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Biologische stabiliteitsparameters in de praktijk

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KWR

Bridging Science to Practice

Rapport

Biologische stabiliteitsparameters in de praktijk

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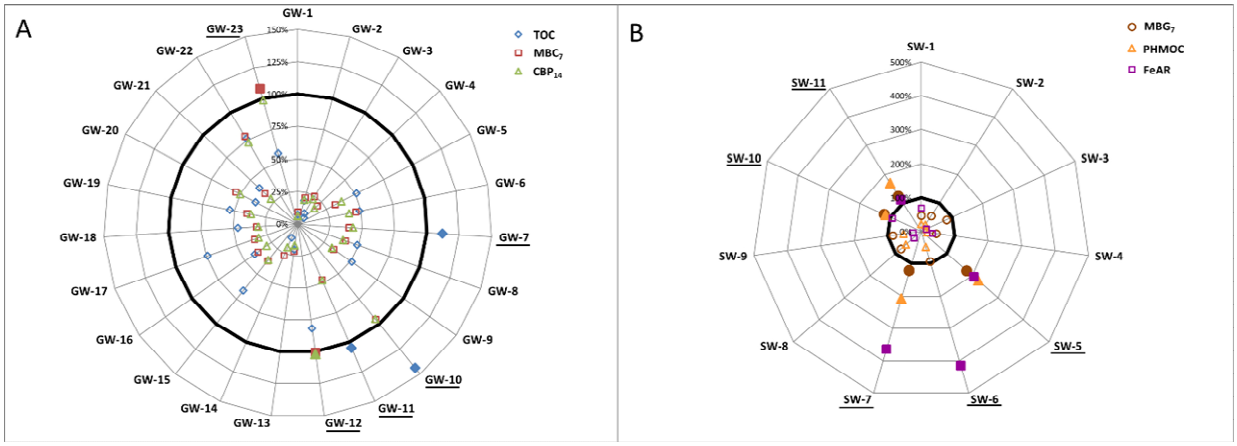
Managementsamenvatting

Biologische stabiliteit beter bewaken met nieuw afgeleide aandachtswaarden voor diverse parameters voor biologische stabiliteit

Auteur dr. Paul van der Wielen

De database voor nieuwe parameters die de groeipotentie en biofilmvormende eigenschappen van drinkwater beschrijven is uitgebreid en gebruikt om aandachtswaarden af te leiden. Aandachtswaarden zijn waarden waarbij de verbetering van de biologische stabiliteit van het drinkwater aandacht vraagt. Op grond van de relatie met KG22 en *Aeromonas* zijn aandachtswaarden voor zes van deze parameters afgeleid (voor drinkwater bereid uit grondwater TOC (4,1 mg C/l), MBC_7 (8,6 ng ATP/l) en CBP_{14} (110 d ng ATP/l), voor drinkwater bereid uit oppervlaktewater MBG_7 (4,5 ng ATP/l), FeAS (0,34 mg Fe m^{-2} dag^{-1}) en PHMOC (47 µg C/l). Door deze aandachtswaarden in de drinkwaterpraktijk te gebruiken is het mogelijk productielocaties met verlaagde biologische stabiliteit te identificeren. Door inzet van een breder scala van nieuwe methoden om de groeipotentie en biofilmvormende eigenschappen van water te beschrijven op dergelijke locaties kan het meest kritische proces in de zuivering worden gedetecteerd. Het rapport bevat aanbevelingen voor toepassing van de nieuw afgeleide aandachtswaarden in de drinkwaterpraktijk.



Radarplots voor aandachtsparameters van reinwater op locaties waar drinkwater uit grondwater (links) of oppervlaktewater (rechts) wordt bereid. Overschrijdingen van de aandachtswaarde zijn in deze radarplots aangegeven met dichte symbolen, waarmee de productielocaties kunnen worden geïdentificeerd waar verbetering van de biologische stabiliteit wenselijk is.

Belang: voor gebruik nieuwe parameters biologische stabiliteit zijn aandachtswaarden nodig

Distributie van drinkwater van hoge kwaliteit, zonder restgehalte desinfectiemiddel is het visitekaartje van de Nederlandse drinkwatervoorziening. Door biologisch stabiel drinkwater te produceren wordt de groei van ongewenste organismen in het drinkwater-distributiesysteem tegengegaan. In de afgelopen vijf tot tien jaar zijn binnen het BTO diverse nieuwe parameters ontwikkeld die de groeipotentie en biofilmvormende eigenschappen van drinkwater volledig beschrijven dan de traditioneel gebruikte parameters AOC (assimileerbaar organisch koolstof) en BVS (biofilmvormingssnelheid). Door voor deze nieuwe parameters aandachtswaarden af te leiden die gerelateerd zijn aan relatief hoge waarden voor de wettelijke parameters voor nagroei, KG22 en *Aeromonas*, wordt het mogelijk scherper in beeld te krijgen waar en hoe de biologische stabiliteit van het geproduceerde water in het gedrang komt.

Aanpak: uitbreiden metingen met nieuwe parameters en relateren aan KG22 en *Aeromonas*

Op 34 locaties met variërende aantallen KG22- en *Aeromonas*- in het distributiesysteem zijn gedurende drie maanden de volgende parameters bepaald van het reinwater: ATP- en TOC-concentratie, biomassaproductiepotentie (BPP)-parameters (MBC_7, MBG_7, CBP_{14}), AOC-A3 concentratie, biomassa- en ijzeraccumulatiesnelheid (BAS, FeAS), deeltjesgebonden en/of hoogmoleculair organisch koolstof (PHMOC) en koolhydraten (PHMCHC) én deeltjesgebonden ijzer (PFe). Daarnaast werd het geometrisch gemiddelde van KG22 en 90-percentiel van *Aeromona*s van het gedistribueerde water berekend. Vervolgens werd onderzocht welke biologische stabiliteitsparameters onderling sterk correleerden en welke biologische stabiliteitsparameters sterk correleerden met KG22 en *Aeromonas*. Daaruit konden vervolgens aandachtswaarden voor de nieuwe parameters worden afgeleid.

Resultaten: aandachtswaarden en correlaties tussen parameters

De verschillende parameters die de groeipotentie en biofilmvormende eigenschappen van het water beschrijven, variëren over een grote range tussen de verschillende productielocaties. Drinkwater bereid uit oxisch grondwater of uit oppervlaktewater na duinfiltratie, vertoont over het algemeen de laagste waarden, terwijl de hoogste waarden zijn aangetroffen voor drinkwater bereid uit oppervlaktewater na reservoir. Locaties die gebruikmaken van anoxisch grondwater variëren voor de meeste parameters van laag naar hoog. Enkele parameters (MBC_7 en CBP_{14}) vertonen onderling een zeer sterk verband, waardoor slechts één van de twee hoeft te worden gemeten. Op basis van locaties met de hoogste *Aeromonas*/KG22 aantallen in het distributiesysteem en enkele/meervoudige correlatieanalyses zijn voor drinkwater bereid uit grondwater aandachtswaarden afgeleid voor TOC (4,1 mg C/l), MBC_7 (8,6 ng ATP/l) en CBP_{14} (11,0 d ng ATP/l). Voor drinkwater bereid uit oppervlaktewater zijn aandachtswaarden afgeleid voor MBG_7 (4,5 ng ATP/l), FeAS (0,34 mg Fe m^{-2} dag^{-1}) en PHMOC (47 µg C/l). Radarplots laten zien dat bij zeven van de 23 locaties, met drinkwaterbereiding uit grondwater, ten minste één van de aandachtswaarden wordt overschreden (zie figuur). Waar drinkwater wordt bereid uit oppervlaktewater overschrijden vijf van de elf onderzochte locaties ten minste één van de aandachtswaarden. Dit zijn ook de locaties waar verhoogde *Aeromonas*- en/of KG22-aantallen in het distributiesysteem zijn waargenomen.

Toepassing: aandachtswaarden inzetten in de drinkwaterpraktijk

De afgeleide aandachtswaarden voor de verschillende parameters kunnen worden ingezet op productielocaties waar zorgen zijn over de biologische stabiliteit of nagroei. Daarvan is een beter beeld te krijgen door de geschikte parameters drie maanden te bepalen en te toetsen aan gemiddelde waarden en de nieuwe aandachtswaarden. Bij overschrijding van één van de aandachtswaarden is de aanbeveling om na elke zuiveringsstap een breed scala aan biologische stabiliteitsparameters te bepalen en zo te bepalen welk zuiveringsproces waarschijnlijk het meest kritisch is en hoe dat geoptimaliseerd kan worden.

Rapport

Dit onderzoek is beschreven in het rapport *Biologische stabiliteitsparameters in de praktijk* (BTO-2023.014).

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Inhoud

1 Introductie

In 2015 t/m 2017 heeft binnen het thematisch onderzoek van het BTO een project gelopen waarin aandachtswaarden zijn afgeleid voor nieuwe biologische stabiliteitsmethoden die door KWR zijn ontwikkeld in relatie tot KG22- en *Aeromonas*-aantallen in het distributiesysteem. De resultaten van dat onderzoek zijn in een rapport gepubliceerd (van der Wielen, 2018). In 2021/2022 heeft binnen het BTO-bedrijfsonderzoek van Evides en Oasen een project gelopen dat als voornaamste doel had om de rapportage uit 2018 om te schrijven tot een wetenschappelijke publicatie. Daarnaast is in dit bedrijfsonderzoekproject ook onderzocht of aandachtswaarden voor de biologische stabiliteitsparameters konden worden afgeleid in relatie tot *Legionella* of dierlijke organismen in het distributiesysteem. Tot slot is ook een workshop gehouden met de verschillende drinkwaterbedrijven waarin verschillende methoden en toepassing van die methoden in de drinkwaterpraktijk of drinkwaterlaboratoria zijn gepresenteerd en waar is gediscussieerd over eventuele nieuwe wensen ten aanzien van biologische stabiliteitsmethoden en factoren die toepassing van de nieuw ontwikkelde methoden in de weg staan.

In deze rapportage worden deze drie onderwerpen gepresenteerd. In hoofdstuk 2 worden alle resultaten, dus de analyse van relaties tussen de nieuwe biologische stabiliteitsparameters en KG22 en *Aeromonas*, maar ook tussen de biologische stabiliteitsparameters en *Legionella* of invertebraten beschreven. Voor die laatste twee bleek geen goede correlatie met de biologische stabiliteitsparameters. In hoofdstuk 3 is het manuscript opgenomen dat is aangeboden aan het wetenschappelijke peer-reviewed tijdschrift Science of the Total Environment en bevat daardoor de resultaten over KG22 en *Aeromonas* die ook in hoofdstuk 2 staan. In hoofdstuk 4 tot slot is een samenvatting van de workshop weergegeven. Daarbij zijn hoofdstuk 2 en 3 in het Engels geschreven, terwijl hoofdstuk 4 in het Nederlands is geschreven.

2 Guidance values for biological stability parameters in relation to HPC22, *Aeromonas*, *Legionella* and invertebrate biomass.

2.1 Introduction

The growth of (micro)organisms in drinking water systems might adversely affect the distribution of safe and impeccable drinking water. Microbial growth can lead to public health issues when opportunistic pathogens, such as *Legionella pneumophila*, *Pseudomonas aeruginosa* and pathogenic nontuberculous mycobacteria multiply in the water system (Falkinham et al. 2015, van der Wielen et al. 2014). Growth can also lead to aesthetic problems and consumer complaints, such as taste and odor issues or growth of invertebrates visible to the naked eye (Christensen et al. 2011, Hambsch et al. 2014, van Lieverloo et al. 2012). Finally, growth in drinking water systems might also result in technical complaints, for instance microbial induced corrosion or clogging of water lines or water meters due to excessive biofilm, sediment or invertebrates (Camper 2014, van der Kooij and van der Wielen 2014).

To prevent such problems, drinking water companies aim to produce and distribute biological stable drinking water. Denmark, the Netherlands, and parts of Belgium, Germany and Switzerland distribute drinking water without a disinfectant residual and produce biological stable drinking water by limiting the nutrient concentration. The drinking water companies in the Netherlands have recently established a working definition for biological stability that they try to establish in their drinking water systems: “Biological stability describes a drinking water system that leads to as little as possible biological changes so that public health risks and/or consumer complaints related to growth of (micro)organisms do not occur” (Hijnen and van der Wielen 2017).

To be able to comply to this working definition, predictive methods must be available to monitor the biological stability of drinking water, and the impact of pipe materials and sediment on microbial growth in drinking water systems. In the 1980s/1990s, two predictive methods were developed to describe the biological stability of drinking water: one that measures the concentration of easily assimilable organic carbon (AOC) and another that determines the biofilm formation rate (BFR) of the drinking water (van der Kooij 1992, van der Kooij et al. 1999, van der Kooij et al. 1982). In the Netherlands, an AOC guideline value of 10 µg C L^{-1} and a BFR guideline value of 10 pg ATP cm^{-2} day^{-1} were deduced based on the relation between AOC of the treated water and heterotrophic plate counts at 22°C (HPC22) in the distributed drinking water, and BFR of the treated water and *Aeromonas* plate counts in the distributed drinking water produced from groundwater (van der Kooij et al. 1999, van der Kooij and Veenendaal 2014). Both HPC22 and *Aeromonas* are indicators for regrowth in the drinking water distribution system and included as legislative parameters in the Dutch drinking water decree (Anonymous 2012). The last two decades, however, it was observed that *Aeromonas* and *Legionella* (another legislative parameter in the Dutch drinking water decree) plate counts in the distribution system of several treatment plants in the Netherlands violated the legislative standard, despite that the treated water at the plant had an AOC concentration and BFR value under these guideline values. These findings indicate that the combination of AOC and BFR measurements are not always adequate to describe the biological stability of drinking water and predict problematic regrowth in relation to legislative microbiological indicator parameters for regrowth.

Recently, our group developed a suite of methods to determine the biological stability in drinking water systems: the biomass production potential test for drinking water (BPP-W), assimilable organic carbon of biopolymers determined with strain A3 (AOC-A3), the continuous biofilm monitor (CBM) and an ultrafiltration (hemoflow)

method to concentrate and quantify particulate and/or high molecular organic carbon (PHMOC)(Hijnen et al. 2018, Sack et al. 2010, 2011, van der Kooij et al. 2015, van der Kooij and Veenendaal 2014). Although these methods have helped improving the drinking water quality at specific production locations (Hijnen et al. 2018, Schurer et al. 2022, van der Kooij et al. 2015), it remains unknown whether the whole suite of methods must be applied nationwide and to what guidance values these new biological stability parameters must comply to prevent regrowth problems in the drinking water distribution systems. Therefore, the objectives of our study are to (i) measure the biological stability of treated water for a wide range of treatment plants, (ii) determine the relations between the different novel biological stability parameters in unchlorinated drinking water produced from different treatment plants, (iii) elucidate which novel biological stability parameter(s) in drinking water is/are related to legal indicators for nuisance regrowth in the distribution system in the Netherlands (HPC22, *Aeromonas* and *Legionella*) and invertebrate biomass and (iv) deduce guidance values for these novel biological stability parameters required to control these regrowth indicators in the distribution system.

2.2 Materials and Methods

2.2.1 Drinking water treatment plants and sampling

During 2010 till 2017, the treated water from 34 different drinking water treatment plants was sampled. Nine of these treatment plants used surface water as source for drinking water production, 23 plants used groundwater and two plants used both surface water and groundwater. The water source used, treatment train applied and the year of sampling for each treatment plant is given in Table S1. Surface water plants were all sampled in the same season (June till October), whereas groundwater plants were not sampled in a specific season.

2.2.2 Predictive biological stability parameters

The methods used to measure the different predictive biological stability parameters are described in detail in the supporting information. Here, a short description of each method will be given.

2.2.2.1 2.2.1 Growth potential tests

MBC_7, MBG_7, CBP_{14} using the BPP-W test

The BPP-W test used in our study was a slightly altered method of the initial BPP-W test published (van der Kooij and Veenendaal 2014). In short, treated drinking water samples (600 ml) were collected in AOC-free flasks to which phosphate and nitrate were added. One ml of a sodium sulfite solution (0.19 M Na_2SO_3) and an inoculum was added to the drinking water when the treated water samples came from treatment plants where filtrate disinfection with chlorine dioxide was used as last treatment step. Flasks were incubated in the dark at 25 ± 2°C for 14 days. In time, subsamples were taken from each bottle and analyzed for the ATP concentration. Three parameters were deduced from the obtained ATP concentrations: (i) the maximal biomass concentration during the first seven days of incubation (MBC_7), (ii) the maximum biomass growth during the first seven days of incubation (MBG_7) and (iii) the cumulative biomass production during 14 days of incubation (CBP_{14}).

AOC-A3

The AOC-A3 concentration was determined using *Flavobacterium johnsoniae* strain A3 (Sack et al. 2010, 2011). In short, treated drinking water samples (600 ml) were taken in AOC-free flasks. Nitrate, phosphate and sodium sulfite were added to the samples in the same manner as described for the BPP-W test. Samples were pasteurized for 30 minutes at 60°C, after which *F. johnsoniae* strain A3 and *Pseudomonas fluorescens* strain P17 were added (starting concentration of approximately 100 cfu/ml). All flasks were incubated in the dark at 15°C ± 1°C. Every two to three days a subsample was taken from each flask and colony counts of strain A3 were determined. This maximum colony count and a previously determined yield factor (1.43×10^7 CFU/µg; (Sack et al. 2011) was used to calculate the AOC-A3 concentrations.

2.2.2.2 Other tests

BAR, FeAR using the CBM

The biomass accumulation rate (BAR) and the iron accumulation rate (FeAR) were determined using a continuous biofilm monitor (CBM)(van der Kooij and Veenendaal 2014). The CBM was connected for three months to the treated water of each investigated treatment plant, resulting in a continuous flow of 10 L h^{-1} through each of four columns containing a glass cylinder with glass beads (Ø2mm). Every two weeks the glass cylinder of two columns was replaced with a new one and the ATP and iron concentration in the biofilm was determined and used to calculate the BAR and FeAR.

PHMOC, PHMCHC, PFe using concentration by ultrafiltration

The particulate and/or high molecular organic carbon (PHMOC), particulate and/or high molecular carbohydrates carbon (PHMCHC) and the particulate iron (PFe) concentrations were obtained using crossflow ultrafiltration. 100 L of the treated water at each plant was concentrated to approximately 500 ml using ultrafiltration. The concentrate was subsequently analyzed for the TOC, carbohydrate and iron concentration. The PHMOC, PHMCHC and PFe concentrations were thereafter calculated by first correcting for the DOC, dissolved carbohydrate or dissolved iron concentration in the drinking water and second using the concentration factor of the crossflow ultrafiltration step.

The parameters obtained from the BPP_{DW} test, AOC-A3, ultrafiltration concentrate, and TOC were determined at three successive months and the value for each parameter was averaged from these three sampling rounds. The CBM parameters and ATP concentration were determined at six successive fortnights and the value for each parameter was averaged from these six sampling rounds.

2.2.3 Analytical analyses

ATP

The ATP concentrations were determined by measuring the amount of light produced in a luciferin-luciferase assay as previously described (van der Wielen and van der Kooij 2010). Briefly, a nucleotide-releasing buffer (NRB, Celsis) was added to the sample to release ATP from the cells. The generated light signal was measured as Relative Light Units (RLU), after a 2 s delay time and a 10 s integration time with a luminometer (Celsis Advance II, Celsis International B.V., Maastricht-Airport, The Netherlands). The concentration of ATP was calculated from the RLU values using a conversion factor determined in calibration measurements. The detection limit of the luminometer is 1 ng ATP l^{-1}.

TOC, DOC and carbohydrates

The TOC concentration was determined by acidifying the samples to a pH between 1 and 2 using 30% HCl. Subsequently, samples and calibration curve standards were measuring using a TOC analyzer (Shimadzu), in which organic carbon is oxidized to carbon dioxide that is successively measured by an infrared detector. The DOC concentration was measured in a similar matter except that water samples were first filtered over a 0.45 µm membrane. The carbohydrate concentration in het hemoflow concentrate was determined by the phenol–sulfuric acid colorimetric assay using a calibration curve with different glucose concentrations (DuBois et al. 1956).

Iron

Samples for iron measurements were acidified to pH < 2,0 using 65% HNO_3 and destructed using a microwave. Subsequently, iron was determined with inductively coupled plasma-mass-spectrometry (ICP-MS) using a calibration curve with different iron concentrations according to NEN-EN-ISO 17294-2.

HPC22, Aeromonas, Legionella and invertebrates

Data for heterotrophic plate counts at 22°C (HPC22), *Aeromonas*, *Legionella* and invertebrates from the distribution system was collected by the drinking water companies in the Netherlands, according to their legislative routine monitoring program. Drinking water samples for HPC22, *Aeromonas* and *Legionella* were taken at different locations in the distribution system and at different time points during the year. These samples were taken at consumers kitchen tap after flushing the water tap till the drinking water temperature is constant for 30 seconds so that the water sample came from the distribution system. HPC22 was determined on glucose-yeast-extract-agar according to NEN-EN-ISO 6222 in which agar plates were incubated at 22°C for 68 ± 4 hours. *Aeromonas* was determined on ampicillin dextrin agar as previously described (Havelaar et al. 1987). *Legionella* was determined on buffered charcoal yeast extract (BCYE) agar with antibiotics according to ISO standard 11731-2.

Samples for invertebrates were taken at different hydrants in the distribution system in September and/or October, when invertebrate numbers are at their maximum, as described by (van Lieverloo et al. 2012). Different invertebrate taxa were identified using microscopic examination as previously described (van Lieverloo et al. 2004). The total invertebrate biomass was subsequently calculated by using the estimated biomass of each invertebrate taxa (van Lieverloo et al. 2012) and the invertebrate biomass is given in mg/m^3.

The data obtained for HPC22, *Aeromonas*, *Legionella*, and invertebrate in the year that the biological stability parameters were determined at a treatment plant, was taken for further analyses. The geometric yearly mean for HPC22 ($HPC22_{gm}$) and Legionella ($Legionella_{gm}$), the yearly 90 percentile for *Aeromonas* ($Aeromonas_{90P}$) and the yearly median or 50-percentile for invertebrate biomass ($invertebrate_{50P}$) was calculated for each distribution system and used in further analyses.

2.2.4 Statistical analyses

Possible outliers were identified by calculating the median, 25 and 75 percentiles of each parameter. Each value for a parameter that were higher or lower than the median value +/- three times the interquartile range was identified if such an extreme value was unexpected, the value was considered an outlier and not included in the statistical analyses.

Different statistical analyses were applied in our study. First, it was determined whether the different biological stability parameters followed a normal distribution using the Shapiro-Wilk test. The results revealed that ATP and TOC were normally distributed. The other parameters (MBC_7, MBG_7, CBP_{14}, AOC-A3, BAR, FeAR, PHMOC, PHMCHC, PFe) were subsequently log-transformed and the log transformed data was tested again using the Shapiro-Wilk test. The log-transformed values of these parameters were all normally distributed.

Differences between treatment plants using groundwater or surface water were statistically tested using the absolute values (ATP, TOC) or log-transformed values (other parameters). Next, it was shown with the Levene's test that all parameters showed equality of variances between groups (groundwater plants versus surface water plants; $p>0.05$). Consequently, statistical difference of these parameters between groundwater and surface water plants was tested with the independent samples t-test and differences were considered statistically significant at the $p<0.05$ level.

Correlation analysis between all parameters (ATP, TOC and log-transformed values of the other parameters) were done using Pearson correlation. Correlations were considered significant at the $p<0.05$ level and for those correlations the R^2 value was calculated. In addition, single linear regression analysis was performed to determine whether the microbiological parameters could be predicted from one or more biological stability parameters. Regression results were considered significant at the $p<0.05$ level and the R^2 of the significant regression models were subsequently calculated. All statistical analyses were performed using SPSS 26.

2.2.5 Calculation of guidance values for predictive biological stability parameters

Guidance values for certain biological stability parameters were determined in relation to the legislative regrowth parameters in the Netherlands (HPC22, *Aeromonas* and *Legionella*) in the distribution system, and in relation to invertebrate biomass in the distribution system. A yearly geometric mean of 20 cfu ml^{-1} for $HPC22_{gm}$, a yearly 90-percentile of 800 cfu 100 ml^{-1} for $Aeromonas_{90P}$, a yearly geometric mean of 100 cfu l^{-1} for $Legionella_{gm}$ and yearly median value of 150 mg m^{-3} for invertebrate $biomass_{50P}$ were used to determine guidance values for the predictive biological stability parameters.

The guidance values for biological stability parameters were determined for all treatment plants together, and the treatment plants that used groundwater or surface water as source. These guidance values were based on values derived from a semi-quantitative and statistical quantitative analysis. In the semi-quantitative analysis, treatment plants were first ranked from highest to lowest value for each of the four biological parameters $HPC22_{gm}$, $Aeromonas_{90P}$, $Legionella_{gm}$ and $invertebrate_{50P}$. It was then established how the top four treatment plants for a biological parameter ranked for each biological stability parameter. A critical biological stability parameter was identified and further investigated when three or four of the top four treatment plants ranked within the top 25% (all treatment plants or groundwater treatment plants) or when two to four of the top four treatment plants ranked within the top 30% (surface water treatment plants) of the plants for a certain biological stability parameter. Subsequently, the semiquantitative threshold value for each identified biological stability parameter was determined. This threshold value was the lowest value from the treatment plants that exceeded the set geometric mean, 90-percentile or median for the four (micro)biological parameters. An example of how this semi-quantitative threshold value was determined is given in the supplemental information.

In the statistical quantitative analyses, biological stability parameters that showed a significant ($p<0.05$) regression with $HPC22_{gm}$, $Aeromonas_{90P}$, $Legionella_{gm}$ or $invertebrate_{50P}$ and that had a $R^2 \geq 0.4$ were investigated in detail. Both the p-value of 0.05 and R^2 value of 0.4 were arbitrarily chosen. The formula describing the regression relation was determined and the established value for each of the four (micro)biological parameters was subsequently used in the formula to derive the statistical quantitative threshold value for the respective biological stability parameter. Thereafter, the guidance value for a certain biological stability parameter was determined by calculating the average value from the threshold values when both the semi-quantitative and statistical quantitative analysis resulted in a threshold value for the respective biological stability parameter.

2.3 Results

2.3.1 Biological stability parameters treated water

Nine novel biological stability parameters and the ATP and TOC concentrations of the treated water from 34 different drinking water treatment plants in the Netherlands were determined and the results are presented in Fig. 1 and Fig. S1 – S4. The ATP concentrations in treated water varied between 0.7 ± 0.6 and 6.6 ± 1.1 ng ATP/L and the TOC concentration between 0.3 ± 0.01 and 5.9 ± 0.3 mg C/L (Fig. S1). The lowest and highest concentrations for both parameters were observed in drinking water produced from groundwater. All three parameters deduced from the BPP_{DW} test showed the lowest values for drinking water produced from groundwater and highest values for drinking water produced from surface water (Fig. 1A & S2). The MBC_7 value ranged between 0.8 ± 0.5 and 13.0 ± 0.04 ng ATP/L, the MBG_7 value between -0.3 ± 0.4 and 8.0 ± 1.4 ng ATP/L and the CBP_{14} value between 6.8 ± 4.1 and 174.8 ± 49.6 d.ng ATP/L. The results from the BPP_{DW} test demonstrates that the microbial growth potential of drinking water in the Netherlands can vary substantially between treatment plants. In general, the AOC-A3 concentrations of the different drinking water types was low with 81% of the treated plants having a concentration below 2.0 µg C/L in their treated water (Figure 1B). The six highest AOC-A3 concentrations varied between 2.1 ± 0.5 and 11.1 ± 1.4 µg C/L and were particularly observed for drinking water produced from surface water.

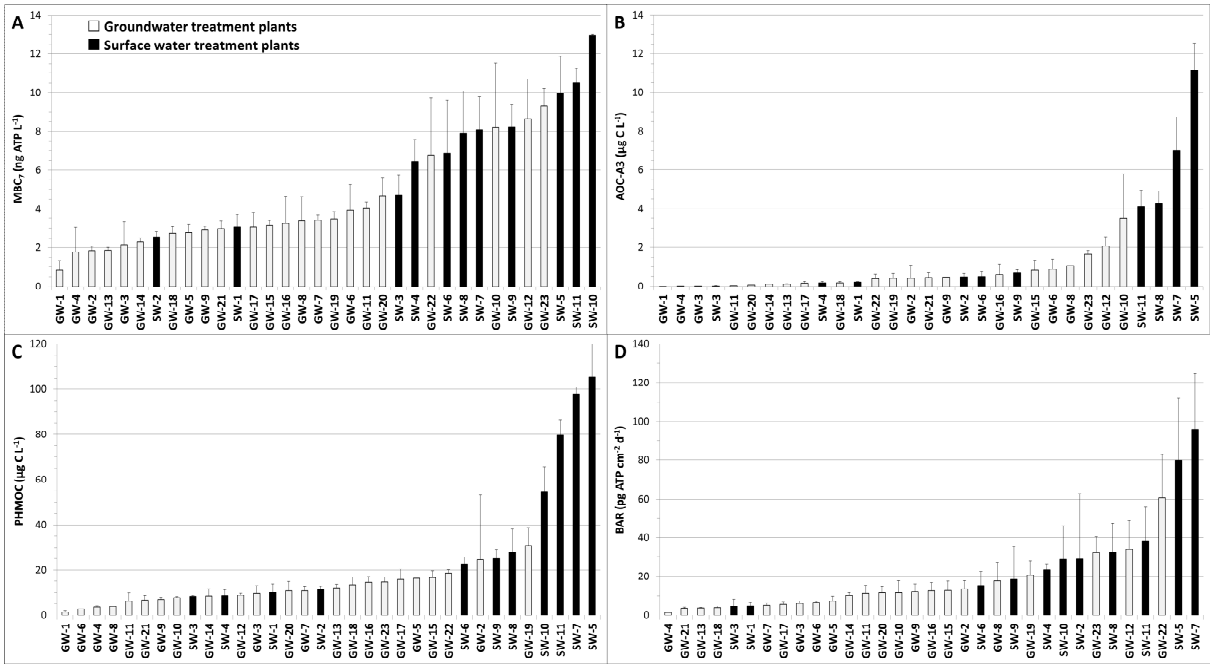

Fig. 1. The mean MBC_7 values (A), AOC-A3 concentrations (B), PHMOC concentrations (C) and BAR values (D) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

A similar observation was made for PHMOC and PHMCHC concentrations in treated water from the different treatment plants, with 82% of the treated water having a PHMOC concentration ≤ 25.0 µg C/L and a PHMCHC concentration < 10.0 µg C/L (Fig. 1C and S3). The six highest concentrations ranged between 28.1 ± 9.8 µg C/L and 105.4 ± 72.2 µg C/L for PHMOC, and between 10.1 ± 3.8 µg C/L and 55.2 ± 45.2 µg C/L for PHMCHC and were mainly observed for drinking water produced from surface water. The high standard deviation observed for the highest PHMOC and PHMCHC concentrations (plant SW-5) also demonstrates that these concentrations can vary considerably over a period of three months. It should be noted, however, that such high standard deviations were not observed for the other treatment plants with high PHMOC and PHMCHC concentrations (Fig. 1C & S3). The PFe concentrations ranged between 0.1 ± 0.1 and 17.1 ± 10.7 µg Fe/L, with the highest concentrations generally observed for drinking water produced from groundwater (Fig. S3).

The BAR determined with the CBM varied between 1.4 ± 0.3 to 96.0 ± 28.8 pg ATP cm^{-2} day^{-1}, with lowest values observed for drinking water produced from groundwater and highest values for drinking water produced from surface water (Fig. 1D). The FeAR ranged from 0.03 ± 0.03 to 3.39 ± 2.24 mg Fe m^{-2} day^{-1}, with the lowest and highest values observed for drinking water produced from groundwater (Fig. S4).

2.3.2 Microbiological parameters distributed drinking water

The routinely measured microbiological parameters in drinking water sampled from the distribution system of 34 treatment plants and invertebrate biomass in flushing water from these distribution systems were analyzed as well (Fig. 2). The geometric mean for HPC22 in distributed drinking water of 34 different treatment plants ranged between 0.4 and 32.2 cfu/mL with the lowest and highest numbers for drinking water produced from groundwater (Fig. 2A). These values are all within the legislative standard of HPC22 in the Netherlands (i.e. geometric year mean < 100 cfu/mL). The 90-percentiles of *Aeromonas* in the distributed drinking water of all plants varied between < 0.1 to 3720 cfu/100 mL (Fig. 2B) and, thus, can differ considerably between treatment plants. The highest 90-percentiles for *Aeromonas* were observed for distributed drinking water from treatment plants that treat surface water. At five plants the 90-percentile of *Aeromonas* in the distribution system exceeds the maximum legislative

Aeromonas standard for the Netherlands (i.e. 1000 cfu/ 100 mL), demonstrating that regrowth in the distribution system is not always under control in the Netherlands.

In 29 of the 34 analyzed distribution systems, the number of cultivable *Legionella* were under the detection limit of 100 cfu/L (Fig. 2C). In the distribution system of the five other plants, that used either groundwater or surface water for their drinking water production, *Legionella* numbers were sporadically above the legislative standard of 100 cfu/L. However, in all cases these *Legionella* colonies belonged to *Legionella* species other than *Legionella pneumophila*. The biomass of invertebrates in samples from these distribution systems varied between < 0.1 mg/m^3 and 326 mg/m^3 (Fig. 2D). The highest concentrations were observed for distributed drinking water produced either from surface water or groundwater.

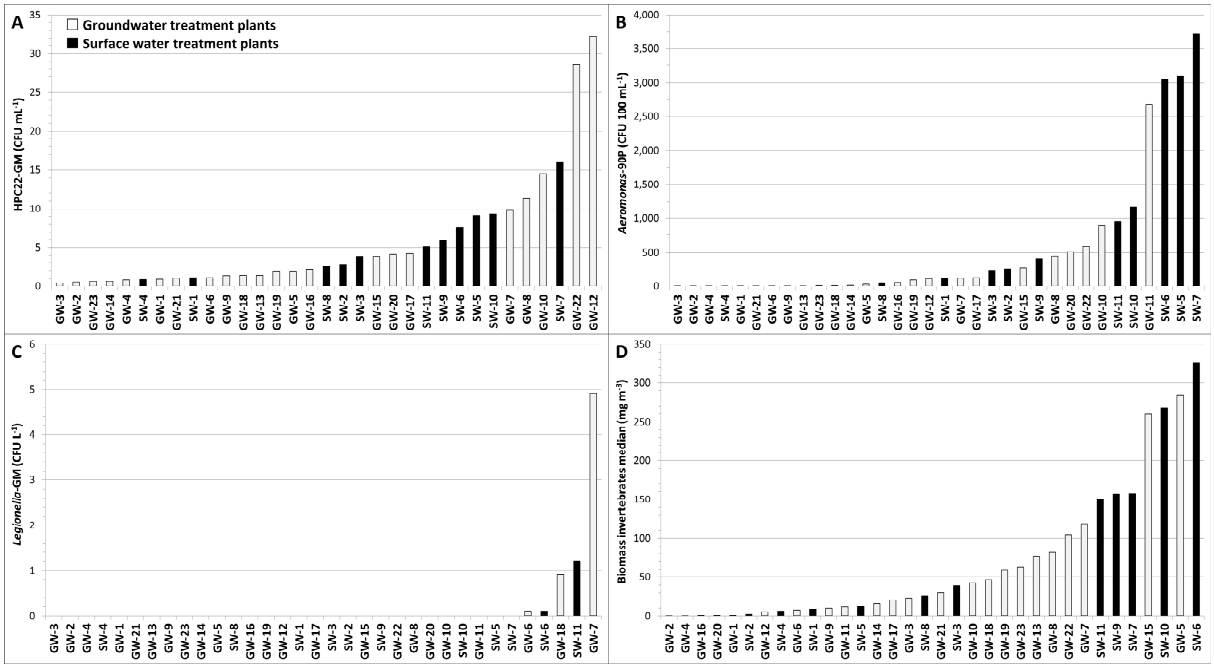

Fig. 2. The geometric mean of HPC22 (A), 90-percentile of *Aeromonas* (B), geometric mean of *Legionella* (C) and median of the invertebrate biomass (D) in drinking water samples from the distribution system of different treatment plants that treat groundwater (open bars) or surface water (closed bars).

2.3.3 Differences between drinking water produced from groundwater and surface water

It was also investigated for each parameter whether the values from drinking water produced from groundwater were significantly different from drinking water produced from surface water. The results demonstrated that the three BPP_{DW} parameters MBC_7, MBG_7 and CBP_{14} were significantly lower in drinking water produced from groundwater than from surface water ($p<0.05$; Table 1). Furthermore, the BAR values, PHMOC and PHMCHC concentrations and *Aeromonas* counts were also significantly lower in drinking water produced from groundwater than from surface water ($p<0.05$; Table 1). These results indicate that, in general, the biological stability of treated water produced from surface water was lower and that the *Aeromonas* regrowth in distribution systems fed with drinking water from surface water was higher than that of drinking water produced from groundwater. Still, it was noted that some plants that treat groundwater to drinking water have biological stability and *Aeromonas* values that are comparable high or higher than values observed for plants that treat surface water and vice versa. This means that care should be taken to generalize these findings to all unchlorinated drinking water types produced from groundwater or surface water.

Table 1 The statistical outcome of comparing (log-transformed) values of different biological stability parameters in treated water and microbiological parameters in distributed drinking water between drinking water produced from groundwater or surface water using the independent samples t-test. The specific p-value and whether differences were significant at the $p<0.05$ level are given as well as whether values for the significant parameters were higher or lower for groundwater treatment plants than those from surface water treatment plants.

Parameter	p-value	Significant	Groundwater plants
ATP	0.95	No	-
Log MBC_7	1.1×10^{-3}	Yes	Lower
Log MBG_7	5.9×10^{-7}	Yes	Lower
Log CBP_{14}	3.2×10^{-3}	Yes	Lower
Log AOC-A3	0.074	No	-
Log BAR	0.013	Yes	Lower
Log FeAR	0.41	No	-
TOC	0.59	No	-
Log PHMOC	1.1×10^{-3}	Yes	Lower
Log PHMCHC	1.4×10^{-5}	Yes	Lower
Log PFe	0.32	No	-
Log HPC_{gg}	0.15	No	-
Log *Aeromonas*$_{90P}$	0.020	Yes	Lower
Log *Legionella*$_{gg}$	0.99	No	-
Log invertebrate$_m$	0.15	No	-

2.3.4 The relation among the different biological stability parameters and among the different (micro)biological parameters

We performed a pair-wise correlation analysis on the obtained data to determine whether the eleven different parameters measured holds unique or common features of the biological stability of treated drinking water. The majority of the 52 pairwise correlations (31 when parameters were not logtransformed and 34 for logtransformed values) were not significant ($p>0.05$) or significant with a relatively low R^2 (<0.4) (Table S2). In the latter case this means that two parameters are significantly related, but that less than 40% of the variance in one parameter could be explained by the variance in the other parameter. In addition, 16 (not logtransformed values) or 20 (logtransformed values) other pair-wise correlations between biological stability parameters were also significant, but had a R^2 between 0.4 and 0.9, indicating a moderate relationship. Finally, two (not logtransformed values) pair-wise correlations (MBC7 – CBP_{14} and PHMOC – PHMCHC) showed, besides significance, a R^2 value higher than 0.9, indicating a strong relationship.

The significant and strong correlation between MBC_7 and CBP_{14} is visualized in Figure 3A and shows that data points were relatively equally ranged over both axes and that the strong correlation is observed for all data and data solely from drinking water produced from groundwater or surface water. The significant and strong correlation between PHMOC and PHCHC shows that four data points were considerably higher than the rest, demonstrating that the data points were not equally ranged over both axes (Fig. 3B) and could be responsible for the relatively high R^2. Moreover, the strong correlation between these two parameters was only observed for all data and data solely from drinking water produced from surface water. When the correlation was calculated for the data from drinking water produced only from groundwater, the relationship was less strong with a R^2 of 0.66.

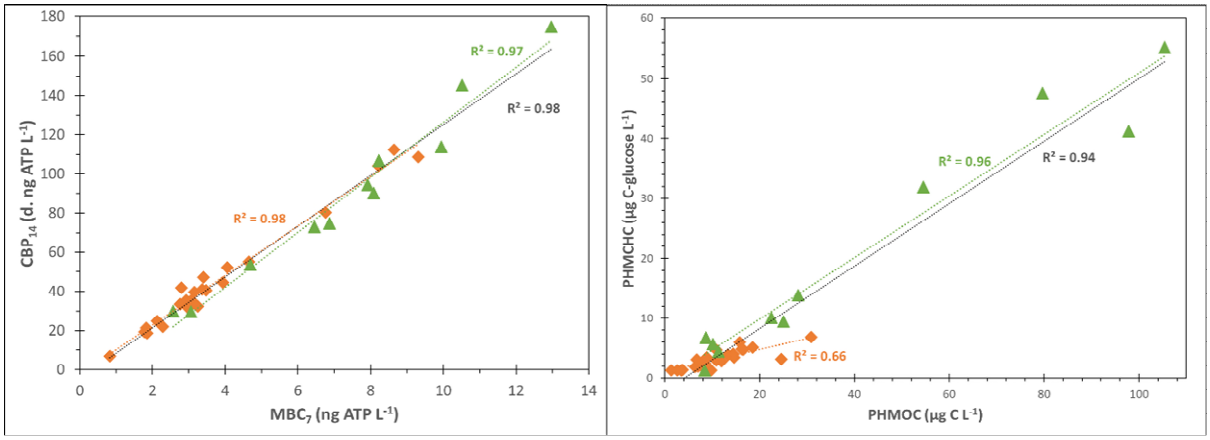

Fig. 3. The correlation between the BPP_{DW} parameters MBC_7 and CPB_{14} (A) and the hemoflow parameters PHMOC and PHMCHC (B) in treated water from 34 different treatment plants. Orange diamonds and line: data for treated water produced from groundwater; green triangles and line: data for treated water produced from surface water; black line: data for all treated water.

The pair-wise correlations between the four different (micro)biological parameters in the distributed drinking water were not significant ($p>0.05$) or significant with a very low R^2 value (<0.2)(Table S3). These results imply that each of these (micro)biological parameters determine another aspect of regrowth in the distribution system.

2.3.5 Guidance values for biological stability

Based on the ranking of the regrowth parameters HPC22, *Aeromonas* and/or invertebrate biomass in the distribution system of 34 treatment plants, six different biological stability parameters could be identified that had a relationship with this ranking (Table S4). Of these six biological stability parameters, MBC_7, AOC-A3, BAR and TOC were related to HPC22, MBG_7 was related to *Aeromonas*, and FeAR was related to invertebrate biomass. Furthermore, none of the biological stability parameters related to the ranking of the treatment plants for *Legionella*. For these six biological stability parameters a threshold value was deduced by taking the lowest value measured for drinking water of the treatment plants that showed an exceedance of the chosen standard for the geometric mean of HPC22, the 90-percentile of *Aeromonas* or the median for the invertebrate biomass in the distribution system (Table S9). The same ranking analysis was done for the 23 groundwater treatment plants only, resulting in nine different biological stability parameters measured for treated water that related to values for the microbial regrowth parameter in the distribution system (Table S5). MBC_7, CPB_{14}, AOC-A3, BAR and TOC were related to HPC22, MBC_7, CPB_{14}, TOC and PFe were related to *Aeromonas*, and FeAR, PHMOC and PHMCHC were related to invertebrate biomass. Furthermore, none of the biological stability parameters related to the ranking of *Legionella*. Subsequently, threshold values based on this ranking analysis were determined for these nine biological stability parameters (Table S10). A similar approach of the 11 surface water treatment plants demonstrated also that nine different biological stability parameters in the treated water had a relationship with the regrowth parameters in the distribution system (Table S6). MBC_7, CBP_{14}, MBG_7, AOC-A3, BAR, FeAR, TOC and PHMOC were related to HPC22 and *Aeromonas*, whereas FeAR, TOC and PFe were related to invertebrate biomass. Additionally, none of the biological stability parameters was associated with the *Legionella* ranking. Threshold values for the biological stability parameters that had a relationship with HPC22 could not be deduced since none of the distribution system of these 11 plants showed an exceedance of the geometric HPC22 standard of 20 cfu/mL. For the biological stability parameters that showed a relationship with *Aeromonas* or invertebrate biomass, threshold values based on this ranking analysis were deduced (Table S11).

Threshold values were also derived based on linear regression between biological stability parameters in treated water and microbial regrowth parameters in the distribution system. A significant ($p<0.05$) regression with a $R^2 >$ 0.4 was observed between the TOC concentration in treated water and log transformed HPC22 numbers and

between the log transformed PHMCHC and log transformed *Aeromonas* numbers in the distribution system when all 34 treatment plants were analysed. Based on the linear regression models, threshold values for the TOC and PHMCHC concentration were deduced (Table S9).

An important observation is that other biological stability parameters significantly correlated to the regrowth parameters in drinking water from groundwater than from surface water (Table S7 and S8). For drinking water produced from groundwater more parameters related to the growth potential of drinking water (namely ATP, TOC, MBC_7 and CBP_{14}) than parameters related to biofouling or biofouling and growth potential parameters (only BAR) showed significant correlations with HPC22 and *Aeromonas*. Furthermore, the strongest relationships ($R^2 > 0.4$) were observed for growth potential parameters only (TOC, MBC_7 and CBP_{14}). In contrast, for drinking water produced from surface water more parameters related to the biofouling potential (PHMOC and FeAR) than to the growth potential parameters (MBG_7) correlated significantly to the regrowth parameters and the strength of all three correlations were strong ($R^2 > 0.4$).

A threshold value for the parameters that showed a significant ($p<0.05$) and strong ($R2 > 0.4$) regression with HPC22 or *Aeromonas* were calculated from the linear regression model (Table S10 and S11). None of the biological stability parameters in treated water from groundwater treatment plants showed a significant linear regression with *Legionella* or invertebrate biomass. In contrast, a significant linear relationship could be observed between log transformed invertebrate biomass and TOC, MBC_7 or CBP_{14} in drinking water from surface water. A threshold value for these three biological stability parameters were calculated based on these regression models with the invertebrate biomass (Table S11).

Final guidance values were calculated for biological stability parameters where a threshold value could be calculated with both the ranking and linear regression analysis (Table S9 – S11). This resulted in a guidance value of 4.1 mg C l^{-1} for the TOC concentration, a value of 8.6 ng ATP l^{-1} for the BPP_{DW} parameter MBC_7 and a value of 110 d.ng ATP l^{-1} for the BPP_{DW} parameter CBP_{14} in treated water produced from groundwater (Table 2). For treated water produced from surface water a value of 2.2 mg C l^{-1} for the TOC concentration, 4.5 ng ATP l^{-1} for the MBG_7 concentration, 47 µg C l^{-1} for the PHMOC concentration and a value of 0.34 mg Fe m^{-2} day^{-1} for the CBM parameter FeAR was calculated (Table 2).

Table 2. Guidance values for several biological stability parameters in treated water, under which the geometric year mean of HPC22, the 90-percentile of *Aeromonas* and the median of invertebrate biomass in the distribution system remain below 20 cfu ml^{-1}, 800 cfu 100 ml^{-1} and 150 mg m^{-3}, respectively.

Parameter	Guidance value
Groundwater treatment plants	
TOC (mg C l^{-1})	4.1
MBC_7 (ng ATP l^{-1})	8.6
CBP_{14} (d.ng ATP l^{-1})	110
Surface water treatment plants	
TOC (mg C l^{-1})	2.2
MBG_7 (ng ATP l^{-1})	4.5
PHMOC (µg C l^{-1})	47
FeAR (mg Fe m^{-2} day^{-1})	0.34

The 23 analysed groundwater and 11 analysed surface water plants were displayed in radar plots to visualize treatment plants that exceed the guidance value of one or more of the selected biological stability parameters (Fig. 4). The radar plot for groundwater treatment plants showed that five plants violated the guidance value for at least one of the three predictive biological stability parameters (Fig. 4A). The TOC concentration of the treated water at the three groundwater treatment plants GW-11, GW-7 and GW-10, that violated the guidance value of 4.1 mg C l^{-1}, were 4.3, 4.6 and 5.9 mg C l^{-1}, respectively. Furthermore, the MBC_7 concentrations at two treatment plants (GW-12 and GW-23), that exceeded the guidance value of 8.6 ng ATP l^{-1}, were 8.6 and 9.2 ng ATP l^{-1}, respectively. GW-12 also slightly exceeded the CBP_{14} guidance value of 110 d.ng ATP l^{-1}, with a CBP_{14} value of 112 d.ng ATP l^{-1}. Six of the 11 surface water treatment plants showed exceedance of the guidance value for at least one of the four predictive biological stability parameters (Fig. 4B). Three of these six plants (SW-5, SW-7 and SW-11) exceeded the guidance value of MBG_7, PHMOC and FeAR, one plant the guidance value of MBG_7 and PHMOC (SW-10), one plant the guidance value of TOC and FeAR (SW-6) and the sixth plant exceeded only the guidance value of TOC (SW-9). The TOC concentration at the two plants that exceeded the guidance value of 2.2 mg C l^{-1} were 3.1 and 3.4 mg C l^{-1}, respectively. The MBG_7 concentration that exceeded the guidance value of 4.5 ng ATP l^{-1} at four plants varied between 5.4 and 8.1 ng ATP l^{-1}. The PHMOC concentration that exceeded the guidance value of 47 µg C l^{-1} at four plants varied between 55 and 105 µg C l^{-1}. Finally, the FeAR that exceeded the guidance value of 0.34 mg Fe m^{-2} day^{-1} at four different plants varied between 0.38 and 1.41 mg Fe m^{-2} day^{-1}.

