH EUB, Dapi).*

	WPCA	R2A	FISH EUB	Dapi
Raw water	3.5×10^4	1.8×10^5	7.5×10^6	1.7×10^7
Filtered water	$> 10^4$	$> 10^4$	2.4×10^5	2.7×10^5
Treated water	$> 10^4$	$> 10^4$	1.5×10^4	4.1×10^4
Furulund	$> 10^4$	$> 10^4$	9.0×10^5	1.4×10^5
Havnabakken	$> 10^4$	$> 10^4$	2.7×10^4	4.1×10^4

Table 5. *Concentration of potential pathogenic and indicator bacteria in biofilm (CFU/cm^2 biofilm).*

	Presumptive *Pseudomonas*	Presumptive *Legionella*	Confirmed *Legionella*	Presumptive *Aeromonas*	Coliform bacteria	*E. coli*
Raw water	30	114	–	6	21	0.1
Filtered water	<1	43	15	<1	<0.1	<0.1
Treated water	<1	<1	<1	<1	<0.1	<0.1
Furulund	<1	28	18	<1	<0.1	<0.1
Havnabakken	<1	47	27	<1	<0.1	<0.1

Table 5 shows the results from analysis of potential pathogens and indicator bacteria by culture on selective media.

Presumptive *Pseudomonas*, presumptive *Aeromonas* and coliform bacteria were detected in biofilm grown in raw water, but neither in biofilm grown in filtered water nor treated waters. Presumptive *Legionella* was detected in all samples except in treated water at the water treatment plant. The presumptive *Legionella* was confirmed both in biofilm grown in filtered water and in the distribution system.

In 2009, shortly before and after the start-up of the new treatment plant, *Legionella* was detected in water samples both in treated water and in the distribution system (Hem et al., 2012). The presence of *Legionella* in biofilm in raw and filtered water as well as during distribution was therefore expected.

Conclusions

The monitored effect of the NOM removal and UV-disinfection on the qualitative and quantitative biofilm formation was:

- 95–99 % reduction in the biofilm formation potential.
- The mould growth in the biofilm changed from excess growth in biofilm grown in raw water to marginal or no growth in filtered, disinfected distributed water.
- The amount of bacteria in the biofilm was considerably reduced during NOM removal, and further reduced during disinfection.
- The presence of possible opportunistic bacteria in the biofilm was reduced during NOM removal and further during disinfection.

The NOM removal by coagulation, sedimentation and filtration was considered the main reason for the quantitative and qualitative reductions in the biofilm formation.

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