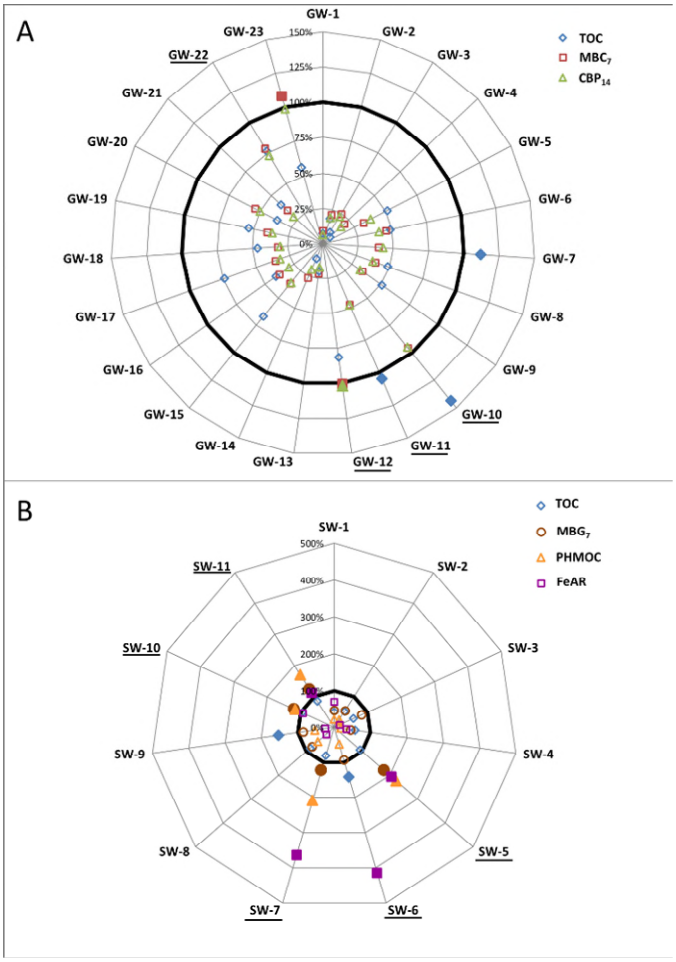

Fig. 4. Radar plots for the selected biological stability parameters in treated water that predict regrowth parameters in the distribution system of groundwater treatment plants (A) and surface water treatment plants (B). The biological stability parameters were expressed relative to the guidance value, which was set at 100% (bold line). The treatment plants that showed a higher geometric mean of 20 cfu ml^{-1} for HPC or a higher 90-percentile of 800 cfu 100 ml^{-1} for *Aeromonas* are underlined.

2.4 Conclusions

The biological stability of unchlorinated drinking water cannot be determined with a sole parameter. Multiple parameters that determine both the growth and biofouling potential of the water are required to reliable describe the biological stability of water.

Unchlorinated drinking water produced from groundwater in the Netherlands has in general a higher biological stability level and lower *Aeromonas* numbers in the distributed drinking water than unchlorinated drinking water produced from surface water.

None of the novel biological stability parameters in the treated water showed a strong relation with HPC22 and/or *Aeromonas* in the distributed water when all treatment plants (groundwater and surface water plants) were analysed.

Growth potential parameters (i.e. TOC, MBC_7, CPB_{14}) dominate the relation with HPC22 and/or *Aeromonas* in drinking water produced from groundwater. In contrast, biofouling potential and growth potential parameters (i.e. MBG_7, PHMOC, FeAR) dominate the relation with HPC22 and/or *Aeromonas* in drinking water produced from surface water.

To remain under 20 cfu mL^{-1} for the yearly geometric mean of HPC22 and under 800 cfu 100 mL^{-1} for the yearly 90-percentile of *Aeromonas* in the distribution systems of treatment plants treating surface water, values for MBG_7, PHMOC and FeAR in treated water should stay below the guidance values of 4.5 ng ATP L^{-1}, 47 µg C L^{-1} and 0.34 mg Fe m^{-2} day^{-1}, respectively.

To remain under 20 cfu mL^{-1} for the yearly geometric mean of HPC22 and under 800 cfu 100 mL^{-1} for the yearly 90-percentile of *Aeromonas* in the distribution systems of treatment plants treating groundwater, values for TOC, MBC_7 and CPB_{14} in treated water should stay below the guidance values of 4.1 mg C L^{-1}, 8.6 ng ATP L^{-1} and 110 d ng ATP L^{-1}, respectively.

Around ten drinking water treatment plants in the Netherlands do not comply to these guidance values for the biological stability parameters inferred in our study. The cause for the non-compliance to the guidance values should be investigated and controlled at these locations, to prevent nuisance growth of the legislative regrowth parameters HPC22 and *Aeromonas*.

The *Legionella* numbers or invertebrate biomass in distributed drinking water did not show clear relations with the novel biological stability parameters determined in treated water at groundwater plants, surface plants or both combined. As a result, no guidance values for these biological stability parameters in relation to *Legionella* or invertebrate biomass could be deduced. However, the TOC concentration in treated water produced from surface water showed a relation with the invertebrate biomass in the distribution system and a guidance value of 2.2 mg C l^{-1} for TOC in treated water from surface water could be deduced. Such a relation between TOC concentration and invertebrate biomass could not be observed for drinking water produced from groundwater.

3 Initiating guidance values for novel biological stability parameters in drinking water to control regrowth in the distribution system

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3.1 Abstract

Nine novel biological stability parameters for drinking water have been developed recently. Here, we report data for these nine parameters in treated water from 34 treatment plants in the Netherlands to deduce guidance values for these parameters. Most parameters did not show a strong correlation with another biological stability parameter in the same sample, demonstrating that most parameters hold different information on the biological stability of drinking water. Furthermore, the novel biological stability parameters in treated water varied considerably between plants and five parameters in treated water were significantly lower for drinking water produced from groundwater than surface water. Two parameters (MBC_7 and CBP_{14}) from the biomass production potential test for drinking water (BPP-W) and the TOC concentration in treated water from groundwater were predictive parameters for HPC22 and *Aeromonas* regrowth in the distribution system. Guidance values of 8.6 ng ATP L^{-1}, 11.0 d.ng ATP L^{-1} and 4.1 mg C L^{-1} could be deduced for these parameters, under which the HPC22 and *Aeromonas* numbers remain at acceptable level. For drinking water from surface water one BPP-W parameter (MBG_7), the particulate and/or high molecular organic carbon and the iron accumulation rate determined with the continuous biofilm monitor were predictive parameters for HPC22 and *Aeromonas* regrowth in the distribution system. Deduced guidance values for these biological stability parameters were 4.5 ng ATP L^{-1}, 47 µg C L^{-1} and 0.34 mg Fe m^{-2} day^{-1}, respectively. We conclude from our study that a multiple parameter assessment is required to reliable describe the biological stability of drinking water, that the biological stability of drinking water produced from groundwater is described with other parameters than the biological stability of drinking water produced from surface water, and that guidance values for predictive biological stability parameters were inferred under which HPC22 and *Aeromonas* regrowth is under control.

Key words: Growth potential; Biofouling potential; biomass production potential test; continuous biofilm monitor; Microbiological water Quality; *Aeromonas*

3.2 Introduction

The growth of (micro)organisms in drinking water systems might adversely affect the distribution of safe and impeccable drinking water. Microbial growth can lead to public health issues when opportunistic pathogens, such as *Legionella pneumophila*, *Pseudomonas aeruginosa* and pathogenic nontuberculous mycobacteria multiply in the water system (Falkinham et al. 2015, van der Wielen et al. 2014). Growth can also lead to aesthetic problems and consumer complaints, such as taste and odour issues or growth of invertebrates visible to the naked eye (Christensen et al. 2011, Hambsch et al. 2014, van Lieverloo et al. 2012). Finally, growth in drinking water systems might also result in technical complaints, for instance microbial induced corrosion or clogging of water lines or water meters due to excessive biofilm, sediment, or invertebrates (Camper 2014, van der Kooij and van der Wielen 2014).

To prevent such problems, drinking water companies aim to produce and distribute biological stable drinking water. Denmark, the Netherlands, and parts of Belgium, Germany and Switzerland distribute drinking water without a disinfectant residual and produce biological stable drinking water by limiting the nutrient concentration. The drinking water companies in the Netherlands have recently established a working definition for biological stability that they try to establish in their drinking water systems: “Biological stability describes a drinking water system from service reservoir to the tap that leads to as little as possible biological changes so that public health risks and/or consumer complaints related to growth of (micro)organisms do not occur” (Hijnen and van der Wielen 2017).

To be able to comply to this working definition, predictive parameters must be available to monitor the biological stability of drinking water, and the impact of pipe materials and sediment on microbial growth in drinking water systems. In the 1980s/1990s, methods were developed to describe the biological stability of drinking water with

two predictive parameters: the concentration of easily assimilable organic carbon using strain P17 and NOX (AOC-P17/NOX) and the biofilm formation rate (BFR) of drinking water (van der Kooij 1992, van der Kooij et al. 1999, van der Kooij et al. 1982). In the Netherlands, an AOC-P17/NOX guideline value of 10 µg C L^{-1} was deduced based on the relation between AOC-P17/NOX of the treated water and heterotrophic plate counts at 22°C (HPC22) in the distributed drinking water. In addition, a BFR guideline value of 10 pg ATP cm^{-2} day^{-1} was inferred between BFR of the treated water and *Aeromonas* plate counts in the distributed drinking water produced from groundwater (van der Kooij et al. 1999, van der Kooij and Veenendaal 2014). Both HPC22 and *Aeromonas* are indicators for regrowth in the drinking water distribution system and included as legislative parameters in the Dutch drinking water decree (Anonymous 2012). The last two decades, however, it was observed that *Aeromonas* plate counts in the distribution system of several treatment plants in the Netherlands violated the legislative standard, although the treated water at the plant had an AOC-P17/NOX concentration and BFR value under these guideline values (van der Wielen 2017). These findings indicate that the combination of AOC-P17/NOX and BFR are not always adequate to describe the biological stability of drinking water and do not always predict problematic regrowth in relation to legislative microbiological indicator parameters for regrowth.

The biological stability of drinking water is influenced by the concentration of biodegradable nutrients in the water, that determines the growth potential, and by the undesirable accumulation of biotic matter on a surface that determines the biofouling potential (Bachmann and Edyvean 2005, van der Kooij and Veenendaal 2014). Recently, our group developed methods to determine a suite of novel biological stability parameters in drinking water systems: the biomass production potential test for water (BPP-W), assimilable organic carbon of biopolymers determined with strain A3 (AOC-A3), the continuous biofilm monitor (CBM) and a crossflow ultrafiltration method to concentrate and quantify particulate and/or high molecular organic carbon (PHMOC)(Hijnen et al. 2018, Sack et al. 2010, 2011, van der Kooij et al. 2015, van der Kooij and Veenendaal 2014). Although these methods have helped to identify components that are responsible for the lower biological stability of drinking water at specific production locations (Hijnen et al. 2018, Schurer et al. 2022, van der Kooij et al. 2015), it remains unknown whether the whole set of parameters must be determined nationwide and to what guidance values these new biological stability parameters must comply to prevent regrowth problems in the drinking water distribution systems. Therefore, the objectives of our study are to (i) measure the biological stability of treated water with the novel parameters for a wide range of treatment plants, (ii) determine the relation between the different novel biological stability parameters in unchlorinated drinking water produced from different treatment plants, (iii) elucidate which novel biological stability parameter(s) in drinking water is/are related to legal indicators for nuisance regrowth in the distribution system in the Netherlands (HPC22 and *Aeromonas*) and (iv) deduce guidance values for these novel biological stability parameters at which these regrowth indicators are under control in the distribution system.

3.3 Materials and Methods

3.3.1 Drinking water treatment plants and sampling

From 2010 till 2017, the treated water from 34 different drinking water treatment plants was sampled three to six times during a three-month period. These treatment plants were chosen because they range from low to high AOC-P17/NOX concentrations and BFR values in the treated water, and from low to high HPC22 and *Aeromonas* numbers in the distributed drinking water obtained during routine monitoring programmes of the drinking water companies. Nine of these treatment plants used surface water as source for drinking water production, 23 plants used groundwater and two plants used both surface water and groundwater. The water source used, treatment train applied and the year of sampling for each treatment plant is given in Table S1. Plants that used surface water were all sampled in the same season (June till October), whereas plants that solely used groundwater were not sampled in a specific season. Furthermore, heterotrophic plate counts at 22°C (HPC22) and *Aeromonas* in drinking water from the corresponding distribution system were determined during the annual legislative routine monitoring program of the drinking water companies.

3.3.2 Predictive biological stability parameters

The methods used to measure the different predictive biological stability parameters are described in detail in the supporting information. Here, a short description of each method will be given.

3.3.2.1 Growth potential tests

MBC_7, MBG_7, CBP_{14} using the BPP-W test

The BPP-W test used in our study was a slightly altered method of the initial BPP-W test published (van der Kooij and Veenendaal 2014). In short, treated drinking water samples (600 mL) were collected in duplicate in AOC-free flasks to which phosphate and nitrate were added. One mL of a sodium sulphite solution (0.19 M Na_2SO_3) and an inoculum was added to the drinking water when the treated water samples came from treatment plants where filtrate disinfection with chlorine dioxide or RO filtration was used as last treatment step. Flasks were incubated in the dark at 25 ± 1°C for 14 days. In time, subsamples were taken from each bottle and analysed for the ATP concentration. Three parameters were deduced from the obtained ATP concentrations: (i) the maximal biomass concentration during day 1 to day 7 of incubation (MBC_7), (ii) the maximum biomass growth during day 1 to day 7 of incubation (MBG_7) and (iii) the cumulative biomass production during 14 days of incubation (CBP_{14}).

AOC-A3

The AOC-A3 concentration was determined using *Flavobacterium johnsoniae* strain A3 (Sack et al. 2010, 2011). In short, treated drinking water samples (600 mL) were taken in duplicate in AOC-free flasks. Nitrate, phosphate, and sodium sulphite were added to the samples in the same manner as described for the BPP-W test. Samples were pasteurized for 30 minutes at 60°C, after which *F. johnsoniae* strain A3 and *Pseudomonas fluorescens* strain P17 were added (starting concentration of approximately 100 cfu mL^{-1}). All flasks were incubated in the dark at 15°C ± 1°C. Every two to three days a subsample was taken from each flask and colony counts of strain A3 were determined. This maximum colony count and previously determined yield factors (1.43×10^7 CFU μg^{-1} when the N_{max} of strain A3 is ≤ 1.5×10^5 cfu mL^{-1} and 0.98×10^7 cfu μg^{-1} when the N_{max} of strain A3 is > 1.5×10^5 cfu mL^{-1}; Sack et al. 2011) were used to calculate the AOC-A3 concentrations.

3.3.2.2 Growth and biofouling potential tests

BAR, FeAR using the CBM

The biomass accumulation rate (BAR) and the iron accumulation rate (FeAR) were determined using a continuous biofilm monitor (CBM)(van der Kooij and Veenendaal 2014). The CBM was connected for three months to the treated water of each investigated treatment plant, resulting in a continuous flow of 10 L h^{-1} through each of four columns containing a glass cylinder with glass beads (Ø 2 mm). Every two weeks the glass cylinders of two columns were replaced with new ones and the ATP and iron concentration in the biofilm was determined and used to calculate the BAR and FeAR.

PHMOC, PHMCHC, PFe using concentration by crossflow ultrafiltration

The particulate and/or high molecular organic carbon (PHMOC), particulate and/or high molecular carbohydrates carbon (PHMCHC) and the particulate iron (PFe) concentrations were obtained using crossflow ultrafiltration. 100 L of the treated water at each plant was concentrated to approximately 500 ml. The concentrate was subsequently analysed for the TOC, carbohydrate, and iron concentration. The PHMOC, PHMCHC and PFe concentrations were thereafter calculated by first correcting for the DOC, dissolved carbohydrate or dissolved iron concentration in the drinking water and second using the concentration factor of the crossflow ultrafiltration step.

3.3.2.3 Sampling moments

The parameters obtained from the BPP-W test, AOC-A3, crossflow ultrafiltration concentrate, and TOC were determined at three successive months and the value for each parameter was averaged from these three sampling rounds. The CBM parameters and ATP concentration were determined at six successive fortnights and the value for each parameter was averaged from these six sampling rounds.

3.3.3 Analytical analyses

3.3.3.1 ATP

The ATP concentrations were determined by measuring the amount of light produced in a luciferin-luciferase assay as previously described (van der Wielen and van der Kooij 2010). Briefly, a nucleotide-releasing buffer (LuminEX, Celsis) was added to the sample to release ATP from the cells. The generated light signal was measured as Relative Light Units (RLU), after a 2 s delay time and a 10 s integration time with a luminometer (Celsis Advance II, Celsis International B.V., Maastricht-Airport, The Netherlands). The concentration of ATP was calculated from the RLU values using a conversion factor determined in calibration measurements. The detection limit of the luminometer is 1 ng ATP L^{-1}.

3.3.3.2 TOC, DOC, and carbohydrates

The TOC concentration was determined by acidifying the samples to a pH between 1 and 2 using 30% HCl. Subsequently, samples and calibration curve standards were measured using a TOC analyser (Shimadzu), in which organic carbon is oxidized to carbon dioxide that is successively measured by an infrared detector. The DOC concentration was measured in a similar matter except that water samples were first filtered over a 0.45 µm membrane. The carbohydrate concentration in the hemoflow concentrate was determined by the phenol–sulfuric acid colorimetric assay using a calibration curve with different glucose concentrations (DuBois et al. 1956).

3.3.3.3 Iron

Samples for iron measurements were acidified to pH < 2,0 using 65% HNO_3 and destructed using a microwave. Subsequently, iron was determined with inductively coupled plasma-mass-spectrometry (ICP-MS) using a calibration curve with different iron concentrations according to NEN-EN-ISO 17294-2.

3.3.3.4 HPC22 and *Aeromonas*

Drinking water samples for HPC22 and *Aeromonas* were taken at different locations in the distribution system and at different time points in the year according to the annual legislative monitoring program of the drinking water companies. These samples were taken at consumers kitchen tap after flushing the water tap till the drinking water temperature is constant for 30 seconds so that the water sample came from the distribution system. HPC22 was determined on glucose-yeast-extract-agar according to NEN-EN-ISO 6222 in which agar plates were incubated at 22°C for 68 ± 4 hours. *Aeromonas* was determined on ampicillin dextrin agar as previously described (Havelaar et al. 1987).

The geometric yearly mean for HPC22 ($HPC22_{gm}$) and the yearly 90 percentile for *Aeromonas* ($Aeromonas_{90P}$) were calculated for each distribution system and for the same year that the biological stability parameters were determined for the treated water of the corresponding treatment plant.

3.3.4 Statistical analyses

Possible outliers were identified by calculating the median, 25 and 75 percentiles of each parameter. Each value for a parameter that were higher or lower than the median value +/- three times the interquartile range was identified and if such an extreme value was unexpected, the value was considered an outlier and not included in the statistical analyses.

Different statistical analyses were applied in our study. First, it was determined whether the different biological stability parameters followed a normal distribution using the Shapiro-Wilk test. The results revealed that ATP and

TOC were normally distributed. The other parameters (MBC_7, MBG_7, CBP_{14}, AOC-A3, BAR, FeAR, PHMOC, PHMCHC, PFe) were subsequently log-transformed and the Shapiro-Wilk test showed that the log transformed data of these parameters were normally distributed.

Differences between treatment plants using groundwater or surface water were statistically tested using the absolute values (ATP, TOC) or log-transformed values (other parameters). Next, it was shown with the Levene's test that all parameters showed equality of variances between groups (groundwater plants versus surface water plants). Consequently, statistical difference of these parameters between groundwater and surface water plants was tested with the independent samples t-test and differences were considered statistically significant at the $p<0.05$ level.

Correlation analysis between all parameters (ATP, TOC, and log-transformed values of the other parameters) were done using Pearson correlation. Correlations were considered significant at the $p<0.05$ level and for those correlations the R^2 value was calculated. In addition, single linear regression analysis was performed to determine whether the microbiological parameters could be predicted from one or more biological stability parameters. Regression results were considered significant at the $p<0.05$ level and the R^2 of the significant regression models were subsequently calculated. All statistical analyses were performed using SPSS 26.

3.3.5 Calculation of guidance values for predictive biological stability parameters

Guidance values for certain biological stability parameters were determined in relation to the legislative regrowth parameters in the Netherlands (HPC22 and *Aeromonas*) in the distribution system. A yearly geometric mean of 20 cfu mL^{-1} for $HPC22_{gm}$, and a yearly 90-percentile of 800 cfu 100 mL^{-1} for $Aeromonas_{90P}$ were used to determine guidance values for the predictive biological stability parameters.

The guidance values for biological stability parameters were determined for the treatment plants that used groundwater or surface water as source. These guidance values were based on threshold values derived from a semi-quantitative and a statistical quantitative analysis. In the semi-quantitative analysis, treatment plants were first ranked from highest to lowest value for each of the two biological parameters $HPC22_{gm}$ and $Aeromonas_{90P}$. It was then established how the top four treatment plants for $HPC22_{gm}$ or $Aeromonas_{90P}$ ranked for each biological stability parameter. A critical biological stability parameter was identified and further investigated when three or four of the top four treatment plants ranked within the top 25% (groundwater treatment plants) or when two to four of the top four treatment plants ranked within the top 30% (surface water treatment plants) of the plants for a certain biological stability parameter. Subsequently, the semiquantitative threshold value for each identified biological stability parameter was determined. This threshold value was the lowest value from the treatment plants that exceeded the 20 cfu mL^{-1} for $HPC22_{gm}$ and/or the 800 cfu 100 mL^{-1} for $Aeromonas_{90p}$. An example of how this semi-quantitative threshold value was determined is given in the supplemental information.

In the statistical quantitative analyses, biological stability parameters that showed a significant ($p<0.05$) regression with $HPC22_{gm}$ or $Aeromonas_{90P}$ and that had a $R^2 \geq 0.4$ were investigated in detail. The formula describing the regression relation was determined and the established value for each of the two microbiological parameters was subsequently used in the formula to derive the statistical quantitative threshold value for the respective biological stability parameter.

A guidance value for a certain biological stability parameter was only calculated when both the semi-quantitative and the quantitative analysis resulted in a threshold value. This guidance value was the average value from the threshold values of the semi-quantitative and statistical quantitative analysis. Finally, threshold values and guidance values for each biological stability parameter were determined separately from the data of drinking water produced from groundwater and from the data of drinking water produced from surface water.

3.4 Results

3.4.1 Biological stability parameters in treated water

Nine novel biological stability parameters and the ATP and TOC concentrations of the treated water from 34 different drinking water treatment plants in the Netherlands were determined and the results are presented in Fig. 1 and Fig. S1 – S4. All three parameters deduced from the BPP-W test showed the lowest values for drinking water produced from groundwater and highest values for drinking water produced from surface water (Fig. 1A & S1). The MBC_7 value ranged between 0.8 ± 0.5 and 13.0 ± 0.04 ng ATP L^{-1}, the MBG_7 value between -0.3 ± 0.4 and 8.0 ± 1.4 ng ATP L^{-1} and the CBP_{14} value between 6.8 ± 4.1 and 174.8 ± 49.6 d.ng ATP L^{-1}. The results from the BPP-W test demonstrates that the microbial growth potential of drinking water in the Netherlands can vary substantially between treatment plants. In general, the AOC-A3 concentrations of the different drinking water types was low with 81% of the treated plants having a concentration below 2.0 µg C L^{-1} in their treated water (Figure 1B). The six highest AOC-A3 concentrations varied between 2.1 ± 0.5 and 11.1 ± 1.4 µg C L^{-1} and were particularly observed for drinking water produced from surface water.

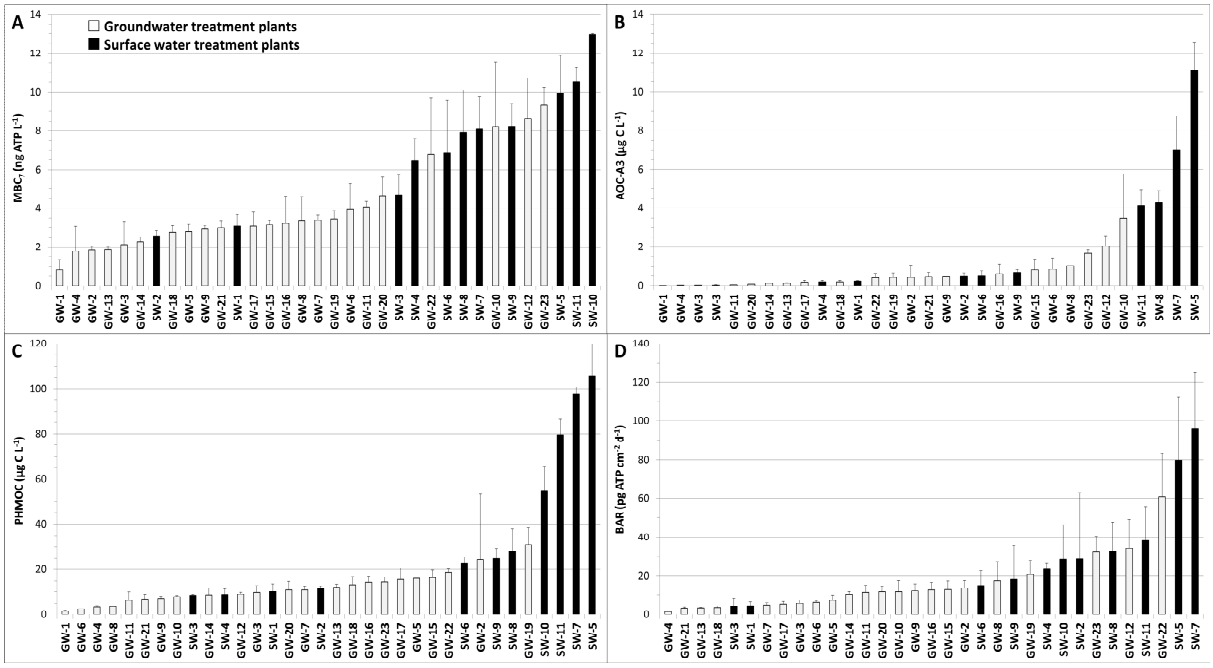

Fig. 1. *The mean MBC_7 values (A), AOC-A3 concentrations (B), PHMOC concentrations (C) and BAR values (D) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.*

A similar observation was made for PHMOC and PHMCHC concentrations in treated water from the different treatment plants, with 82% of the treated water having a PHMOC concentration ≤ 25.0 µg C L^{-1} and a PHMCHC concentration < 10.0 µg C L^{-1} (Fig. 1C and S2). The six highest concentrations ranged between 28.1 ± 9.8 µg C L^{-1} and 105.4 ± 72.2 µg C L^{-1} for PHMOC, and between 10.1 ± 3.8 µg C L^{-1} and 55.2 ± 45.2 µg C L^{-1} for PHMCHC. These highest concentrations were mainly observed for drinking water produced from surface water. The high standard deviation observed for the highest PHMOC and PHMCHC concentrations (plant SW-5) also demonstrates that these concentrations can vary considerably over a period of three months. It should be noted, however, that such high standard deviations were not observed for the other treatment plants with high PHMOC and PHMCHC concentrations (Fig. 1C & S2). The PFe concentrations ranged between 0.1 ± 0.1 and 17.1 ± 10.7 µg Fe L^{-1}, with the highest concentrations generally observed for drinking water produced from groundwater (Fig. S2).

The BAR determined with the CBM varied between 1.4 ± 0.3 to 96.0 ± 28.8 pg ATP cm^{-2} day^{-1}, with lowest values observed for drinking water produced from groundwater and highest values for drinking water produced from surface water (Fig. 1D). The FeAR ranged from 0.03 ± 0.03 to 3.39 ± 2.24 mg Fe m^{-2} day^{-1}, with the lowest and highest values observed for drinking water produced from groundwater (Fig. S3).

The ATP concentrations in treated water varied between 0.7 ± 0.6 and 6.6 ± 1.1 ng ATP L^{-1} and the TOC concentration between 0.3 ± 0.01 and 5.9 ± 0.3 mg C L^{-1} (Fig. S10). The lowest and highest concentrations for both parameters were observed in drinking water produced from groundwater.

3.4.2 Microbiological parameters in distributed drinking water

The routinely measured microbiological parameters in drinking water sampled from the distribution system of 34 treatment plants were analysed as well (Fig. 2). The geometric mean for HPC22 in distributed drinking water of 34 different treatment plants ranged between 0.4 and 32.2 cfu mL^{-1} with the lowest and highest numbers for drinking water produced from groundwater (Fig. 2A). These values are all within the legislative standard of HPC22 in the Netherlands (i.e. geometric year mean < 100 cfu mL^{-1}). The 90-percentiles of *Aeromonas* in the distributed drinking water of all plants varied between < 0.1 and 3720 cfu 100 mL^{-1} (Fig. 2B) and, thus, can differ considerably between treatment plants. The highest 90-percentiles for *Aeromonas* were observed for distributed drinking water from treatment plants that treat surface water. At five plants the 90-percentile of *Aeromonas* in the distribution system exceeds the maximum legislative *Aeromonas* standard for the Netherlands (i.e. 1000 cfu 100 mL^{-1}), demonstrating that regrowth in the distribution system is not always under control in the Netherlands.

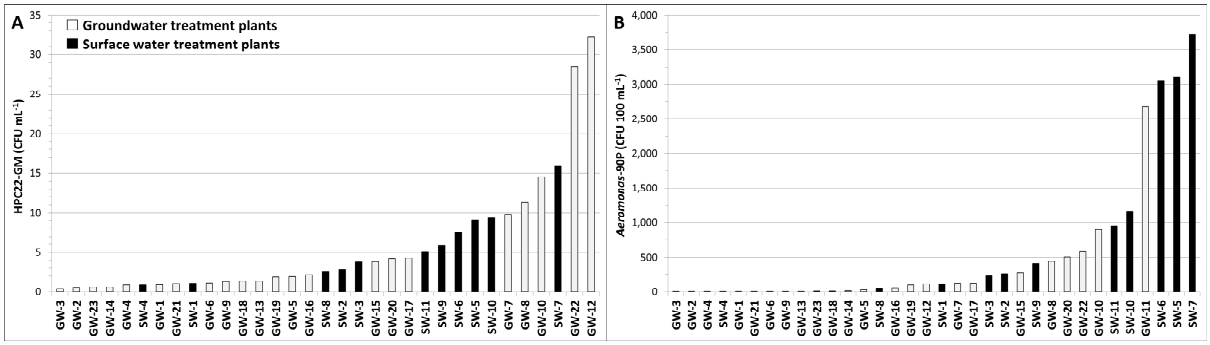

Fig. 2. *The geometric mean of HPC22 (A) and 90-percentile of Aeromonas (B) in drinking water samples from the distribution system of different treatment plants that treat groundwater (open bars) or surface water (closed bars).*

3.4.3 Differences between drinking water produced from groundwater and surface water

It was also investigated for each parameter whether the values from drinking water produced from groundwater were significantly different from drinking water produced from surface water. The results demonstrated that the three BPP-W parameters MBC_7, MBG_7 and CBP_{14} were significantly lower in drinking water produced from groundwater than from surface water ($p<0.05$; Table 1). Furthermore, the BAR values, PHMOC and PHMCHC concentrations and *Aeromonas* counts were also significantly lower in drinking water produced from groundwater than from surface water ($p<0.05$; Table 1). These results indicate that, in general, the biological stability of treated water produced from surface water was lower and that the *Aeromonas* regrowth in distribution systems fed with drinking water from surface water was higher than that of drinking water produced from groundwater. Still, it was noted that some plants that treat groundwater to drinking water have biological stability and *Aeromonas* values that are comparable high or higher than values observed for plants that treat surface water and vice versa (Fig. 1 & 2 and Fig. S1 to S4). This means that care should be taken to generalize these findings to all unchlorinated drinking water types produced from groundwater or surface water.

Table 1. *The statistical outcome of comparing (log-transformed) values of different biological stability parameters in treated water and microbiological parameters in distributed drinking water between drinking water produced from groundwater or surface water using the independent samples t-test. The specific p-value and whether differences were significant at the p<0.05 level are given, as well as whether values for the significant parameters were higher or lower for groundwater treatment plants than those from surface water treatment plants.*

Parameter	p-value	Significant	Groundwater plants
ATP	0.95	No	-
Log MBC_7	1.1×10^{-3}	Yes	Lower
Log MBG_7	5.9×10^{-7}	Yes	Lower
Log CBP_{14}	3.2×10^{-3}	Yes	Lower
Log AOC-A3	0.074	No	-
Log BAR	0.013	Yes	Lower
Log FeAR	0.41	No	-
TOC	0.59	No	-
Log PHMOC	1.1×10^{-3}	Yes	Lower
Log PHMCHC	1.4×10^{-5}	Yes	Lower
Log PFe	0.32	No	-
Log HPC_{gg}	0.15	No	-
Log *Aeromonas*$_{90P}$	0.020	Yes	Lower

3.4.4 The relation among the different biological stability parameters and among the different (micro)biological parameters

We performed a pair-wise correlation analysis on the obtained data to determine whether the eleven different parameters measured holds unique or common features of the biological stability of treated drinking water. The majority of the pairwise correlations (37 out of 55) were not significant ($p>0.05$) or significant with a relatively low R^2 (<0.4) when all treatment plants were included (Table S2). In the latter case this means that two parameters are significantly related, but that less than 40% of the variance in one parameter could be explained by the variance in the other parameter. In addition, 17 other pair-wise correlations between biological stability parameters were also significant, but had a R^2 between 0.4 and 0.9, indicating a moderate relationship. Finally, one pair-wise correlation (MBC_7 – CBP_{14}) showed, besides significance, a R^2 value higher than 0.9, indicating a strong relationship. The significant and strong correlation between MBC_7 and CBP_{14} is visualized in Figure 3A and shows that data points were relatively equally ranged over both axes and that the strong correlation is observed for all data and data solely from drinking water produced from groundwater or surface water.

Similar findings were also observed when pair-wise correlations were determined between the biological stability parameters obtained from treated water of the groundwater treatment plants or surface water treatment plants (Table S2). An important exception was the observation that in contrast to all treatment plants or groundwater treatment plants, the data from the surface water treatment plants showed a significant correlation between PHMOC and PHMCHC concentrations in treated water with a R^2 value ≥ 0.9. This correlation between PHMOC and PHMCHC is visualized for all, groundwater and surface water treatment plants (Fig. 3B) and shows that the high R^2 value for the data from the surface water plants is probably caused by the four surface water treatment plants that showed high PHMOC and PHMCHC values.

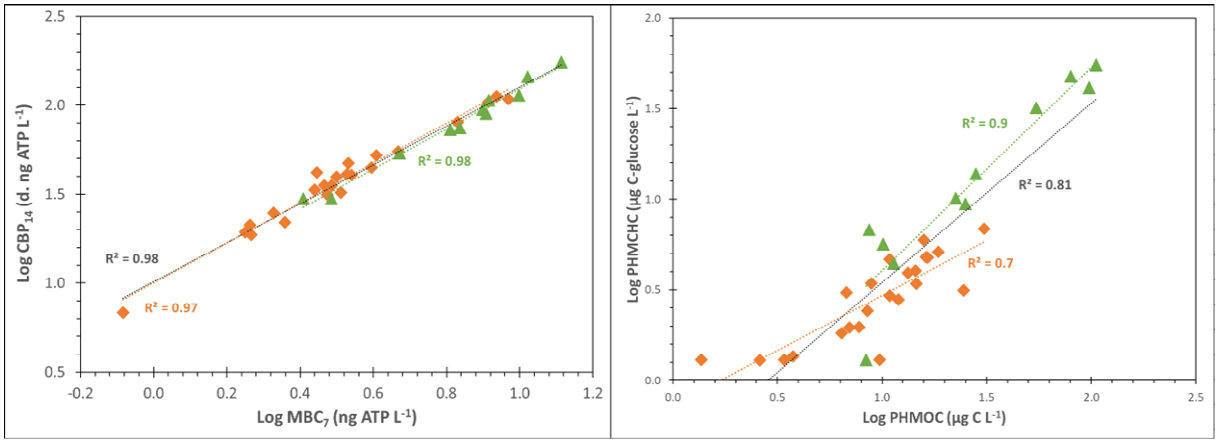

Fig. 3. *The correlation between the log transformed BPP-W parameters MBC_7 and CBP_{14} (A) and the log transformed hemoflow parameters PHMOC and PHMCHC (B) in treated water from 34 different treatment plants. Orange diamonds and line: data for treated water produced from groundwater; green triangles and line: data for treated water produced from surface water; black line: data for all treated water.*

The pair-wise correlations between the two (micro)biological parameters HPC22 and *Aeromonas* in the distributed drinking water were significant with a R^2 value between 0.62 and 0.75 (Table S3). These results imply that part (~30% of the variance) of these (micro)biological parameters determine other aspects of regrowth in the distribution system.

3.4.5 Guidance values for biological stability

Due to the significant differences observed for certain novel biological stability parameters or *Aeromonas* between drinking water produced from groundwater or surface water, separate guidance values were calculated for drinking water produced from groundwater and from surface water. Based on the semiquantitative ranking analysis of the regrowth parameters HPC22 and *Aeromonas* in the distribution system of 23 treatment plants that process groundwater, six different biological stability parameters in treated water showed a relation with values for the microbial regrowth parameter in the distribution system (Table S4). MBC_7, CBP_{14}, AOC-A3, BAR and TOC were related to HPC22, and MBC_7, CBP_{14}, TOC and PFe were related to *Aeromonas*. Subsequently, a threshold value based on this ranking analysis was determined for these six biological stability parameters (Table S8). A similar approach of the 11 surface water treatment plants demonstrated that eight different biological stability parameters in the treated water had a relationship with the regrowth parameters in the distribution system (Table S5). MBC_7, CBP_{14}, MBG_7, AOC-A3, BAR, FeAR, TOC and PHMOC were related to HPC22 and *Aeromonas*. Threshold values for the biological stability parameters that had a relationship with HPC22 could not be deduced since none of the distribution system of these 11 plants showed an exceedance of the geometric HPC22 standard of 20 cfu mL^{-1} (Fig. 2). For the biological stability parameters that showed a relationship with *Aeromonas*, threshold values based on this ranking analysis were deduced (Table S9).

Threshold values were also derived based on linear regression between biological stability parameters in treated water and microbial regrowth parameters in the distribution system. An important observation is that other biological stability parameters showed significant regression with the regrowth parameters in drinking water from groundwater than from surface water (Table S6 and S7). For drinking water produced from groundwater more of these significant parameters related to the growth potential of drinking water (namely ATP, TOC, MBC_7 and CBP_{14}) than parameters related to biofouling or biofouling and growth potential parameters (only BAR). Furthermore, the strongest relationships ($R^2 > 0.4$) were observed for growth potential parameters only (TOC, MBC_7 and CBP_{14}). In contrast, for drinking water produced from surface water more of the significant parameters related to the biofouling potential (PHMOC and FeAR) than to the growth potential parameters (MBG_7) and the strength of all three correlations were strong ($R^2 > 0.4$). A threshold value for the parameters that showed a significant ($p<0.05$)

and strong (R^2 > 0.4) regression with HPC22 or *Aeromonas* were calculated from the linear regression model (Table S8 and S9).

Final guidance values were calculated for biological stability parameters where a threshold value could be calculated with both ranking and linear regression analysis (Table S8 and S9). This resulted in a guidance value of 4.1 mg C L^{-1} for the TOC concentration, a value of 8.6 ng ATP L^{-1} for the BPP-W parameter MBC_7 and a value of 110 d.ng ATP L^{-1} for the BPP-W parameter CBP_{14} in treated water produced from groundwater (Table 2). For treated water produced from surface water a value of 4.5 ng ATP L^{-1} for the MBG_7 concentration, 47 µg C L^{-1} for the PHMOC concentration and a value of 0.34 mg Fe m^{-2} day^{-1} for the CBM parameter FeAR was calculated (Table 2).

Table 2. *Guidance values for several biological stability parameters in treated water, under which the geometric year mean of HPC22 and the 90-percentile of Aeromonas in the distribution system remain below 20 cfu mL^{-1} and 800 cfu 100 mL^{-1}, respectively.*

Parameter	Guidance value
Groundwater treatment plants	
TOC (mg C L^{-1})	4.1
MBC_7 (ng ATP L^{-1})	8.6
CBP_{14} (d.ng ATP L^{-1})	110
Surface water treatment plants	
MBG_7 (ng ATP L^{-1})	4.5
PHMOC (µg C L^{-1})	47
FeAR (mg Fe m^{-2} day^{-1})	0.34

The 23 analysed groundwater and 11 analysed surface water plants were displayed in radar plots to visualize treatment plants that exceed the guidance value of one or more of the selected biological stability parameters (Fig. 4). The radar plot for groundwater treatment plants showed that five plants violated the guidance value for at least one of the three predictive biological stability parameters (Fig. 4A). The TOC concentration of the treated water at the three groundwater treatment plants GW-11, GW-7 and GW-10 that violated the guidance value of 4.1 mg C L^{-1}, were 4.3, 4.6 and 5.9 mg C L^{-1}, respectively. Furthermore, the MBC_7 concentrations at two treatment plants (GW-12 and GW-23) that exceeded the guidance value of 8.6 ng ATP L^{-1}, were 8.6 and 9.2 ng ATP L^{-1}, respectively. GW-12 also slightly exceeded the CBP_{14} guidance value of 110 d.ng ATP L^{-1}, with a CBP_{14} value of 112 d.ng ATP L^{-1}. Five of the 11 surface water treatment plants showed exceedance of the guidance value for at least one of the three predictive biological stability parameters (Fig. 4B). Three of these five plants (SW-5, SW-7 and SW-11) exceeded the guidance value of MBG_7, PHMOC and FeAR, one plant the guidance value of MBG_7 and PHMOC (SW-10), and one plant the guidance value of FeAR (SW-6). The MBG_7 concentration that exceeded the guidance value of 4.5 ng ATP L^{-1} at four plants varied between 5.4 and 8.1 ng ATP L^{-1}. The PHMOC concentration that exceeded the guidance value of 47 µg C L^{-1} at four plants varied between 55 and 105 µg C L^{-1}. Finally, the FeAR that exceeded the guidance value of 0.34 mg Fe m^{-2} day^{-1} at four different plants varied between 0.38 and 1.41 mg Fe m^{-2} day^{-1}.

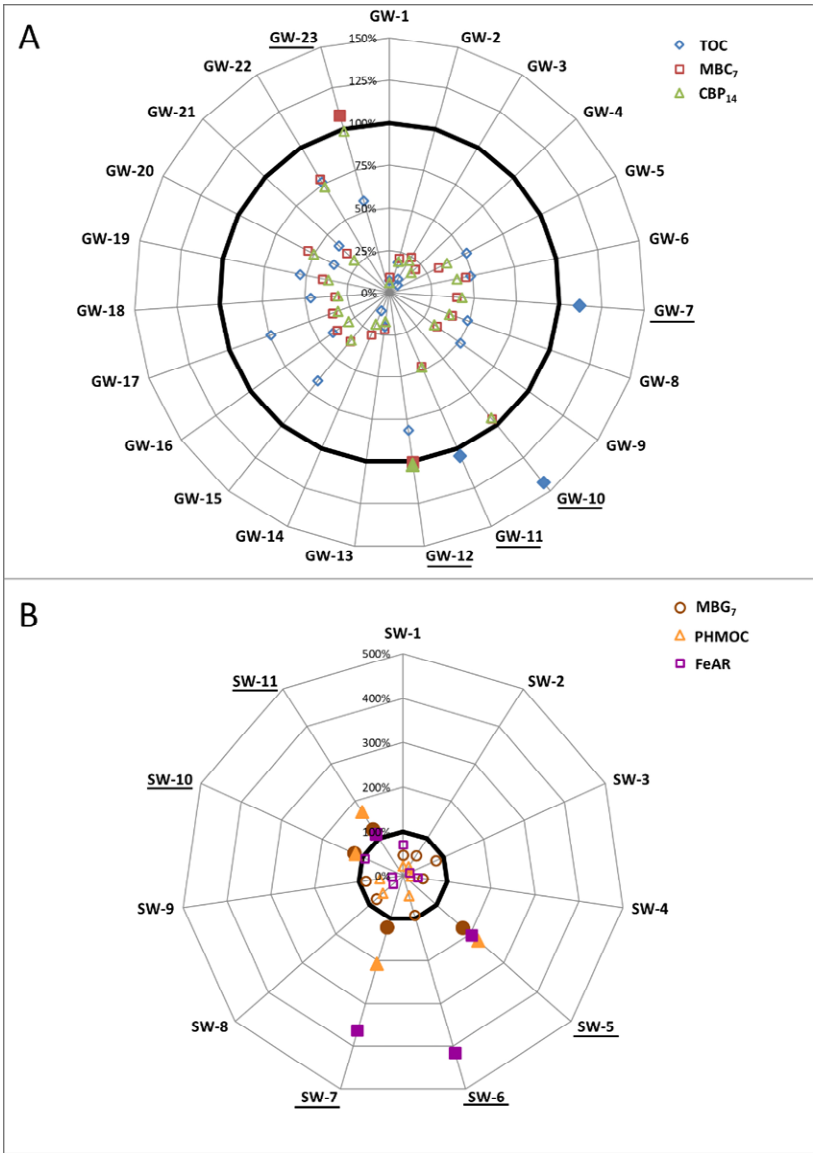

Fig. 4. *Radar plots for the selected biological stability parameters in treated water that predict regrowth parameters in the distribution system of groundwater treatment plants (A) and surface water treatment plants (B). The biological stability parameters were expressed relative to the guidance value, which was set at 100% (bold line). The treatment plants that showed a higher geometric mean of 20 cfu mL^{-1} for HPC or a higher 90-percentile of 800 cfu 100 mL^{-1} for Aeromonas are underlined.*

3.5 Discussion

3.5.1 Growth potential and biofouling potential of drinking water

We have measured nine relatively novel biological stability parameters in drinking water from 34 different treatment plants in the Netherlands, determined possible relationships among these nine parameters and with HPC22 or *Aeromonas*, and deduced guidance values for some of these novel biological stability parameters. The novel parameters MBC_7, MBG_7, CBP_{14} and AOC-A3 are parameters that are indicators for the growth potential of the drinking water, the novel parameters FeAR and PFe are indicators for the biofouling potential of the drinking water and the novel parameters BAR, PHMOC and PHMCHC are indicators for both the growth and biofouling potential.

3.5.1.1 Growth potential of drinking water

The BPP test was one of the methods used to determine the growth potential of the drinking water and three different parameters (MBC_7, MBG_7 and CBP_{14}) were deduced from this test. It was observed that the MBC_7 and

CBP_{14}, both obtained with the BPP-W test, were very strongly correlated. Such a correlation has already been reported for drinking water produced from surface water that had slow sand filtration as final step in treatment (van der Kooij et al. 2017b). Here we show that this strong relationship also applies to unchlorinated drinking water produced from different sources (groundwater and surface water) and from different treatment trains. It, thus, seems that MBC_7 and CBP_{14} hold similar information on the microbial growth potential of drinking water. The strong correlation between both parameters makes it feasible to include only one of these two in the set of parameters to describe the biological stability of drinking water. An argument to use MBC_7 instead of CBP_{14} is that the incubation of the BPP-W test can then be reduced to seven instead of 14 days. An argument to use CBP_{14} is that this parameter is the integrated ATP production during 14 days of incubations and, thus, a measure of total biodegradable matter in drinking water, whereas the MBC_7 is the maximum ATP concentration during the first seven days, and, thus, only a measure for the easily biodegradable matter.

In contrast to the strong correlation between MBC_7 and CBP_{14}, the third parameter obtained from the BPP-W test (MBG_7) was not strongly correlated with the MBC_7 or CBP_{14}. The MBG_7 parameter takes only microbial growth during the first seven days of incubation into account, whereas CBP_{14} include microbial growth and maintenance processes of the microorganisms (Schurer et al. 2022). It has been shown that, especially at low substrate concentrations, maintenance and not growth can be a dominant energy consuming process by microorganisms in drinking water (Schurer et al. 2022, van der Kooij et al. 2017b). The lack of a strong correlation between MBG_7 and MBC_7 or CBP_{14} might indicate that in drinking water with a very low MBG_7 (e.g. < 1.0 ng ATP L^{-1}) maintenance is the dominant microbial process, whereas in drinking water types with higher MBG_7 concentrations growth is the dominant microbial process in the BPP test.

AOC-A3 is another indicator for the growth potential of drinking water, but the AOC-A3 concentration did not correlate strongly with the BPP parameters. The lack of correlation is probably because AOC-A3 is a specific indicator for slowly biodegradable biopolymers (Sack et al. 2010, 2011), whereas the BPP parameters are only partly determined by the biopolymer concentration. Although the BAR, PHMOC and PHMCHC are also influenced by the growth potential of the drinking water, none of these parameters correlated strongly with the BPP parameters or AOC-A3, likely because the BAR, PHMOC and PHMCHC are also influenced by the biofouling potential of the water.

3.5.1.2 Biofouling potential of drinking water

The FeAR and PFe are both indicators for the (bio)fouling potential of drinking water, but these two parameters are not strongly correlated with each other, although both are influenced by iron in the drinking water. The FeAR parameter is determined by the iron adsorbed to the biofilm developed on the glass pearls and the particle-associated iron that is strained by the glass pearls in the CBM. This means that the FeAR is also dependent on the amount of biofilm formed on the glass pearls. The PFe concentration is determined by particle-associated iron concentrated by the crossflow ultrafilter and thus independent from the biofilm formed. This discrepancy between the two parameters might be the cause for the lack of correlation between the two parameters.

The BAR, PHMOC and PHMCHC are also influenced by the biofouling potential of the drinking water, but none of these parameters correlate strongly with the FeAR or PFe, probably because the first three parameters are also influenced by the growth potential of the drinking water.

3.5.2 Biological stability

The traditional AOC-P17/NOX and the BFR of the drinking water types were not analysed. Although their use in the 1980s and 1990s have improved the drinking water quality, the AOC-P17/NOX and BFR methods are very laborious. The original AOC-P17/NOX method requires pasteurization, inoculation with test strains that must be pregrown in minimal medium, undefined incubation time and regular plating and counting of these test strains on agar medium (van der Kooij 2002). Moreover, the BFR method results in only a single BFR value after ~ 100 days of incubation (van der Kooij et al. 2003), and as a result has not been regularly used in drinking water research. In addition,

guideline values already exist for these parameters (van der Kooij and Veenendaal 2014). Current drinking water quality in the Netherlands comply with those guideline values, but *Aeromonas* still exceed the legislative standard in several distribution systems (Anonymous 2020). The predictive biological stability parameters obtained in our study for regrowth of HPC22 and *Aeromonas* in the distribution system, i.e. MBC_7, CBP_{14}, MBG_7, TOC, PHMOC and FeAR, are obtained from methods that are less laborious than the original AOC-P17/NOX and BFR method. We recommend, therefore, to replace the original AOC-P17/NOX and BFR method with the BPP-W, CBM, PHMOC and TOC assays to determine the biological stability of unchlorinated drinking water.

3.5.2.1 Multiple parameters determine biological stability of drinking water

The biological stability parameters applied in our study have been used before to investigate the biological stability of water from different stages in the drinking water treatment train at treatment plants that experience *Aeromonas* regrowth above the legal standard (Hijnen et al. 2018, Schurer et al. 2022, van der Kooij et al. 2015). We extended the use of these parameters to estimate the biological stability of treated unchlorinated drinking water to a wider range of treatment plants (n=34) that vary in the level of regrowth in the distribution system. The results demonstrated that most biological stability parameters did not show a significant or strong correlation ($p<0.05$ and $R^2 > 0.9$) with each other, meaning that almost each parameter holds other information on the biological stability of drinking water in the Netherlands. Consequently, the biological stability of drinking water cannot reliably be determined with a sole parameter, but a suite of methods that measures both the growth and biofouling potential of drinking water needs to be applied. Research groups have developed growth potential tests that are comparable to the BPP-W-test developed in our group (Farhat et al. 2018, Prest et al. 2016, Sousi et al. 2018). It was suggested in several studies that the biological stability of drinking water can be determined by the application of such a sole growth potential test (de Vera and Wert 2019, Farhat et al. 2018, Hou et al. 2022, Nescerecka et al. 2018, Pick et al. 2019, Pick et al. 2021a, Prest et al. 2016, Sousi et al. 2020). Others, however, have already concluded that a low microbial growth potential in drinking water observed with a growth potential test, does not have to be equivalent to biological stability of drinking water (van der Kooij et al. 2017b). Our observation that most biological stability parameters are not strongly correlated confirm this last conclusion and encourage research groups to use a more extensive set of parameters to reliable describe the biological stability of drinking water. The results from our study can help to select the right biological stability assays for that purpose.

As stated before, a similar suite of biological stability parameters was used to determine the biological stability in drinking water produced from surface water after reservoir passage and where high numbers of *Aeromonas* are observed in the distributed drinking water (Hijnen et al. 2018, van der Kooij et al. 2015). The values for the biological stability parameters in drinking water reported for a treatment plant (van der Kooij et al. 2015) were comparable to the values obtained in our study for that same plant. The values for some of the biological stability parameters in drinking water from three different plants obtained by Hijnen et al. (2018), however, were in general higher than values observed in our study. This apparent discrepancy is probably caused by a difference in sampling period. In our study drinking water samples from plants treating surface water were taken from June till October, whereas in the study of Hijnen et al. (2018) samples were taken throughout the whole year. Some of the biological stability parameters showed higher values in the winter than in the warmer seasons (June till October) (Schurer et al. 2022), resulting in higher average values.

3.5.2.2 Drinking water produced from groundwater versus surface water

Our study demonstrated that six of the nine biological stability parameters were significantly lower in drinking water produced from groundwater than from surface water. These six biological stability parameters were used as a measure for easily and/or slowly biodegradable matter and determined both the growth and biofouling potential of drinking water. This indicates that drinking water produced from groundwater has in general a higher biological stability than drinking water produced from surface water, which coincidences with significant lower *Aeromonas* numbers in distributed drinking water produced from groundwater than surface water. Similarly, lower AOC-P17/NOX levels in unchlorinated drinking water produced from groundwater compared to surface water have been

reported in the past (Park et al. 2021, van der Kooij 1992). Although several new methods to determine the regrowth potential of drinking water were introduced during the last decade (Farhat et al. 2018, Prest et al. 2016, Sousi et al. 2018), none of these methods have been utilized to compare drinking water from different sources, making it impossible to determine whether similar findings have been observed in other parts of the world. An important difference between groundwater and surface water is that primary production in groundwater is low compared to surface water because phototrophic organisms do not grow in groundwater. Furthermore, groundwater generally has a long residence time in the underground (up to more than 60 years) before it is abstracted for drinking water production, whereas the retention time of surface water in reservoirs is relatively short (months rather than years) (Hijnen et al. 2018, Visser et al. 2013). The long residence time of groundwater gives microorganisms in the underground ample time to degrade the biodegradable organic fraction, making organic matter much more recalcitrant in groundwater than in surface water. This higher recalcitrant organic matter concentration in groundwater is the likely cause for the in general higher biological stability in drinking water produced from groundwater than from surface water. Still, drinking water produced at some groundwater locations has a lower biological stability and relatively high numbers of *Aeromonas* in the distribution system. The raw groundwater quality at these locations is characterized as aNOXic with high methane, ammonia, iron, and manganese concentrations, which results in relatively high biomass concentrations in the rapid sand filters treating the water and parts of this biomass ends up in the treated water (van der Wielen and van der Kooij 2010, van Lieverloo et al. 2012, Wullings et al. 2011). The biomass in the treated water might subsequently be responsible for the lower biological stability at these locations.

3.5.3 Predictive biological stability parameters for nuisance growth

3.5.3.1 Novel parameters predictive for HPC22 and *Aeromonas* numbers in distribution system

The main purpose to produce biological stable drinking water is to prevent nuisance growth in the distribution system. Therefore, biological stability tools are needed that predict nuisance growth in the distribution system. In the past, it was observed that the traditional AOC-P17/NOX and the biofilm formation of treated water related to regrowth of HPC22, *Aeromonas* and coliforms in the distribution system (LeChevallier et al. 1996, van der Kooij et al. 1999, van der Kooij and Veenendaal 2014). However, reducing AOC-P17/NOX and the biofilm formation rate of treated water did not always result in reduction of *Aeromonas* (van der Wielen 2017), demonstrating that these two parameters are not always reliable predictive parameters for *Aeromonas* regrowth in the distribution system. Moreover, many papers describe different biological stability methods for drinking water, but these studies have not investigated the predictive potential of these methods for nuisance regrowth (de Vera and Wert 2019, Farhat et al. 2018, Favere et al. 2021, Hammes and Egli 2005, Nescerecka et al. 2018, Pick et al. 2019, Pick et al. 2021a, Prest et al. 2016, Servais et al. 1987, Sousi et al. 2020, Weinrich et al. 2011). Consequently, the predicted value of such methods in relation to nuisance growth in the distribution system is unknown. Here, we specifically investigated whether parameters of the biological stability methods developed in our group can be used to predict HPC22 and *Aeromonas* in the distribution system. The significant difference for many biological stability parameters between drinking water produced from groundwater and surface water, made us decide to determine possible relationships between the biological stability parameters in the treated water and the regrowth parameters in the distribution system separately for drinking water produced from groundwater and from surface water. The results from these analyses demonstrated that growth potential parameters (TOC, MBC_7, CBP_{14}) seem to dominate the correlations with HPC22 and *Aeromonas* for drinking water produced from groundwater. In contrast, biofouling potential parameters (PHMOC, FeAR) seem to dominate the correlations with the microbiological parameters for drinking water produced from surface water, although PHMOC also holds information on the growth potential and one growth potential parameter (MBG_7) showed a significant and strong relation with HPC22 and *Aeromonas* as well.

Biofouling of drinking water distribution systems, defined as undesirable accumulation of biotic matter on surface in the distribution system (Bachmann and Edyvean 2005), has been identified as an important factor in regrowth and drinking water quality (reviewed in Bachmann and Edyvean 2005, Cowle et al. 2014). The

importance of biofouling on regrowth in drinking water systems has thus been acknowledged, but methods to determine the biofouling potential of drinking water have only been used sporadically. Mostly, monitoring devices were developed that focus on biofilm formation (Boe-Hansen et al. 2003, Carter et al. 2000, Deines et al. 2010, Delahaye et al. 2006, Donlan et al. 1994, Juhna et al. 2007, Keinanen-Toivola et al. 2006, Pick et al. 2021b, van der Kooij et al. 1995, van der Kooij et al. 2003). Furthermore, these devices are often less suited for routine monitoring and provide only a single or few values over a long monitoring period. In addition, studies have shown the importance of metals (e.g. iron, manganese) on biofouling properties in drinking water distribution systems (Ginige et al. 2011, Liu et al. 2017, Sly et al. 1990). Based on these observations, we developed parameters to monitor the biofouling potential of drinking water. The CBM includes the BAR (consisting of biomass accumulation due to biofilm formation and retaining biomass) and FeAR (consisting of iron accumulation due to adsorption and retaining iron) that can be routinely measured biweekly for an endless time (van der Kooij et al. 2015). Furthermore, the PHMOC, PHMCHC and PFe contain organic carbon and iron attached to particles and high molecular weight organic carbon (Schurer et al. 2022). Recently, it was shown that also the PHMOC fraction in drinking water can contribute significantly as biofouling factor to regrowth in the drinking water distribution system (Hijnen et al. 2018, Schurer et al. 2022). In addition, loose deposits in drinking water systems are an important factor in biofouling of the drinking water distribution system (Bachmann and Edyvean 2005) and *Aeromonas* occurs mainly in the loose deposits (Liu et al. 2017, van der Wielen and Lut 2016). Because *Aeromona*s correlated well with some biofouling potential parameters for drinking water (i.e. FeAR, PHMOC and PHMCHC) produced from surface water in our study, these biofouling potential parameters might be suitable indicators for biofouling in distribution systems where drinking water produced from surface water is transported. The absence of relationships between these biofouling potential parameters and HPC22 and *Aeromonas* at plants that produce drinking water from groundwater is, however, surprising as this drinking water type can also contain relatively high iron concentrations (Liu et al. 2017, Makris et al. 2014, Vreeburg and Boxall 2007) and have the highest PFe concentrations and FeAR values in our study.

It was observed that more biological stability parameters in treated water correlated to *Aeromonas* than HPC22 numbers in the distributed drinking water. Regrowth of *Aeromonas*, thus, seems better related to the biological stability of drinking water. This suggests that *Aeromonas* might be a stricter regrowth indicator than HPC22 in unchlorinated drinking water in the Netherlands as has been concluded before (van der Kooij et al. 2015). Concomitant, more guidance values for the biological stability parameters were directly related to *Aeromonas* than to HPC22. As far as we are aware, the Netherlands is the only country that has both HPC22 and *Aeromonas* as regrowth indicators in the Drinking Water Decree, other countries have mainly HPC22 as regrowth indicator in the drinking water legislation. Since *Aeromonas* seems to be a stricter regrowth parameter than HPC22, drinking water in the Netherlands requires a higher biological stability level compared to other countries. Such a higher biological stability level for drinking water in the Netherlands is also necessary, since drinking water in the Netherlands is distributed without a disinfectant residual. Most other countries rely on a disinfectant residual in drinking water rather than a high biological stability of drinking water to control regrowth in the distribution system (van der Kooij 2003). Still, some countries besides the Netherlands also supply drinking water without a disinfectant residual, but they do not have *Aeromonas* in their legislation and, consequently, could accept a lower biological stability of their drinking water than the Netherlands. It is, therefore, recommended to investigate whether the biological stability of drinking water in those countries is lower than in the Netherlands and, if so, whether nuisance growth in the distribution system occurs more regularly than in the Netherlands.

Others have claimed that ATP concentrations, cell numbers or bacterial community composition in treated and distributed drinking water predict the biological stability of drinking water or nuisance growth, but data supporting that claim is missing in those studies (Favere et al. 2021, Hammes et al. 2010, Lautenschlager et al. 2013). Here, we demonstrate that the ATP concentration in treated water did not show any relation with HPC22 or *Aeromonas* numbers in the distributed drinking water. This was not surprising as it was shown that ATP, cell numbers or community composition in drinking water are poor indicators for regrowth and, therefore, did not show a relation with HPC22 or *Aeromonas* in distribution systems (Roeselers et al. 2015, van der Wielen et al. 2016). Furthermore,

it has also been shown that parameters like ATP and cell numbers do not relate to numbers of opportunistic pathogens in unchlorinated drinking water systems (van der Wielen and van der Kooij 2013, van der Wielen et al. 2016, van der Wielen and Lut 2016), demonstrating that these general microbiological parameters are not only poor indicators for *Aeromonas* and HPC22 regrowth, but also for regrowth of opportunistic pathogens in drinking water in the Netherlands. Similarly, HPC22 and *Aeromonas* were poor indicators for opportunistic pathogens in unchlorinated drinking water as well (van der Wielen and Lut 2016), demonstrating that the guidance values for biological stability parameters deduced in our study do not automatically apply to prevent growth of opportunistic pathogens in drinking water systems. Others showed that the traditional AOC-P17/NOX guideline of 10 µg C L^{-1} does not prevent growth of *Legionella pneumophila* in drinking water systems and that a ten times stricter guideline value of 1 µg C L^{-1} was necessary (van der Kooij et al. 2017a). It is not unlikely, that separate and possible more strict guidance values are also required for the biological stability parameters investigated in our study to prevent growth of opportunistic pathogens like *L. pneumophila* in unchlorinated drinking water systems.

3.5.3.2 Exceedance of the guidance values

Nine of the 34 treatment plants (26%) investigated exceeded the guidance values for the inferred predictive biological stability parameters. The treatment plants included in our study ranged from low to high traditional AOC-P17/NOX concentrations and BFR values in treated drinking water, and from low to high HPC22 and *Aeromonas* numbers in the distributed drinking water. Almost all plants with high traditional AOC-P17/NOX concentrations, BFR values, and/or HPC22 and *Aeromonas* numbers were included in our study. It is, therefore, reasonable to assume that in total only around 10 treatment plants in the Netherlands exceed these guidance values, demonstrating that the biological stability of unchlorinated drinking water in the Netherlands is in general high. It is recommended to identify and eradicate possible causes in treatment or distribution responsible for the lower biological stability at the locations that exceed these established guidance values for the predictive biological stability parameters. The whole range of biological stability parameters described in our study seems to be especially suited to identify such causes and to monitor how well possible solutions work to eradicate these causes. At some plants that exceed the guidance values such research was already initiated and the results of those studies have been published for plant SW-11 (van der Kooij et al. 2015) and plants SW-5 and SW-7 (Hijnen et al. 2018, Schurer et al. 2022). These previous studies suggested that only PHMOC and AOC-A3 concentrations in treated water were predictive parameters for *Aeromonas* in the distribution system. Our study also showed that PHMOC was related to *Aeromonas*, but AOC-A3 was not. It is likely that AOC-A3 was predictive for *Aeromonas* regrowth in the distribution system of these specific treatment plants, but that this relationship does not hold when all drinking water types produced from surface water in the Netherlands are taken into consideration. The results from our study showed that when a wider range of surface water plants were included, MBG_7 and FeAR were also predictive parameters for HPC22 and *Aeromonas* regrowth. These observations, thus, show that besides the nationwide guidance values established in our study, more treatment plant specific guidance values can be established that might help improve the biological stability of drinking water at a single plant (Hijnen et al. 2018, Schurer et al. 2022, van der Kooij et al. 2015).

3.5.3.3 Drinking water with a disinfectant residual

The original AOC-P17/NOX test was also used to determine the biological stability in chlorinated drinking water by quenching free chlorine using thiosulphate before the AOC strains P17 and NOX were added (Kaplan et al. 1993, LeChevallier et al. 1993, van der Kooij 2002). Previous research showed that the BPP-W and AOC-A3 test can also be applied to chlorinated drinking water when free chlorine is quenched using sulphite or thiosulfate (de Vera and Wert 2019, van der Kooij et al. 2015). In addition, TOC and PHMOC measurements are not inhibited by a disinfectant residual in drinking water, making these four assays also suitable for drinking water with a disinfectant residual. Whether the BAR and FeAR of chlorinated drinking water can be determined using the CBM has still to be established as biofilm formation can be inhibited by a disinfectant residual. Furthermore, additional experiments are required to determine guidance values for these novel parameters in drinking water with a disinfectant residual, because in chlorinated drinking water systems regrowth is mainly controlled by the disinfectant residual.

Moreover, certain drinking water companies applying secondary infection are investigating ways to decrease disinfectant use because of sustainability goals, consumer complaints and prevention of by product formation. The novel biological stability methods presented in our study seem to be very well suited to investigate to what extend (i) secondary disinfection can be reduced and (ii) treatment processes reduce biodegradable matter in treated water. Consequently, it seems worthwhile studying these novel biological stability methods also in chlorinated drinking water systems and determine guidance values for chlorinated drinking water in relation to nuisance growth.

3.6 Conclusions

- The biological stability of unchlorinated drinking water cannot be determined with a sole parameter. Multiple parameters that determine both the growth and biofouling potential of the water are required to reliable describe the biological stability of water.

- Unchlorinated drinking water produced from groundwater in the Netherlands has in general a higher biological stability level and lower *Aeromonas* numbers in the distributed drinking water than unchlorinated drinking water produced from surface water.

- Growth potential parameters (i.e. TOC, MBC_7, CBP_{14}) dominate the relation with HPC22 and/or *Aeromonas* in drinking water produced from groundwater. In contrast, biofouling potential and growth potential parameters (i.e. MBG_7, PHMOC, FeAR) dominate the relation with HPC22 and/or *Aeromonas* in drinking water produced from surface water.

- To remain under 20 cfu mL^{-1} for the yearly geometric mean of HPC22 and under 800 cfu 100 mL^{-1} for the yearly 90-percentile of *Aeromonas* in the distribution systems of treatment plants treating surface water, values for MBG_7, PHMOC and FeAR in treated water should stay below the guidance values of 4.5 ng ATP L^{-1}, 47 µg C L^{-1} and 0.34 mg Fe m^{-2} day^{-1}, respectively.

- To remain under 20 cfu mL^{-1} for the yearly geometric mean of HPC22 and under 800 cfu 100 mL^{-1} for the yearly 90-percentile of *Aeromonas* in the distribution systems of treatment plants treating groundwater, values for TOC, MBC_7 and CBP_{14} in treated water should stay below the guidance values of 4.1 mg C L^{-1}, 8.6 ng ATP L^{-1} and 110 d ng ATP L^{-1}, respectively.

- Around ten drinking water treatment plants in the Netherlands do not comply to these guidance values for the biological stability parameters inferred in our study. The cause for the non-compliance to the guidance values should be investigated and controlled at these locations, to prevent nuisance growth of the legislative regrowth parameters HPC22 and *Aeromonas*.

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Declaration of competing interest

The authors declare no competing financial interests.

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4 Workshop: Biologische-stabiliteitsparameters in de praktijk – 03-10-2022

4.1 Uitnodiging en programma

ACHTERGROND

Het produceren van biologisch stabiel drinkwater is voor de Nederlandse drinkwaterbedrijven een voorwaarde om drinkwater zonder desinfectieresidu te distribueren naar hun klanten. Binnen KWR is decennialang BTO-onderzoek naar biologische stabiliteit uitgevoerd, waarbij ook allerlei biologische stabiliteitsmethoden zijn ontwikkeld. Door deze biologische stabiliteitsparameters van het reinwater te bepalen, bijvoorbeeld na aanpassing van de drinkwaterzuivering, kan worden voorspeld wat het verwachte effect is van het distribueren van dit reinwater op de twee wettelijke parameters voor nagroei: koloniegetal bij 22°C (KG22) en *Aeromonas*. Oorspronkelijk zijn de biologische stabiliteitsparameters AOC-P17/Nox en BVS hiervoor ontwikkeld. Deze twee methoden bleken echter niet in staat om de overschrijding van de Nederlandse *Aeromonas*-norm op alle locaties te voorspellen. Sinds 2010 zijn daarom nieuwe voorspellende biologische stabiliteitsmethoden ontwikkeld (BPP-test, AOC-A3, CBM en PHMOC-bepaling na hemoflow) en is een groot landelijk onderzoek uitgevoerd op 37 productielocaties (23 grondwater en 14 oppervlaktewater) die een variërende mate van nagroei hebben in het distributiesysteem. De resultaten van die studie zijn gebruikt om voor het reinwater aandachtswaarden voor deze nieuwe methoden af te leiden, die voorspellend blijken te zijn voor hoge aantallen van KG22 en *Aeromonas* in het distributiesysteem (van der Wielen, 2018). Daarbij bleek dat voor drinkwater bereid uit oppervlaktewater andere aandachtswaarden voorspellend waren dan voor drinkwater bereid uit grondwater. Toepassing van deze nieuwe biologische stabiliteitsparameters en hun aandachtswaarden in de dagelijkse drinkwaterpraktijk lijkt echter nog beperkt en de indruk is dat ze minder vaak worden gebruikt dan de oude biologische stabiliteitsparameter AOC P17/NOX in het verleden.

Binnen het BTO-bedrijfsonderzoek van Evides en Oasen wordt een workshop op maandag 3 oktober 2022 georganiseerd op locatie Berenplaat van Evides (Berenplaat 10, Spijkenisse). Tijdens de workshop worden de nieuwe biologische stabiliteitsparameters en hun aandachtswaarden toegelicht, wordt de toepassing van deze en/of andere methoden die de biologische stabiliteit van drinkwater bepalen bij verschillende drinkwaterbedrijven gepresenteerd en wordt de implementatie van sommige methoden bij een drinkwaterlaboratorium besproken. Daarnaast is een discussiesessie ingeroosterd waar op basis van stellingen wordt gediscussieerd wat de behoefte van de drinkwaterbedrijven is om de biologische stabiliteit op productielocaties te verbeteren en om biologische stabiliteitsmonitoring uit te voeren, de toepassing van de nieuwe methoden in de praktijk, de wensen ten aanzien van eventuele optimalisatie van de huidige methoden of wensen voor nieuwe methoden en de implementatie van de methoden bij de drinkwaterlaboratoria, waarbij de resultaten kunnen worden gebruikt om toekomstig onderzoek binnen het BTO te definiëren. Tot slot is gekozen dat het Nederlands de voertaal zal zijn tijdens de workshop.

PROGRAMMA

9:30 – 10:00 Inloop met koffie
10:00 – 10:10 Introductie – Wim Hijnen, Evides
10:10 – 10:40 Nieuwe biologische stabiliteitsparameters en aandachtswaarden – Paul van der Wielen, KWR
10:40 – 11:10 Meten van biologische stabiliteit in praktijk van DPW-bedrijven – Eline Stroobach, HWL
11:10 – 11:30 Koffie
11:30 – 12:00 Biologische stabiliteit bij Oasen. Een nieuw proces, nog stabieler? – Maarten Lut, Oasen
12:00 – 13:00 Lunch
13:00 – 13:30 Betekenis van de nieuwe biostabiliteitsmethoden voor het onderzoek naar ongewenste nagroei bij Evides – Wim Hijnen/Rinnert Schurer, Evides
13:30 – 14:00 Biologische stabiliteit bepaling bij PWN: combinatie van laboratorium en on-site metingen – Emmanuelle Prest, PWNT
14:00 – 14:30 Break
14:30 – 16:00 Discussiesessie
16:00 - … Borrel

De workshop zal plaatsvinden op productielocatie Berenplaat van Evides, Berenplaat 10 te Spijkenisse.

4.2 Introductie – Wim Hijnen

Zie bijlage III voor de presentatieslides.

4.3 Nieuwe biologische stabiliteitsparameters en aandachtswaarden – Paul van der Wielen

Zie bijlage IV voor de presentatieslides.

Vragen:

- Worden CBP_{14} bepalingen gedaan ten opzichte van de 0 waarde?
  Antwoord: Ja
- Er is een correlatie tussen CPB_{14} en MBC_7, maar zijn dit wel andere waarden, gaat de lijn door de 0?
  Antwoord: De correlatie laat zien dat deze twee parameters in drinkwater een sterke relatie hebben, het maakt daarbij niet uit of ze door de 0 gaan. Door de sterke correlatie is het misschien niet nodig om beide parameters te bepalen met de BPP test. De correlatie is wel gebaseerd op data verkregen met reinwater en is misschien minder sterk voor ruwwater of water na verschillende zuiveringsstappen.
- Waar ligt het drinkwater geproduceerd uit infiltratiewater in de grafieken?
  Antwoord: Deze zijn weergegeven als oppervlaktewater, waarbij het voornamelijk de oppervlaktewaterlocaties zijn waarvan de biologische stabiliteitswaarden tussen de grondwaterlocaties vallen. Locaties met menging oppervlakte- en grondwater zijn ook als oppervlaktewater weergegeven. Dit is beter gesplitst weergegeven in het rapport.
- Er zijn drie parameters gevonden voor drinkwater bereid uit oppervlaktewater waarvoor een aandachtswaarde kon worden afgeleid, welke van deze drie is het belangrijkste?

Antwoord: Ze zijn eigenlijk allemaal even belangrijk. Er is geprobeerd te achterhalen welke meer invloed zou hebben in een meervoudig regressiemodel, maar hier kwam er niet één sterker naar voren dan de andere.

- Vraag: Is er enig beeld van extrapolatie van deze waarden naar het buitenland?
  Antwoord: Waarschijnlijk redelijk te extrapoleren naar bijvoorbeeld Denemarken en Zwitserland etc. aangezien daar ook geen chloor wordt gedoseerd, maar daar staat *Aeromonas* niet in de wet. Paul is zelf meer benieuwd naar extrapolatie van de nieuwe parameters naar locaties met gechloreerd drinkwater.
- Vraag: Correleren andere groeiproblemen ook met de nieuwe parameters?
  Antwoord: Invertebratenbiomassa in drinkwater bereid uit oppervlaktewater correleerde met TOC. Geen correlatie met *Legionella*, maar voor die parameter te veel nulmetingen om dat betrouwbaar vast te stellen, dit gaat misschien beter als data worden gebruikt van drinkwaterinstallaties in gebouwen. Niet gekeken naar andere opportunistische pathogenen, maar is nog wel een openstaande vraag.
- Vraag: Zijn de nieuwe parameters bepaald in het distributiesysteem?
  Antwoord: Alleen gekeken naar reinwater, hoewel KWR voor PWN en Evides wel in het distributiesysteem heeft gemeten.
- Vraag: Is temperatuur een belangrijke factor?
  Antwoord: Speelt zeker een rol bij drinkwater bereid uit oppervlaktewater. Voor de studie zijn de biologische stabiliteitsparameters in drinkwater bereid uit oppervlaktewater ook alleen bepaald in dezelfde periode van het jaar (mei t/m oktober)

4.4 Meten van biologische stabiliteit in praktijk van DPW-bedrijven – Eline Stroobach

Zie bijlage V voor de presentatieslides.

Vragen:

- Vraag: Bij de entingstudie van de BPP zijn soms alleen KWR of alleen HWL gemeten?
  Antwoord: Ja, sommige condities zijn alleen gedaan door één instituut.
- Vraag: Bij de vergelijking van het aantal analyses dat wordt gedaan, wordt de AOC soms vervangen door de BPP? Worden er minder AOC's gedaan?
  Antwoord: Er wordt nog steeds veel AOC P17/NOX gedaan. Dunea doet wel meer BPP-metingen, PWN gebruikt BPP alleen bij projectmatig onderzoek.
- Vraag: In hoeverre mate zouden BPP-testen gedaan moeten worden voor verschillende stappen in de zuivering?
  Antwoord: Niet nodig om routine te doen, maar kan wel nuttig zijn voor onderzoek om het proces in kaart te brengen. Routinematig kan de BPP beter op drinkwater worden gedaan.

4.5 Biologische stabiliteit bij Oasen. Een nieuw proces, nog stabieler? – Maarten Lut

Zie bijlage VI voor de presentatieslides.

Vragen:

- Bevat het RO-permeaat nog sulfaat of nitraat?
  Antwoord: Nee, alleen ammonium en methaan.
- Zou de lage nutriëntconcentratie in RO-water kunnen leiden tot het loslaten van de biofilm op de leidingwand?
  Antwoord: Daar is veel onderzoek naar gedaan en daaruit is gekomen dat het geen probleem zou moeten zijn, maar het zal wel gemonitord worden wanneer fullscale wordt overgeschakeld. Ook is duidelijk dat RO-water een andere bacteriepopulatie bevat dan drinkwater uit een andere zuivering.
- Zal er in de praktijk RO-water gemengd gedistribueerd worden?

Antwoord: RO-water zal ongemengd gedistribueerd worden.
- Is er overwogen om het ontgassingsproces voor het RO-membraan te laten plaatsvinden?
  Antwoord: Ja, het nadeel is dat dan meer fouling op het RO-membraan voorkomt. Het zuiveringsproces zal per locatie bekeken moeten worden, zo zou op bepaalde locaties bijvoorbeeld eerst een voorzuivering met ontgassing en ontijzering moeten plaatsvinden.
- De visie van het proces is om het zo anaeroob mogelijk te doen tot de ontgassing?
  Antwoord: Ja, dit om de groei te limiteren.
- Er waren toch verhoogde KG22 en ATP in het zuiveringsconcept met RO?
- Antwoord: Er was waarschijnlijk toch wat zuurstoflekkage. Zuurstof werd ook wel gemonitord in de proefinstallatie, maar er werden geen maatregelen tegen genomen. De spoeling van het calciet was ook een bron van zuurstof. Daar moet in de fullscale bedrijfsvoering dus op gelet worden.

4.6 Betekenis van de nieuwe biostabiliteitsmethoden voor het onderzoek naar ongewenste nagroei bij Evides – Wim Hijnen/Rinnert Schurer

Zie bijlage VII voor de presentatieslides.

Vragen:
- Waar in het leidingnet zijn de monsters genomen voor de BPP-bepalingen, dichtbij of veraf?
  Antwoord: Weergegeven data zijn het gemiddelde over het leidingnet, er was weinig variatie tussen verschillende afstanden.
- Is de daling van de biofilmvorming in de CBM bij Hoeksche Waard niet het effect van de deeltjesverwijdering door het UF-membraan?
  Antwoord: Gedeeltelijk wel, deze adsorberen niet meer, maar ook door de verbetering van de biologische stabiliteit en een verminderde groei.
- Zijn de weergegeven resultaten van de ijzermetingen in het distributienet van Oud-Beijerland van het net dat wel of niet gereinigd was?
  Antwoord: Gereinigd en niet gereinigd zijn hierbij gecombineerd.
- Is er een idee van de rol van de configuratie van het leidingnet op de biologische stabiliteit en hoe zich dit verhoudt tot Andijk.
  Antwoord: Zowel de configuratie als het verbruikspatroon lijken belangrijk te zijn, maar de precieze rol blijft onduidelijk. De configuratie zou vergeleken kunnen worden met Andijk.
- Aanwezigheid van *Asellus* is de belangrijkste factor in relatie tot *Aeromonas*-overschrijdingen, *Asellus* is ook niet aanwezig in Braakman (waar lagere *Aeromonas*-aantallen zijn). *Asellus* is moeilijk uit het leidingnet te krijgen, maar als er te weinig zijn zou de vermeerdering kunnen stoppen door een te lage kans op ontmoeting. *Asellus*-aantallen zijn wellicht ook gedeeltelijk afhankelijk van het verbruikspatroon, maar dit is nog onduidelijk.
- Is *Asellus* minder vaak aanwezig in het oppervlaktewater in het gebied van Braakman, zodat het leidingnet Braakman niet is geïnoculeerd met Asellus?
  Antwoord: Dit wordt onderzocht bij de WUR, waar ze onderzoeken hoe *Asellus* in het leidingnet komt.
- Wellicht was er wel een effect van het UF-membraan en de reiniging op de biofilmvorming totdat er weer voldoende micro-organismen in het water zaten om biofilmgroei te krijgen of meet de CBM alleen verwijdering van deeltjes die niet meer hechten en niet de groei van de biofilm?
  Antwoord: CBM meet ook biofilmvorming door groei. Membraanfouling van het UF treedt al op bij 1 µg/l acetaat, maar de belangrijkste factor zijn de biopolymeren die achterblijven, wat leidt tot minder biofilmvorming met het permeaat. Er is te veel focus geweest op AOC en niet genoeg op de biopolymeren die wel belangrijk zijn. Als deze biopolymeren geladen zijn kunnen ze hechten aan de biofilm en dan dienen als voedselbron.
- Benieuwd naar het effect van een nog fijner membraan (huidig UF-membraan was 150kDa)
  Antwoord: We gaan een jaar een test doen met een 10kDa membraan.

- Het is nog steeds onbekend wat exact gebeurt in het leidingnet. Komt het ijzer van de zuivering of komt het van roest van afsluiters?
  Antwoord: Dit is misschien te achterhalen met een isotopenonderzoek. Het is belangrijk om te weten zodat de focus op de zuiveringsprocessen eventueel kan worden aangepast.
- Wordt het leidingnet nog een keer gereinigd voordat het 10kDa membraan in gebruik gaat?
  Antwoord: Dat staat momenteel niet gepland, maar daar gaat nog over gesproken worden om misschien wel te reinigen aangezien het wel een effect kan hebben.

4.7 Biologische stabiliteit bepaling bij PWN: combinatie van laboratorium en on-site metingen – Emmanuelle Prest

Zie bijlage VIII voor de presentatieslides.

Vragen:

- Hoeveel water wordt er gebruikt voor de monstername van de zakfilters?
  Antwoord: 30 m^3 per week gaat door de zakfilters. Per locatie zitten er twee achter elkaar.
- Zitten de zakfilters op een vaste locatie?
  Antwoord: Ja, ze zitten op een vaste locatie, maar de exacte plaats is afhankelijk van de meetlocatie
- Wat is de toegevoegde Q in de grafieken van de studie naar groei van nutriëntlimitatie?
  Antwoord: Quenching van het disinfectieresidu
- KWR heeft gekeken naar een link tussen hydraulica en biofilmconcentratie en -samenstelling, maar daar werden geen significante effecten van hydraulica waargenomen. Speelt hydraulica wel een belangrijke rol?
  Antwoord: Er is meer een link met het sediment door de opwerveling van deeltjes
  Discussie: Er zijn echter ook plaatsen met veel sediment, maar weinig microbiologie.
  Antwoord: PWN heeft specifieke plekken met veel sediment, waar ook problemen zijn met de microbiologie
- Hoe is de legionelladata verzameld in de studie van van der Lugt et al.? Zijn specifieke metingen opgezet? Komt het van zorginstellingen?
  Antwoord: Wilco van der Lugt heeft deze data verzameld en bestaat uit routinematige metingen van de gebouwbeheerders die *Legionella* meten in het drinkwater.
- De Watergroep heeft ook veel legionelladata, maar kunnen deze niet linken aan de biologische stabiliteit aangezien biologische stabiliteitsparameters weinig worden gemeten. Is er een correlatie?
  Antwoord: Uit de studie van van der Lugt et al. komt naar voren dat gebieden met veel *Legionella* in drinkwater gebouwen, de gebieden zijn met biologisch minder stabiel water, maar dit is niet expliciet onderzocht.
- Voor het karakteriseren van water zijn bepaalde parameters belangrijk, voor het begrijpen van wat er gebeurd zijn andere analyses belangrijk. Hoe zie je de toekomst voor het onderzoek?
  Antwoord: In het drinkwater zijn veel stoffen aanwezig in een lage concentratie waar geen methodes voor zijn om deze goed te meten. Wij staan open voor nieuwe methoden om deze te kunnen meten.
- De microbiologen hebben in het verleden de chemici uitgedaagd om met methoden te komen die dit zouden kunnen meten. Zo kan met chemische methoden wel microverontreinigingen op ng/l niveau worden gemeten, maar de stoffen gerelateerd aan biologische stabiliteit kunnen niet betrouwbaar worden bepaald. Het is de hoop dat dergelijke chemische methoden wel beschikbaar gaan komen.
  Antwoord: Stoffen gerelateerd aan biologische stabiliteit zijn lastig te meten. Omdat er zoveel verschillende stoffen zijn is het lastig om één methode te ontwikkelen die alles omvangt. Het zou misschien kunnen met hoogresolutiemassaspectrometrie, maar dan moet er ook iets goeds zijn om de resultaten op te valideren, dus de groeiproeven zijn belangrijk in dat proces. Onderzocht moet worden of de chemische veranderingen die optreden tijdens een groeiproef kunnen worden gedetecteerd.

- De aandacht voor het sediment komt goed naar voren. Evides ziet meer sediment in Braakman, maar deze locatie heeft biologisch stabieler water dan de locaties waar minder sediment (maar meer *Asellus*) wordt aangetroffen. De hypothese van Evides is dat *Asellus* het sediment verbruikt, waardoor de sedimentconcentratie afneemt, wat het een complexe situatie maakt.
- Heeft Evides het sediment ook onder de microscoop bekeken voor de samenstelling?
  Antwoord: Soms zien we veel fecale pellets van *Asellus* op locaties waar weinig *Asellus* gevonden wordt en soms is de omgekeerde situatie te zien. Het sediment lijkt zich op bepaalde plekken op te bouwen.

4.8 Discussiesessie

Tijdens de discussiesessie werden een aantal vragen met multiple-choiceantwoorden voorgelegd waar eenieder via de mobiel op kon stemmen. De uitkomst werd vervolgens gedeeld en op basis daarvan getracht een verdere discussie op te bouwen. Op een aantal vragen ontlokte deze aanpak een discussie, maar soms was de respons (en daarmee de discussie) beperkt. In deze paragraaf worden de vragen en de uitkomst van de poll weergegeven, direct per vraag gevolgd door de discussie die het ontlokte.

Vraag 1: Heeft uw bedrijf of klant behoefte om de biologische stabiliteit te verbeteren?

Ja:	23
Nee:	0
Weet ik niet:	1

Discussie:
De Watergroep heeft aangegeven “Weet ik niet”, omdat bij de Watergroep nog onduidelijkheid is over de biologische stabiliteit van het huidige drinkwater, waardoor ook nog onbekend is of er behoefte voor verbetering is.

Vraag 2: Bepaalt uw bedrijf of klant BPP, AOC-A3, CBM of PHMOC?

Ja, routinematig:	7
Ja, onderzoek:	15
Nee:	3

Discussie:
Waternet geeft aan dat de biologische-stabiliteitsparameters een goede onderzoekstool zijn, maar dat zij deze momenteel niet toepassen, omdat er geen onderzoek loopt naar biologische stabiliteit. Waternet gebruikt *Aeromonas* als bedrijfstechnische parameter en heeft in het verleden de zuivering geoptimaliseerd om *Aeromonas*-aantallen in het distributiesysteem zo laag mogelijk te krijgen. De huidige situatie is dat bij lange verblijftijden hoge aantallen *Aeromonas* aangetroffen kunnen worden, maar dat is elk jaar een consistent beeld. Waternet grijpt pas in als er onverwachte verhoging plaatsvindt, maar dat is de laatste jaren niet opgetreden. Daarnaast volgt Waternet wel de laatste inzichten op gebied van biologische stabiliteit en heeft daarbij gezien dat een verschuiving heeft plaatsgevonden van focus op AOC naar focus op biopolymeren, maar deze laatste groep van stoffen wordt door langzame zandfiltratie (dat door Waternet wordt toegepast in de zuivering) goed verwijderd. Waternet bepaalt nog wel AOC P17/Nox als routineparameter. Ze hebben overwogen om over te schakelen op de BPP ipv AOC P17/Nox, maar ze willen hun gegevens graag vergelijken met de oude gegevens.

Evides geeft aan dat hun focus op *Aeromonas* in de toekomst afhangt van de nut of noodzaak van *Aeromonas* als indicatorparameter. Wanneer bijvoorbeeld alle nagroeiproblemen zijn opgelost, maar *Aeromonas* overschrijdt nog steeds de norm, dan is er weinig motivatie om bijvoorbeeld een extra processtap aan de zuivering toe te voegen. Vooralsnog is echter onduidelijk of problemen in de binnenhuisinstallatie optreden als *Aeromonas*-aantallen in het distributiesysteem hoog zijn. Binnen het BTO zou daarom onderzoek gedaan moeten naar *Aeromonas* als kwaliteitsparameter. Evides mat vroeger AOC P17/Nox routinematig, maar gaat binnenkort

overschakelen naar BPP als routineparameter. AOC P17/Nox zal dan alleen nog voor bepaalde (onderzoeks)projecten worden bepaald.

Samenvatting:

- Een belemmering om over te stappen naar de nieuwe parameters is het niet kunnen vergelijken met oude gegevens (trendbreuk).
- Er moet nagedacht worden over *Aeromonas* als kwaliteitsparameter en of *Aeromonas*-aantallen verder verlaagd moeten worden als andere nagroeiproblemen niet plaatsvinden.

Vraag 3: Welke organische stoffracties bepaalt u in water?

PHMOC met hemoflow:	7
LC-OCD:	13
Andere methode:	1
Geen:	3

Vraag 4: Welke groeipotentiebepaling (waterfase) voert u uit?

AOC P17/NOX:	15
AOC-A3:	11
BPP:	18
Geen:	3

Vraag 5: Welke groeipotentiebepaling (op oppervlakken) voert u uit?

BVS met biofilmmonitor:	4
BAS/BVS met CBM:	11
Andere methode:	4
Geen:	7

Discussie:

PWN geeft aan dat ze biofilm direct van de leidingen swabben en daar de ATP-concentratie van bepalen om de biofilmconcentratie op de leidingen te weten.

Vraag 6: Welke belemmering ervaart u bij de implementatie in uw laboratorium?

Te complex binnen routinelab:	12
Te weinig vraag bij klanten:	10
Onvoldoende gevalideerd:	8
Geen:	4

Discussie:

KWR ondervindt uiteraard geen belemmering bij het toepassen van deze methoden, omdat ze door KWR zijn ontwikkeld. Pidpa geeft aan dat de analyses sterk afwijken van andere routineanalyses in het drinkwaterlaboratorium. Het is veel werk om ze te implementeren en zo is er bijvoorbeeld een aparte ruimte nodig om contaminatie van de drinkwatermonsters met vluchtige stoffen zoals ethanol te voorkomen. Aan de hand van dit voorbeeld werd de vraag gesteld of het ook mogelijk is om een simpelere methode te ontwikkelen, die kwalitatief slechter is, maar wel een richting qua resultaat laat zien. Aangegeven wordt dat de LC-OCD bijvoorbeeld een versimpeling is van de biopolymerenbepaling met AOC-A3 of PHMOC. Opgemerkt wordt dat LC-OCD niet gevalideerd is en daardoor geen betrouwbaar alternatief. Andere onderschrijven dat de LC-OCD niet voldoende is gevalideerd, hoewel sommige aangeven dat de LC-OCD data wel een beeld geeft over hoe de biopolymerenconcentratie verandert na bepaalde zuiveringsprocessen. Het wordt wel belangrijk gevonden dat methoden betrouwbaar zijn en daar hoort een validatie bij. Een belangrijk aspect is ook de

interlaboratoriumvariatie van methoden. Zo heeft Aqualab Zuid de BPP geïmplementeerd, maar weken de getallen voor de BPP-parameters af van de getallen die door KWR werden verkregen, waarschijnlijk omdat ATP met een andere methode/apparaat werd bepaald. Ook de implementatie van de LC-OCD bij Aqualab Zuid liet zien dat getallen voor de verschillende fracties anders waren dan wanneer het Duitse laboratorium Doc Labor deze bepaalde. Een openstaande vraag is daarom hoe we de verschillende methoden op een betrouwbare manier geïmplementeerd kunnen krijgen bij de drinkwaterlaboratoria. De discussie vervolgde met de vraag of het ook nodig is dat alle drinkwaterlaboratoria alle biologische stabiliteitsmetingen implementeren. De drinkwaterlaboratoria geven aan dat ze pas gaan implementeren als er voldoende vraag naar is, oftewel de businesscase om een nieuwe methode in te brengen moet positief zijn. Daarnaast moet ook duidelijk zijn dat de nieuwe methoden niet de routinemethoden verstoren en moeten methoden voldoende gevalideerd zijn. Uiteindelijk wordt geconcludeerd dat het belangrijk is dat KWR en de drinkwaterlaboratoria gesprekken voeren over welke methoden implementeerbaar zijn voor de drinkwaterlaboratoria en welke methoden specifiek bij KWR kunnen blijven. Het ideaalplaatje is overigens dat we nog maar één laboratorium hebben, met bijvoorbeeld een routinematige dependance in noorden, midden en zuiden van het land en een onderzoekslaboratorium (zoals KWR) in het midden van het land. Tevens wordt opgemerkt dat de mate van urgentie ook bepaald of een methode (versnelt) wordt gestandaardiseerd en momenteel is de urgentie op het gebied van biologische stabiliteit te laag hiervoor. Tot slot wordt aangegeven dat het van belang is dat, wanneer methoden zijn geïmplementeerd bij de drinkwaterlaboratoria, er ook ringonderzoeken plaatsvinden.

Samenvatting:

- De complexiteit van de methoden (gerelateerd aan biologische stabiliteit) zit hem in de omgeving die ervoor gecreëerd moet worden.
- De LC-OCD is een interessante methode, maar moet gevalideerd worden.
- Een methode moet een getal leveren wat begrepen wordt door iedereen die ermee werkt en waar iets mee gedaan kan worden zonder verdere bewerkingen.
- Er is samenwerking en overleg nodig tussen KWR en de drinkwaterlaboratoria om methodes geïmplementeerd te krijgen en sturing vanuit de drinkwaterbedrijven over wat ze eigenlijk willen.
- Specifieke methodes voor bijvoorbeeld onderzoek bij KWR houden en de routineanalyses implementeren bij de drinkwaterlaboratoria.
- Biologische stabiliteit wordt momenteel niet gezien als een groot genoeg probleem om snel gestandaardiseerde methoden voor te ontwikkelen.

Vraag 7: Welke kritische kanttekeningen heeft u bij genoemde nieuwe methoden?

Nut nog onvoldoende duidelijk:	10
Geen duidelijke aandachtswaarden:	10
Het aantal parameters wordt te veel:	9
Te bewerkelijk en te duur:	11

Discussie:

Het grote aantal parameters is met name een kostenprobleem. Wanneer voor bijvoorbeeld 50 locaties meerdere parameters moeten worden bepaald, dan worden de kosten erg hoog. Daarbij geldt ook nog dat om een betrouwbaar beeld te krijgen meerdere keren per jaar de parameters bepaald moeten worden. Opgemerkt wordt dat dit ondervangen kan worden door onderscheid te maken tussen routine en onderzoek. De vraag is of routinematig alle parameters overal bepaald moeten worden, waarschijnlijk is het voldoende om een beperkt aantal parameters bij een beperkt aantal locaties te bepalen. Andere geven aan dat hoge kosten vaak wordt genoemd, maar de BPP-test is bijvoorbeeld goedkoper dan de vaker routinematig toegepaste AOC P17/Nox. De duurdere kosten zitten voornamelijk in het inzetten van de nieuwe én oude parameter gedurende een tijd (schaduwdraaien), maar daar zo op voorhand over nagedacht kunnen worden. De drinkwaterbedrijven zien echter het verlies van historische AOC-data als belemmering om over te schakelen.

Evides geeft aan dat de aandachtswaarden nog te weinig houvast bieden. Door bepaalde maatregelen verbeterde de biologische stabiliteitsparameters maar leidde dat niet tot acceptabele *Aeromonas*-aantallen. KWR geeft aan dat uit de presentatie van Evides is gebleken dat de biologische stabiliteitswaarden van de verschillende parameters verbeterde, maar dat deze nog niet allemaal onder de aandachtswaarden lagen, waardoor ook verwacht mag worden dat *Aeromonas*-aantallen nog niet acceptabel zijn. Opgemerkt wordt dat ook andere factoren die niet met de biologische-stabiliteitsmethoden worden bepaald (bijvoorbeeld distributiesysteemtechnische factoren) een rol hebben, waardoor verbetering van de biologische stabiliteit van het drinkwater misschien niet altijd hoeft te leiden tot minder nagroei. Tevens wordt opgemerkt dat de interpretatie van de CBM-data lastig blijft en dat een CBM na een zuiveringsstap waar bacteriën worden geïnactiveerd of verwijderd minder betrouwbare data geeft. Evides denkt dat een leidingsimulator een goede tool is om biofilmvorming en de invloed van verschillende factoren op deze biofilmvorming te onderzoeken. KWR heeft een dergelijke leidingsimulator (KIVODIS), maar daar werd geen effect gezien van hydraulica op biofilmconcentratie of -samenstelling, dus vooralsnog lijkt de invloed beperkt, maar verder onderzoek hiernaar is nodig. Uiteindelijk wordt opgemerkt dat biofilmvorming een complex fenomeen is, waarvan gedacht werd dat het met een CBM op een relatief eenvoudige manier kon worden bepaald. Aanvullend onderzoek is echter nodig om de relatie tussen CBM-data, leidingsimulatordata en distributiesysteemdata te achterhalen.

Samenvatting:

- Keuzes voor biologische-stabiliteitsparameters moeten gemaakt worden in onderzoek, zeker wanneer veel locaties gemeten worden, maar daar is soms voorkennis voor nodig wat het lastig maakt.
- Keuzes voor biologische-stabiliteitsparameters moeten ook gemaakt worden om kosten te drukken.
- Een belemmering om over te stappen naar de nieuwe parameters is het kunnen vergelijken met oude gegevens, daarvoor moet worden geschaduwdraaid dat hoge kosten met zich meebrengt.
- Plaatsing van een CBM moet goed doordacht worden om een representatief beeld te krijgen.
- Een leidingsimulator om distributiefactoren in relatie tot biologische stabiliteit en nagroei mee te nemen zou interessant kunnen zijn. Eerder onderzoek daarmee zag echter een beperkt effect en een leidingsimulator neemt ook niet alle factoren van een distributiesysteem mee.

Vraag 8: Vindt u verdere optimalisatie in BTO-verband zinvol?

Ja: 8
Nee: 0

Vraag 9: Waar ziet u een duidelijke rol bij de uitvoering van de bepalingsmethoden?

Drinkwaterlaboratoria: 3
KWR: 4
Allebei: 11

Discussie:

De implementatie moet worden uitgewerkt bij KWR, de routineanalyses moeten worden gedaan door de drinkwaterlaboratoria. Als een methode routine wordt, is het bij KWR te duur om de methode te laten uitvoeren en routineanalyses zijn ook niet de taak van KWR. De methoden kunnen echter ook in onderzoeksprojecten worden toegepast, maar ook dan is het duur om meerdere biologische-stabiliteitsmethoden te laten uitvoeren. Opgemerkt wordt dat het beter is om bij de ontwikkeling van een nieuwe methode al na te denken of de methode bij KWR blijft of naar een routinelaboratorium gaat. Nu ontwikkelt KWR een methode en wordt die vervolgens bij de drinkwaterlaboratoria in de schoot geworpen. Daarnaast wordt bij urgente problematiek gezien dat ieder laboratorium afzonderlijk een methode gaat ontwikkelen, waardoor de kans bestaat dat er meerdere methoden worden ontwikkeld. Het is dus noodzakelijk dat meer afstemming tussen KWR en de drinkwaterlaboratoria plaatsvindt bij de ontwikkeling van methoden.

Vraag 10: Zou uw bedrijf onderzoek naar biologische activiteit stoppen als overheid en klanten het niet als een probleem ervaren?

Ja: 5
Nee: 13

Discussie:
Sommigen zouden overwegen te stoppen, maar wel de vinger aan de pols blijven houden ten aanzien van de pathogenen die zich in drinkwater kunnen vermeerderen. Het operationele beeld is ook dat klanten met name klagen over bijvoorbeeld troebelheid en niet over bepaalde micro-organismen, dus het drinkwaterbedrijf moet ook die klachten oplossen. Die klanten vertellen dat het water dan wel wat troebel is, maar dat het bacteriologisch wel betrouwbaar is, is daarbij onvoldoende. Andere merken op dat biologische stabiliteit iets vertelt over hoe de bron, de zuivering en distributie de waterkwaliteit beïnvloeden en dat het daarom belangrijk blijft om onderzoek naar biologische stabiliteit te blijven doen.

Samenvatting:
- Onderzoek doe je niet alleen om huidige problemen op te lossen, maar ook om te achterhalen hoe je systeem precies werkt. Deze kennis kan een handelingsperspectief geven als er een nieuwe situatie of een nieuw probleem zich voordoet.
- Biologische stabiliteit geeft een geheel beeld van je systeem. Zowel de bron, zuivering en distributie spelen een rol.

Vraag 13: Welke kennis over biologische activiteit mist u nu?

Discussie:
Evides geeft aan dat ze kennis over de invloed van de infrastructuur op de biologische stabiliteit missen alsook wat de rol van *Asellus* is op de biologische stabiliteit of nagroei en waar *Asellus* op groeit. Dit is echter deels al onderzocht, waarbij is gevonden dat *Asellus* zich voedt met sediment. Ook is Evides geïnteresseerd in de relatie tussen bacteriën en ongewervelde dieren en tot welke nagroeiproblemen leidt die relatie. Doordat de workshop op zijn einde loopt, zijn een aantal deelnemers vertrokken, waardoor deze discussie voornamelijk onderzoeksvragen vanuit Evides oplevert. Dat maakt het lastig om te achterhalen of de onderzoeksvragen naar *Asellus* bijvoorbeeld breed in het BTO moet worden opgenomen. Deelnemers die bij andere bedrijven werken en nog aanwezig zijn, vragen zich inderdaad af of het nodig is om het hele voedselweb te begrijpen om in te kunnen grijpen wanneer er bijvoorbeeld veel *Aeromonas*-overschrijdingen worden waargenomen. Het is waarschijnlijk belangrijker om de ecologie van opportunistische ziekteverwekkers te begrijpen en de relatie van die micro-organismen met *Aeromonas*, ongewervelde dieren en biologische stabiliteit. Een ander aspect dat aandacht nodig heeft is de invloed van klimaatverandering (hogere drinkwatertemperatuur) op de biologische stabiliteit en activiteit (met name opportunistische ziekteverwekkers) in het drinkwaterdistributiesysteem.

Samenvatting:
- Kennis over de invloed van de infrastructuur op de biologische stabiliteit wordt nog gemist.
- Kennis over de rol van *Asellus* op de biologische stabiliteit en nagroei wordt nog gemist.
- Kennis over de rol van biologische stabiliteit op opportunistische pathogenen wordt gemist.
- Kennis over de link tussen biologische stabiliteit en zowel gezondheidsrisico's als esthetische problemen wordt gemist.
- Kennis over de effecten van klimaatverandering op de biologische stabiliteit en opportunistische ziekteverwekkers wordt gemist.
- De verschillende drinkwaterbedrijven hebben focus op andere vragen in relatie tot biologische stabiliteit en nagroei. Daarom moet achterhaald worden wat de gezamenlijke onderzoeksvragen zijn, zodat die in het thematisch BTO kunnen worden opgepakt. Deze exercitie vindt momenteel plaats bij het opstellen van het nieuwe zesjarenplan voor het thema Biologische Veiligheid binnen het BTO. De

afzonderlijke onderzoeksvragen kunnen dan in het bedrijfsonderzoek van het BTO (of in een andere vorm) worden opgepakt.

I Supplemental information Chapter 2

I.I Detailed description of the biological stability methods used in this study

MBC_7, MBG_7, CBP_{14}

The BPP test that was used in our study was a slightly altered method of the initial BPP test published by (van der Kooij and Veenendaal 2014). Treated drinking water samples (600 ml) were collected in AOC-free glass-stoppered Pyrex-glass Erlenmeyer flasks that had been cleaned using a standard washing procedure, subsequently flushed with demineralized water and finally heated for 4 h at 550°C. To prevent nitrate or phosphate limitation, 0.6 ml of a phosphate/nitrate solution (11.0 mM KH_2PO_4; 59.4 mM KNO_3) was added to the drinking water samples (end concentration: 0.9 mg/600 ml KH_2PO_4, 3.6 mg/600 ml KNO_3). When the treated water samples came from treatment plants where the last step in the treatment was a disinfection step with chlorine dioxide (plants SW1, SW2, SW5, SW7, SW8, SW10, SW11), then 1.0 ml of a sodium sulfite solution (0.19 M Na_2SO_3) was added to the drinking water samples (end concentration 24 mg/600 ml) as well as the effluent (1 ml) of the biological active carbon filtration of the same treatment plant, which served as an inoculum. In all other cases, no sodium sulfite or inoculum were added. Subsequently, all bottles were incubated in the dark at 25 ± 2°C for 14 days. At day 0, 1, 2, 4, 7, 9, 11 and 14 a subsample was taken from each bottle and analyzed for the ATP concentration. Three different parameters were deduced from the obtained ATP concentrations. First, the MBC_7 (previously named BP_7; (Hijnen et al. 2018, Schurer et al. 2022, van der Kooij et al. 2015) which is the maximal biomass concentration during the first seven days of incubation and is given in ng ATP/l. Second, the MBG_7, which is the maximum biomass growth during the first seven days of incubation, also given in ng ATP/l. The MBG_7 is calculated according to the following formula:

$$MBG_7 = ATP_{max,7} - ATP_{start,7}$$

With $ATP_{max,7}$, the maximum ATP concentration during the first seven days of incubation and $ATP_{start,7}$, the ATP concentration at start (day 0) of the BPP-test. Third, the CBP_{14} (previously named BPC_{14}; (Hijnen et al. 2018, Schurer et al. 2022, van der Kooij et al. 2015) (which is the cumulative biomass production during 14 days of incubation and is given in d.ng ATP/l. The CBP_{14} was calculated using the following formula:

$$CBP_{14} = \sum_{t=0}^{t} ATP(t) \times \Delta t.$$

AOC-A3

The AOC-A3 concentration was determined using Flavobacterium johnsoniae strain A3 (Sack et al. 2010, 2011). Treated drinking water samples (600 ml) were taken at each treatment plant in the same AOC-free glass-stoppered Pyrex-glass Erlenmeyer as described above. Nitrate and phosphate were added to the samples in the same manner as described for the BPP-test. Sodium sulfite was also added in the same manner as described above for the treated water samples from plants SW1, SW2, SW5, SW7, SW8, SW10, SW11. Subsequently, samples were pasteurized for 30 minutes at 60°C and cooled to room temperature by storing flasks in melting ice. The samples were then inoculated with F. johnsoniae strain A3 precultured in minimal medium with amylopectine and Pseudomonas fluorescens strain P17 precultured in minimal medium with acetate to obtain a starting concentration of approximately 100 cfu/ml. All flasks were incubated in the dark at 15°C ± 1°C. Every two to three days a subsample was taken from each flask and colony counts of strain A3 were determined using Lab-Lemco (LLA) agar streak plates (Oxoid), which were incubated for three days at 25°C, until the maximum colony count was obtained. AOC-A3 concentrations, expressed as µg C-biopolymer equivalents per liter, were calculated by applying a previously determined yield factor (1.43×10^7 CFU/µg; (Sack et al. 2011) using the following formula:

$$AOC_{A3} = \frac{N_{max} \times 1000}{Y}$$

with N_{max} the maximum colony count of A3, and Y the yield factor.

BAR, FeAR

The biomass accumulation rate (BAR) and the iron accumulation rate (FeAR) were determined using a continuous biofilm monitor (CBM)(van der Kooij and Veenendaal 2014). The CBM consists of five parallel columns, with four columns containing glass containers filled with 1.69 g glass beads (2 mm) that corresponds to an exposed glass bead surface of 19.844 cm^2 in each glass container (Figure S1). The CBM was connected for three months to the treated water of each investigated treatment plant, resulting in a continuous flow of 10 L/h through each column containing a glass cylinder. The flow rate over the fifth column, without a glass container, was kept at 40 L/h. After two weeks the glass cylinder of the first two columns were replaced, and after four week the glass cylinder of columns 3 and 4 were replaced with new glass cylinders containing the same amount of glass beads. This replacement process was repeated until the end of the measuring period. In this manner, the first two glass cylinders with glass beads were exposed to continuously flowing water for two weeks (which was discarded from further analysis), whereas all other glass cylinders obtained from the CBM had been exposed to continuously flowing water for four weeks. The glass beads from the removed columns were transferred to 10 ml sterilized drinking water and transported at 4°C to the lab. In the laboratory, glass beads were 2 min treated with low-energy ultrasound sonication using a water bath (Branson Sonication Unit 5050). This procedure was repeated twice using fresh sterilized drinking water each time. The three obtained suspensions were pooled (30 ml total volume) and analyzed for the ATP and iron concentration. The BAR, expressed as pg ATP cm^{-2} day^{-1} was calculated from the ATP concentrations according to the following formula:

$$BAR = \frac{(ATP \times 30)/_{SA}}{t}$$

with ATP being the ATP concentration of the pooled suspension in pg/ml, SA the surface area of the glass beads (19.844 cm^2) and t the period that the glass beads were exposed to the flowing water (28 days). The FeAR, expressed as mg Fe m^{-2} day^{-1}, was calculated in a similar manner with the measured iron concentration of the pooled suspension.

PHMOC, PHMCHC, PFe

The particulate and/or high molecular organic carbon (PHMOC), particulate and/or high molecular carbohydrates carbon (PHMCHC) and the particulate iron (PFe) concentrations were obtained using crossflow ultrafiltration. 100 L of the treated water at each plant was supplied to a hemoflow membrane HF80S (Fresenius Medical Care) at a flow rate of 900 mL/min to concentrate the water to approximately 500 ml. The molecular weight cut-off of the membrane was 30 kDa (0.01 µm particle size), retaining organic molecules above 30 kDa and particles and/or colloids above 0.01 µm in the concentrate. The concentrate was subsequently analyzed for the TOC concentration, the carbohydrate concentration and iron as described below. The PHMOC, PHMCHC and PFe concentrations were thereafter calculated by first correcting for the DOC, dissolved carbohydrate or dissolved iron concentration in the drinking water and second using the concentration factor of the crossflow ultrafiltration step. PHMOC are expressed as µg C/l, PHMCHC as µg glucose-C equivalents/l and PFe as µg Fe/l.

I.II Example for calculation of guidance value for TOC in relation to HPC-22 for all treatment plants

First a value was derived using the semi-quantitative analysis. The four treatment plants with the highest HPC-22_{gg} were determined, which were treatment plants GW-12 (32.2 cfu/ml), GW-22 (28.5 cfu/ml), SW-7 (16.0 cfu/ml) and GW-10 (14.5 cfu/ml). Next, the ranking of these four plants compared to all treatment plants was determined for the TOC concentration. This comparison showed that plant GW-12 ranked 5, GW-22 ranked 6, SW-7 ranked 21 and GW-10 ranked 1 for the TOC-concentration in the treated water. Three of these four plants ranked within the top 25% of all plants (top 8 of all treatment plants). The lowest TOC concentration at a treatment plant that exceeded the established value of 20 cfu/ml for HPC-22_{gg} (i.e. GW-12 and GW-22) was 3,1 mg C/l and consequently this TOC concentration was used as the value obtained with the semi-quantitative analysis.

Second, a value was derived using the statistical quantitative analysis. The TOC concentration of the drinking water from each treatment plant and the HPC-22_{gg} of each treatment plant showed a significant regression ($p<0.05$) with a R^2 of 0.46. The formula describing this relationship was:

$$Log(HPC\text{-}22_{gg}) = 0.3424 \times TOC - 0.2039$$

Using the established value of 20 cfu/ml for HPC-22_{gg} resulted in a TOC concentration of 4.4 mg C/l and this TOC concentration was used as value obtained with the statistical quantitative analysis.

The guidance value for TOC was subsequently calculated as the average value of 3,1 mg C/l (derived from semi-quantitative analysis) and 4.4 mg C/l (derived from statistical quantitative analysis), which is 3.7 mg C/l.

Table S1. The source, treatment and years sampled for the 34 different treatment plants analyzed.

Plant	Source	Treatment[†]	Year sampled
GW-1	Oxic groundwater	A, M	2011
GW-2	Oxic groundwater	A, RSF, M	2015
GW-3	Oxic groundwater	A, RSF	2017
GW-4	Oxic groundwater	M	2017
GW-5	Anoxic groundwater	A, RSF	2011
GW-6	Anoxic groundwater	A, RSF, A, RSF	2009
GW-7	Anoxic groundwater	A, RSF, S, RSF, IEX	2009
GW-8	Anoxic groundwater	A, RSF, A, RSF	2009
GW-9	Anoxic groundwater	A, RSF	2010
GW-10	Anoxic groundwater	A, RSF, A, S, RSF	2010
GW-11	Anoxic groundwater	NF[*], A, RSF, RSF	2010
GW-12	Anoxic groundwater	I[#]: A, RSF, KMnO4, RSF, ACF, UV II: A, S, RSF	2012
GW-13	Anoxic groundwater	M, A, RSF	2016
GW-14	Anoxic groundwater	A, RSF	2016
GW-15	Anoxic groundwater	A, RSF	2015
GW-16	Anoxic groundwater	A, RSF, A	2015
GW-17	Anoxic groundwater	A, RSF, S, RSF	2015
GW-18	Anoxic groundwater	A, RSF, S, RSF	2016
GW-19	Anoxic groundwater	A, RSF	2016
GW-20	Anoxic groundwater	A, RSF, A, RSF	2016
GW-21	Anoxic groundwater	S, A, RSF	2015
GW-22	Anoxic groundwater	A, S, RSF	2016
GW-23	Anoxic groundwater	RO[^], A, RSF, RSF, S, RSF, ACF, A, UV	2016
SW-1	Surface water	CA, RSF, UV/H2O2 (60%) followed by ACF, DI, A, RSF, or UF+RO (40%)[$], ClO2[&]	2015
SW-2	Surface water	CA, RSF, ACF, UV/H2O2 (75%) followed by ACF, DI, S, A, RSF, or UF+RO (25%)[$], ClO2[&]	2015
SW-3	Surface water	CA, RSF, DI, RSF, O, S, ACF, SSF	2017
SW-4	Surface water	DI, A, RSF, ACF, UV	2016
SW-5	Surface water	R, CA, RSF, UV, ACF, ClO2[&]	2012
SW-6	Surface water/groundwater	I[@]: R, CA, RSF, A, ACF, RSF, SSF, UV II: A, RSF, A	2016
SW-7	Surface water	R, CA, RSF, UV, ACF, ClO2[&]	2015
SW-8	Surface water	R, CA, O, RSF, ACF, ClO2[&]	2012
SW-9	Surface water	R, CA, RSF, O, S, ACF, SSF	2017
SW-10	Surface water/groundwater	I[@]: R, CA, RSF, O, ACF, ClO2[&] II: A, RSF, S, RSF	2013
SW-11	Surface water	R, S, CA, RSF, UV/H2O2, ACF, ClO2[&]	2013

[†] M = marble filtration, A = aeration, RSF = rapid sand filtration, S = softening, IEX = ion exchange, KMnO4 = KMnO4 dosing, NF = nanofiltration, ACF = active carbon filtration, UV = UV disinfection, CA = coagulation followed by sedimentation, flocculation and/or flotation, UV/H2O2 = UV + hydrogen peroxide disinfection, DI = dune infiltration, UF = ultrafiltration, RO = reverse osmosis filtration, O = ozone disinfection, SSF = slow sand filtration, ClO2 = chlorine dioxide disinfection,

[*] 40% treated with nanofiltration, 60% bypass

[#] Two different water types with different water quality are treated in separate treatment trains before being mixed at the end of the treatment

[^] 13% treated with RO, 87% bypass

[&] ClO2 concentration dosed is low, resulting in undetectable chlorine concentrations in the treated water leaving the plant, except for SW-11 where undetectable chlorine concentrations are observed after short residence time in the transport pipe of the distribution system

[$] After first RSF 60% (SW1) or 75% (SW2) of water is treated by UV/H2O2 followed by ACF, DI, A and RSF, the other 40% (SW1) or 25% (SW2) by UF followed by RO. The partial flows are subsequently mixed before ClO2.

[@] Surface water and groundwater are used for drinking water production. Surface water is treated according to treatment train I, groundwater is treated according to treatment train II. After treatment both water types are mixed.

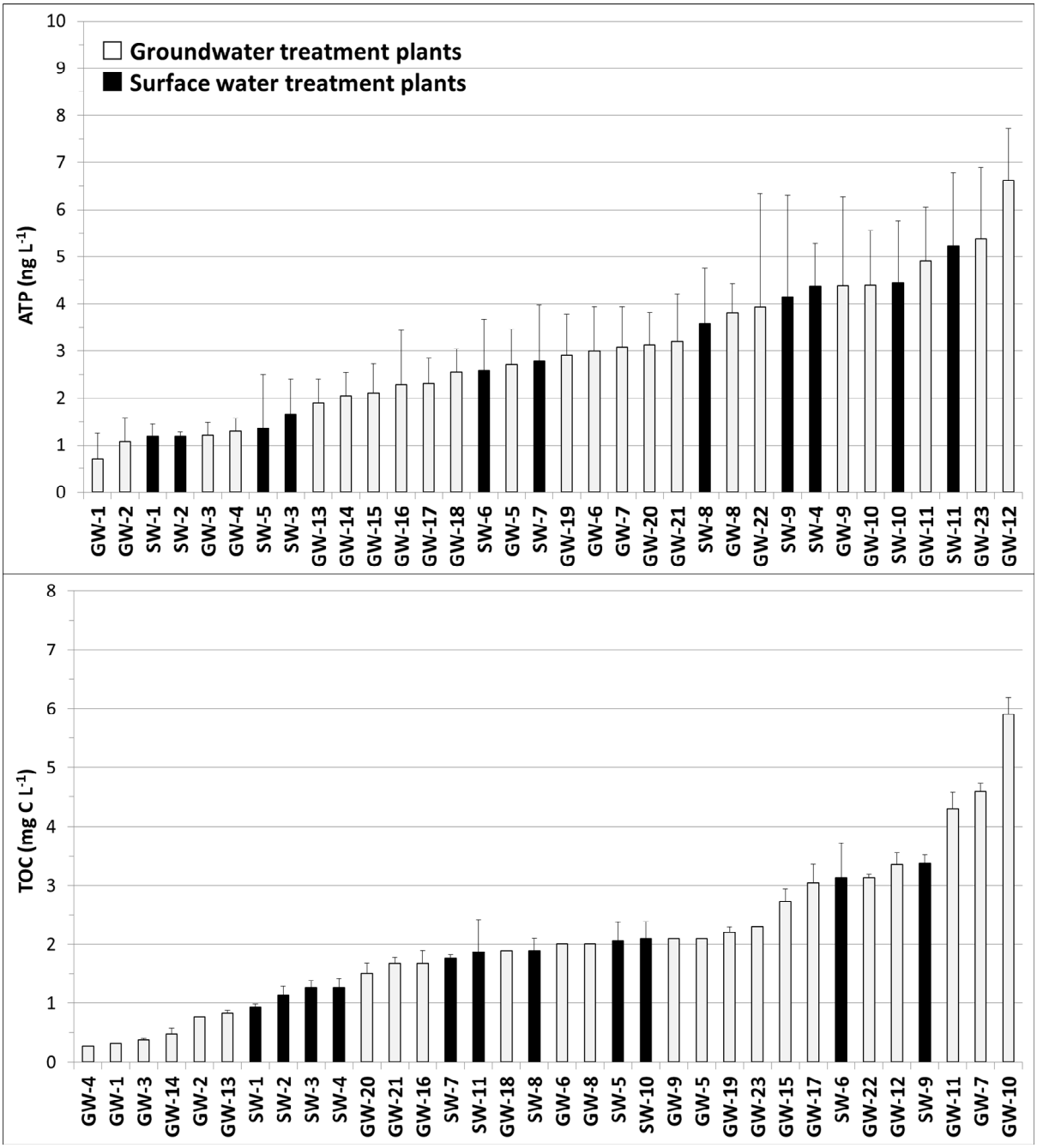

Figure S1. The mean ATP (top) and TOC concentrations (bottom) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

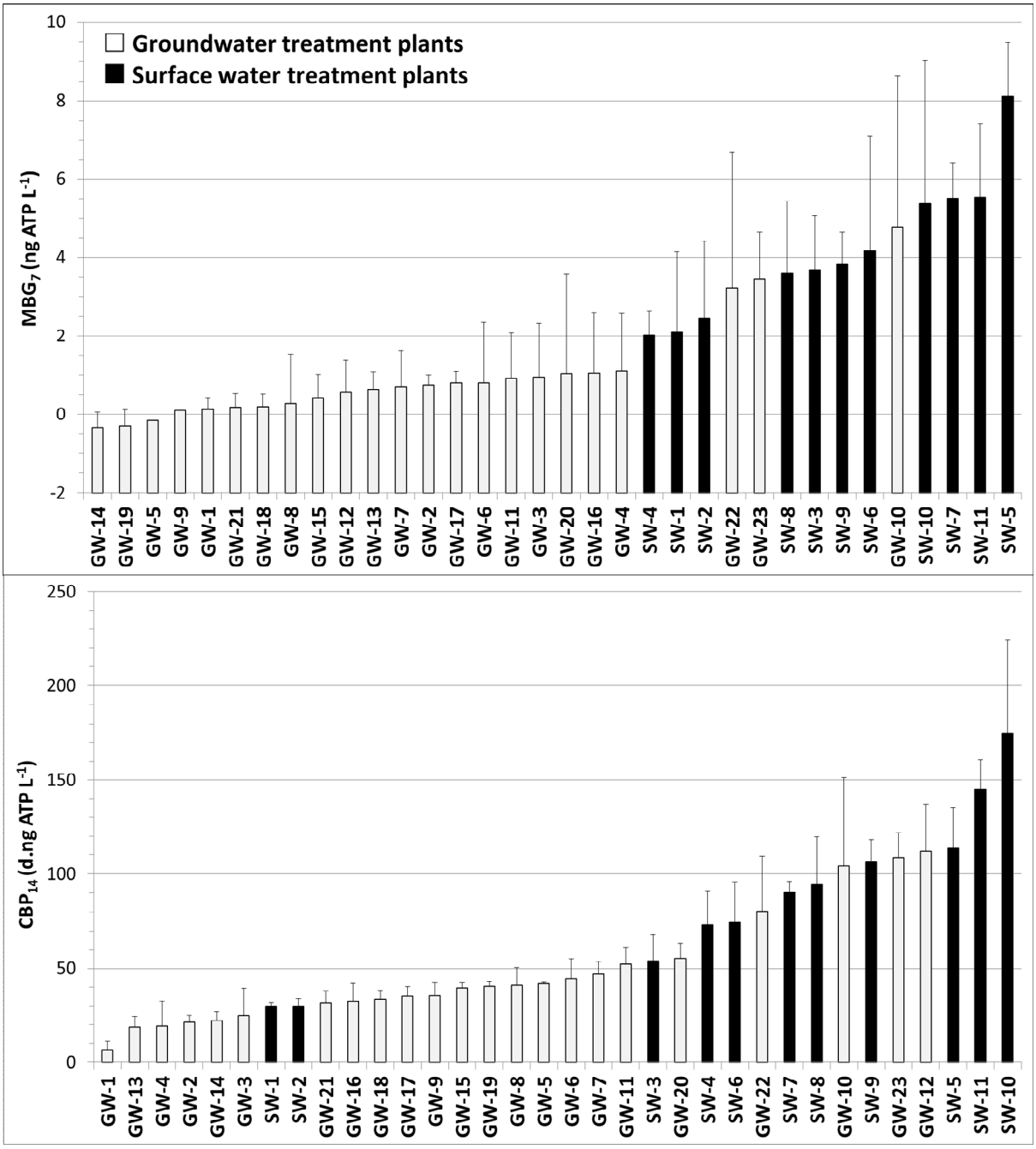

Figure S2. The mean MBG_7 (top) and CBP_{14} values (bottom) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

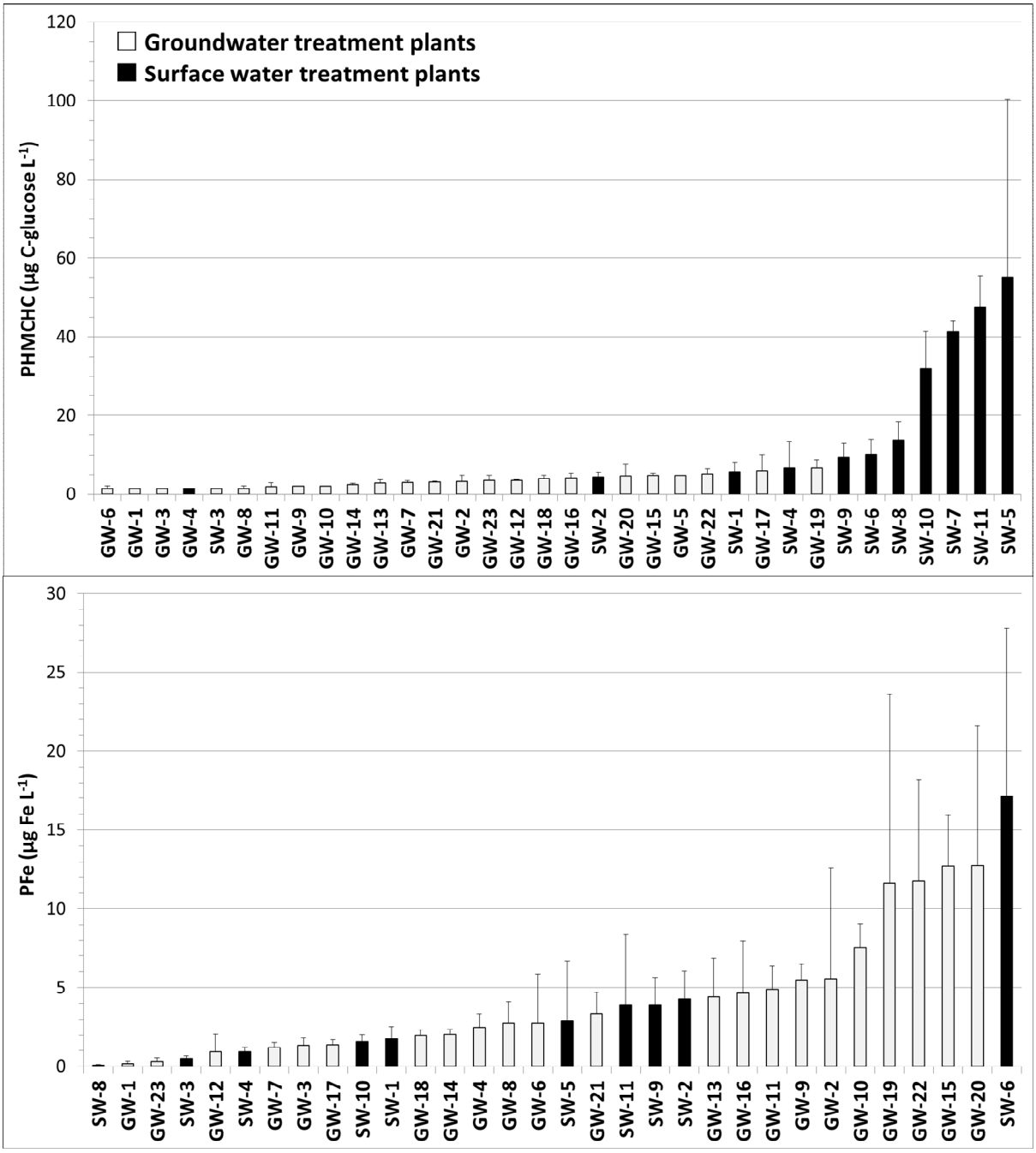

Figure S3. The mean PHMCHC (top) and PFe concentrations (bottom) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

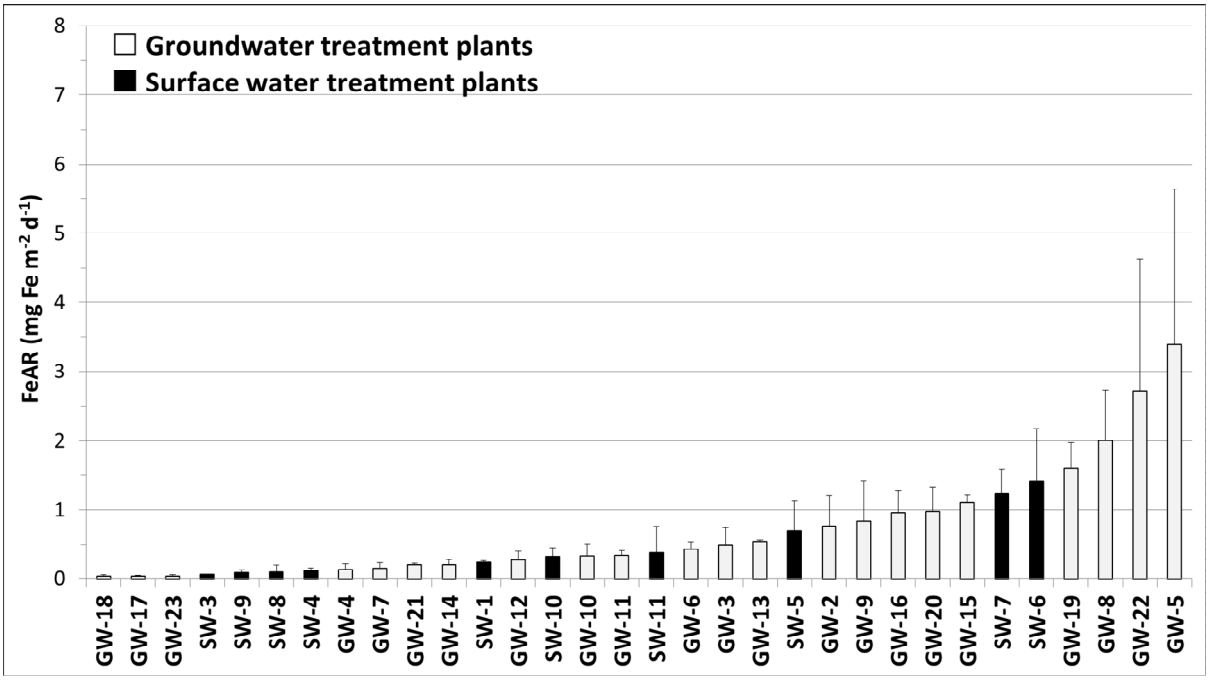

Figure S4. The mean FeAR values ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

Table S2. The R^2 values of the pair-wise correlation analysis between the different biological stability parameters (absolute and log-transformed values) and the different microbiological parameters. Light blue cells: $p<0.05$ & $R^2 < 0.4$; green cells: $p<0.05$ & $0.4 < R^2 < 0.9$; orange cells: $p<0.05$ & $R^2 > 0.9$.

	ATP	MBC_7	MBG_7	CBP_{14}	AOC-A3	BAR	FeAR	TOC	PHMOC	PHMCHC	PFe	$HPC22_{gm}$	*Aeromonas* $_{90P}$	*Legionella* $_{gm}$	$Invertebrate_m$
ATP	1.00	0.39	0.03	0.43	0.01	0.04	0.00	0.34	0.00	0.01	0.00	0.25	0.01	0.00	0.02
MBC_7		1.00	0.67	0.98	0.45	0.39	0.01	0.16	0.39	0.45	0.00	0.24	0.21	0.00	0.12
MBG_7			1.00	0.61	0.60	0.47	0.01	0.05	0.56	0.61	0.00	0.10	0.39	0.00	0.07
CBP_{14}				1.00	0.40	0.32	0.01	0.18	0.36	0.44	0.00	0.24	0.17	0.00	0.15
AOC-A3					1.00	0.63	0.00	0.04	0.75	0.76	0.01	0.09	0.35	0.01	0.01
BAR						1.00	0.05	0.00	0.65	0.56	0.00	0.26	0.36	0.01	0.02
FeAR							1.00	0.00	0.01	0.00	0.47	0.07	0.02	0.03	0.21
TOC								1.00	0.00	0.00	0.07	0.33	0.09	0.11	0.08
PHMOC									1.00	0.94	0.00	0.05	0.42	0.00	0.09
PHMCHC										1.00	0.00	0.04	0.39	0.00	0.08
PFe											1.00	0.05	0.16	0.02	0.22
$HPC22_{gm}$												1.00	0.16	0.01	0.05
Aeromonas $_{90P}$													1.00	0.00	0.14
Legionella $_{gm}$														1.00	0.02
$Invertebrate_m$															1.00

	Lg ATP	Lg MBC_7	Lg MBG_7	Lg CBP_{14}	Lg AOC-A3	Lg BAR	Lg FeAR	Lg TOC	Lg PHMOC	Lg PHMCHC	Lg PFe	Lg $HPC22_{gm}$	Lg *Aeromonas* $_{90P}$	Lg *Legionella* $_{gm}$	Lg $Invertebrate_m$
Lg ATP	1.00	0.49	0.03	0.54	0.23	0.14	0.00	0.53	0.04	0.05	0.01	0.21	0.14	0.02	0.18
Lg MBC_7		1.00	0.54	0.98	0.53	0.51	0.01	0.43	0.39	0.43	0.00	0.37	0.40	0.00	0.21
Lg MBG_7			1.00	0.49	0.24	0.27	0.02	0.07	0.30	0.36	0.01	0.20	0.24	0.00	0.05
Lg CBP_{14}				1.00	0.52	0.50	0.00	0.48	0.39	0.41	0.00	0.39	0.41	0.01	0.24
Lg AOC-A3					1.00	0.54	0.05	0.36	0.41	0.44	0.01	0.27	0.24	0.03	0.16
Lg BAR						1.00	0.11	0.14	0.43	0.44	0.00	0.26	0.27	0.02	0.04
Lg FeAR							1.00	0.00	0.03	0.01	0.51	0.07	0.06	0.05	0.01
Lg TOC								1.00	0.11	0.08	0.08	0.47	0.44	0.06	0.26
Lg PHMOC									1.00	0.81	0.04	0.14	0.28	0.01	0.21
Lg PHMCHC										1.00	0.01	0.17	0.27	0.01	0.15
Lg PFe											1.00	0.06	0.11	0.00	0.02
Lg $HPC22_{gm}$												1.00	0.64	0.02	0.16
Lg *Aeromonas* $_{90P}$													1.00	0.01	0.23
Lg *Legionella* $_{gm}$														1.00	0.07
Lg $Invertebrate_m$															1.00

Table S3 Top 4 ranking of drinking water treatment plants for HPC22, *Aeromonas*, *Legionella* and invertebrate biomass and the ranking of these top 4 plants for each biological stability parameter when all 34 treatment plants are included. When at least three of the top 4 treatment plants rank within the top 25% for a biological stability parameter, then this parameter is highlighted in grey.

Treatment plant	HPC22 (cfu/ml) Geomean	ATP Rank	MBC_7 Rank	MBG_7 Rank	AOC-A3 Rank	BAR Rank	FeAR Rank	TOC Rank	PHMOC Rank	PFe Rank
GW-12	32.2	1	5	25	6	5	20	5	23	28
GW-22	28.5	10	11	11	19	3	2	6	10	4
SW-7	16.0	18	8	3	2	1	6	21	2	33
GW-10	14.5	6	7	5	5	19	18	1	27	6
Treatment plant	*Aeromonas* (cfu/100 ml) 90-Percentile	ATP Rank	MBC_7 Rank	MBG_7 Rank	AOC-A3 Rank	BAR Rank	FeAR Rank	TOC Rank	PHMOC Rank	PFe Rank
SW-7	3,720	18	8	3	2	1	6	21	2	33
SW-5	3,100	28	3	1	1	1	12	15	1	16
SW-6	3,050	20	10	6	13	14	5	7	9	1
GW-11	2,680	4	15	19	27	21	17	3	30	9
Treatment plant	*Legionella* (cfu/l) Geomean	ATP Rank	MBC_7 Rank	MBG_7 Rank	AOC-A3 Rank	BAR Rank	FeAR Rank	TOC Rank	PHMOC Rank	PFe Rank
GW-7	5.01	15	18	23	33	27	24	2	19	26
SW-11	1.30	3	2	2	4	4	16	20	3	14
GW-18	1.00	21	27	28	21	30	32	19	16	21
SW-6	0.20	20	10	6	13	14	5	7	9	1
Treatment plant	Invertebrates (mg/m^3) Geomean	ATP Rank	MBC_7 Rank	MBG_7 Rank	AOC-A3 Rank	BAR Rank	FeAR Rank	TOC Rank	PHMOC Rank	PFe Rank
SW-6	325.8	20	10	6	13	14	5	7	9	1
GW-5	284.7	19	26	32	34	23	1	12	12	34
SW-10	267.5	5	1	4	32	9	19	14	4	23
GW-15	260.0	24	21	26	10	16	7	9	11	3

Table S4 Top 4 ranking of drinking water treatment plants for HPC22, *Aeromonas*, *Legionella* and invertebrate biomass and the ranking of these top 4 plants for each biological stability parameter when only the 23 groundwater treatment plants are included. When at least three of the top 4 treatment plants rank within the top 25% of all groundwater treatment plants for a biological stability parameter, then this parameter is highlighted in grey.

Treatment plant	HPC22 (cfu/ml) Geomean	ATP Rank	MBC_7 Rank	MBG_7 Rank	AOC-A3 Rank	BAR Rank	FeAR Rank	TOC Rank	PHMOC Rank	PHMCHC Rank	PFe Rank
GW-12	32.2	1	2	14	2	2	15	4	14	9	20
GW-22	28.5	6	4	3	12	1	2	5	3	3	3
GW-10	14.5	4	3	1	1	10	14	1	16	16	5
GW-8	11.3	7	10	16	4	5	3	12	20	19	13
Treatment plant	*Aeromonas* (cfu/100 ml) 90-Percentile	ATP Rank	MBC_7 Rank	MBG_7 Rank	AOC-A3 Rank	BAR Rank	FeAR Rank	TOC Rank	PHMOC Rank	PHMCHC Rank	PFe Rank
GW-11	2,680	3	6	8	18	12	13	3	19	18	8
GW-10	900	4	3	1	1	10	14	1	16	16	5
GW-22	585	6	4	3	12	1	2	5	3	3	3
GW-20	510	9	5	6	17	11	6	17	12	6	1
Treatment plant	*Legionella* (cfu/l) Geomean	ATP Rank	MBC_7 Rank	MBG_7 Rank	AOC-A3 Rank	BAR Rank	FeAR Rank	TOC Rank	PHMOC Rank	PHMCHC Rank	PFe Rank
GW-7	5.01	10	9	12	22	18	18	2	11	13	19
GW-18	1.00	14	17	17	13	19	22	14	9	8	16
GW-6	0.20	11	7	9	5	15	12	13	22	23	12
Treatment plant	Invertebrates (mg/m^3) Geomean	ATP Rank	MBC_7 Rank	MBG_7 Rank	AOC-A3 Rank	BAR Rank	FeAR Rank	TOC Rank	PHMOC Rank	PHMCHC Rank	PFe Rank
GW-5	284.7	13	16	21	23	14	1	10	5	4	23
GW-15	260.0	17	12	15	6	7	5	7	4	5	2
GW-7	118.6	10	9	12	22	18	18	2	11	13	19
GW-22	104.1	6	4	3	12	1	2	5	3	3	3

Table S5 Top 4 ranking of drinking water treatment plants for HPC22, *Aeromonas*, *Legionella* and invertebrate biomass and the ranking of these top 4 plants for each biological stability parameter when only the 11 surface water treatment plants are included. When at least two of the top 4 treatment plants rank within the top 30% of all surface water treatment plants for a biological stability parameter, then this parameter is highlighted in grey.

Treatment	HPC22 (cfu/ml)	ATP	MBC_7	MBG_7	AOC-A3	BAR	FeAR	TOC	PHMOC	PFe
plant	Geomean	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank
SW-7	16.0	6	5	3	2	1	2	7	2	11
SW-10	9.3	9	1	4	11	6	5	3	4	7
SW-5	9.1	2	3	1	1	2	3	4	1	5
SW-6	7.6	7	7	5	6	9	1	2	7	1
Treatment	*Aeromonas* (cfu/100 ml)	ATP	MBC_7	MBG_7	AOC-A3	BAR	FeAR	TOC	PHMOC	PFe
plant	90-Percentile	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank
SW-7	3,720	6	5	3	2	1	2	7	2	11
SW-5	3,100	2	3	1	1	2	3	4	1	5
SW-6	3,050	7	7	5	6	9	1	2	7	1
SW-10	1,170	9	1	4	11	6	5	3	4	7
Treatment	*Legionella* (cfu/l)	ATP	MBC_7	MBG_7	AOC-A3	BAR	FeAR	TOC	PHMOC	PFe
plant	Geomean	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank
SW-11	1.30	1	2	2	4	3	4	6	3	4
SW-6	0.20	7	7	5	6	9	1	2	7	1
Treatment	Invertebrates (mg/m^3)	ATP	MBC_7	MBG_7	AOC-A3	BAR	FeAR	TOC	PHMOC	PFe
plant	Geomean	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank
SW-6	325.8	7	7	5	6	9	1	2	7	1
SW-10	267.5	2	1	4	11	6	5	3	4	7
SW-7	158.1	6	5	3	2	1	2	7	2	11
SW-9	157.7	4	4	6	5	8	9	1	6	3

Table S6. Threshold and guidance values based on data from 34 different treatment plants for biological stability parameters in treated water related to HPC22 (geometric mean of 20 cfu/ml), *Aeromonas* (90-percentile of 800 cfu/100 ml), *Legionella* (geometric mean of 100 cfu/l) and invertebrate biomass (median of 150 mg/m3) in the distribution system. The threshold values were based on ranking and regressions analysis of the relationship between biological stability parameters in treated water and the biological parameters in the distribution system. The final guidance value is the mean of the threshold values based on ranking and regression.

Parameter	Ranking	Regression	Final
Related to HPC22			
TOC (mg C l^{-1})	3.1	4.9	4.0
MBC_7 (ng ATP l^{-1})	6.8	N.C.*	N.C.&
AOC-A3 (µg C l^{-1})	2.1	N.C.	N.C.
BAR (pg ATP cm^{-2} day^{-1})	34	N.C.	N.C.
Related to Aeromonas			
MBG_7 (ng ATP l^{-1})	4.2	N.C.	N.C.
Related to Legionella			
	N.C.#	N.C.	N.C.
Related to invertebrate biomass			
FeAR (mg Fe m^{-2} day^{-1})	1.1	N.C.	N.C.

* N.C. is not calculated, because regression was not significant (p>0.05) or R^2 value was low (< 0.4)
& N.C. is not calculated, because ranking and/or regression did not have a reliable value
# N.C. is not calculated, because value could not be deduced based on ranking method

Table S7. Guidance values based on data from 23 different groundwater treatment plants for biological stability parameters in treated water related to HPC22 (geometric mean of 20 cfu/ml), *Aeromonas* (90-percentile of 800 cfu/100 ml), *Legionella* (geometric mean of 100 cfu/l) and invertebrate biomass (median of 150 mg/m3) in the distribution system. The guidance values were based on ranking and regressions analysis of the relationship between biological stability parameters in treated water and the biological parameters in the distribution system. The final guidance value is the mean of the guidance values based on ranking and regression.

Parameter	Ranking	Regression	Final
Related to HPC22			
TOC (mg C L^{-1})	3.1	5.2	4.1
MBC_7 (ng ATP L^{-1})	6.8	N.C.*	N.C.&
AOC-A3 (µg C L^{-1})	2.1	N.C.	N.C.
BAR (pg ATP cm^{-2} day^{-1})	34	N.C.	N.C.
Related to Aeromonas			
MBC_7 (ng ATP L^{-1})	8.2	9.0	8.6
TOC (mg C L^{-1})	5.9	2.3	4.1
PFe (µg Fe L^{-1})	4.9	N.C.	N.C.
Related to Legionella			
	N.C.#	N.C.	N.C.
Related to invertebrate biomass			
PHMOC (µg C L^{-1})	16	N.C.	N.C.
PHMCHC (µg C L^{-1})	4.8	N.C.	N.C.
FeAR (mg Fe m^{-2} day^{-1})	1.1	N.C.	N.C.

* N.C. is not calculated, because regression was not significant (p>0.05) or R^2 value was low (< 0.4)
& N.C. is not calculated, because ranking and/or regression did not have a reliable value
# N.C. is not calculated, because value could not be deduced based on ranking method

Table S8. Guidance values based on data from 11 different surface water treatment plants for biological stability parameters in treated water related to HPC22 (geometric mean of 20 cfu/ml), *Aeromonas* (90-percentile of 800 cfu/100 ml), *Legionella* (geometric mean of 100 cfu/l) and invertebrate biomass (median of 150 mg/m3) in the distribution system. The guidance values were based on ranking and regressions analysis of the relationship between biological stability parameters in treated water and the biological parameters in the distribution system. The final guidance value is the mean of the guidance values based on ranking and regression.

Parameter	Ranking	Regression	Final
Related to HPC22			
MBG_7 (ng ATP L^{-1})	N.C.[#]	8.8	N.C.[&]
PHMOC (µg C L^{-1})	N.C.	231	N.C.
Related to Aeromonas			
MBG_7 (ng ATP L^{-1})	4.2	4.7	4.5
PHMOC (µg C L^{-1})	54	40	47
FeAR (mg Fe m^{-2} day^{-1})	0.32	0.36	0.34
MBC_7 (ng ATP L^{-1})	6.9	N.C.[*]	N.C.
AOC-A3 (µg C L^{-1})	4.1	N.C.	N.C.
TOC (mg C L^{-1})	1.8	N.C.	N.C.
BAR (pg ATP cm^{-2} day^{-1})	15	N.C.	N.C.
Related to Legionella			
	N.C.	N.C.	N.C.
Related to invertebrate biomass			
TOC (mg C L^{-1})	1.8	2.7	2.2
MBC_7 (ng ATP L^{-1})	N.C.	12.0	N.C.
FeAR (mg Fe m^{-2} day^{-1})	0.32	N.C.	N.C.

[*] N.C. is not calculated, because regression was not significant ($p>0.05$) or R^2 value was low (< 0.4)
[&] N.C. is not calculated, because ranking and/or regression did not have a reliable value
[#] N.C. is not calculated, because value could not be deduced based on ranking method

II Supplemental information for Chapter 3

II.I Detailed description of the biological stability methods used in this study

MBC_7, MBG_7, CBP_{14}

The BPP test that was used in our study was a slightly altered method of the initial BPP test published by (van der Kooij and Veenendaal 2014). Treated drinking water samples (600 mL) were collected in duplicate in AOC-free glass-stoppered Pyrex-glass Erlenmeyer flasks that had been cleaned using a standard washing procedure, subsequently flushed with demineralized water, and finally heated for 4 h at 550°C. To prevent nitrate or phosphate limitation, 0.6 mL of a phosphate/nitrate solution (11.0 mM KH_2PO_4; 59.4 mM KNO_3) was added to the drinking water samples (end concentration: 0.9 mg 600 mL^{-1} KH_2PO_4, 3.6 mg 600 mL^{-1} KNO_3). When the treated water samples came from treatment plants where the last step in the treatment was a disinfection step with chlorine dioxide (plants SW-1, SW-2, SW-5, SW-7, SW-8, SW-10, SW-11), then 1.0 mL of a sodium sulphite solution (0.19 M Na_2SO_3) was added to the drinking water samples (end concentration 24 mg 600 mL^{-1}) as well as the effluent (1 mL) of the biological active carbon filtration of the same treatment plant, which served as an inoculum. In all other cases, no sodium sulphite or inoculum were added. Subsequently, all bottles were incubated in the dark at 25 ± 1°C for 14 days. At day 0, 1, 2, 4 or 5, 7, 9, 11 or 12 and 14 a subsample was taken from each bottle and analysed for the ATP concentration. Three different parameters were deduced from the obtained ATP concentrations. First, the MBC_7 (previously named BP_7; (Hijnen et al. 2018, Schurer et al. 2022, van der Kooij et al. 2015), which is the maximal biomass concentration from day to day 7 during incubation and is given in ng ATP L^{-1}. Second, the MBG_7, which is the maximum biomass growth during the first seven days of incubation, also given in ng ATP L^{-1}. The MBG_7 is calculated according to the following formula:

$$MBG_7 = MBC_7 - ATP_{start}$$

With $ATP_{max,7}$, the maximum ATP concentration during the first seven days of incubation and ATP_{start}, the ATP concentration at start (day 0) of the BPP-test. Third, the CBP_{14} (previously named BPC_{14}; (Hijnen et al. 2018, Schurer et al. 2022, van der Kooij et al. 2015), which is the cumulative biomass production during 14 days of incubation and is given in d.ng ATP/l. The CBP_{14} was calculated using the following formula:

$$CBP_{14} = \sum_{t=0}^{t} ATP(t) \times \Delta t.$$

AOC-A3

The AOC-A3 concentration was determined using *Flavobacterium johnsoniae* strain A3 (Sack et al. 2010, 2011). Treated drinking water samples (600 mL) were taken in duplicate at each treatment plant in the same AOC-free glass-stoppered Pyrex-glass Erlenmeyer as described above. Nitrate and phosphate were added to the samples in the same manner as described for the BPP-test. Sodium sulphite was also added in the same manner as described above for the treated water samples from plants SW-1, SW-2, SW-5, SW-7, SW-8, SW-10, SW-11. Subsequently, samples were pasteurized for 30 minutes at 60°C and cooled to room temperature by storing flasks in melting ice. The samples were then inoculated with *F. johnsoniae* strain A3 precultured in minimal medium with amylopectine and *Pseudomonas fluorescens* strain P17 precultured in minimal medium with acetate to obtain a starting concentration of approximately 100 cfu mL^{-1}. All flasks were incubated in the dark at 15°C ± 1°C. Every two to three days a subsample was taken from each flask and colony counts of strain A3 were determined using Lab-Lemco (LLA) agar streak plates (Oxoid), which were incubated for three days at 25 ± 1°C, until the maximum colony count was obtained. AOC-A3 concentrations, expressed as µg C-biopolymer equivalents per litre, were calculated by applying previously determined yield factors (1.43×10^7 CFU μg^{-1} when the N_{max} of strain A3 is ≤ 1.5×10^5 cfu mL^{-1} and 0.98×10^7 cfu μg^{-1} when the N_{max} of strain A3 is > 1.5×10^5 cfu mL^{-1}; Sack et al. 2011) using the following formula:

$$AOC_{A3} = \frac{N_{max}\times 1000}{Y}$$

with N_{max} the maximum colony count of A3, and Y the yield factor.

BAR, FeAR

The biomass accumulation rate (BAR) and the iron accumulation rate (FeAR) were determined using a continuous biofilm monitor (CBM)(van der Kooij and Veenendaal 2014). The CBM consists of five parallel columns, with four columns containing glass containers filled with 1.69 g glass beads (Ø 2 mm) that corresponds to an exposed glass bead surface of 19.844 cm^2 in each glass container (Figure S1). The CBM was connected for three months to the treated water of each investigated treatment plant, resulting in a continuous flow of 10 L h^{-1} through each column containing a glass cylinder. The flow rate over the fifth column, without a glass container, was kept at 40 L h^{-1}. After two weeks the glass cylinder of the first two columns were replaced, and after four

week the glass cylinder of columns 3 and 4 were replaced with new glass cylinders containing the same amount of glass beads. This replacement process was repeated until the end of the measuring period. In this manner, the first two glass cylinders with glass beads were exposed to continuously flowing water for two weeks (which was discarded from further analysis), whereas all other glass cylinders obtained from the CBM had been exposed to continuously flowing water for four weeks. The glass beads from the removed columns were transferred to 10 mL sterilized drinking water and transported at 4°C to the lab. In the laboratory, glass beads were 2 min treated with low-energy ultrasound sonication using a water bath (Branson Sonication Unit 5050). This procedure was done three times using fresh sterilized drinking water each time. The three obtained suspensions were pooled (30 mL total volume) and analysed for the ATP and iron concentration. The BAR, expressed as pg ATP cm^{-2} day^{-1} was calculated from the ATP concentrations according to the following formula:

$$BAR = \frac{(ATP \times 30)/_{SA}}{t}$$

with ATP being the ATP concentration of the pooled suspension in pg mL^{-1}, SA the surface area of the glass beads (19.844 cm^2) and t the period that the glass beads were exposed to the flowing water (28 days). The FeAR, expressed as mg Fe m^{-2} day^{-1}, was calculated in a similar manner with the measured iron concentration of the pooled suspension.

PHMOC, PHMCHC, PFe

The particulate and/or high molecular organic carbon (PHMOC), particulate and/or high molecular carbohydrates carbon (PHMCHC) and the particulate iron (PFe) concentrations were obtained using crossflow ultrafiltration. 100 L of the treated water at each plant was supplied to a hemoflow crossflow ultrafiltration membrane HF80S (Fresenius Medical Care) at a flow rate of 900 mL min^{-1} to concentrate the water to approximately 500 mL. The molecular weight cut-off of the membrane was 30 kDa (0.01 µm particle size), retaining organic molecules above 30 kDa and particles and/or colloids above 0.01 µm in the concentrate. The concentrate was subsequently analysed for the TOC concentration, the carbohydrate concentration and iron as described below. The PHMOC, PHMCHC and PFe concentrations were thereafter calculated by first correcting for the DOC, dissolved carbohydrate or dissolved iron concentration in the drinking water and second using the concentration factor of the crossflow ultrafiltration step. PHMOC are expressed as µg C L^{-1}, PHMCHC as µg glucose-C equivalents L^{-1} and PFe as µg Fe L^{-1}.

II.II Example for calculation of guidance value for TOC in relation to HPC22 for all treatment plants

First a value was derived using the semi-quantitative analysis. The four treatment plants with the highest $HPC22_{gg}$ were determined, which were treatment plants GW-12 (32.2 cfu mL^{-1}), GW-22 (28.5 cfu mL^{-1}), SW-7 (16.0 cfu mL^{-1}) and GW-10 (14.5 cfu mL^{-1}). Next, the ranking of these four plants compared to all treatment plants was determined for the TOC concentration. This comparison showed that plant GW-12 ranked 5, GW-22 ranked 6, SW-7 ranked 21 and GW-10 ranked 1 for the TOC-concentration in the treated water. Three of these four plants ranked within the top 25% of all plants (top 8 of all treatment plants). The lowest TOC concentration at a treatment plant that exceeded the established value of 20 cfu mL^{-1} for $HPC22_{gg}$ (i.e. GW-12 and GW-22) was 3.1 mg C L^{-1} and consequently this TOC concentration was used as the value obtained with the semi-quantitative analysis.

Second, a value was derived using the statistical quantitative analysis. The TOC concentration of the drinking water from each treatment plant and the $HPC22_{gg}$ of each treatment plant showed a significant regression ($p<0.05$) with a R^2 of 0.46. The formula describing this relationship was:

$Log(HPC22_{gg}) = 0.3424 \times TOC - 0.2039$

Using the established value of 20 cfu mL^{-1} for $HPC\text{-}22_{gg}$ resulted in a TOC concentration of 4.4 mg C L^{-1} and this TOC concentration was used as value obtained with the statistical quantitative analysis.

The guidance value for TOC was subsequently calculated as the average value of 3.1 mg C L^{-1} (derived from semi-quantitative analysis) and 4.4 mg C L^{-1} (derived from statistical quantitative analysis), which is 3.7 mg C L^{-1}.

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Table S1. The source, treatment and years sampled for the 34 different treatment plants analyzed.

Plant	Source	Treatment[†]	Year sampled
GW-1	Oxic groundwater	A, M	2011
GW-2	Oxic groundwater	A, RSF, M	2015
GW-3	Oxic groundwater	A, RSF	2017
GW-4	Oxic groundwater	M	2017
GW-5	Anoxic groundwater	A, RSF	2011
GW-6	Anoxic groundwater	A, RSF, A, RSF	2009
GW-7	Anoxic groundwater	A, RSF, S, RSF, IEX	2009
GW-8	Anoxic groundwater	A, RSF, A, RSF	2009
GW-9	Anoxic groundwater	A, RSF	2010
GW-10	Anoxic groundwater	A, RSF, A, S, RSF	2010
GW-11	Anoxic groundwater	NF[*], A, RSF, RSF	2010
GW-12	Anoxic groundwater	I[#]: A, RSF, KMnO4, RSF, ACF, UV II: A, S, RSF	2012
GW-13	Anoxic groundwater	M, A, RSF	2016
GW-14	Anoxic groundwater	A, RSF	2016
GW-15	Anoxic groundwater	A, RSF	2015
GW-16	Anoxic groundwater	A, RSF, A	2015
GW-17	Anoxic groundwater	A, RSF, S, RSF	2015
GW-18	Anoxic groundwater	A, RSF, S, RSF	2016
GW-19	Anoxic groundwater	A, RSF	2016
GW-20	Anoxic groundwater	A, RSF, A, RSF	2016
GW-21	Anoxic groundwater	S, A, RSF	2015
GW-22	Anoxic groundwater	A, S, RSF	2016
GW-23	Anoxic groundwater	RO[^], A, RSF, RSF, S, RSF, ACF, A, UV	2016
SW-1	Surface water	CA, RSF, UV/H2O2 (60%) followed by ACF, DI, A, RSF, or UF+RO (40%)[$], ClO2[&]	2015
SW-2	Surface water	CA, RSF, ACF, UV/H2O2 (75%) followed by ACF, DI, S, A, RSF, or UF+RO (25%)[$], ClO2[&]	2015
SW-3	Surface water	CA, RSF, DI, RSF, O, S, ACF, SSF	2017
SW-4	Surface water	DI, A, RSF, ACF, UV	2016
SW-5	Surface water	R, CA, RSF, UV, ACF, ClO2[&]	2012
SW-6	Surface water/groundwater	I[@]: R, CA, RSF, A, ACF, RSF, SSF, UV II: A, RSF, A	2016
SW-7	Surface water	R, CA, RSF, UV, ACF, ClO2[&]	2015
SW-8	Surface water	R, CA, O, RSF, ACF, ClO2[&]	2012
SW-9	Surface water	R, CA, RSF, O, S, ACF, SSF	2017
SW-10	Surface water/groundwater	I[@]: R, CA, RSF, O, ACF, ClO2[&] II: A, RSF, S, RSF	2013
SW-11	Surface water	R, S, CA, RSF, UV/H2O2, ACF, ClO2[&]	2013

[†] M = marble filtration, A = aeration, RSF = rapid sand filtration, S = softening, IEX = ion exchange, KMnO4 = KMnO4 dosing, NF = nanofiltration, ACF = active carbon filtration, UV = UV disinfection, CA = coagulation followed by sedimentation, flocculation and/or flotation, UV/H2O2 = UV + hydrogen peroxide disinfection, DI = dune infiltration, UF = ultrafiltration, RO = reverse osmosis filtration, O = ozone disinfection, SSF = slow sand filtration, ClO2 = chlorine dioxide disinfection,

[*] 40% treated with nanofiltration, 60% bypass

[#] Two different water types with different water quality are treated in separate treatment trains before being mixed at the end of the treatment

[^] 13% treated with RO, 87% bypass

[&] ClO2 concentration dosed is low, resulting in undetectable chlorine concentrations in the treated water leaving the plant, except for SW-11 where undetectable chlorine concentrations are observed after short residence time in the transport pipe of the distribution system

[$] After first RSF 60% (SW1) or 75% (SW2) of water is treated by UV/H2O2 followed by ACF, DI, A and RSF, the other 40% (SW1) or 25% (SW2) by UF followed by RO. The partial flows are subsequently mixed before ClO2.

[@] Surface water and groundwater are used for drinking water production. Surface water is treated according to treatment train I, groundwater is treated according to treatment train II. After treatment both water types are mixed.

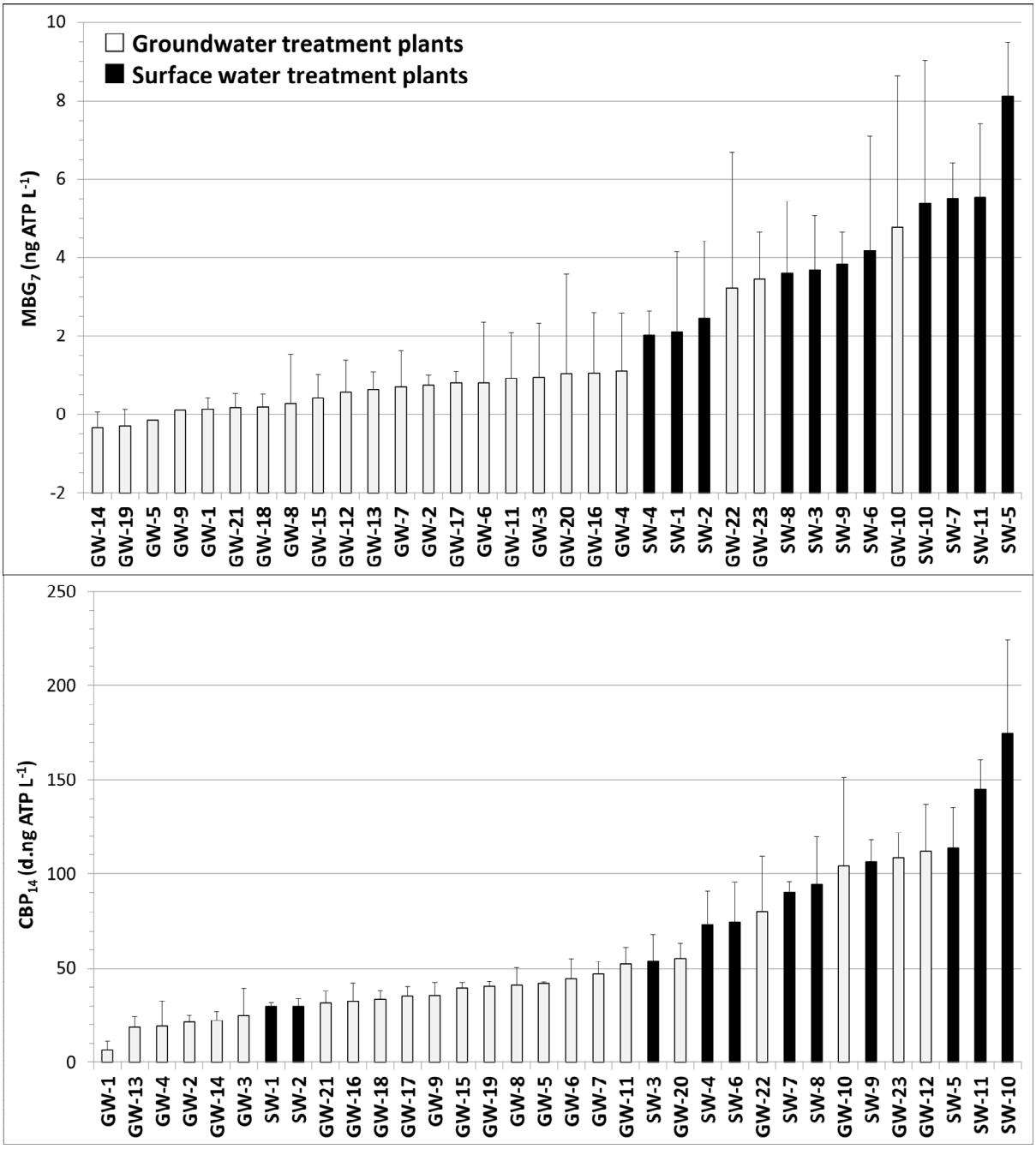


Figure S1. The mean MBG_7 (top) and CBP_{14} values (bottom) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

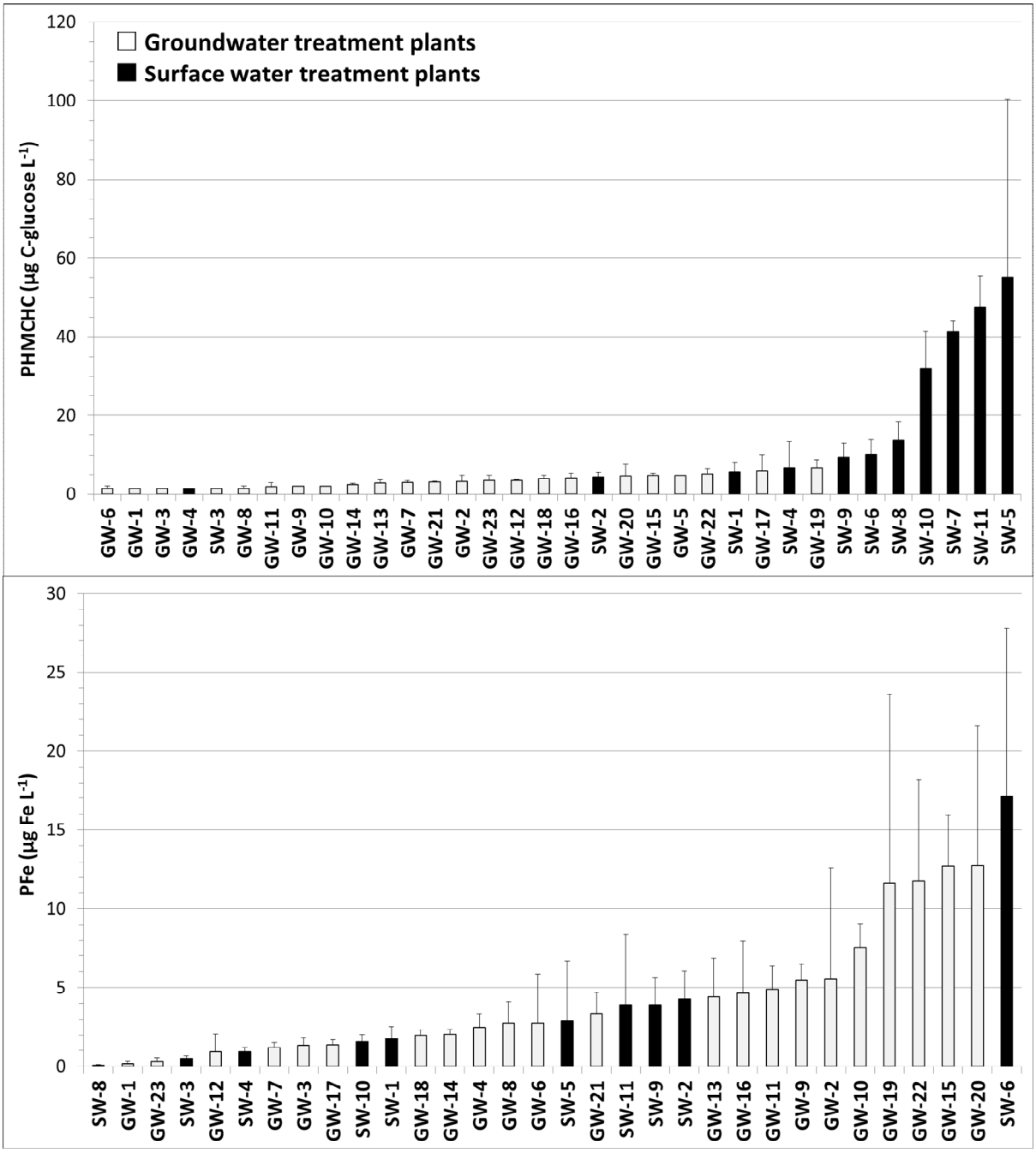


Figure S2. The mean PHMCHC (top) and PFe concentrations (bottom) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

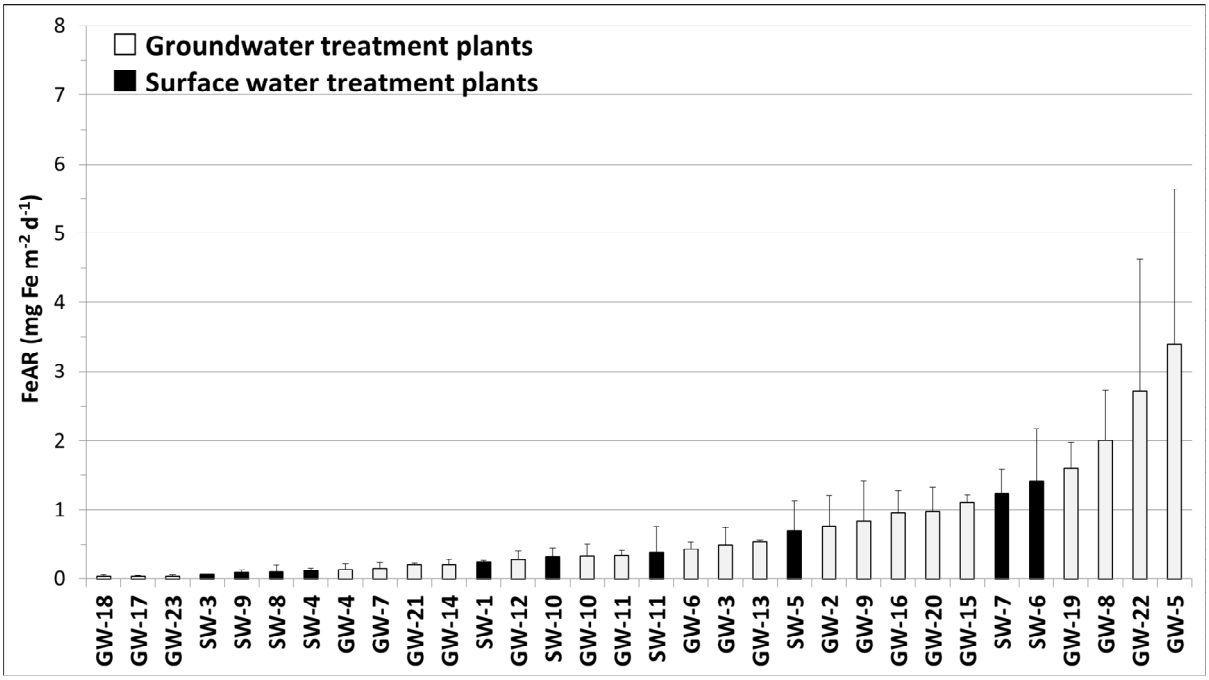


Figure S3. The mean FeAR values ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

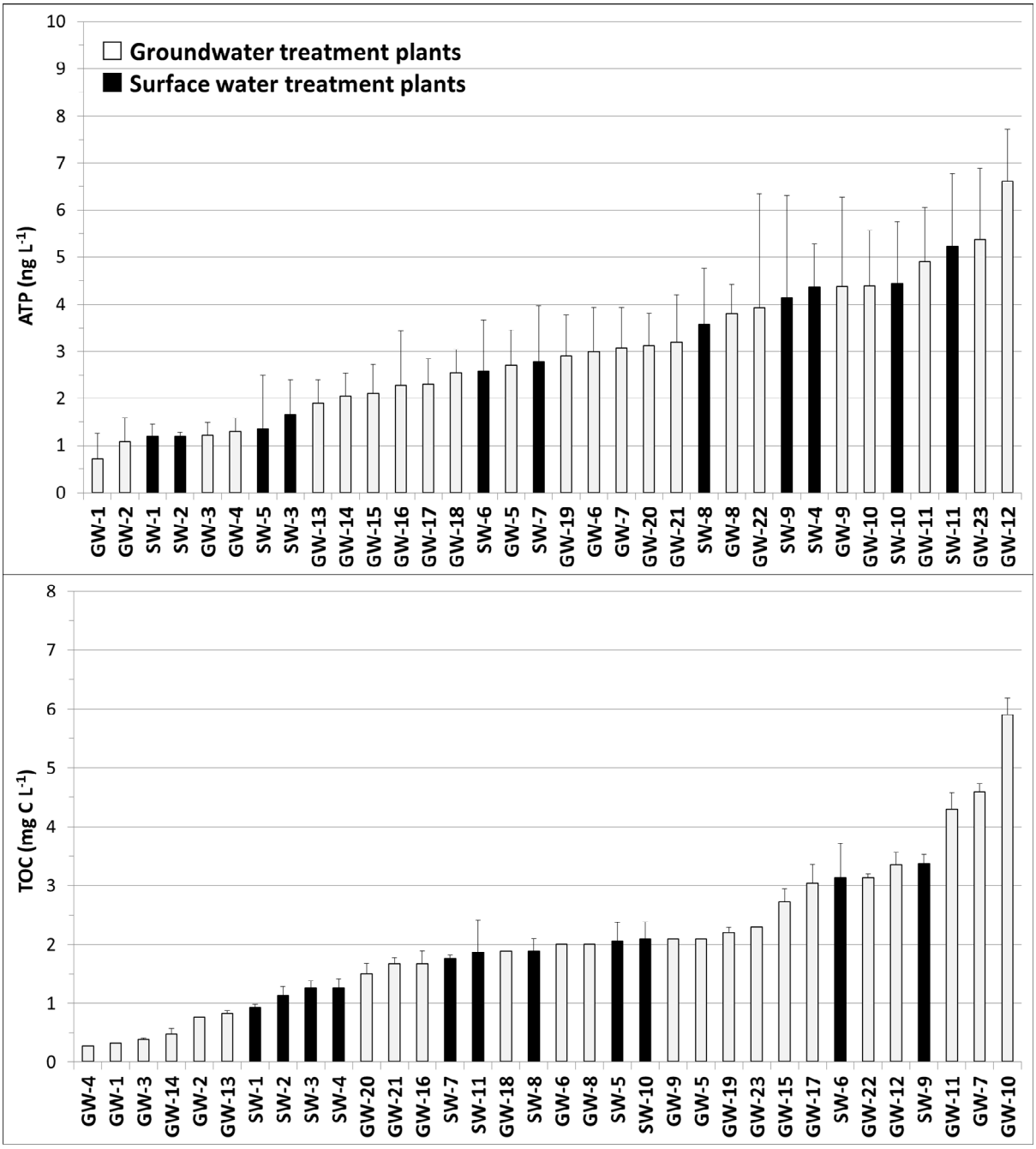


Figure S4. The mean ATP (top) and TOC concentrations (bottom) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized above.

Table S2. The R^2 values of the pair-wise correlation analysis between the different biological stability parameters (absolute and log-transformed values) measured in treated water of all 34 different treatment plants (top), 23 groundwater treatment plants (middle) and 11 surface water treatment plants (bottom). Light blue cells: $p<0.05$ & $R^2 < 0.4$; green cells: $p<0.05$ & $0.4 < R^2 < 0.9$; orange cells: $p<0.05$ & $R^2 > 0.9$.

All locations	ATP	Lg MBC_7	Lg CBP_{14}	Lg MBG_7	Lg AOC-A3	Lg BAR	Lg FeAR	TOC	Lg PHMOC	Lg PHMCHC	Lg PFe
ATP	1.00	0.46	0.50	0.03	0.19	0.17	0.01	0.35	0.02	0.03	0.00
Lg MBC_7		1.00	0.98	0.54	0.53	0.51	0.01	0.24	0.39	0.43	0.00
Lg CBP_{14}			1.00	0.49	0.52	0.50	0.00	0.20	0.39	0.41	0.00
Lg MBG_7				1.00	0.24	0.27	0.02	0.02	0.30	0.36	0.01
Lg AOC-A3					1.00	0.54	0.05	0.15	0.41	0.44	0.01
Lg BAR						1.00	0.11	0.06	0.43	0.44	0.00
Lg FeAR							1.00	0.00	0.03	0.01	0.51
TOC								1.00	0.05	0.04	0.04
Lg PHMOC									1.00	0.81	0.04
Lg PHMCHC										1.00	0.01
Lg PFe											1.00

Groundwater locations	ATP	Lg MBC_7	Lg CBP_{14}	Lg MBG_7	Lg AOC-A3	Lg BAR	Lg FeAR	TOC	Lg PHMOC	Lg PHMCHC	Lg PFe
ATP	1.00	0.73	0.72	0.11	0.39	0.36	0.00	0.43	0.01	0.02	0.00
Lg MBC_7		1.00	0.97	0.33	0.53	0.47	0.00	0.49	0.11	0.11	0.04
Lg CBP_{14}			1.00	0.29	0.53	0.45	0.00	0.54	0.13	0.12	0.05
Lg MBG_7				1.00	0.09	0.06	0.03	0.20	0.00	0.01	0.00
Lg AOC-A3					1.00	0.33	0.02	0.35	0.12	0.10	0.05
Lg BAR						1.00	0.16	0.10	0.14	0.11	0.02
Lg FeAR							1.00	0.00	0.02	0.01	0.53
TOC								1.00	0.03	0.05	0.04
Lg PHMOC									1.00	0.70	0.20
Lg PHMCHC										1.00	0.14
Lg PFe											1.00

Surface water locations	ATP	Lg MBC_7	Lg CBP_{14}	Lg MBG_7	Lg AOC-A3	Lg BAR	Lg FeAR	TOC	Lg PHMOC	Lg PHMCHC	Lg PFe
ATP	1.00	0.48	0.54	0.02	0.05	0.08	0.03	0.14	0.09	0.16	0.01
Lg MBC_7		1.00	0.98	0.56	0.41	0.31	0.09	0.34	0.58	0.56	0.00
Lg CBP_{14}			1.00	0.54	0.39	0.30	0.04	0.34	0.56	0.53	0.00
Lg MBG_7				1.00	0.50	0.37	0.30	0.21	0.81	0.55	0.04
Lg AOC-A3					1.00	0.78	0.31	0.09	0.84	0.88	0.00
Lg BAR						1.00	0.27	0.05	0.63	0.67	0.01
Lg FeAR							1.00	0.06	0.41	0.42	0.52
TOC								1.00	0.15	0.11	0.18
Lg PHMOC									1.00	0.90	0.04
Lg PHMCHC										1.00	0.04
Lg PFe											1.00

Table S3. The R^2 values of the pair-wise correlation analysis between the different microbiological parameters (absolute and log-transformed values) measured in distributed water of all 34 different treatment plants (top), 23 groundwater treatment plants (middle) and 11 surface water treatment plants (bottom). Green cells: $p<0.05$ & $0.4 < R^2 < 0.9$

All locations	**Lg $HPC22_{gm}$**	**Lg *Aeromonas* $_{90P}$**
Lg $HPC22_{gm}$	**1.00**	**0.64**
Lg *Aeromonas* $_{90P}$		**1.00**
Groundwater locations	**Lg $HPC22_{gm}$**	**Lg *Aeromonas* $_{90P}$**
Lg $HPC22_{gm}$	**1.00**	**0.62**
Lg *Aeromonas* $_{90P}$		**1.00**
Surface water locations	**Lg $HPC22_{gm}$**	**Lg *Aeromonas* $_{90P}$**
Lg $HPC22_{gm}$	**1.00**	**0.75**
Lg *Aeromonas* $_{90P}$		**1.00**

Table S4. Top 4 ranking of drinking water treatment plants for HPC22 and *Aeromonas* and the ranking of these top 4 plants for each biological stability parameter when only the 23 groundwater treatment plants are included. When at least three of the top 4 treatment plants rank within the top 25% of all groundwater treatment plants for a biological stability parameter, then this parameter is highlighted in grey.

Treatment	HPC22 (cfu/ml)	ATP	MBC_7	CBP_{14}	MBG_7	AOC-A3	BAR	FeAR	TOC	PHMOC	PHMCHC	PFe
plant	Geomean	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank
GW-12	32.2	1	2	1	14	2	2	15	4	14	9	20
GW-22	28.5	6	4	4	3	12	1	2	5	3	3	3
GW-10	14.5	4	3	3	1	1	10	14	1	16	16	5
GW-8	11.3	7	10	10	16	4	5	3	12	20	19	13
Treatment	*Aeromonas* (cfu/100 ml)	ATP	MBC_7	CBP_{14}	MBG_7	AOC-A3	BAR	FeAR	TOC	PHMOC	PHMCHC	PFe
plant	90-Percentile	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank
GW-11	2,680	3	6	6	8	18	12	13	3	19	18	8
GW-10	900	4	3	3	1	1	10	14	1	16	16	5
GW-22	585	6	4	4	3	12	1	2	5	3	3	3
GW-20	510	9	5	5	6	17	11	6	17	12	6	1

Table S5. Top 4 ranking of drinking water treatment plants for HPC22 and *Aeromonas* and the ranking of these top 4 plants for each biological stability parameter when only the 11 surface water treatment plants are included. When at least two of the top 4 treatment plants rank within the top 30% of all surface water treatment plants for a biological stability parameter, then this parameter is highlighted in grey.

Treatment	HPC22 (cfu/ml)	ATP	MBC_7	CBP_{14}	MBG_7	AOC-A3	BAR	FeAR	TOC	PHMOC	PHMCHC	PFe
plant	Geomean	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank
SW-7	16.0	6	5	6	3	2	1	2	7	2	3	11
SW-10	9.3	9	1	1	4	11	6	5	3	4	4	7
SW-5	9.1	2	3	3	1	1	2	3	4	1	1	5
SW-6	7.6	7	7	7	5	6	9	1	2	7	6	1
Treatment	*Aeromonas* (cfu/100 ml)	ATP	MBC_7	CBP_{14}	MBG_7	AOC-A3	BAR	FeAR	TOC	PHMOC	PHMCHC	PFe
plant	90-Percentile	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank	Rank
SW-7	3,720	6	5	6	3	2	1	2	7	2	3	11
SW-5	3,100	2	3	3	1	1	2	3	4	1	1	5
SW-6	3,050	7	7	7	5	6	9	1	2	7	6	1
SW-10	1,170	9	1	1	4	11	6	5	3	4	4	7

Table S6. The significance and R^2 values of the correlations between log $HPC22_{gm}$ and the different biological stability parameters in the treated water

	Drinking water produced from groundwater		Drinking water produced from surface water	
Parameter	P value	R^2	P value	R^2
ATP	< 0.05	0.34	> 0.05	-
TOC	< 0.05	0.56	> 0.05	-
Log MBC_7	< 0.05	0.35	> 0.05	-
Log MBG_7	> 0.05	-	< 0.05	0.76
Log CBP_{14}	< 0.05	0.37	> 0.05	-
Log AOC-A3	< 0.05	0.23	> 0.05	-
Log PHMOC	> 0.05	-	< 0.05	0.62
Log PHMCHC	> 0.05	-	> 0.05	-
Log PFe	> 0.05	-	> 0.05	-
Log BAR	< 0.05	0.21	> 0.05	-
Log FeAR	> 0.05	-	> 0.05	-

Table S7. The significance and R^2 values of the correlations between log *Aeromonas*$_{90p}$ and the different biological stability parameters in the treated water

	Drinking water produced from groundwater		Drinking water produced from surface water	
Parameter	P value	R^2	P value	R^2
ATP	< 0.05	0.33	> 0.05	-
TOC	< 0.05	0.53	> 0.05	-
Log MBC_7	< 0.05	0.43	> 0.05	-
Log MBG_7	> 0.05	-	< 0.05	0.56
Log CBP_{14}	< 0.05	0.45	> 0.05	-
Log AOC-A3	> 0.05	-	> 0.05	-
Log PHMOC	> 0.05	-	< 0.05	0.46
Log PHMCHC	> 0.05	-	> 0.05	-
Log PFe	> 0.05	-	> 0.05	-
Log BAR	< 0.05	0.28	> 0.05	-
Log FeAR	> 0.05	-	< 0.05	0.69

Table S8. Threshold and guidance values based on data from 23 different groundwater treatment plants for biological stability parameters in treated water related to HPC22 (geometric mean of 20 cfu mL^{-1}) and *Aeromonas* (90-percentile of 800 cfu 100 mL^{-1}) in the distribution system. The threshold values were based on ranking or regression of the relationship between biological stability parameters in treated water and the biological parameters in the distribution system. The guidance value is the mean of the threshold values based on ranking and regression.

	Threshold value		Guidance value
Parameter	Ranking	Regression	Final
Related to HPC22			
TOC (mg C L^{-1})	3.1	5.2	4.1
MBC_7 (ng ATP L^{-1})	6.8	N.C.*	N.C.&
CBP_{14} (d.ng ATP L^{-1})	80	N.C.	N.C.
AOC-A3 (µg C L^{-1})	2.1	N.C.	N.C.
BAR (pg ATP cm^{-2} day^{-1})	34	N.C.	N.C.
Related to Aeromonas			
MBC_7 (ng ATP L^{-1})	8.2	9.0	8.6
CBP_{14} (d.ng ATP L^{-1})	104	116	110
TOC (mg C L^{-1})	5.9	2.3	4.1
PFe (µg Fe L^{-1})	4.9	N.C.	N.C.

* N.C. is not calculated, because regression was not significant ($p>0.05$) or R^2 value was low (< 0.4)
& N.C. is not calculated, because ranking and/or regression did not have a reliable value
# N.C. is not calculated, because value could not be deduced based on ranking method

Table S9. Threshold and guidance values based on data from 11 different surface water treatment plants for biological stability parameters in treated water related to HPC22 (geometric mean of 20 cfu mL^{-1}) and *Aeromonas* (90-percentile of 800 cfu 100 mL^{-1}) in the distribution system. The threshold values were based on ranking or regression of the relationship between biological stability parameters in treated water and the biological parameters in the distribution system. The guidance value is the mean of the threshold values based on ranking and regression.

	Threshold value		Guidance value
Parameter	Ranking	Regression	Final
Related to HPC22			
MBG_7 (ng ATP L^{-1})	N.C.[#]	8.8	N.C.[&]
PHMOC (µg C L^{-1})	N.C.	231	N.C.
Related to Aeromonas			
MBG_7 (ng ATP L^{-1})	4.2	4.7	4.5
PHMOC (µg C L^{-1})	54	40	47
PHMCHC (µg C L^{-1})	32	N.C.[*]	N.C.
FeAR (mg Fe m^{-2} day^{-1})	0.32	0.36	0.34
MBC_7 (ng ATP L^{-1})	6.9	N.C.	N.C.
CBP_{14} (d.ng ATP L^{-1})	75	N.C.	N.C.
AOC-A3 (µg C L^{-1})	4.1	N.C.	N.C.
TOC (mg C L^{-1})	1.8	N.C.	N.C.
BAR (pg ATP cm^{-2} day^{-1})	15	N.C.	N.C.

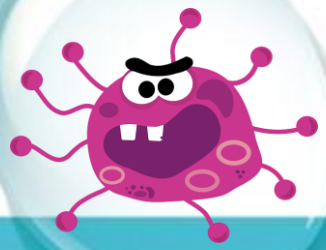
[*] N.C. is not calculated, because regression was not significant ($p>0.05$) or R^2 value was low (< 0.4)
[&] N.C. is not calculated, because ranking and/or regression did not have a reliable value
[#] N.C. is not calculated, because value could not be deduced based on ranking method

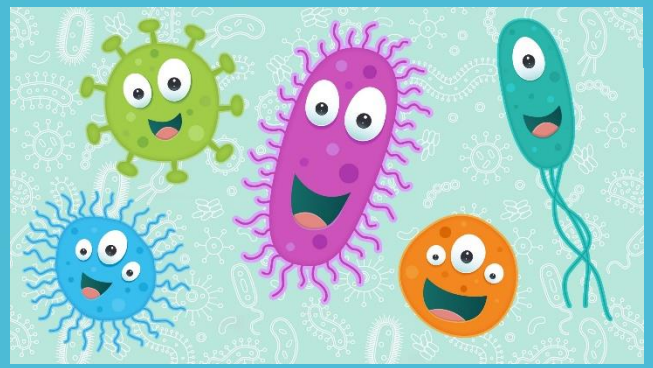
III Presentaties van de workshop

III Presentation Introductie – Wim Hijnen

Biologische stabiliteit parameters in de praktijk

Introductie





KWR workshop 3 oktober 2022

Wim Hijnen

Groei van micro-organismen in drinkwater distributiesystemen

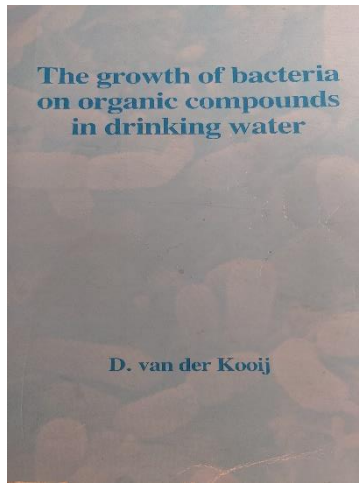
- Bedrijfsonderzoek 1972 Hoofdafdeling Speurwerk, KIWA N.V. Instituut v/d Drinkwaterbedrijven: 1978, Colloquium KIWA/VWN

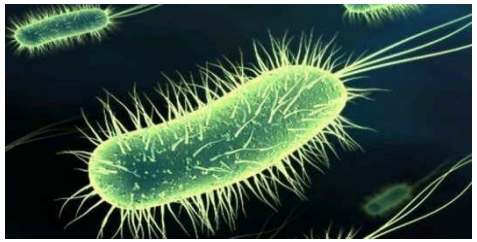
  ‘Methodenontwikkeling t.b.v. waterkwaliteitsmeting’

- Nagroei:
  1. Hogere organismen
  2. Reuk/smaak
  3. Materialen aantasting
  4. Kwaliteitsbeoordeling
  5. Opportunistisch pathogenen
- Dick van der Kooij, 1978
  - Groei bacterien in drinkwater
  - Methode groeibevorderende stoffen in water: AOC







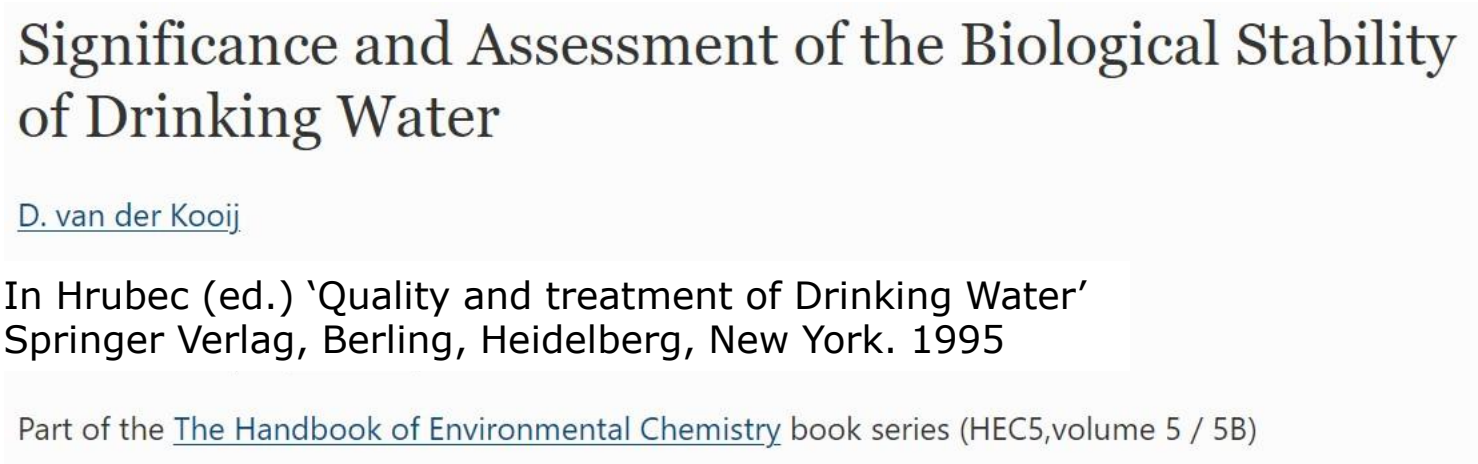

AOC bepaling

- Proefschrift Dick van der Kooij stelling 1984: ‘De kinetiek van de groei van een micro-organisme op een groeibeperkend substraat kan met behulp van batch-cultures op een eenvoudige wijze worden vastgetseld, mits de groei van het organisme kan worden gemeten met behulp van koloniegetalbepalingen’
- AOC methode met reincultures: *Pseudomonas fluorescens* P17 en later voor ozonbehandeld water *Spirillum* NOX
- Internationaal geaccepteerd en toegepast: Researchgate

Biologische stabiliteit

- Nagroei - regrowth: nieuwe term wordt biologische stabiliteit
- Rittman, B.E. and Snoeyink, V.L. (1984) Achieving biological stable drinking water. J. Am. Wat.Works Assoc. 76(10), 106-114.

'Biologisch stabiel drinkwater bevordert geen overmatige groei van micro-organismen.'



Significance and Assessment of the Biological Stability of Drinking Water

D. van der Kooij

In Hrubec (ed.) 'Quality and treatment of Drinking Water'
Springer Verlag, Berling, Heidelberg, New York. 1995

Part of the The Handbook of Environmental Chemistry book series (HEC5,volume 5 / 5B)

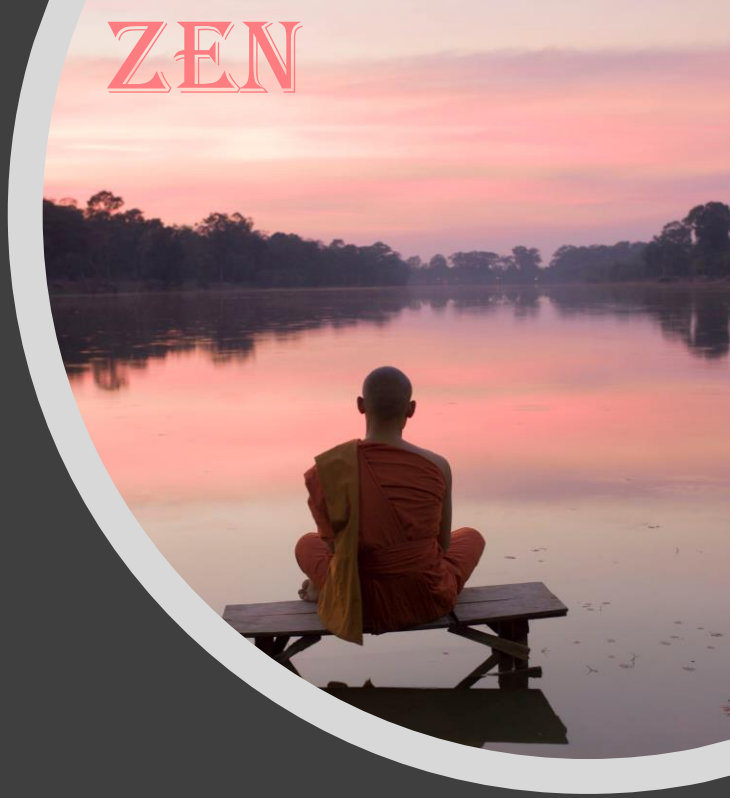
Biologische stabiliteit: definitie bedrijfstak

Biologische stabiliteit van drinkwater beschrijft een drinkwatersysteem dat tot een zo min mogelijke biologische verandering leidt, zodat gezondheidsrisico's en/of consumentenklachten, gerelateerd aan groei van (micro-)organismen, niet kunnen optreden.

Het gaat over de drinkwaterkwaliteit in een distributiegebied

biologisch stabiel drinkwatersysteem = biologisch probleemloos systeem

Wens van de bedrijfstak

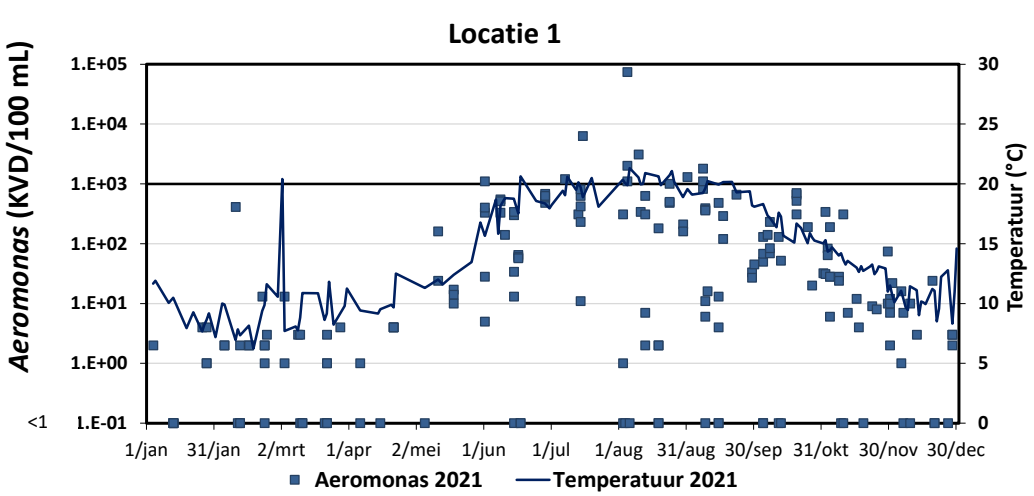


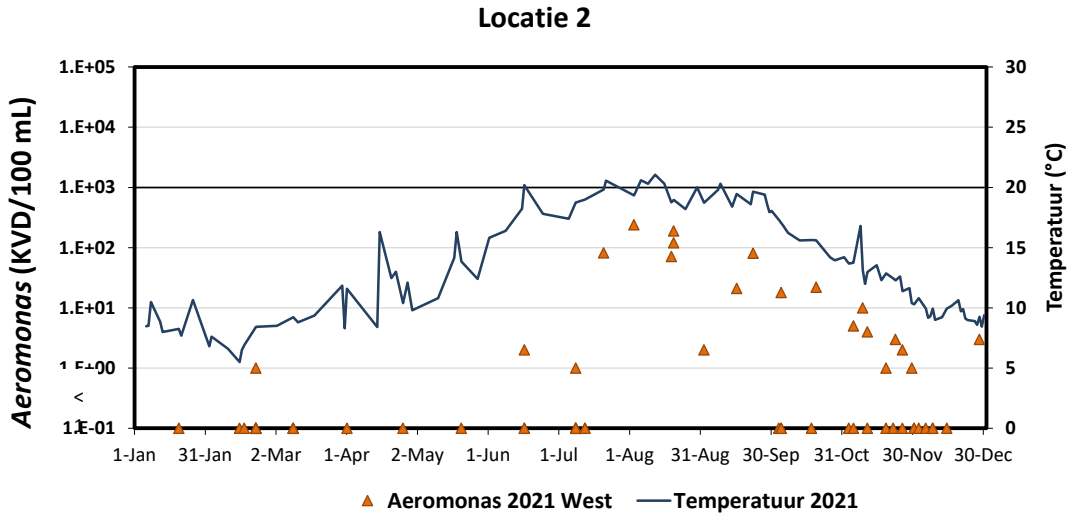
Stabiliteit, evenwicht, veiligheid

Biologische stabiliteit = preventief net als AMVD

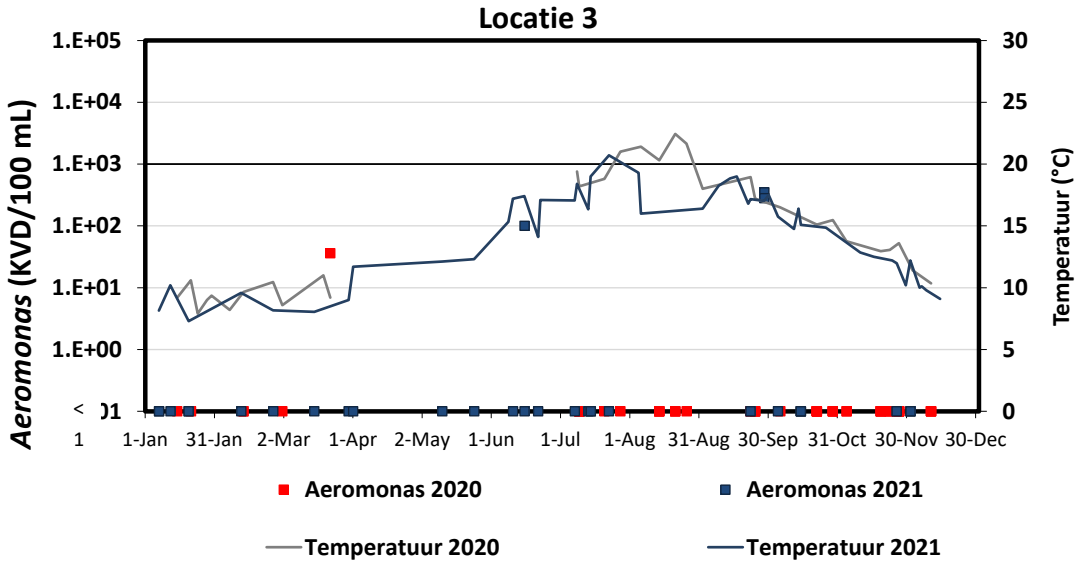
Biologisch stabiel distributiesysteem

- Instabiel, stabieler en stabielst ……..





- Locatie 3 >locatie 2 >locatie 3
- Locatie 3: meerdere nagroeiproblemen



Methoden

Momenteel: biologische stabiliteit is voornamelijk drinkwater kwaliteit

- 1928 – 2014 e.v. gepubliceerde methoden met aanpassingen

TABEL 1 OVERZICHT VAN DE VERSCHILLENDE METHODEN ONTWIKKELD OM DE BACTERIEGROEI BEVORDERENDE EIGENSCHAPPEN VAN (DRINK)WATER TE KWANTIFICEREN

Jaar	Auteurs	Principe
1928	Heymann	Afname $KMnO_4$ van water in zandfilter
1979, 1982	van der Kooij et al.	KVE reincultuur P17; ijklijn acetaat; AOC
1984	van der Kooij Hijnen	KVE reinculturen P17/Nox; ijklijn/acetaat AOC
1985	Werner	Troebelheidstoename
1986	Joret et Lévi	DOC afname bij incubatie met zand; BDOC
1987	Servais	BDOC zonder zand
1988	Standfield Jago	ATP productie en ijklijn op acetaat-C
1993	LeChevallier et al.	AOC reincultuur; ATP i.p.v. koloniegetal; ijklijn acetaat
1990	Lucena et al.	DOC afname door kolom met glasparels; BDOC
2004	Haddix et al.	AOC reinculturen; fluorescentie meting; ijklijn acetaat
2005	Hammes Egli	Celtelling FCM; ijklijn op acetaat-C
2009	Weinrich et al.	AOC reincultuur; luminescentie, aectaat ijklijn
2009	van der Kooij Veenendaal	Zeewater AOC; reincultuur *Halomonas cupida*
2011	Weinrich et al.	Zeewater AOC; reincultuur *Vibrio harvey*; ijklijn acetaat
2011	Sack et al.	KVE reincultuur A3, ijklijn µg biopolymeren-C/l
2014	van der Kooij Veenendaal	BPP test voor water cumulatieve ATP productie (d.ng/l)

Bron: Hijnen en van der Wielen, 2017

Methoden

Momenteel: biologische stabiliteit voornamelijk gericht op drinkwater kwaliteit en materialen

- 1928 – 2014 e.v. detectie van ….

Biomassagroei
Reincultuur/ijklijn water (1982)
ATP water +/- materialen (1988-2014)
ATP biofilm (1995)
Celtelling water (2005)

- Toekomst: naast biologisch stabiel drinkwater ook infrastructurele voorwaarden?

State of the art 2022: deze workshop

- Breed scala aan methoden/parameters
- Oude richtwaarden (AOC, BDOC) onvoldoende
- Nieuwe NL methoden sinds 2014

Aandachtswaarden voor biologisch stabiel drinkwater: grondwater en oppervlaktewater

Programma

PROGRAMMA

9:30 – 10:00	Inloop met koffie
10:00 – 10:10	Introductie – Wim Hijnen, Evides
10:10 – 10:40	Nieuwe biologische stabiliteitsparameters en aandachtswaaren – Paul van der Wielen, KWR
10:40 – 11:10	Meten van biologische stabiliteit in praktijk van DPW-bedrijven – Aleksandra Knezev, HWL
11:10 – 11:30	Koffie
11:30 – 12:00	Biologische stabiliteit bij Oasen. Een nieuw proces, nog stabieler? – Maarten Lut, Oasen
12:00 – 13:00	Lunch
13:00 – 13:30	Betekenis van de nieuwe biostabiliteitsmethoden voor het onderzoek naar ongewenste nagroei bij Evides – Wim Hijnen/Rinnert Schurer, Evides
13:30 – 14:00	Biologische stabiliteit bepaling bij PWN: combinatie van laboratorium en on-site metingen – Emmanuelle Prest, PWNT
14:00 – 14:30	Break
14:30 – 16:00	Discussiesessie
16:00 - …	Borrel

IV Presentation Nieuwe biologische stabiliteitsparameters en aandachtswaarden – Paul van der Wielen

3 oktober 2022 – Workshop biologische stabiliteitsparameters in de praktijk

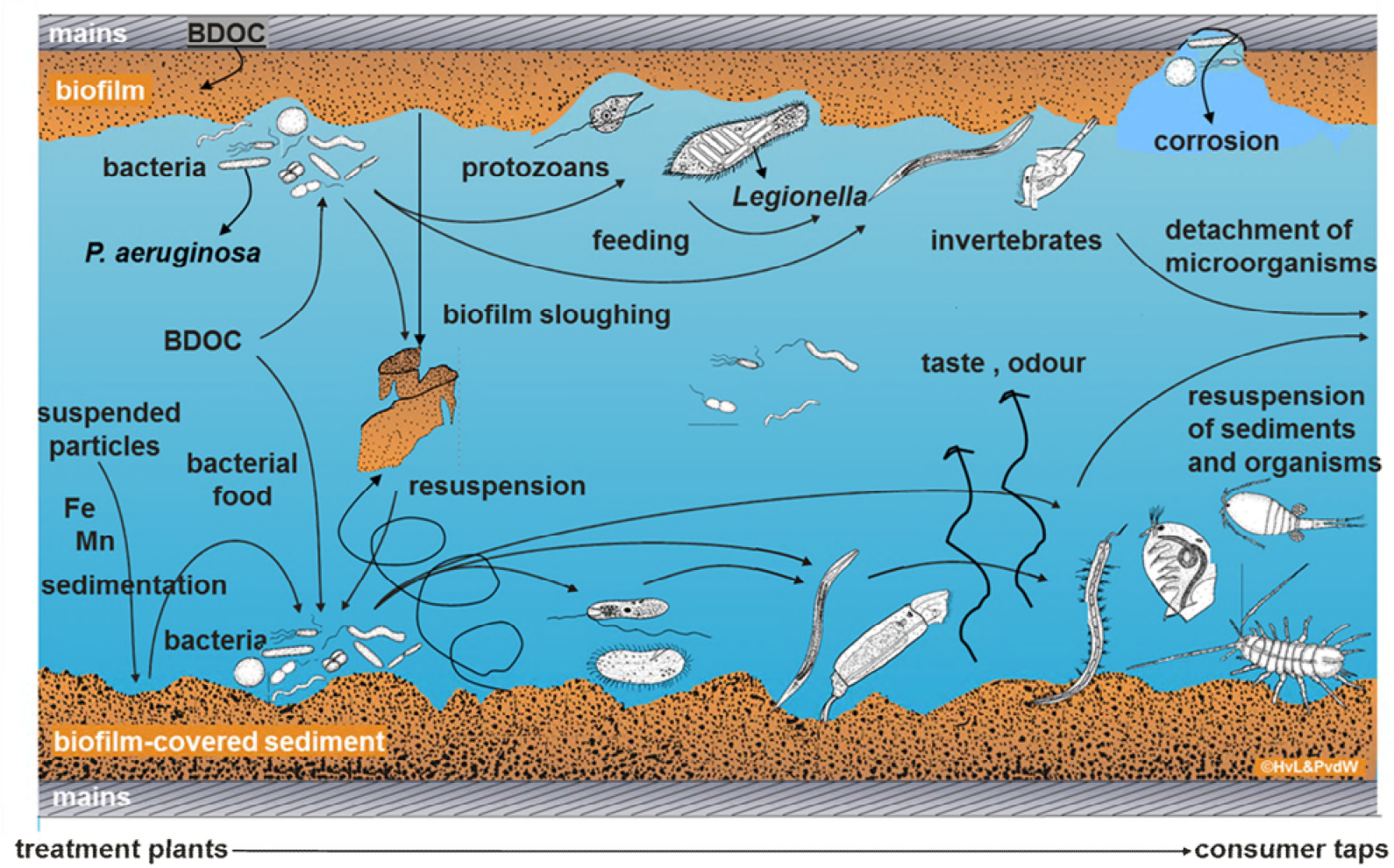
Aandachtswaarden biologische stabiliteits-parameters

Paul van der Wielen

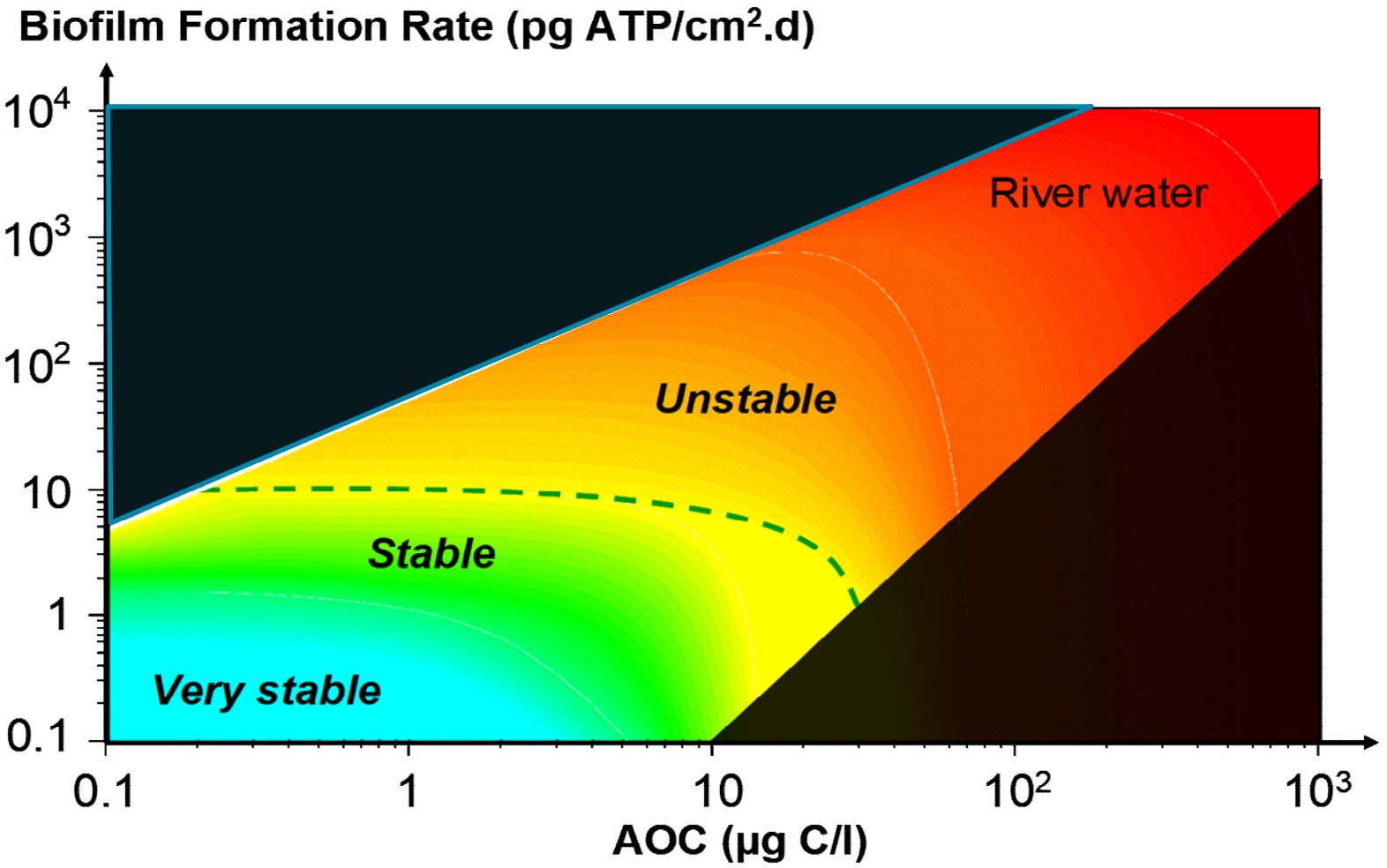
KWR

Bridging Science to Practice

Nagroei in drinkwaterdistributiesysteem



Biologische stabiliteit was twee dimensies

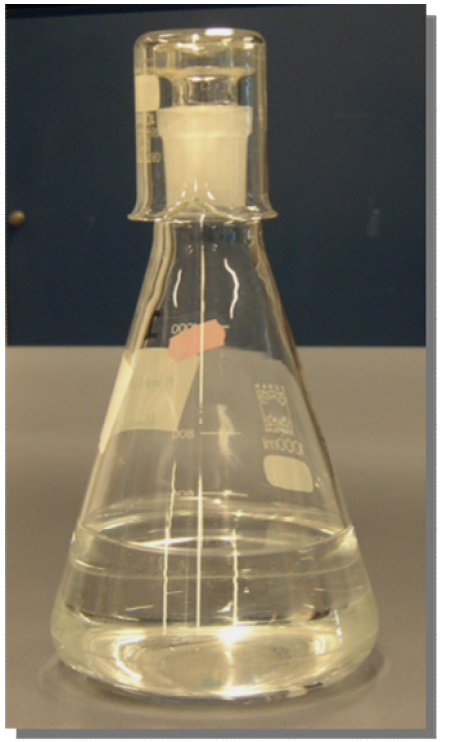


Nieuwe methoden biologische stabiliteit – waarom

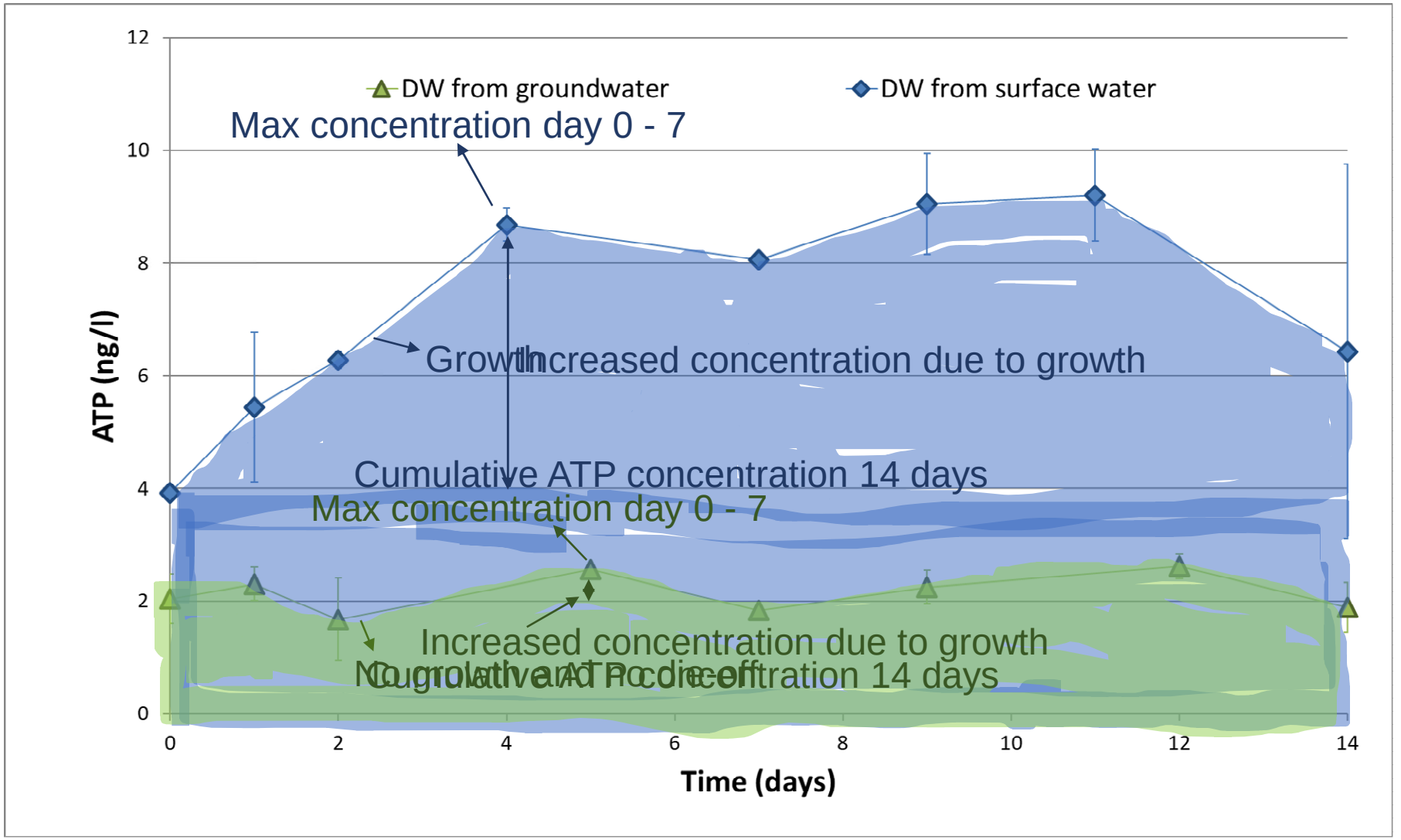
- AOC en BVS beschreven de biologische stabiliteit en waren gerelateerd aan KG22 en *Aeromonas* in distributiesysteem
- Drinkwaterbedrijven zuivering geoptimaliseerd zodat AOC en BVS aan richtwaarde voldeed, verwachting dat KG22 en *Aeromonas* zouden afnemen
- Klopt voor KG22, maar niet voor *Aeromonas*
- Onduidelijk waarom, maar misschien dat ook moeilijk assimileerbaar organisch koolstof een belangrijke rol speelt
- Nieuwe methoden voor biologische stabiliteit ontwikkelt en toegepast tijdens de periode 2010 – 2020
- Deze nieuwe methoden bepalen de groeipotentie, de biofoulingspotentie of beide van het drinkwater

Biomassaproductiepotentie (BBP) test

- Drinkwatermonsters worden genomen in AOC-vrije flessen
- Monsters worden niet gepasteuriseerd en de van nature aanwezige microbiële gemeenschap wordt gebruikt in de analyse
- Flessen worden 14 dagen geïncubeerd bij 25°C
- Op specifieke dagen worden monsters genomen en de ATP concentratie gemeten



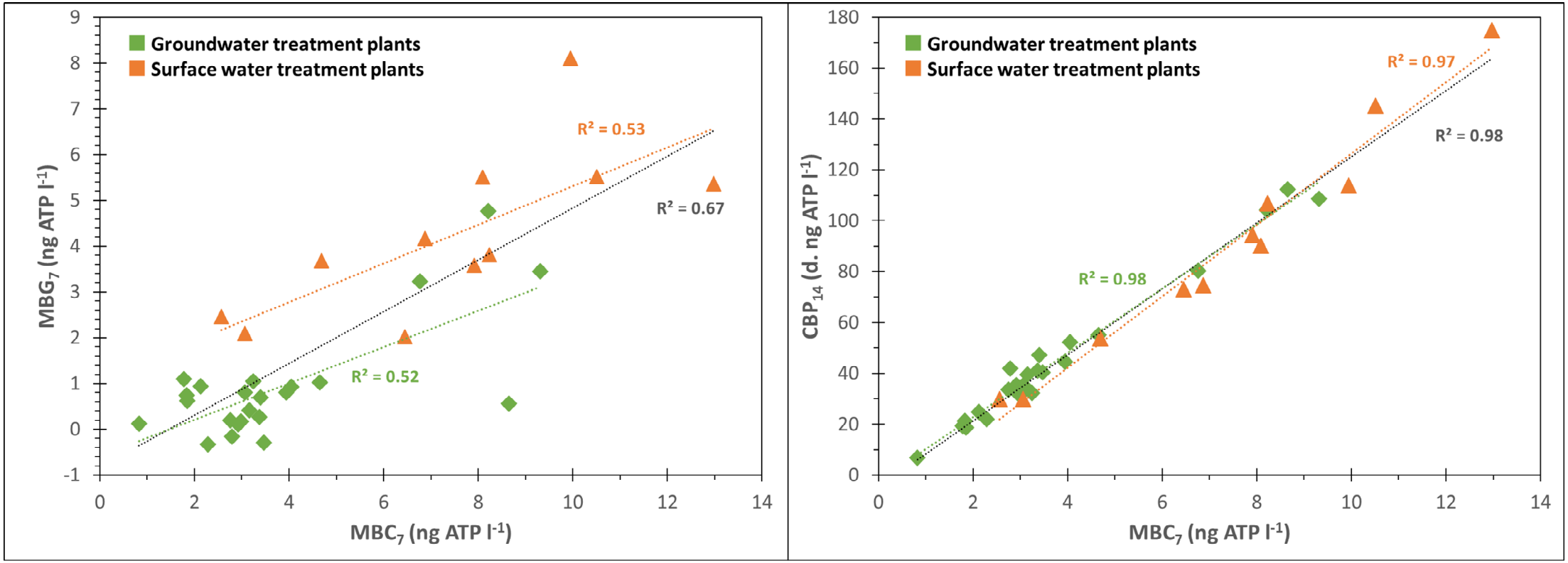
Biomassaproductiepotentie (BBP) test



Parameters van de BPP-test

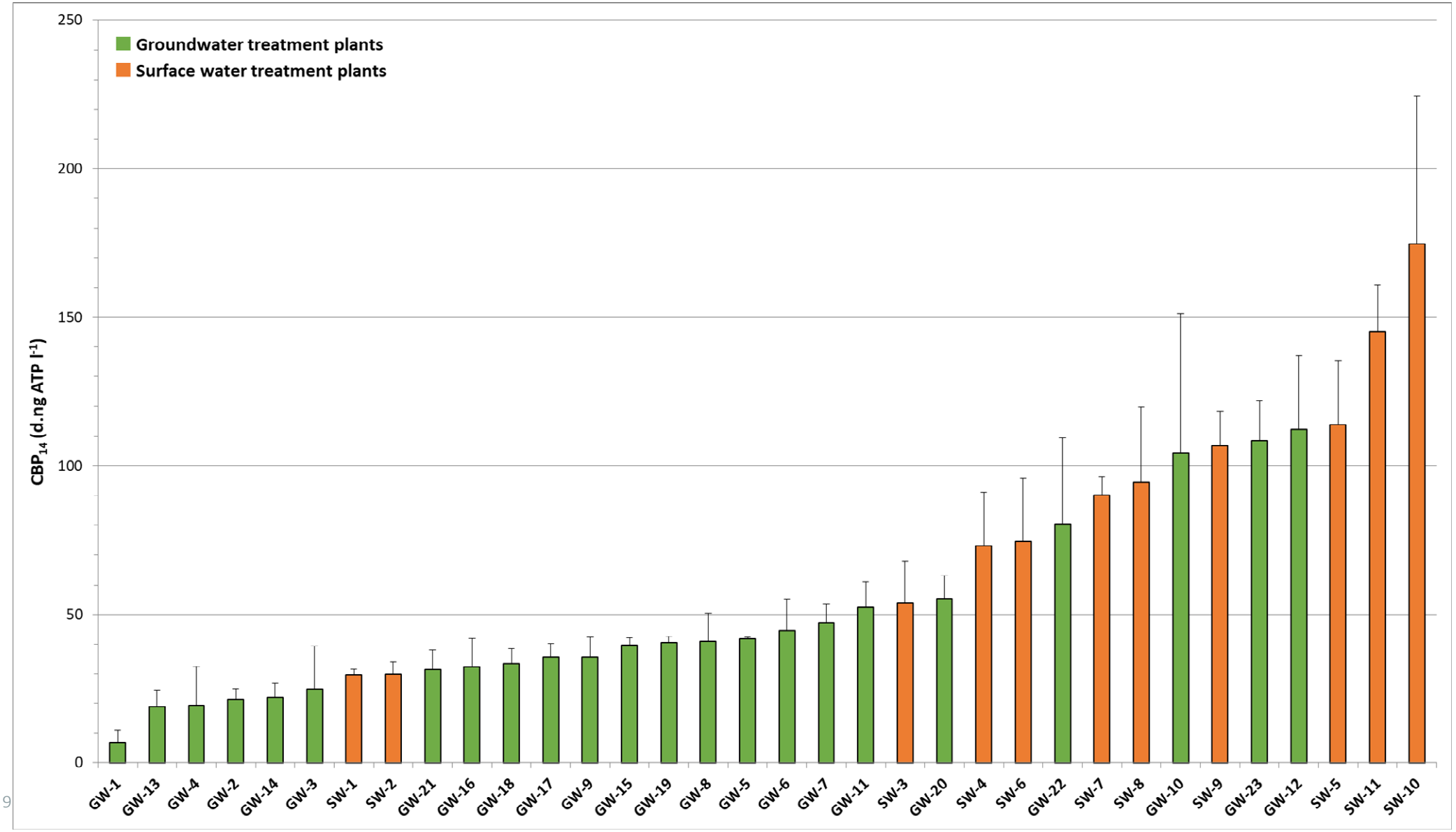
- De volgende parameters kunnen uit de BPP-test worden afgeleid:
  - MBC_7 (ng ATP/L) (= BP_7)
    - Maximum biomassaconcentratie gedurende de eerste 7 dagen van de incubatie
    - Indicatie voor gemakkelijk afbreekbare verbindingen ?
  - MBG_7 (ng ATP/L) (= $BP_7 – BP_0$)
    - Maximum biomassagroei gedurende de eerste 7 dagen van de incubatie
    - Een (betere?) indicatie voor gemakkelijk afbreekbare verbindingen
  - CBP_{14} (d.ng ATP/L) (= BPC_{14})
    - Cumulatieve biomassaproductie gedurende 14 dagen incubatie
    - Indicatie voor totale concentratie afbreekbare verbindingen

Relatie tussen parameters BPP test

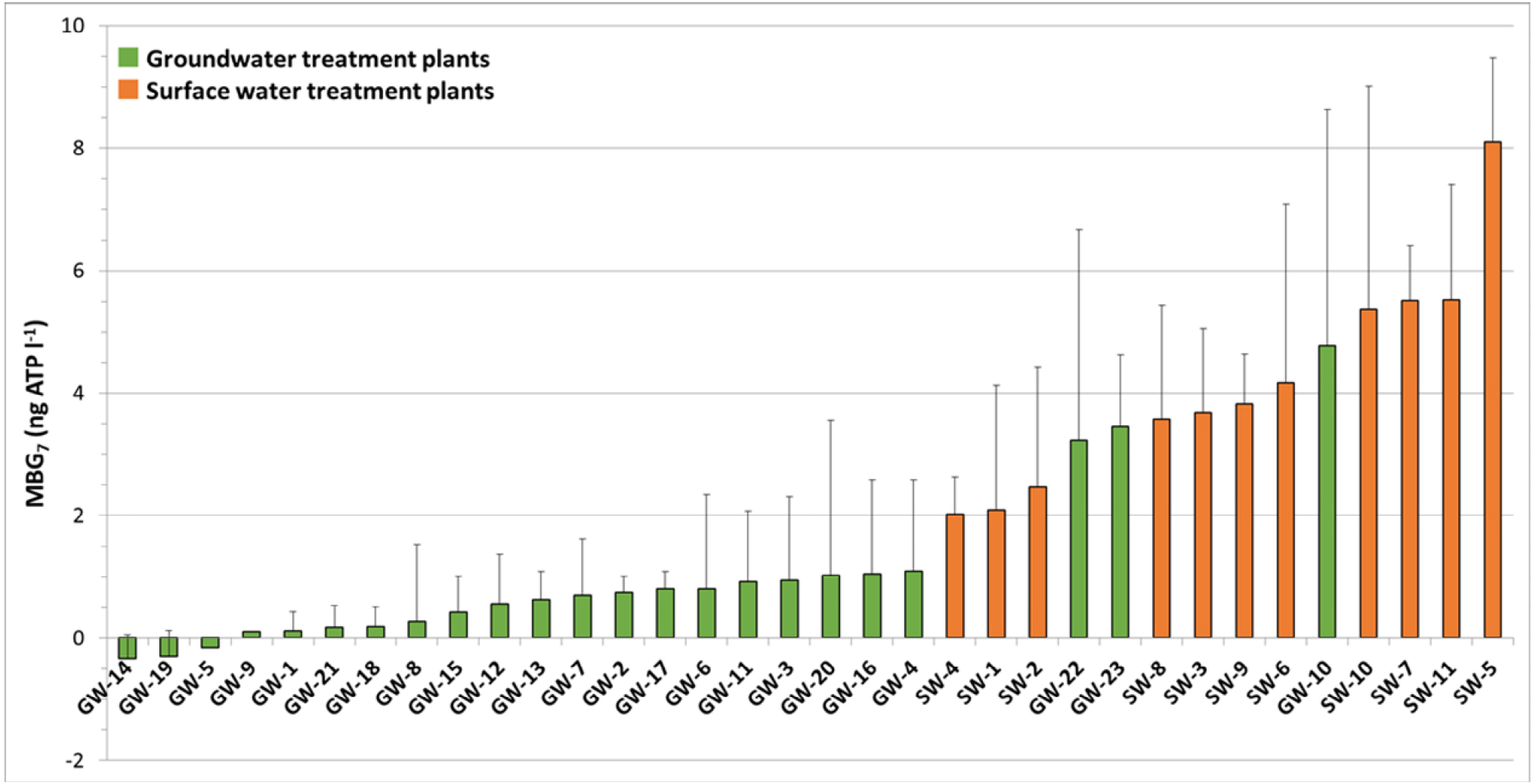


- MBC_7 en MBG_7 niet sterk gecorreleerd, beide parameters bruikbaar
- MBC_7 en CBP_{14} sterk gecorreleerd, één van de twee is voldoende

CBP_{14} in reinwater 34 verschillende locaties

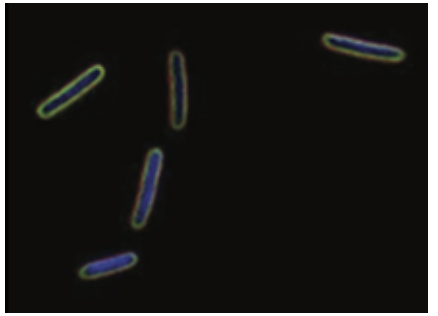


MBG_7 in reinwater 34 verschillende locaties

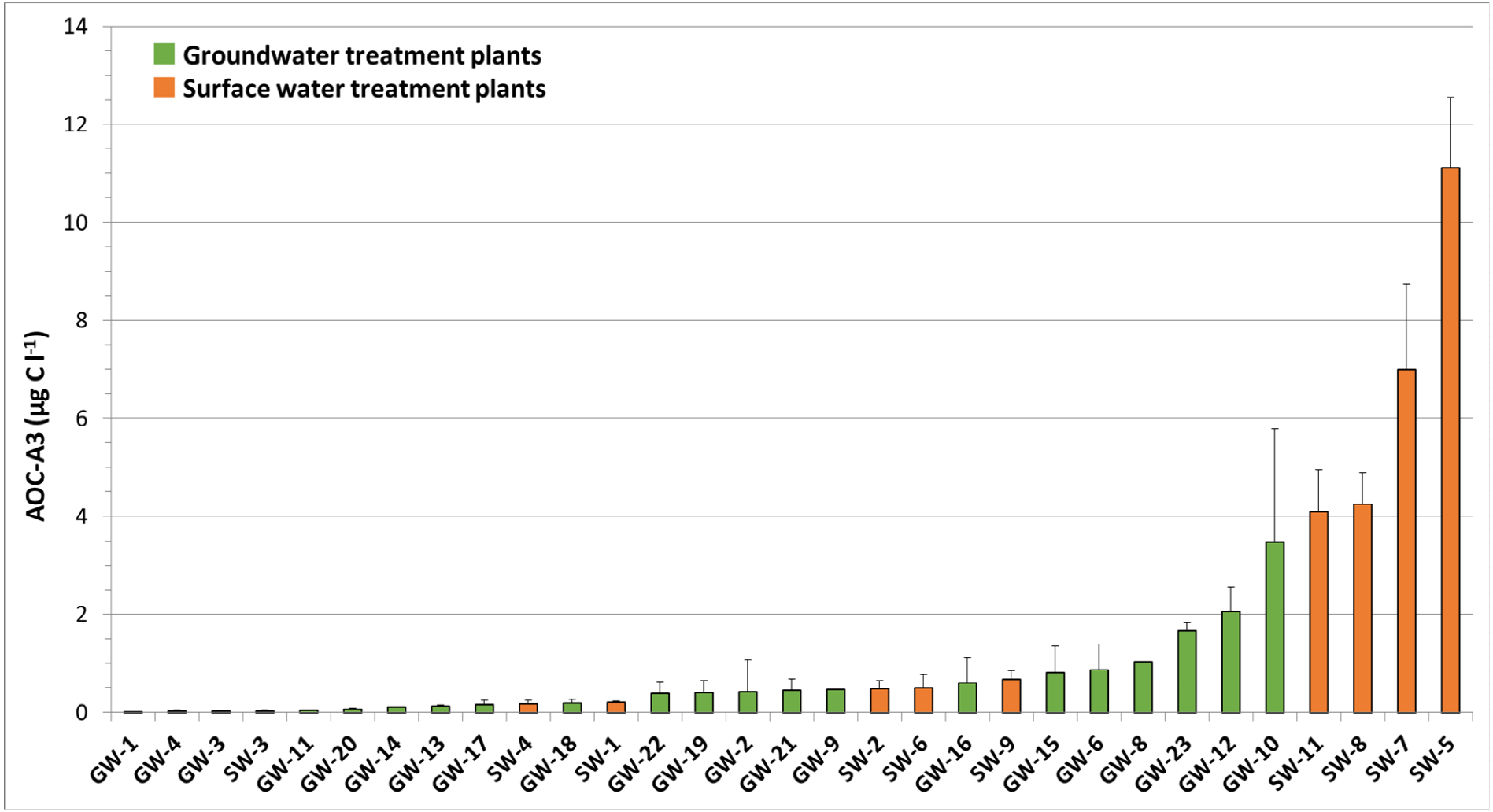


AOC-A3

- Traditionele AOC (P17/Nox) bepaalt carbonzuren en aminozuren, maar niet biopolymeren zoals suikers en eiwitten
- Stam *Flavobacterium johnsonii* A3 geïsoleerd die gespecialiseerd is in afbraak van biopolymeren (maar kan ook aminozuren benutten)
- In AOC-A3 test worden stam A3 en P17 (gespecialiseerd in aminozuurafbraak) toegevoegd
  - Stam P17 groeit sneller op aminozuren en worden door P17 benut voordat A3 ze kan benutten
  - Stam A3 groeit daardoor alleen op biopolymeren
- Maximum kolonieaantallen van A3 worden gebruikt om AOC-A3 concentraties te berekenen met de yield factor van stam A3
- Concentraties worden uitgedrukt als µg biopolymeren-C/L



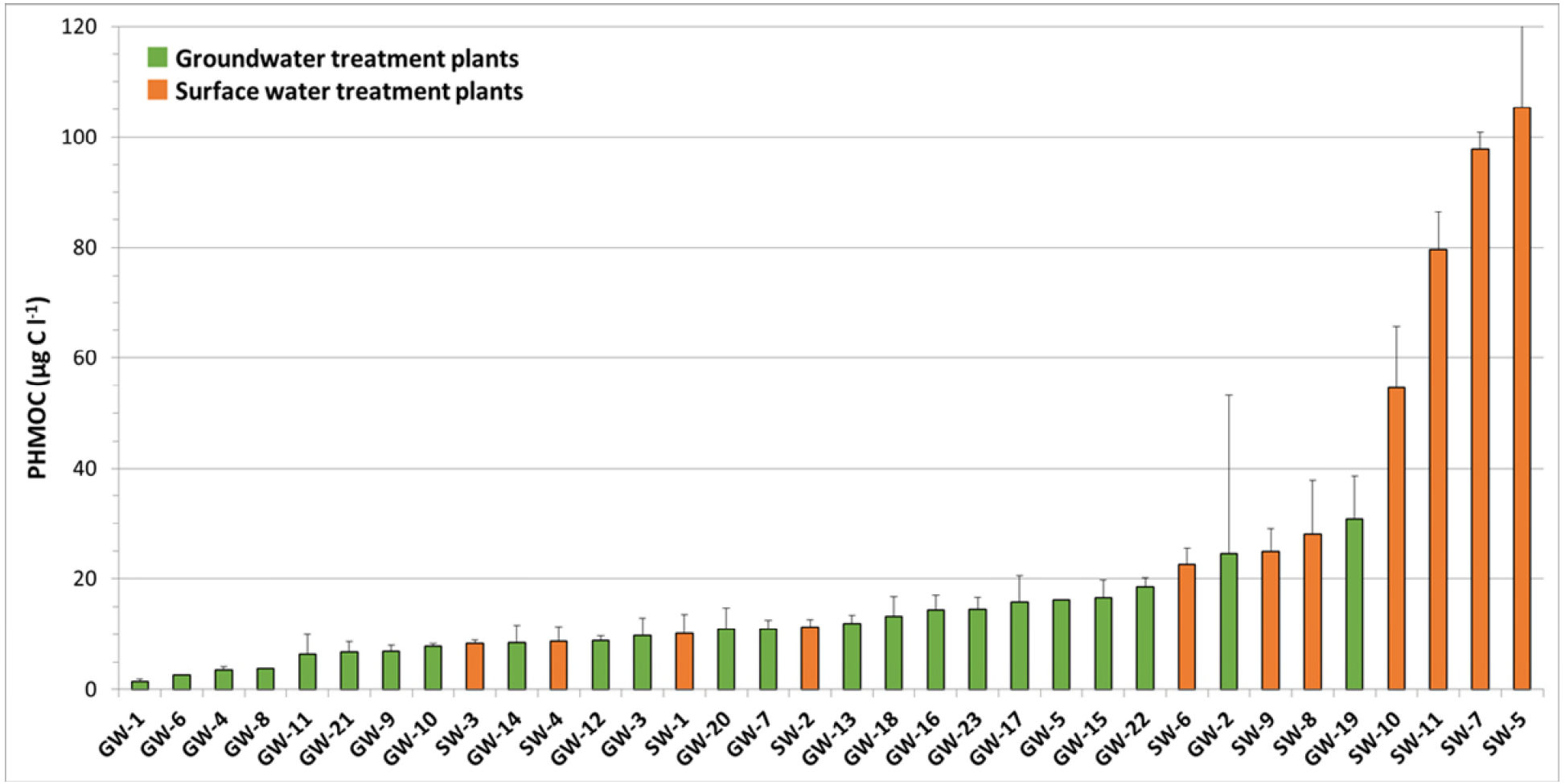
AOC-A3 in reinwater 34 verschillende locaties



PHMOC

- Particulair gebonden en/of hoogmoleculaire organisch koolstof (PHMOC)
- Maat voor biomassa en langzaam afbreekbaar organisch koolstof
- Methode
  - Drinkwater wordt geconcentreerd met hemoflowfiltratie (normaal gebruikt voor dialyse nierpatiënten)
  - Het is een ultrafiltratiemembraan met cut-off van 35 kDa
  - 100 liter water wordt geconcentreerd naar 500 ml
  - TOC-concentratie in het concentraat en DOC-concentratie in drinkwater wordt bepaald
  - $PHMOC = TOC_{concentrate} - DOC_{drinking\ water}$
- Concentraties worden uitgedrukt in µg C per liter
- Particulair ijzer (PFe) concentratie wordt ook bepaald

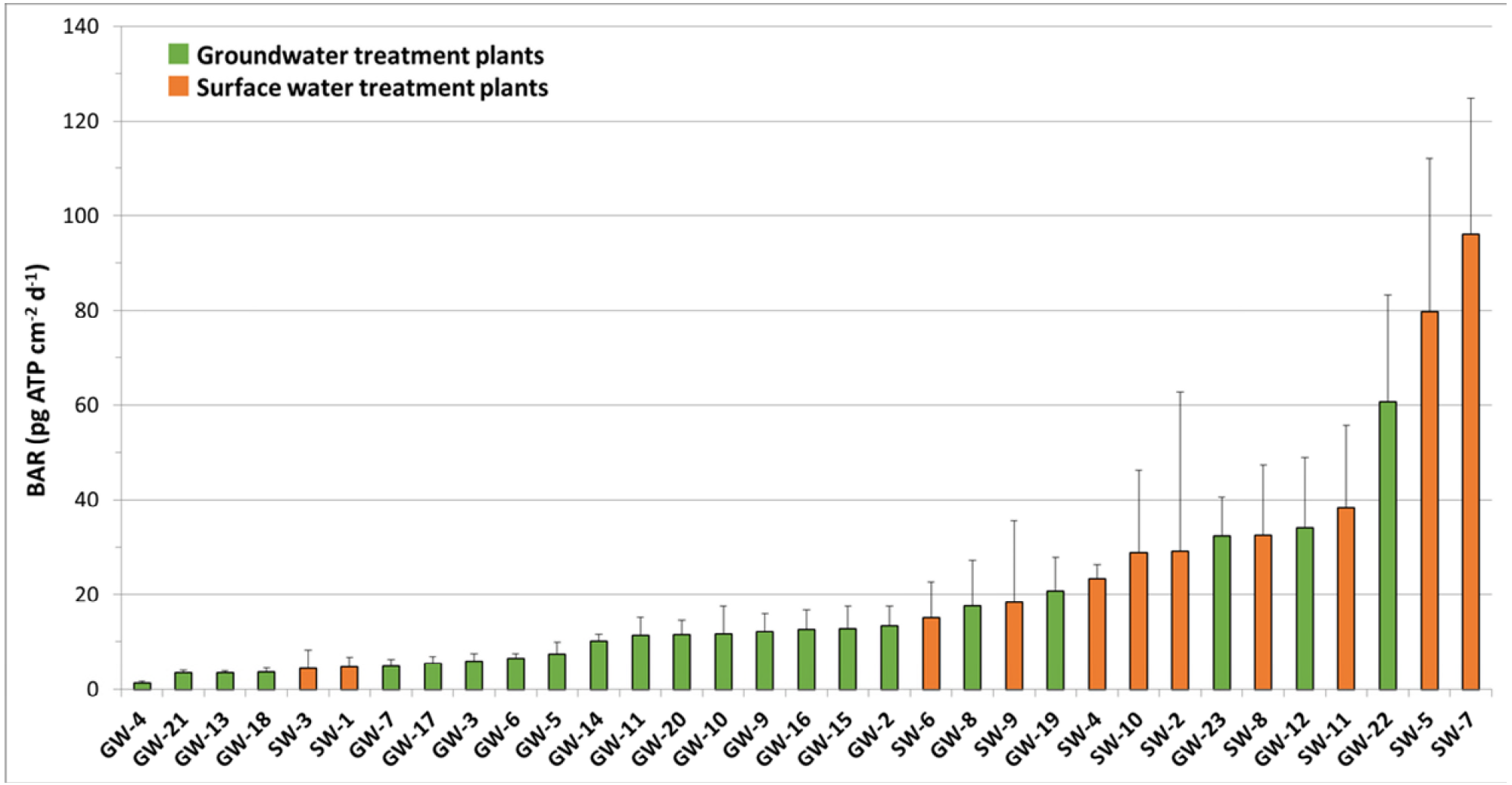
PHMOC in reinwater 34 verschillende locaties



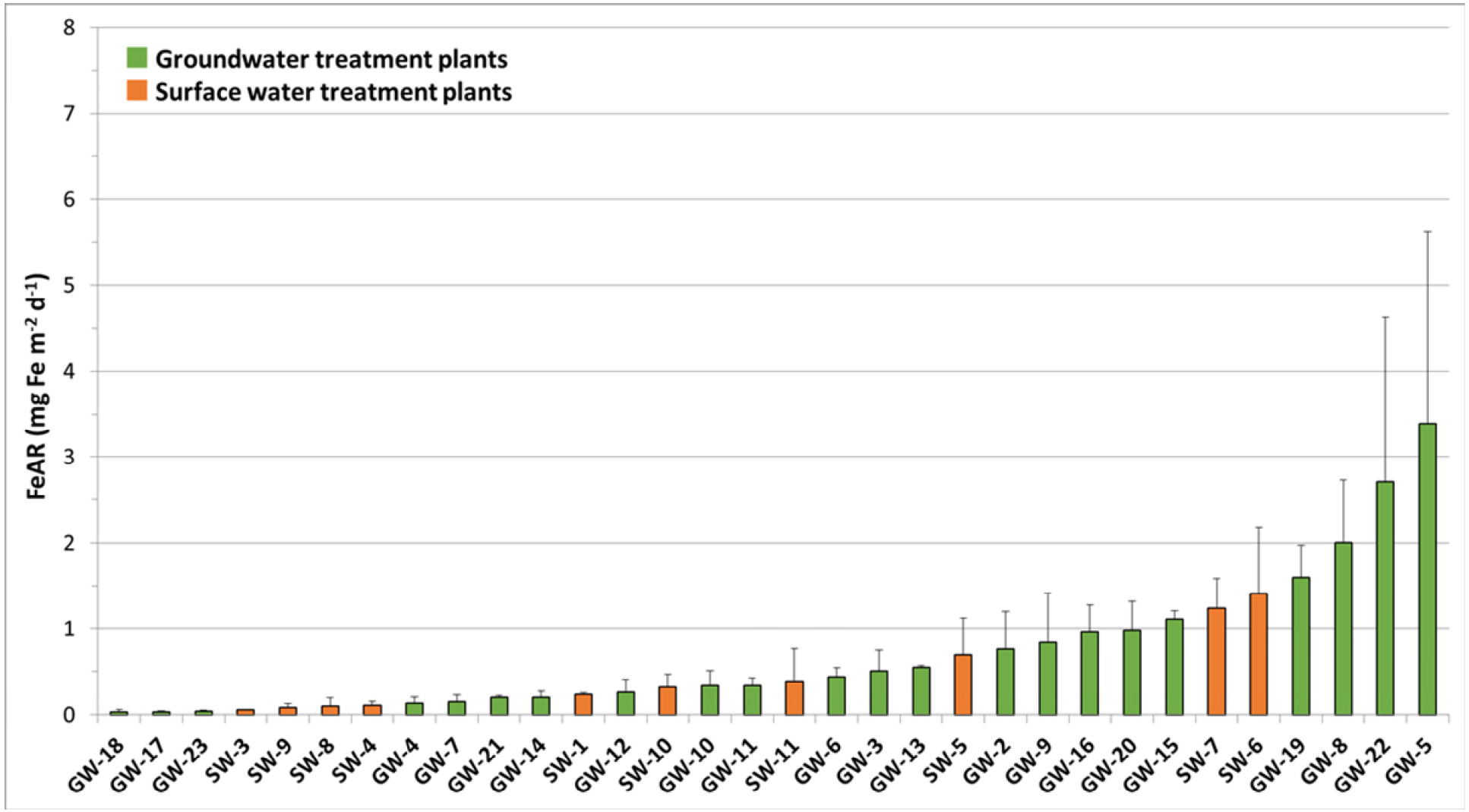
Continue biofilmmonitor (CBM)

- Bevat vier cuvetten met glasparels of -coupons
- 80 liter water per uur stroomt door de monitor
- Elke twee weken worden twee cuvetten vervangen door twee nieuwe cuvetten
- Behalve voor het eerste paar cuvetten, zitten de cuvetten dus vier weken in de monitor
- De ATP- en ijzerconcentratie van de biofilm op de glasparels of -coupons worden bepaald
- Door deze opzet wordt tweewekelijks een biomassa- en ijzeraccumulatiesnelheid verkregen

BAS in reinwater 34 verschillende locaties



FeAS in reinwater 34 verschillende locaties



Nieuwe parameters biologische stabiliteit

- Een hele range van nieuwe parameters: MBC_7, MBG_7, CBP_{14}, AOC-A3, PHMOC, PFe, BAS, FeAS
  - Correlatieanalyse tussen parameters
    - MBC_7 en CPB_{14} sterk gecorreleerd (R^2=0,98)
    - Overige parameters hebben geen sterke correlatie met elkaar
    - Iedere parameter bepaalt blijkbaar een ander aspect van de biologische stabiliteit van drinkwater
    - MBC_7, MBG_7, CBP_{14} en AOC-A3 parameters voor groeipotentie drinkwater
    - PFe en FeAS zijn parameters voor biofoulingpotentie drinkwater
    - PHMOC en BAS zijn parameters voor groei- en biofoulingpotentie drinkwater

- Conclusie: Er is niet een enkele parameter die de biologische stabiliteit van drinkwater beschrijft, multiple parameters zijn nodig!

Biologische stabiliteit is een meerkoppig monster

Wanneer is de biologische stabiliteit voldoende?

- Fijn dat we zoveel verschillende biologische stabiliteitsparameters voor drinkwater hebben, maar belangrijker is om vast te stellen wanneer de biologische stabiliteit voldoende is

- Om deze vraag te beantwoorden is het nodig om te weten wanneer de microbiologische waterkwaliteit voldoende is, bijvoorbeeld
  - Wanneer deze voldoet aan de wettelijke eisen, KG22 en *Aeromonas*
  - Dat pathogenen zich niet kunnen vermeerderen
  - Dat voor de mens waarneembare invertebraten niet aanwezig zijn
  - Et cetera

- Op zijn minst zou de drinkwaterkwaliteit moeten voldoen aan de wettelijke eisen

Wanneer is de biologische stabiliteit voldoende?

- Biologische stabiliteitsparameters bepaald van reinwater van 34 verschillende productielocaties in Nederland
- Tevens KG22 en *Aeromonas* aantallen in distributiesysteem van deze locaties opgevraagd
- Relaties tussen deze wettelijke parameters en biologische stabiliteitsparameters bepaald
- Significante verschillen voor aantal biologische stabiliteitsparameters tussen drinkwater van grondwater en oppervlaktewater waargenomen
- Daarom werden de relaties apart bepaald voor grondwater- en oppervlaktewaterlocaties
- Op basis van de waargenomen relaties zijn aandachtswaarden afgeleid voor parameters die voorspellend zijn voor KG22 en *Aeromonas*

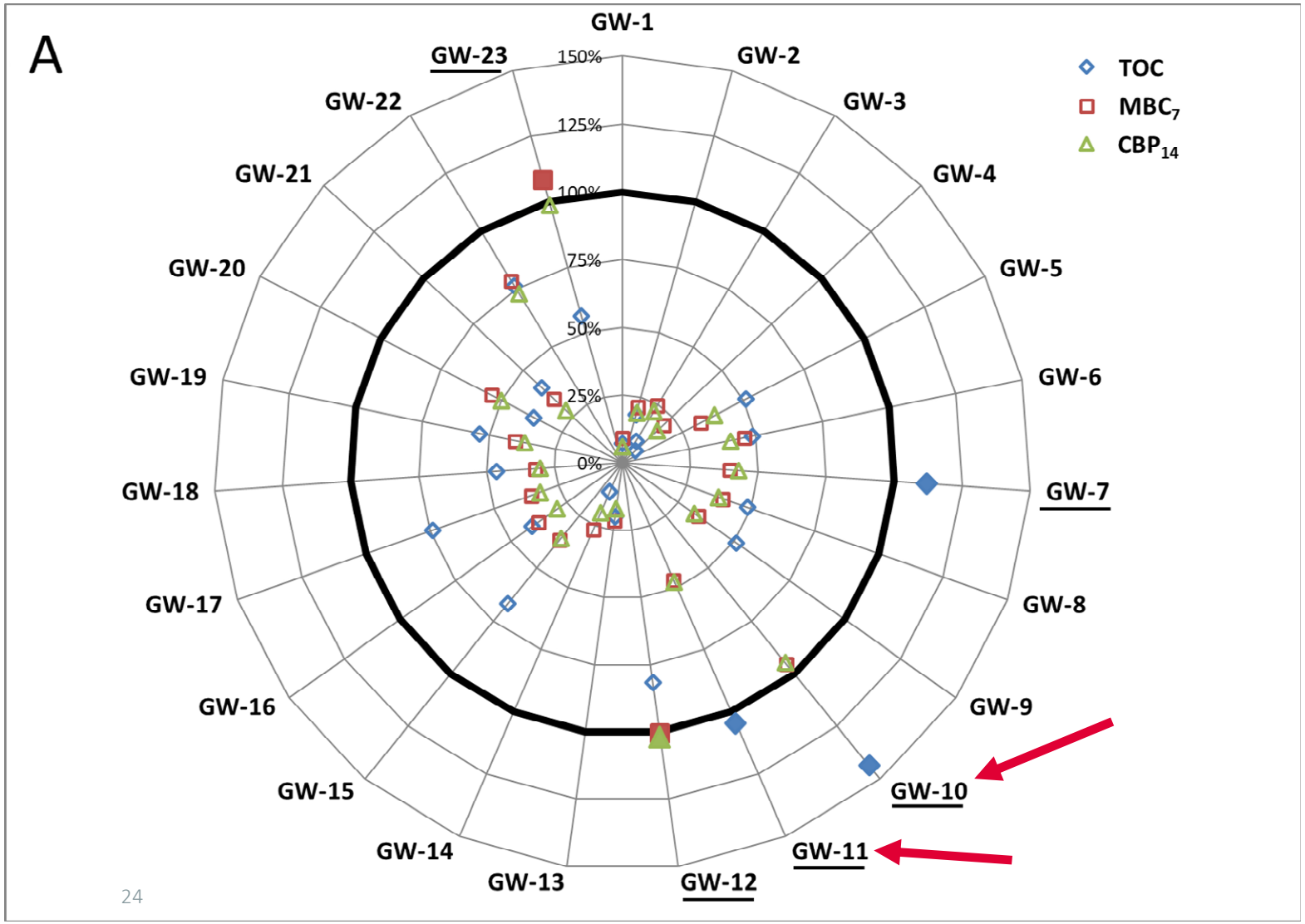
Aandachtswaarden reinwater uit grondwater

Parameter	Ranking	Regressie	Final
Gerelateerd aan KG22			
TOC (mg C L^{-1})	3.1	5.2	4.1
MBC_7 (ng ATP L^{-1})	6.8	N.C.	N.C.
CBP_{14} (d.ng ATP L^{-1})	80	N.C.	N.C.
AOC-A3 (µg C L^{-1})	2.1	N.C.	N.C.
BAS (pg ATP cm^{-2} dag^{-1})	34	N.C.	N.C.
Gerelateerd aan Aeromonas			
MBC_7 (ng ATP L^{-1})	8.2	9.0	8.6
CBP_{14} (d.ng ATP L^{-1})	104	116	110
TOC (mg C L^{-1})	5.9	2.3	4.1
PFe (µg Fe L^{-1})	4.9	N.C.	N.C.

Aandachtswaarden reinwater uit oppervlaktewater

Parameter	Ranking	Regressie	Final
Gerelateerd aan KG22			
MBG_7 (ng ATP L^{-1})	N.C.	8.8	N.C.
PHMOC (µg C L^{-1})	N.C.	231	N.C.
Gerelateerd aan Aeromonas			
MBG_7 (ng ATP L^{-1})	4.2	4.7	4.5
PHMOC (µg C L^{-1})	54	40	47
FeAS (mg Fe m^{-2} dag^{-1})	0.32	0.36	0.34
MBC_7 (ng ATP L^{-1})	6.9	N.C.	N.C.
CBP_{14} (d.ng ATP L^{-1})	75	N.C.	N.C.
AOC-A3 (µg C L^{-1})	4.1	N.C.	N.C.
TOC (mg C L^{-1})	1.8	N.C.	N.C.
BAS (pg ATP cm^{-2} dag^{-1})	15	N.C.	N.C.

Aandachtswaarden grondwaterlocaties



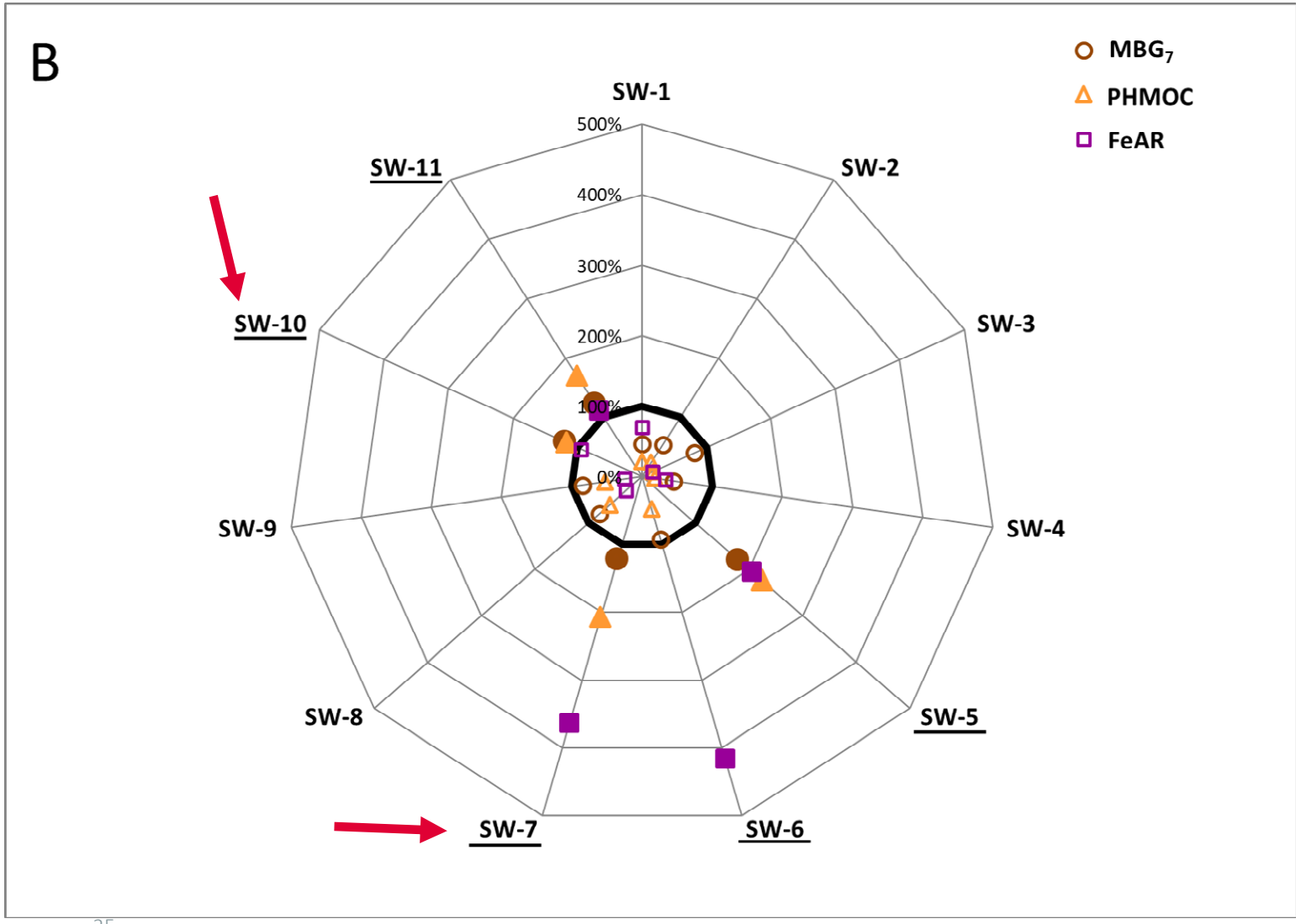
GW-10

	Aandachts waarde	GW-10
TOC	4,1	***5,9***
MBC_7	8,6	8,2
CBP_{14}	110	104

GW-11

	Aandachts waarde	GW-11
TOC	4,1	***4,3***
MBC_7	8,6	4,1
CBP_{14}	110	52

Aandachtswaarden oppervlaktewaterlocaties



- SW-10

	Aandachts waarde	SW-10
PHMOC	47	***55***
MBG_7	4,5	***5,4***
FeAS	0,34	0,32

- SW-7

	Aandachts waarde	SW-7
PHMOC	47	***100***
MBG_7	4,5	***5,5***
FeAS	0,34	***1,24***

Conclusies

- Met de nieuwe biologische stabiliteitsparameters wordt een vollediger beeld van de biologische stabiliteit van drinkwater verkregen dan met de traditionele parameters AOC-P17/Nox en BVS
- Biologische stabiliteit van drinkwater kan niet met een enkele parameter worden beschreven, een range van parameters die de groeipotentie en/of biofouling potentie van het water beschrijven, is nodig om de biologische stabiliteit van drinkwater betrouwbaar te beschrijven
- Biologische stabiliteitsparameters verschillen significant in drinkwater bereid uit grondwater of oppervlaktewater en aandachtswaarden voorspellend voor KG22 en *Aeromonas* kon voor elk watertype worden afgeleid:
  - Grondwater: TOC < 4,1 mg C/l; MBC_7 < 8,6 ng ATP/l; CBP_{14} < 110 d.ng ATP/l
  - Oppervlaktewater: PHMOC < 47,0 µg C/l; MBG_7 < 4,5 ng ATP/l; FeAS < 0,34 mg Fe m^{-2} dag^{-1}

Acknowledgements

- Dick van der Kooij
- Wim Hijnen
- Anke Brouwer
- Ronald Italiaander
- KWR laboratory technicians

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V Presentation Meten van biologische stabiliteit in praktijk van DPW-bedrijven – Eline Stroobach

Het Waterlaboratorium

Meten van biologische stabiliteit in praktijk van DPW-bedrijven

Implementatie en toepassing van BPP analyse

BTO-Workshop Biologische stabiliteitsparameters in de praktijk, 3 oktober 2022

E. Stroobach en A. Knezev

Het Waterlaboratorium

Bron: Vewin

Historie biologische stabiliteit analyses bij DPW-labs

- DPW-laboratoria midden jaren 1990
  - AOC standaard uitgevoerd bij alle drie laboratoria
  - ATP bij PWN en Waternet
- Vanaf 2003 – Het Waterlaboratorium opgericht
  - AOC
  - ATP – luciferine/luciferase (snelle reactie)
  - DCT (microscopie)
  - BVS (Biofilmmonitor)

Verbetering klassieke methoden biologische stabiliteit

- Klassieke AOC analyse
  - *Pseudomonas fluorescens* P17/ *Spirillum* sp. NOX
  - Meet niet alle beschikbare koolstof
  - Equivalent Acetaat
  - Zeer bewerkelijk (en dus duur)
  - Storende componenten: ClO_2 , H_2O_2

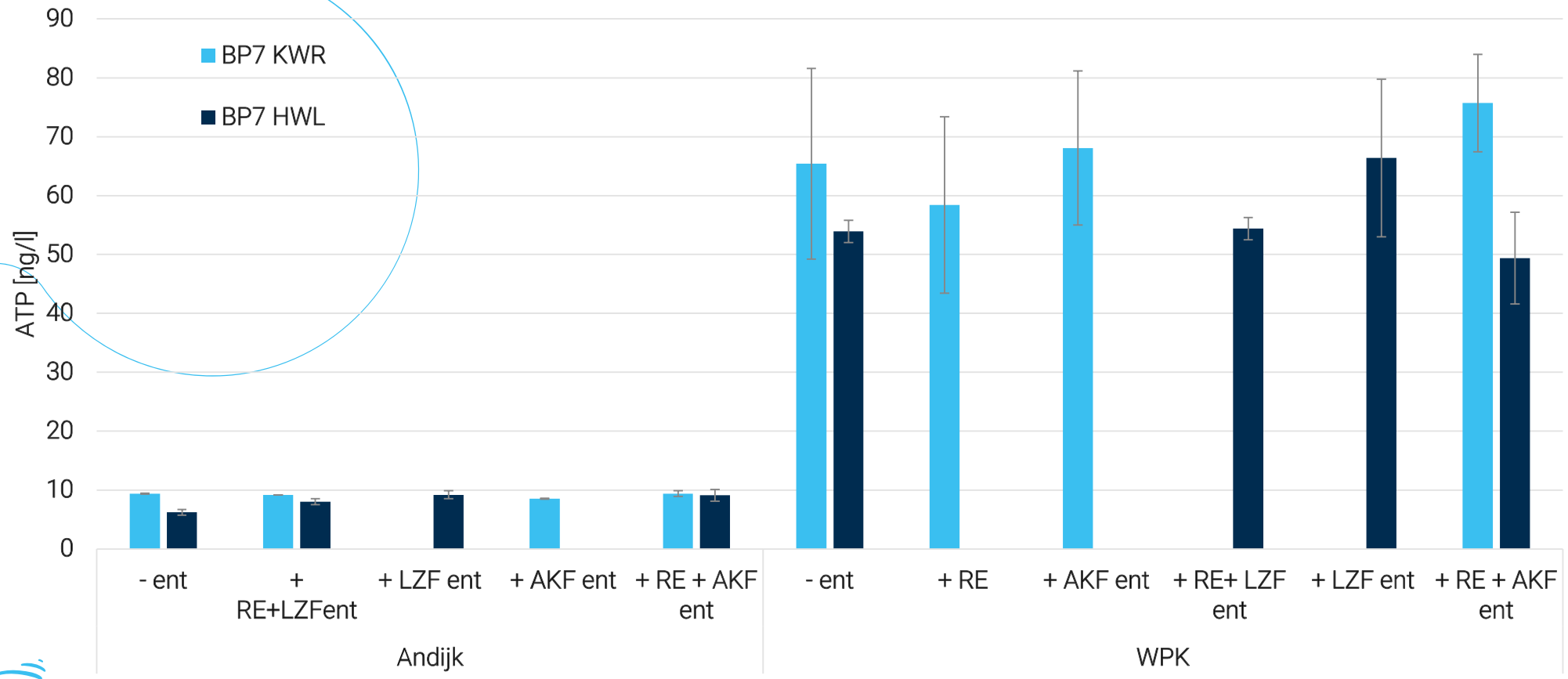
- BTO → AOC-A3, en BPP
  - *Flavobacterium johnsoniae* A3: lange koolstofketens
  - BPP: autochtone flora, ATP productie

Implementatie BPP bij HWL

2014: Vergelijkend onderzoek BPP met KWR

- Effecten ent-materiaal
- Weesperkarspel na O_3 en Andijk drinkwater (ClO_2)
- Effect van volume

Effect type ent-materiaal BP7



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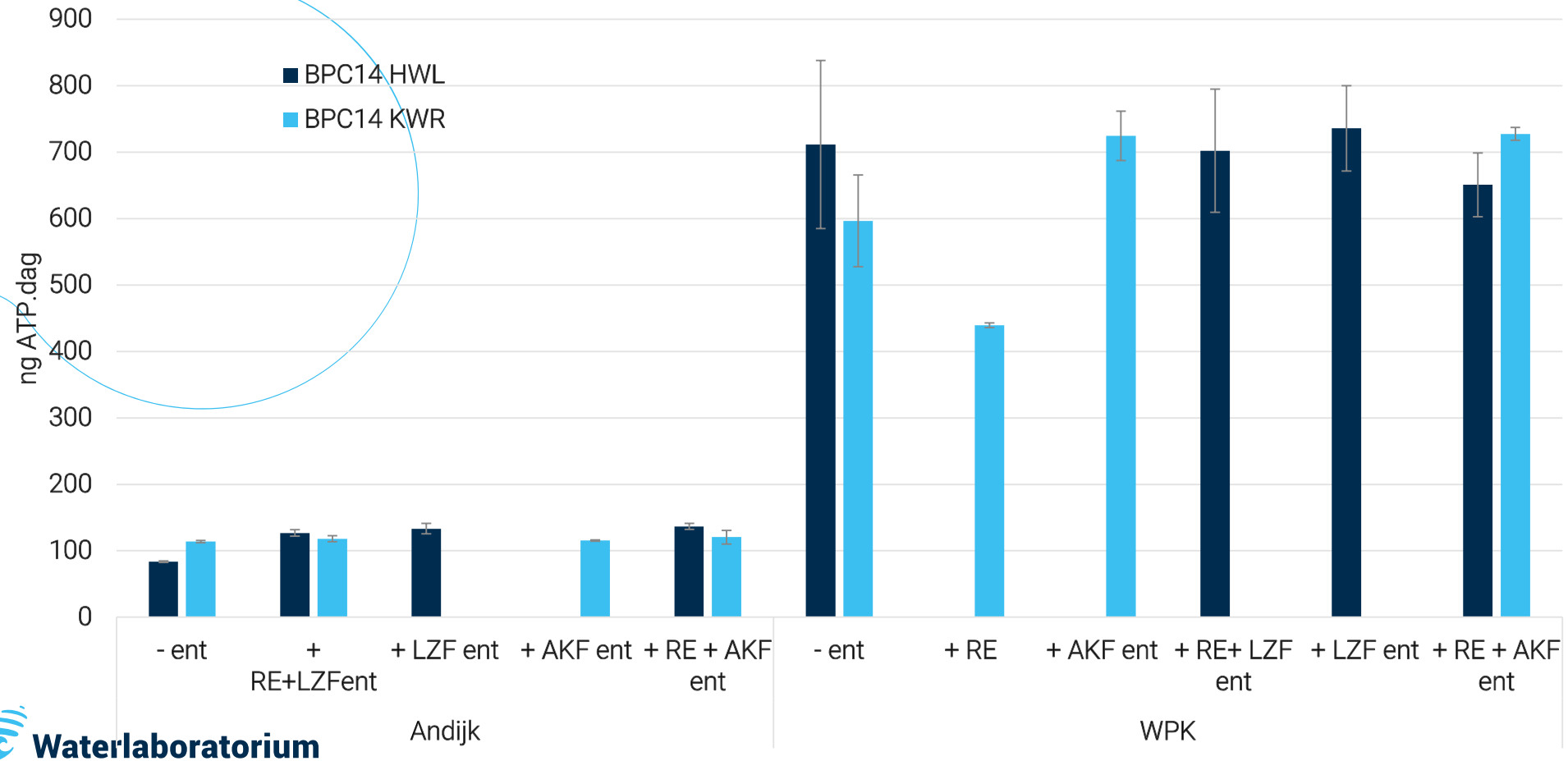
Effect type ent-materiaal BPC14



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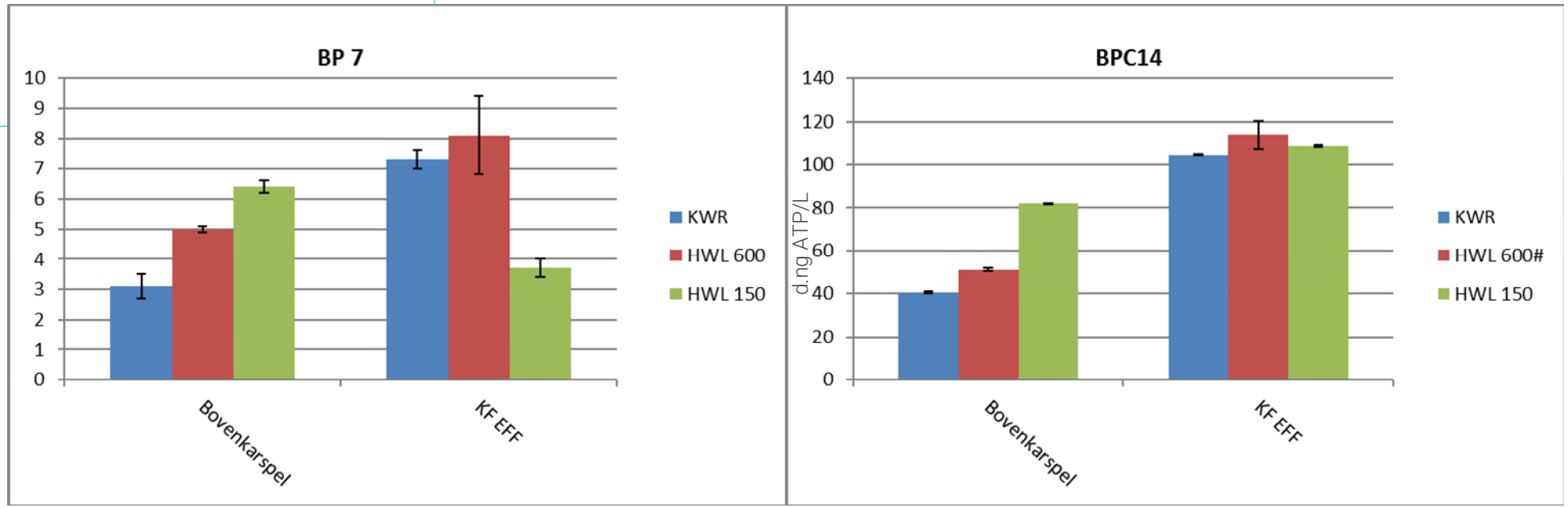
Effect monster volume

- 600 ml vs 150 ml



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Toepassing nieuwe methoden bij DPW

Dunea:

- 2015 BPP en CBM ingezet in zuivering ter verkenning
- 2017 – 2021 GOBAM

PWN: onderzoek naar biologische stabiliteit in distributienet:

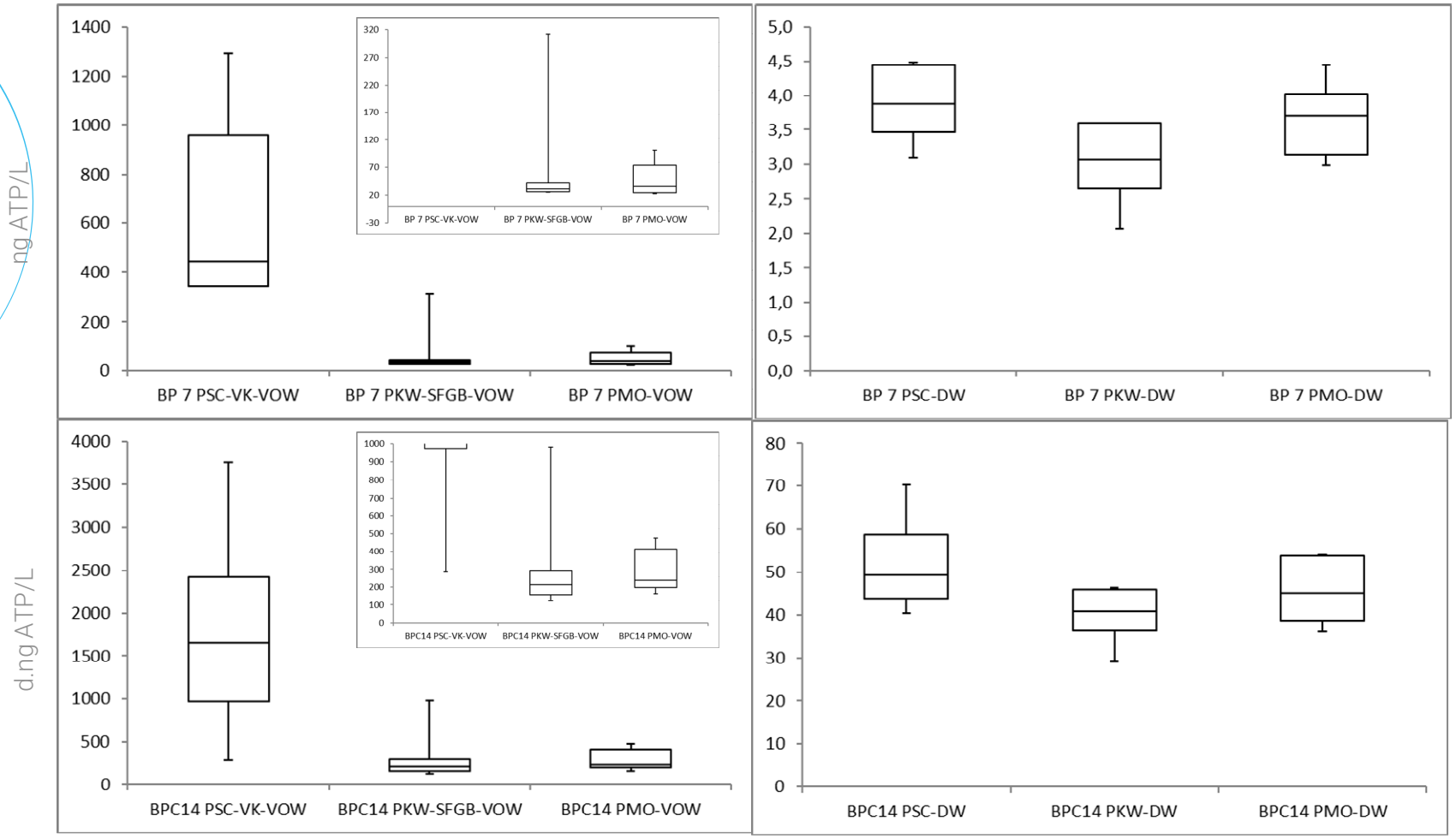
- DCT
- Vergelijking BPP op basis van ATP en DCT (E. Prest)

Waternet:

- In samenwerking met KWR

Verkenning zuivering Dunea 2015

- 2015 BPP in duinfiltraat en drinkwater 13 x per jaar
- Verschillen tussen de locaties
- Verlaging BPP door de zuivering
- Seizoensvariatie



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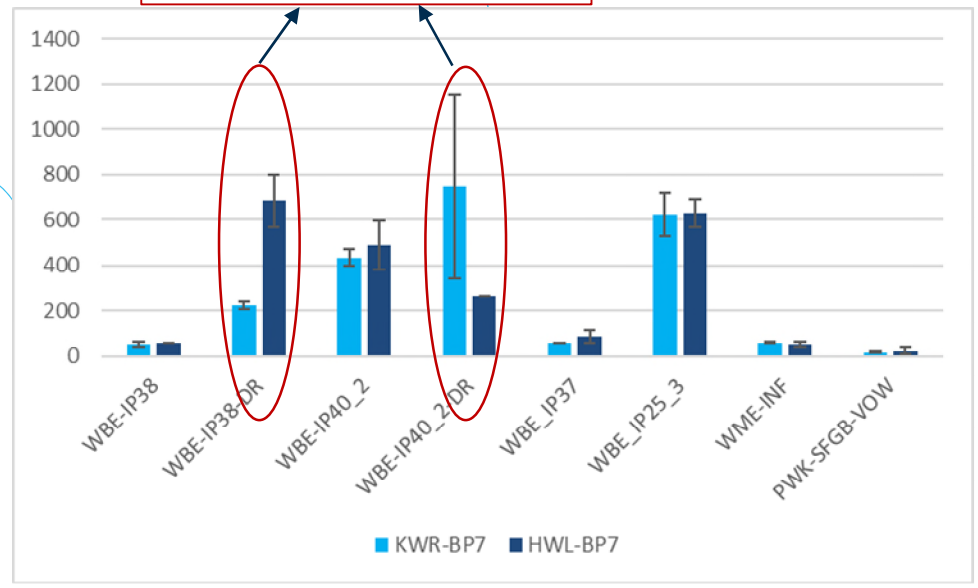
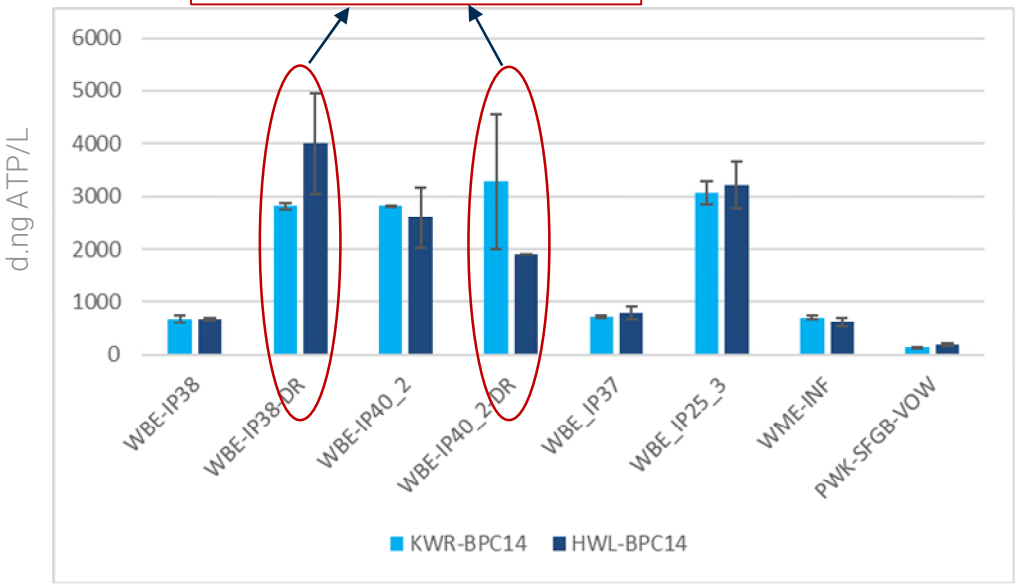
Vergelijkend onderzoek KWR-HWL infiltratieplassen en winningen Dunea (2016)

Grote variatie duplo



Grote variatie duplo



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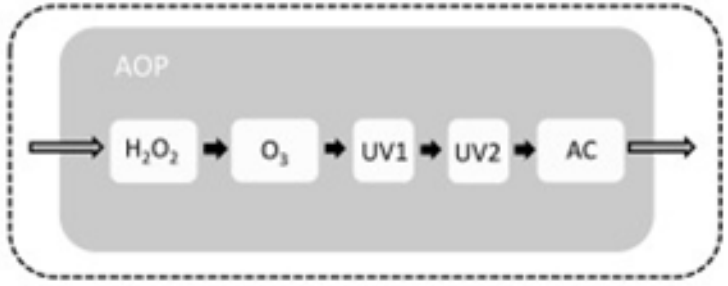
ELSEVIER

Journal of Hazardous Materials

Volume 429, 5 May 2022, 128346

Improved drinking water quality after adding advanced oxidation for organic micropollutant removal to pretreatment of river water undergoing dune infiltration near The Hague, Netherlands

Peer H.A. Timmers [a], T. Slootweg [b], A. Knezev [b], M. van der Schans [a], L. Zandvliet [b], A. Reus [a], D. Vughs [a], L. Heijnen [a], T. Knol [c], J. El Majjaoui [c], P. van der Wielen [a, d], P.J. Stuyfzand [a, e], K. Lekkerkerker-Teunissen [c]



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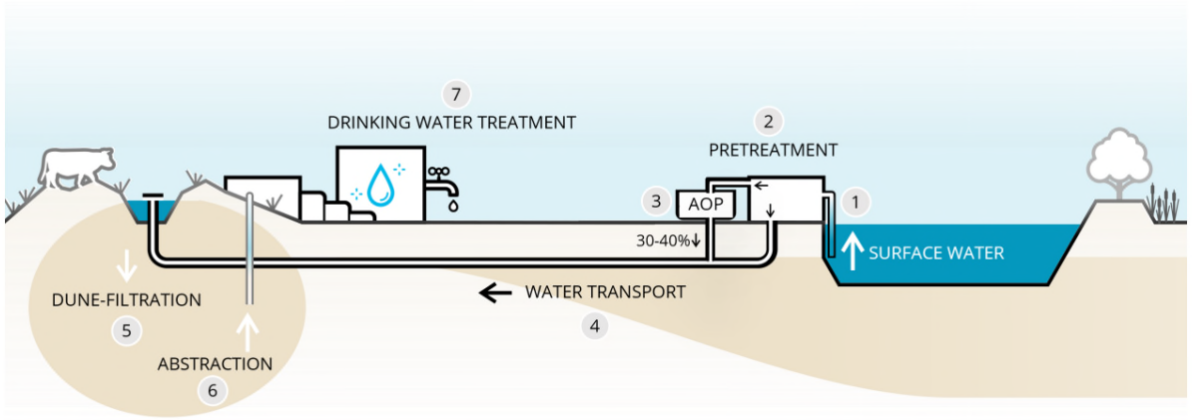
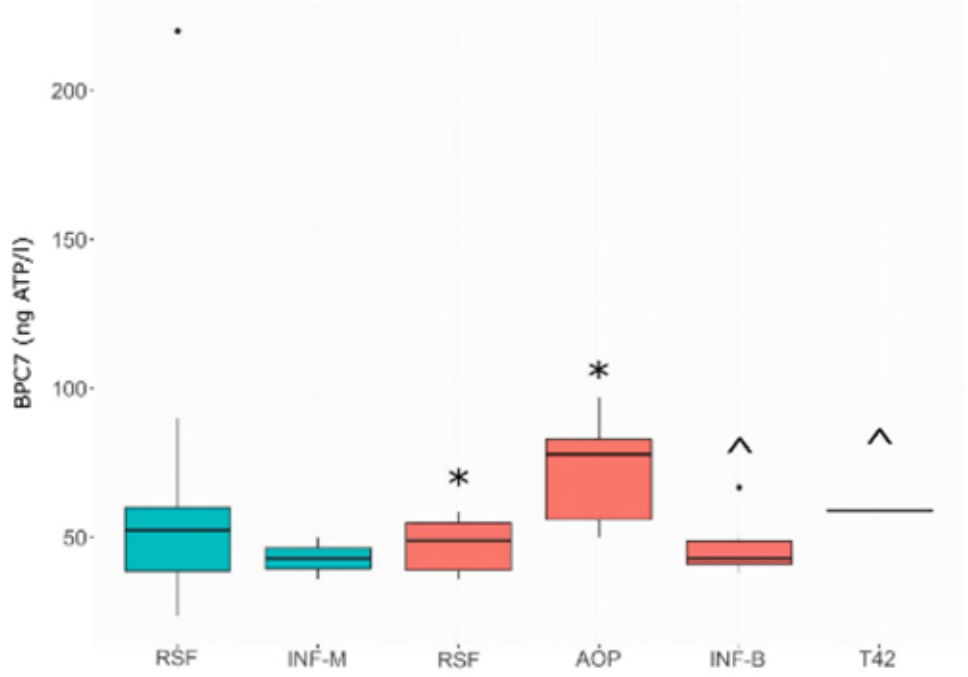
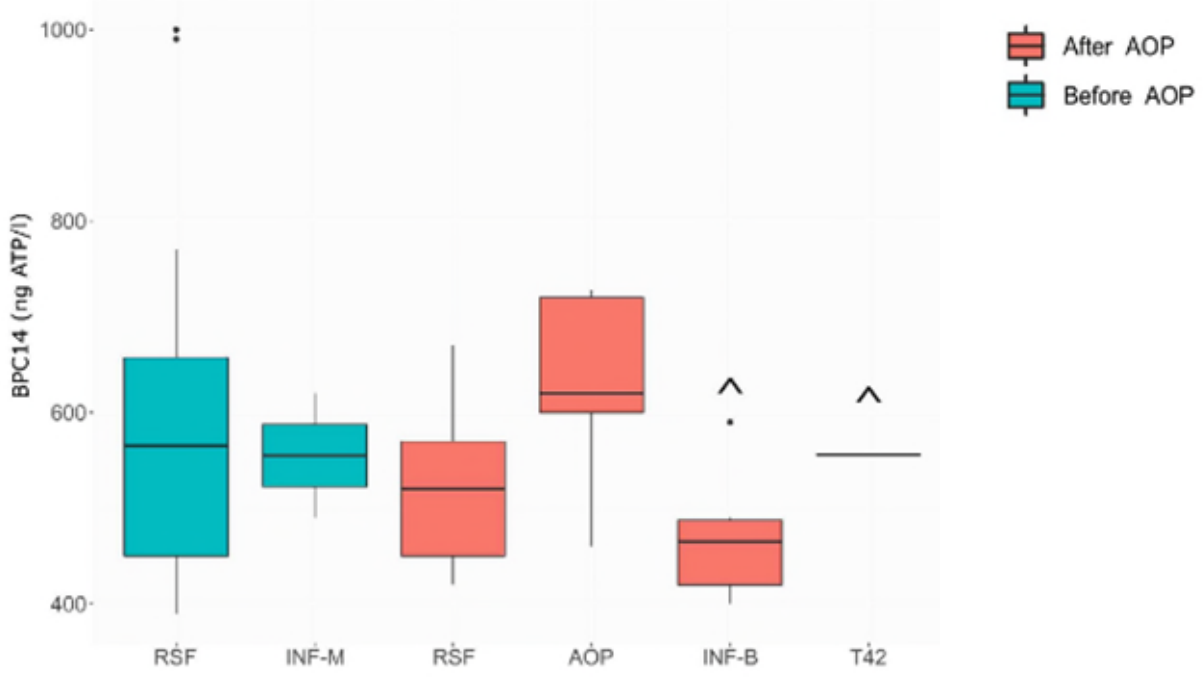
Onderzoek effectmeting GOBAM



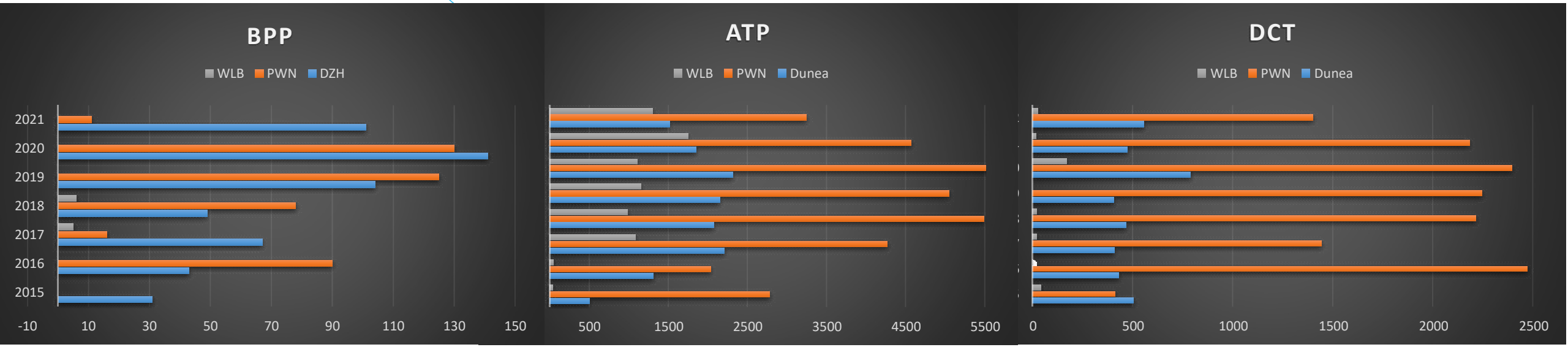




Het Waterlaboratorium

Tot slot – waar staan we nu?

Meetprogramma's DPW 2015 t/m 2021



- ATP en DCT worden grootschalig gebruikt in de routine en op projectmatige basis
- BPP voornamelijk op projectmatige basis (Dunea en PWN)
- Deze parameters, vooral in combinatie, zijn zeer nuttig voor de beoordeling en het begrijpen van microbiologische processen tijdens drinkwaterproductie en -distributie

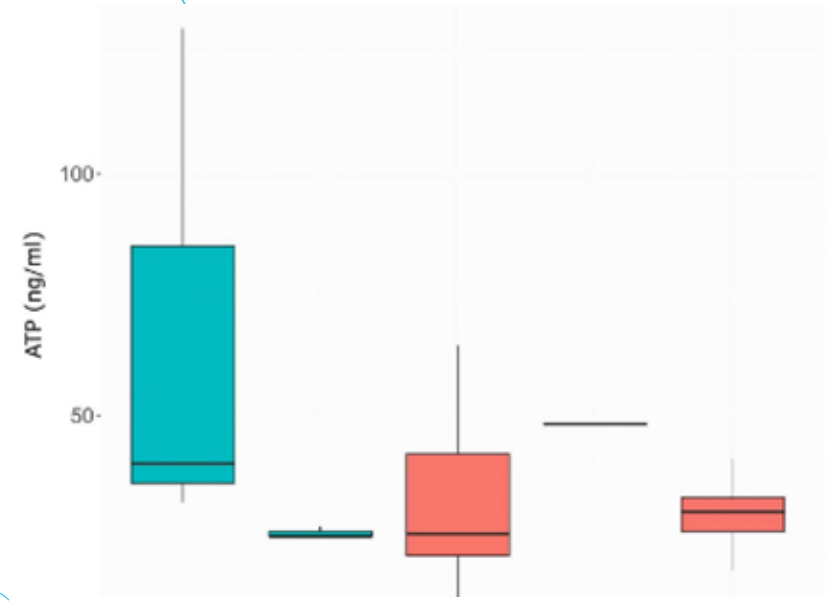
Het Waterlaboratorium

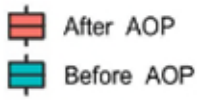
Het Waterlaboratorium

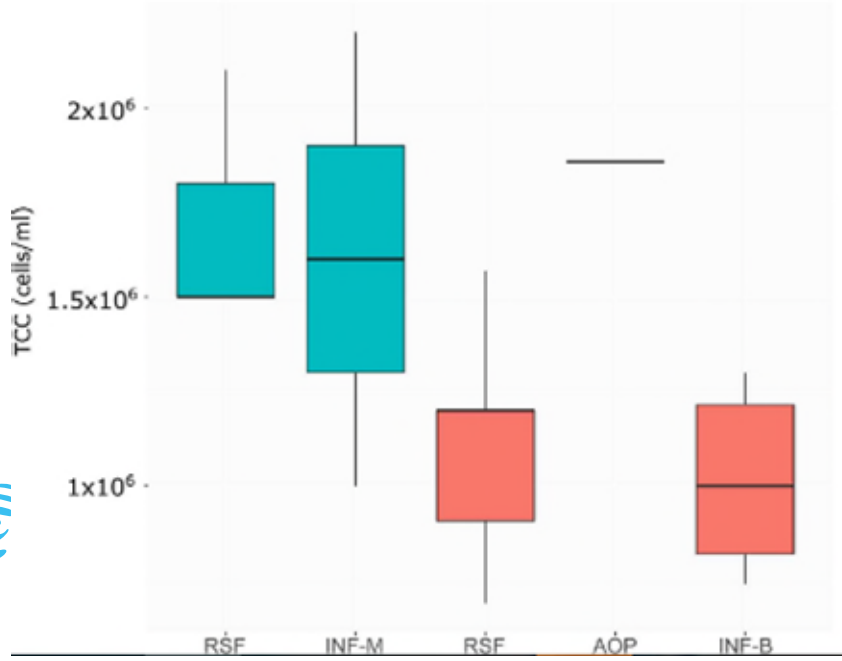
Vragen?

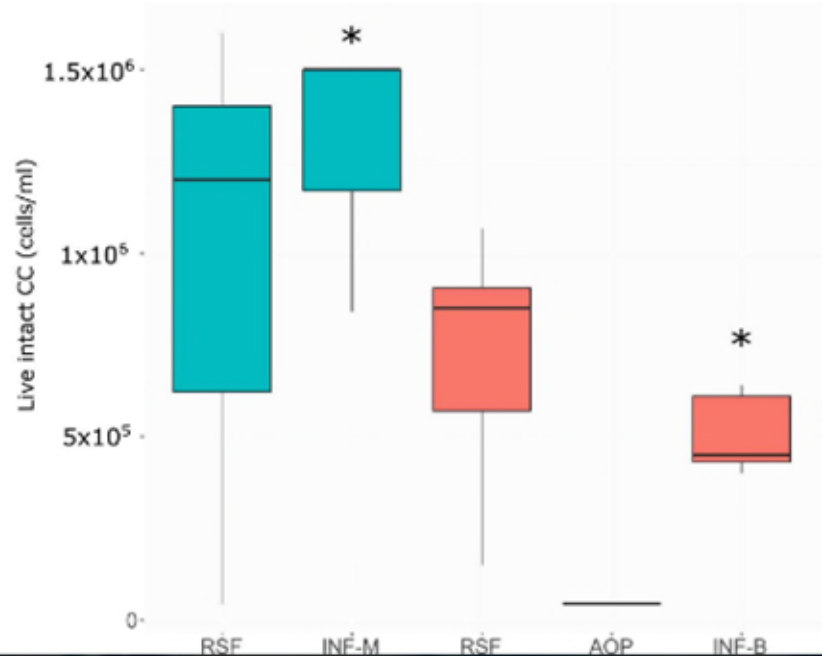
Tot slot – waar staan we nu?

- ATP en DCT worden grootschalig gebruikt in de routine en op projectmatige basis
- BPP voornamelijk op projectmatige basis (Dunea en PWN)
- Deze parameters, vooral in combinatie, zijn zeer nuttig voor de beoordeling en het begrijpen van microbiologische processen tijdens drinkwaterproductie en -distributie


ATP (ng/ml)
100
50


After AOP
Before AOP


TCC (cells/ml)
2x10⁶
1.5x10⁶
1x10⁶
RSF
INF-M
RSF
AOP
INF-B


Live intact CC (cells/ml)
1.5x10⁶
1x10⁵
5x10⁵
0
*
*
RSF
INF-M
RSF
AOP
INF-B

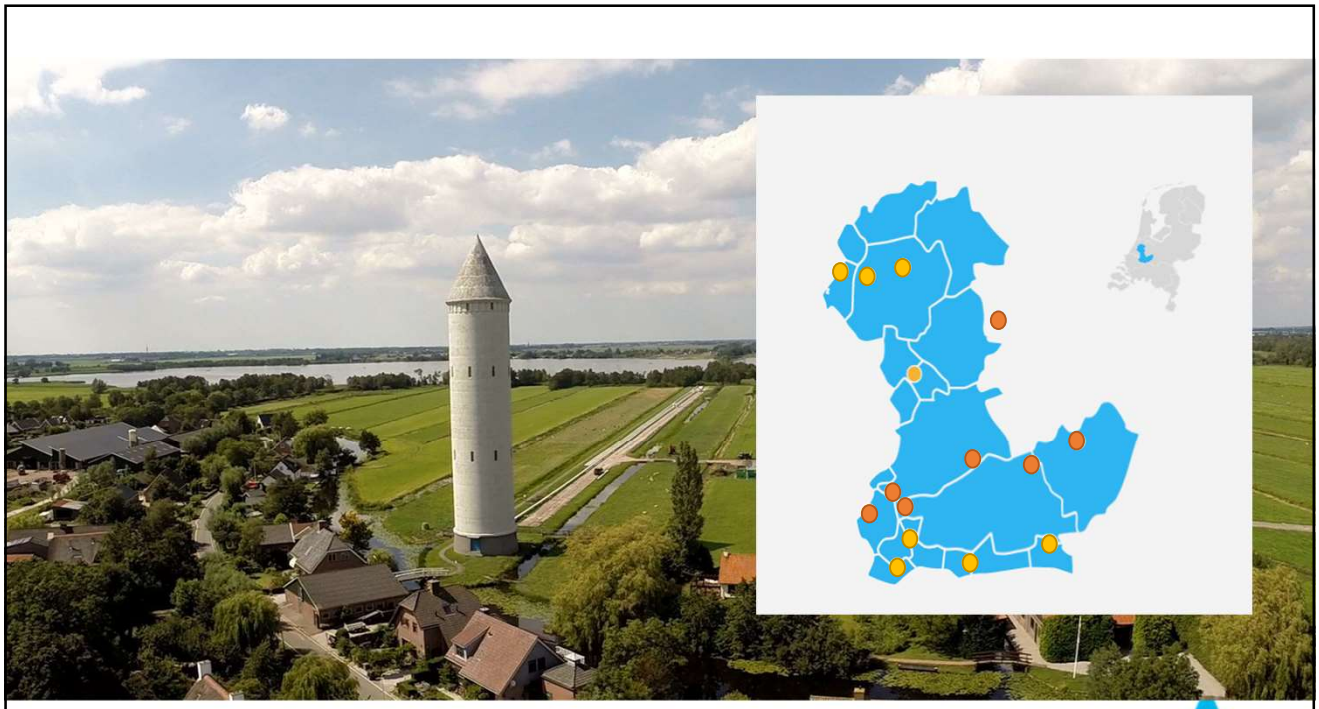
VI Presentation Biologische stabiliteit bij Oasen. Een nieuw proces, nog stabieler? – Maarten Lut

Biologische stabiliteit bij Oasen.

Een nieuw proces, nog stabieler?

Maarten Lut | 3 oktober 2022

1

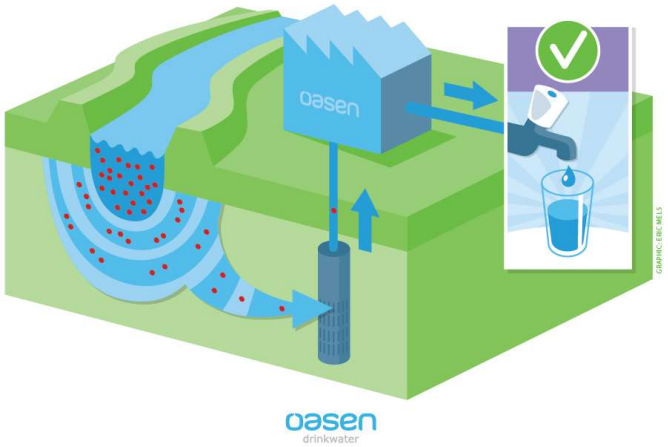


2

Oevergrondwater - De rivier als bron

Bodempassage:

- Microbiologische veilig water
- Verwijdering en afvlakking van verontreinigingen
- Toename van o.a. Ammonium, Ijzer, Mangaan



oasen drinkwater

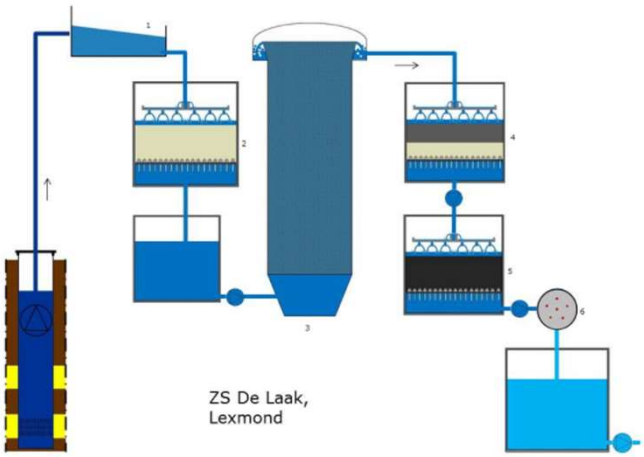
3

Drinkwater uit oevergrondwater – Klassieke zuivering

Zuiveringsproces:

- (Oever) Grondwater
- Beluchting/Ontgassing
- Voorfiltratie (1-2x)
- Ontharding
- Carry over filtratie
- Aktief koolfiltratie

Veel microbiologie in de processen
(voorfiltratie en AK filters)



oasen drinkwater

4

Een nieuw proces o.b.v. volstroom RO

- Oevergrondwater is best beschikbare bron, maar de kwaliteit staat onder druk:
  - Verzilting
  - Industriële lozingen

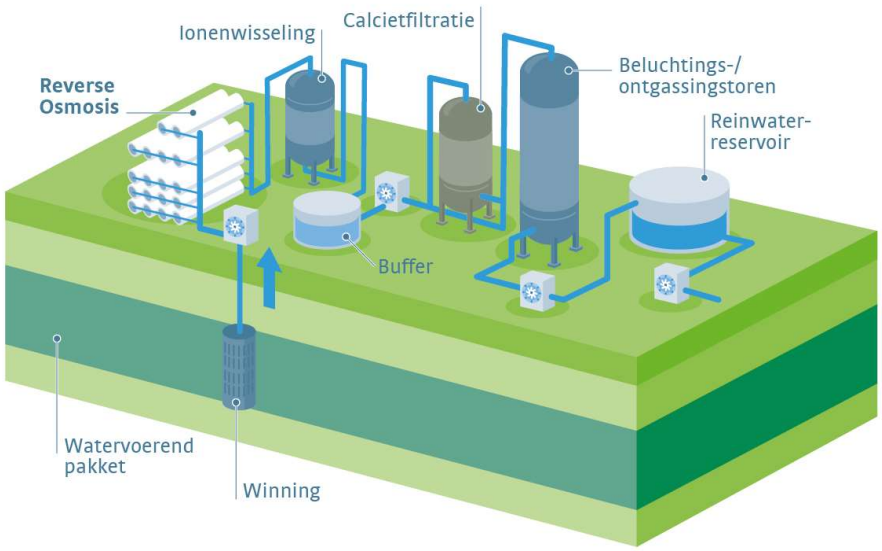
- Bronbescherming is daarom belangrijk.
- Maar daarnaast kijken we ook naar nieuwe technieken om de beste waterkwaliteit te kunnen maken.

- Met volstroom RO kunnen we zout en verontreinigingen verwijderen.

oasen drinkwater

5

Nieuw zuiveringsconcept – volstroom RO



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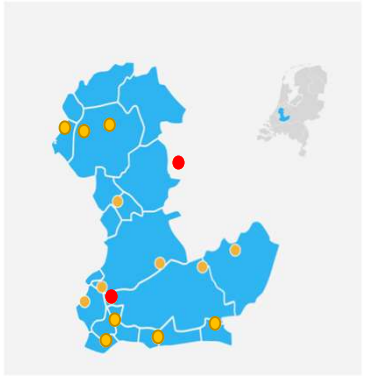
6

Nieuwbouw RO zuivering

ZS De Put (Nieuw-Lekkerland)

ZS De Hooge Boom (Kamerik)



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7

Onderzoek biologische stabiliteit (ca. 2013 – 2017)

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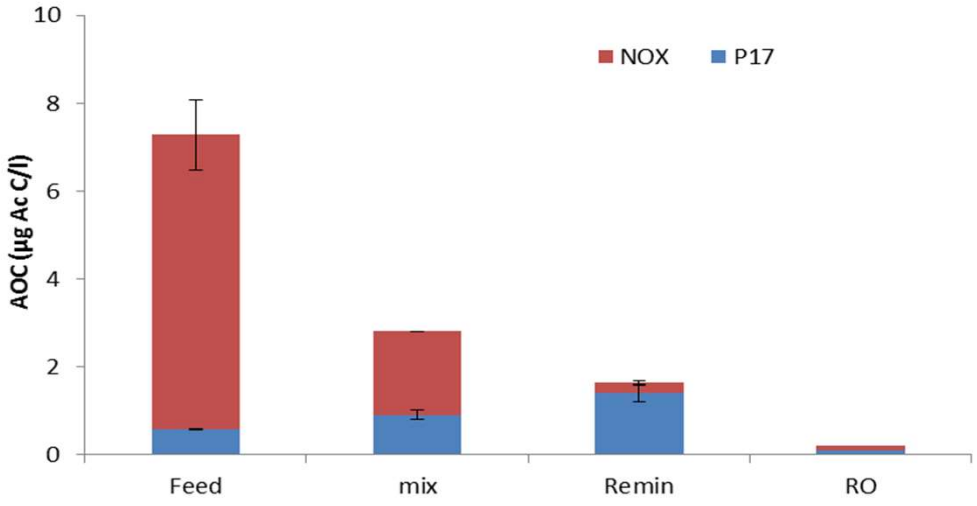
8

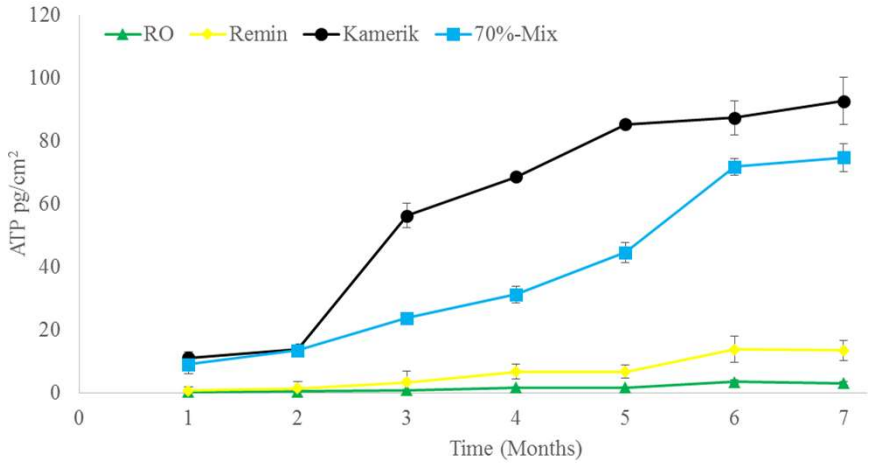
Hoe biologisch stabiel is het water?



9

Hoe biologisch stabiel is het water?

Biofilm in Distributiepilot



10

Legionellagroeipotentie [BTO 2016.202(s)]

Het geproduceerde drinkwater met de huidige zuivering van zs Kamerik heeft een relatief hoge legionellagroeipotentie. In een binneninstallatie kan dit water dus groei van *Legionella pneumophila* veroorzaken, ongeacht het leidingmateriaal van de binneninstallatie

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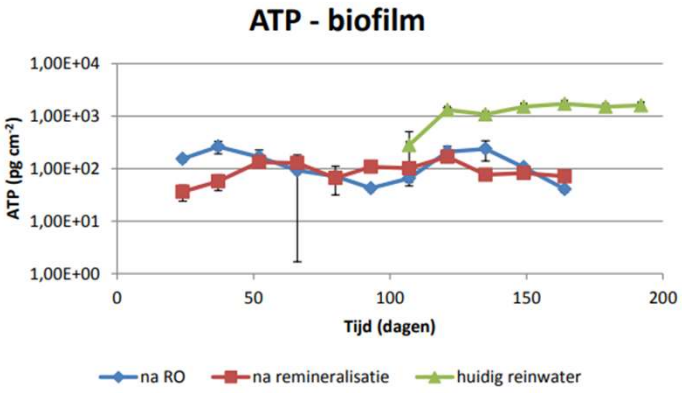
11

Legionellagroeipotentie [BTO 2016.202(s)]



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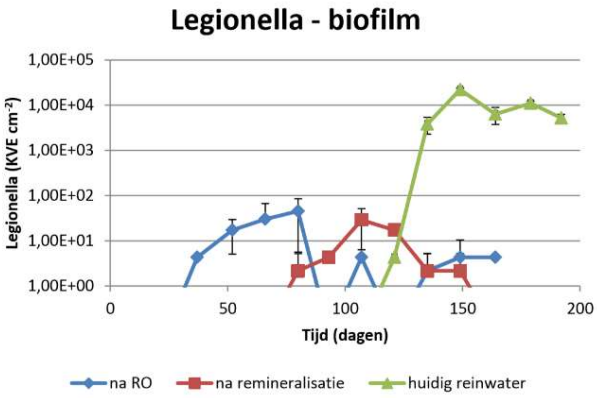
12

Legionellagroeipotentie [BTO 2016.202(s)]

Resultaten: LGP in (geremineraliseerd) RO-water ligt lager dan in reinwater

Uit het onderzoek blijkt dat zowel RO-water als geremineraliseerd RO-water een lagere legionellagroeipotentie (LGP) hebben dan het huidige reinwater van het zuiveringsstation van Kamerik. De LGP van het (geremineraliseerde) RO-water is vergelijkbaar met de laagste waarde die tot nu toe in het Nederlandse drinkwater is waargenomen.



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13

Impact bedrijfsvoering op biologische stabiliteit (2019-2022)

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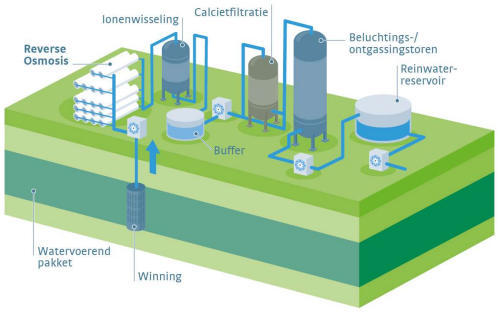
14

De pilot een paar jaar later

- Hoofdonderzoek is afgerond
- Pilot draait nog i.v.m. aanvullend onderzoek
  - Focus op RO proces
  - Geen focus op remineralisatie en microbiologie
- Bedrijfsvoering niet optimaal m.b.t. microbiologie



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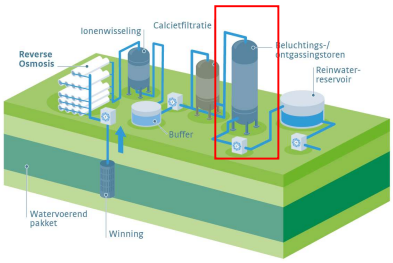
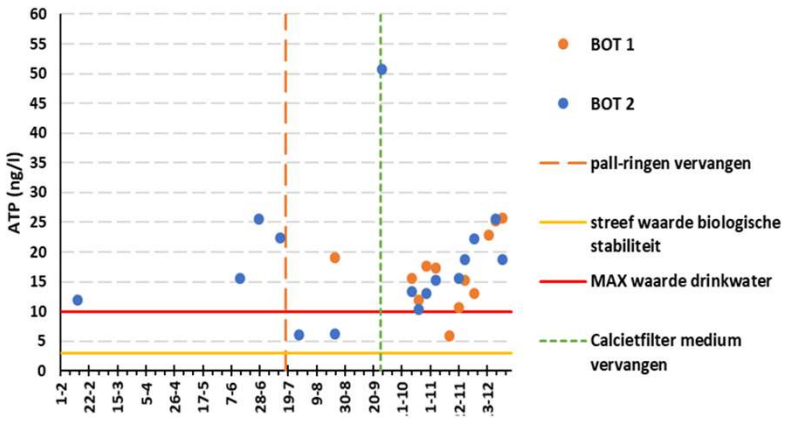
15

Hoge waardes ATP na de beluchtingstoren.





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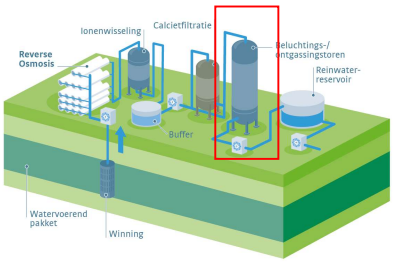
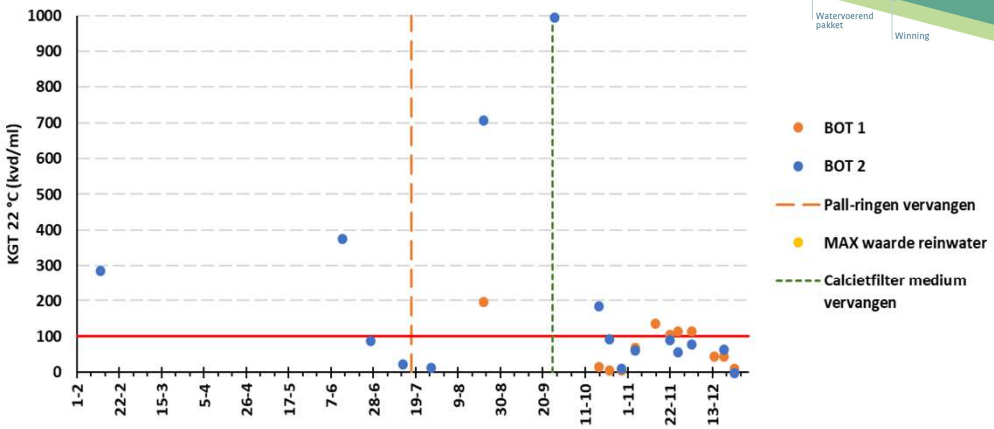
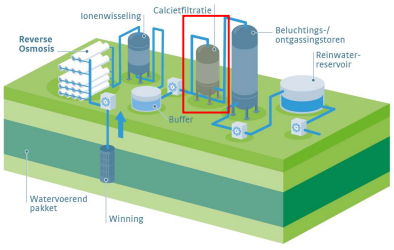
16

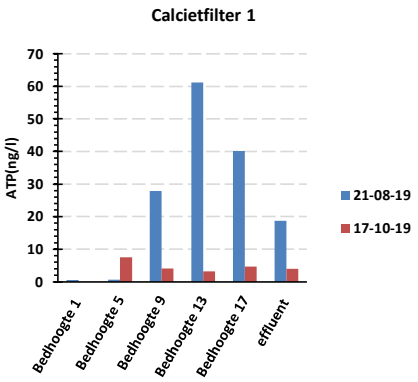
Ook koloniegetal hoog na de beluchtingstoren

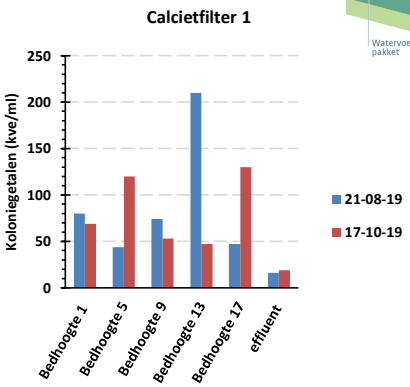




Ook de calcietfilters laten verhoogde waardes zien

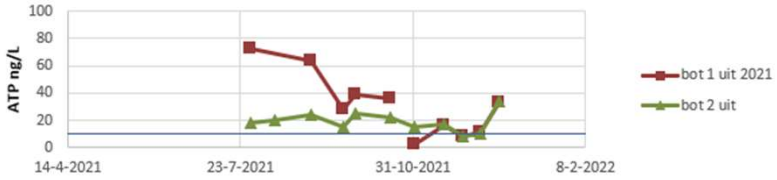


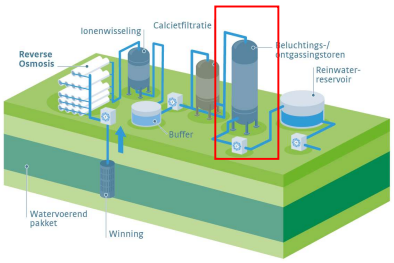




Na vervangen filtermateriaal lijkt de situatie verbeterd

In 2021 nog steeds hoge waardes uit BOT

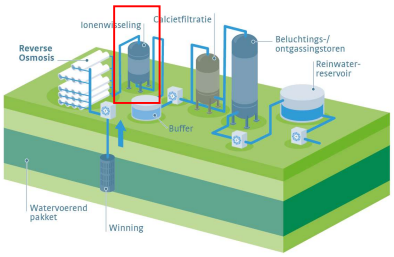


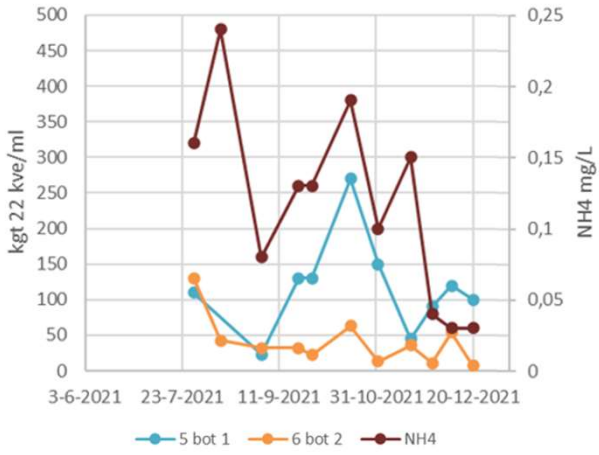


oasen drinkwater

19

Ionenwisseling speelt een rol – Doorslag NH_4





oasen drinkwater

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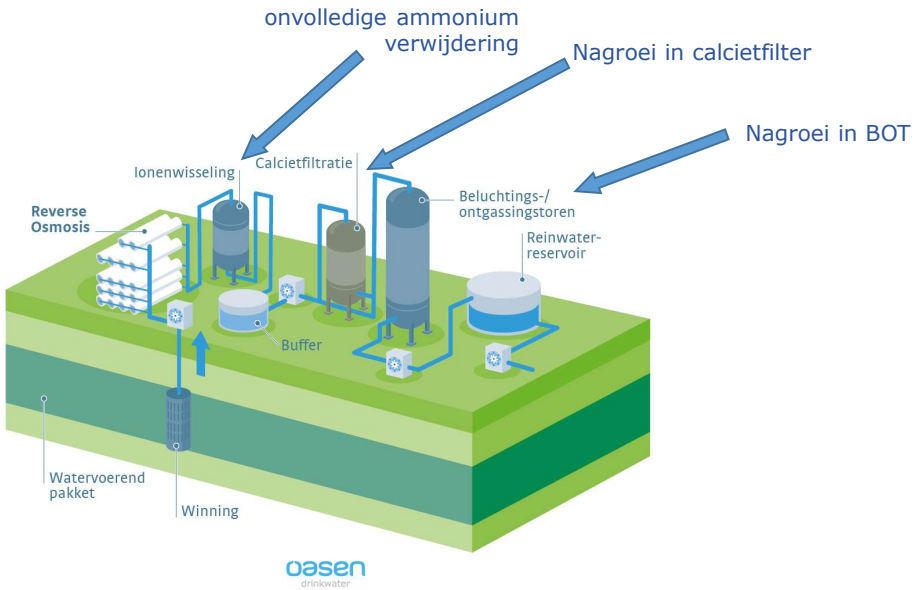
Optimalisatie proces

21

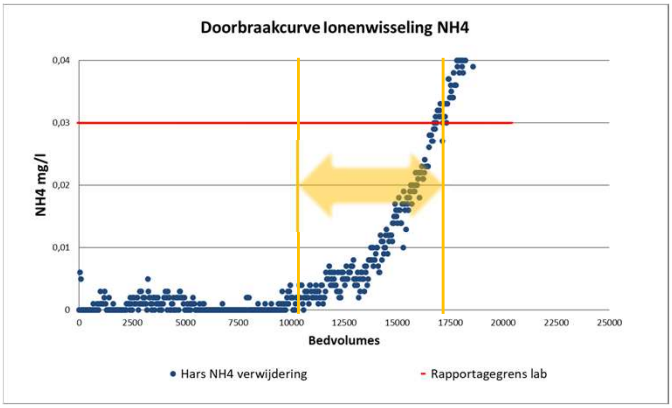
Aandachtspunten conditionering m.b.t. biologische stabiliteit



22

Regeneratieproces Ionenwisseling verbeterd

- Op tijd regenereren
- Zoutconcentratie niet te laag



Ondervangen in full-scale installatie door:

- ✓ Continue monitoring NH4 concentratie in voeding en effluent
- ✓ Geautomatiseerd regeneratieproces
- ✓ Optimum zoeken i.r.t. water- en chemicalienverbruik

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Spoelen calcietfilters verbeterd

- (Langdurige) stilstand kan leiden tot bacteriegroei
- Na stilstand is goed spoelen belangrijk
- Na het spoelen kan er (kortdurig) doorslag van microbiologie optreden (KGT>1000 kve/ml)
- Door frequent spoelen minder groei en ophoping

Ondervangen in full-scale installatie door:

- ✓ Zo min mogelijk en geen langdurige stilstand
- ✓ Optimale spoeling i.r.t. andere aspecten zoals spoelwaterverlies

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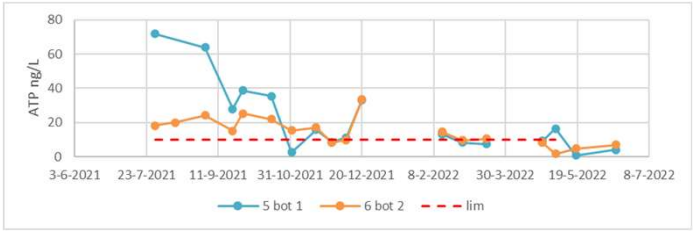
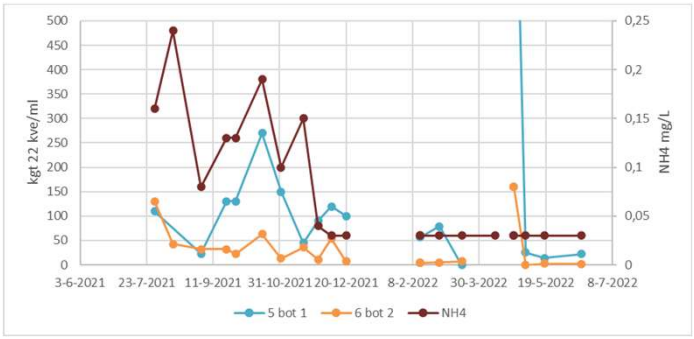
Optimalisaties hebben geholpen. Waterkwaliteit verbeterd.





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Bevestiging met 'Nieuwe' Meetmethodes

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Aantonen en bewaken biologische stabiliteit

We willen een toolset beschikbaar hebben om de biologische stabiliteit van het zuiveringsproces aan te tonen en te bewaken.

Naast KGT22 en ATP zijn dat:

- Flowcytometrie (ICC)
- AOC – A3 (biopolymeren)
- BPP test (BP7 en BPC14) i.p.v. AOC P17/Nox
- Legionellagroeipotentie (éénmalig per full-scale installatie)

Deze tools hebben we getest in onze proefhal en gaan we ook toepassen in onze nieuwe zuiveringsstations.

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Goede resultaten celtellingen, BP7, BPC14 en AOC-A3

ICC/ml	4-5-2022		
iex	15300	iex	15300
caf 2	40980		
bot 2	54390	bot 1	20420
ICC/ml	18-5-2022		
iex	1070	iex	1070
caf 2	68970		
bot 2	57280	bot 1	20210
ICC/ml	15-6-2022		
iex	160	iex	160
caf 2	69450	caf 1	42970
bot 2	67290	bot 1	44710

Analyse	Eenheid	IEX	CaF 2	BOT 1	BOT 2
AOC Stam A3	µg biop.-C/l	< 0,03	0,05	< 0,03	0,70
AOC Stam A3 SD	µg biop.-C/l	-	0,03	-	0,08
BP7	ng/l	3,1	2,2	1,7	2,2
BP7 SD	ng/l	0,1	0,4	0,3	0,7
BPC14	S.14 (d.ng AT	40	29	19	23
BPC14 SD	S.14 (d.ng AT	2,5	8,9	3,0	0,5

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Conclusies

- De biologische stabiliteit van het water in onze proefhal blijkt goed te zijn, ondanks dat we een periode last hebben gehad van verhoogde ATP en KGT22 waardes.
- De bedrijfsvoering van de installatie is belangrijk om nagroei te beperken
- De gebruikte meetmethodes kunnen we inzetten om de prestaties van de nieuwe zuiveringen te monitoren

oasen drinkwater

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VII Presentation Toepassing en betekenis van de biologische stabiliteit methoden bij Evides 2012 - 2020 – Wim Hijnen

Toepassing en betekenis van de biologisch stabiliteit methoden bij Evides 2012-2020

KWR workshop 3 oktober 2022

Wim Hijnen en Rinnert Schurer

Bijdragen

Evides Projectgroep Strategoo (strategisch onderzoek Oud-Beijeland)

- Andries van Eckeveld (PM, sinds kort werkzaam elders)
- Leonie Marang
- Julia Wunderer
- Giovanni Sandrini
- Friso Snijder

Evides collega's van regio, Infra, operationeel beheer, operationele waterkwaliteit, Controle centrum

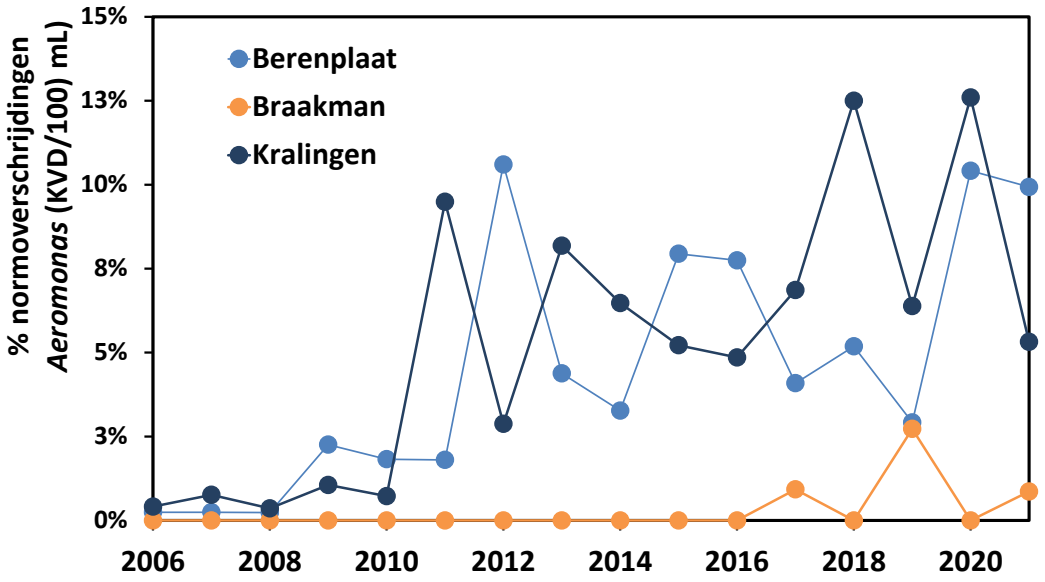
Collega's van KWR en AQZ

Inhoud

- Aanleiding en probleembeschrijving Evides
- Verband met biologische stabiliteit parameters
- Maatregelen onderzoek: veldstudies verbeteren biologische stabiliteit en reinigen
- Conclusies

Probleembeschrijving

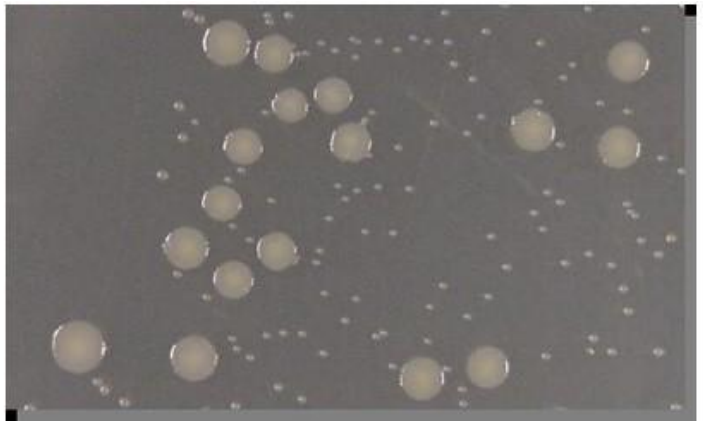
- 2006: stoppen met distributie chloorhoudend drinkwater uit oppervlaktewater
- Nagroei tijdens distributie Berenplaat/Kralingen: verhoging indicatoren KG22/*Aeromonas* (overschrijding norm), coliformen nagroei, beperkt aantal reuk/smaak klachten en aanwezigheid zichtbare organismen (e.g. *Asellus*)
- Niet bij locatie Braakman
- Landelijk beeld 2017 *Aeromonas* overschrijdingen per 10^6 m^3 bij de 10 bedrijven:
  - 6: <0,1
  - 3: 0,1-0,2 (Evides)
  - 1: >0,1



Biologische stabiliteit drinkwater 1.0

Groei potentie drinkwater: AOC P17/NOX

(µg C/L)

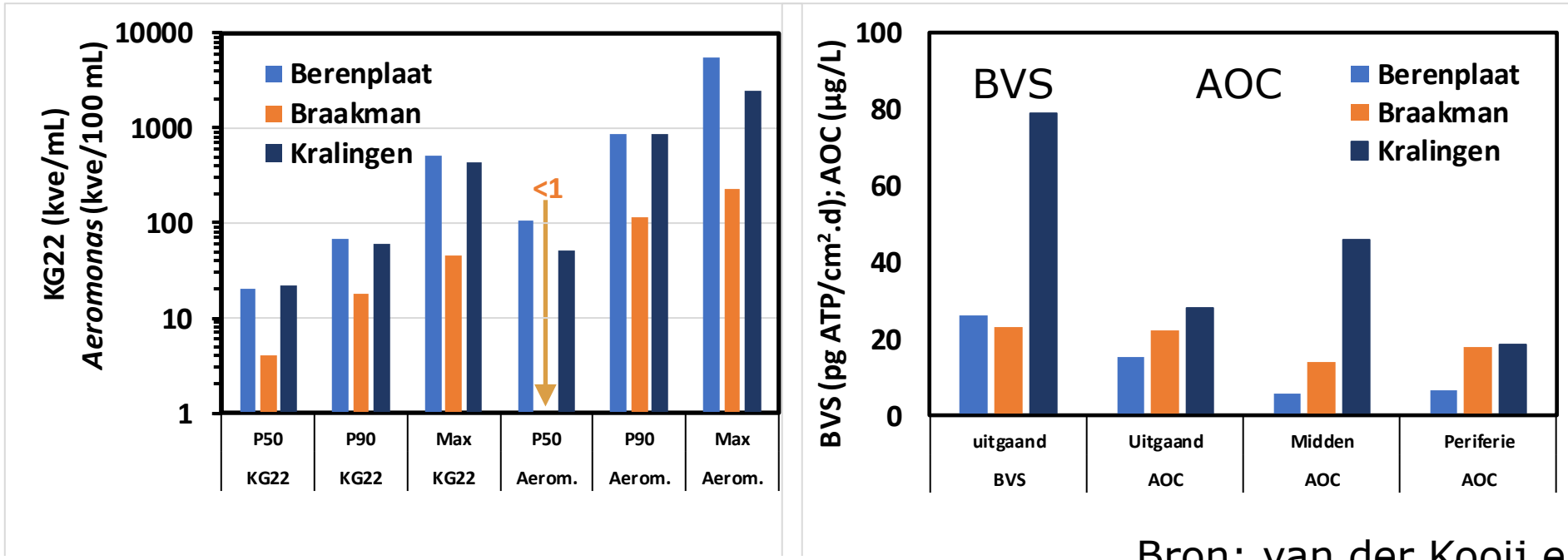


Biofilmvorming: biofilmvormingssnelheid BVS

(pg ATP/cm^2.d)

Probleembeschrijving Evides

- Drie locaties: eutroof reservoir water BPL, BRA, KRA
- Conventionele zuivering: coagualtie, flocculatie, snelfiltratie, UV, BACF
- Koloniegetal 22°C toename: BPL,KRA > BRA (geen overschrijdingen)
- BPL, KRA normoverschrijdingen *Aeromonas,* coliformen, *Legionella;* BRA niet
- Biologische stabiliteit traditionele parameter: AOC-P17/NOX
  en Biofilmvormingssnelheid (BVS) **niet onderscheidend**



Bron: van der Kooij e.a., 2008

Biologische stabiliteit parameters 2.0 bij Evides

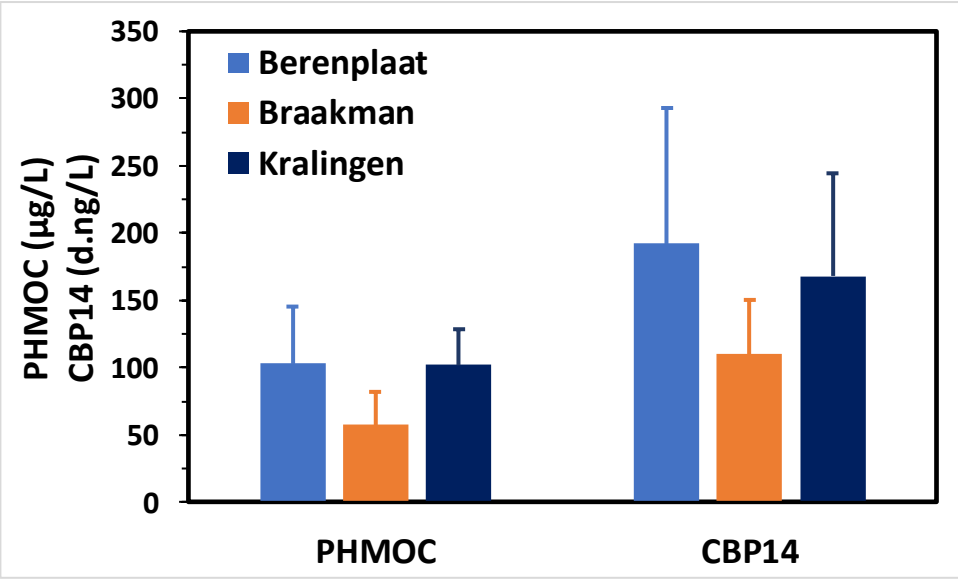
- Uitvoerig onderzoek sinds 2012 in zuivering en DWDS, breed instrumentarium:
- HMWOC, OC: PHMOC, LC-OCD (biopolymeren fractie)
- Assimileerbaar OC: AOC-P17/NOX, AOC-A3
- Groeipotentie: BPP (CPB_{14})
- Biomassa: ATP, TCC (flowcytometrie)
- Microbioom: NGS
- Biofilm: CBM: BAS, FeAS
- Buisuitnames: ATP, TOC, Fe
- Wettelijke parameters: *Aeromonas*, koloniegetal, coliformen,
- Sediment/Invertebraten: 30, 100, 500 µm spuimonsters

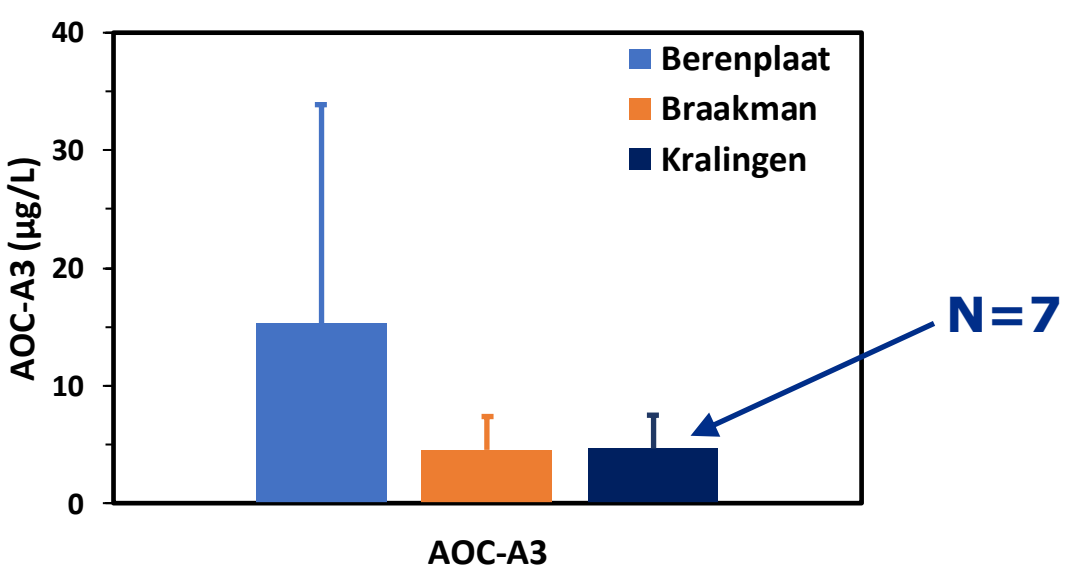
‘voorspellende’ parameters

‘effect’ parameters

Nieuwe methoden

- Evides meetcampagne: 2012-2015
- Particulate and high-molecular weight OC (biomass, biopolymers; PHMOC)
- Biomassa productie potentie (BPP, CBP_{14}): autochtone bacterien
- AOC-A3: biopolymeren (poly-saccharіden, eiwitten, EPS)





Bron: Hijnen e.a., 2018

Biologische stabiliteit Evides locaties: 2012-2020

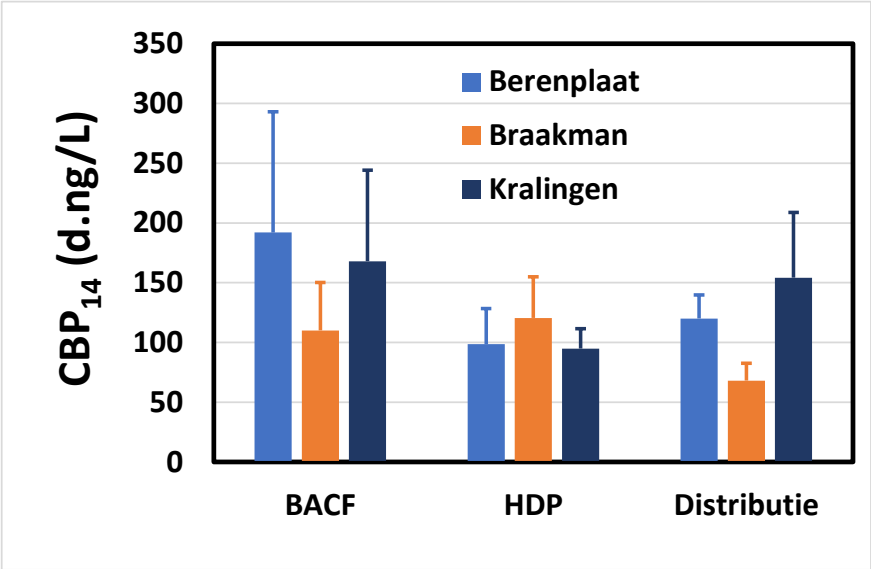
- Gemiddelden: grondwaterlocaties GW beperkte aantal metingen; oppervlaktewater OW groter aantal metingen
- Duidelijk verschil biostab parameters: OW > GW
- Humuszuren GW met nagroei > OW

		Nagroei	DOC	Humuszuren	PHMOC ***	CBP14	AOC-A3
			mg/L	Mg/L	ug/L	d.ng/L	ug/L
GW3	Ossendrecht	-	1.2	0.42	7	30	0.7
DW2	Haamstede	-	1.4	0.8	4	39	0.7
GW1	Huijbergen	(+)*	3.3	1.7**	2	47	0.6
GW2	Halsteren	(+)*	3.1	1.9**	2	52	1.6
SW2	Braakman	-	2	0.87	56	103	4.6
SW3	Kralingen	+	2.1	1.08	113	168	7.6
SW1	Berenplaat	+	2.1	0.95	98	182	8.1
SW4+GW	Baanhoek	+	2.8	1.28	56	191	4.9

← Kralingen

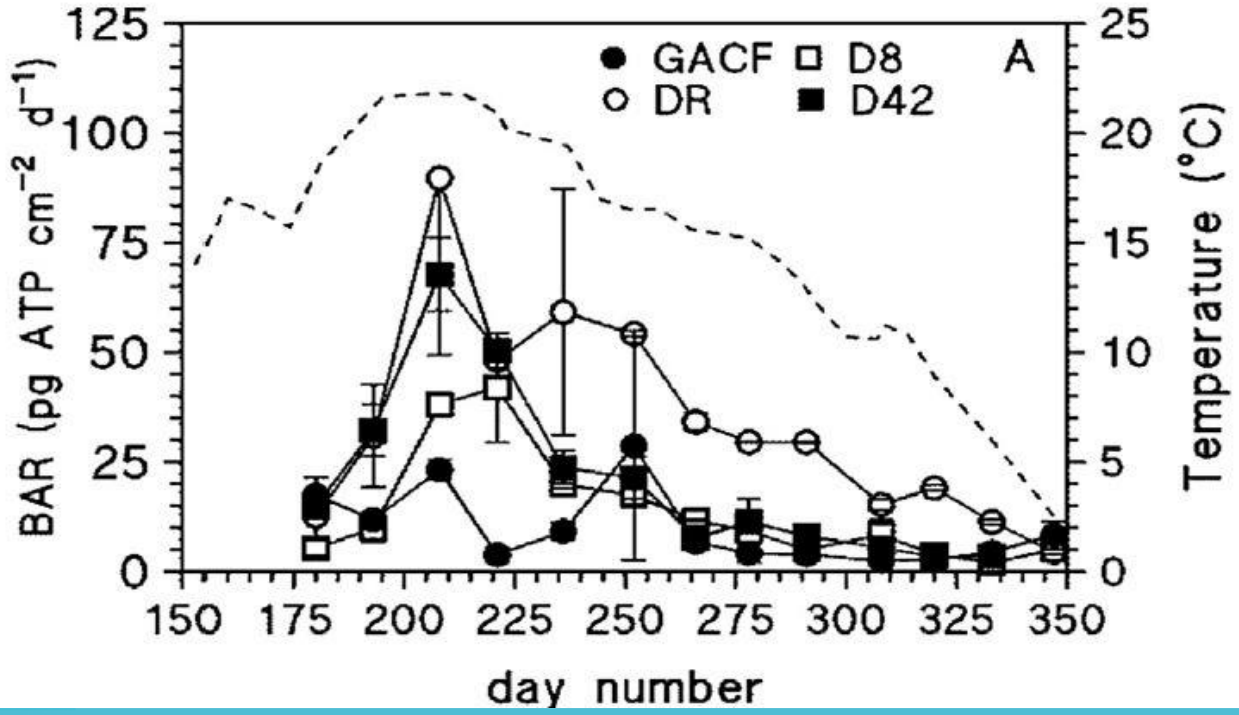
Biologische stabiliteit in leidingnet

- Metingen: BACF – HDP (ClO_2 + RW) – Distributie (dichtbij-midden-ver)
- PHMOC = geen significant meetbare effecten (hoge waarden versus lage reductie)
- CBP_{14}:
  - BACF – HDP = afname bij BPL/KRA
  - HDP – distributie: toename Berenplaat/Kralingen; afname bij Braakman
  - Gemiddelde distributie waarde Braakman < Berenplaat en Kralingen
  - Dynamiek houdt mogelijk verband met biomassa concentratie (Schurer e.a., 2021)



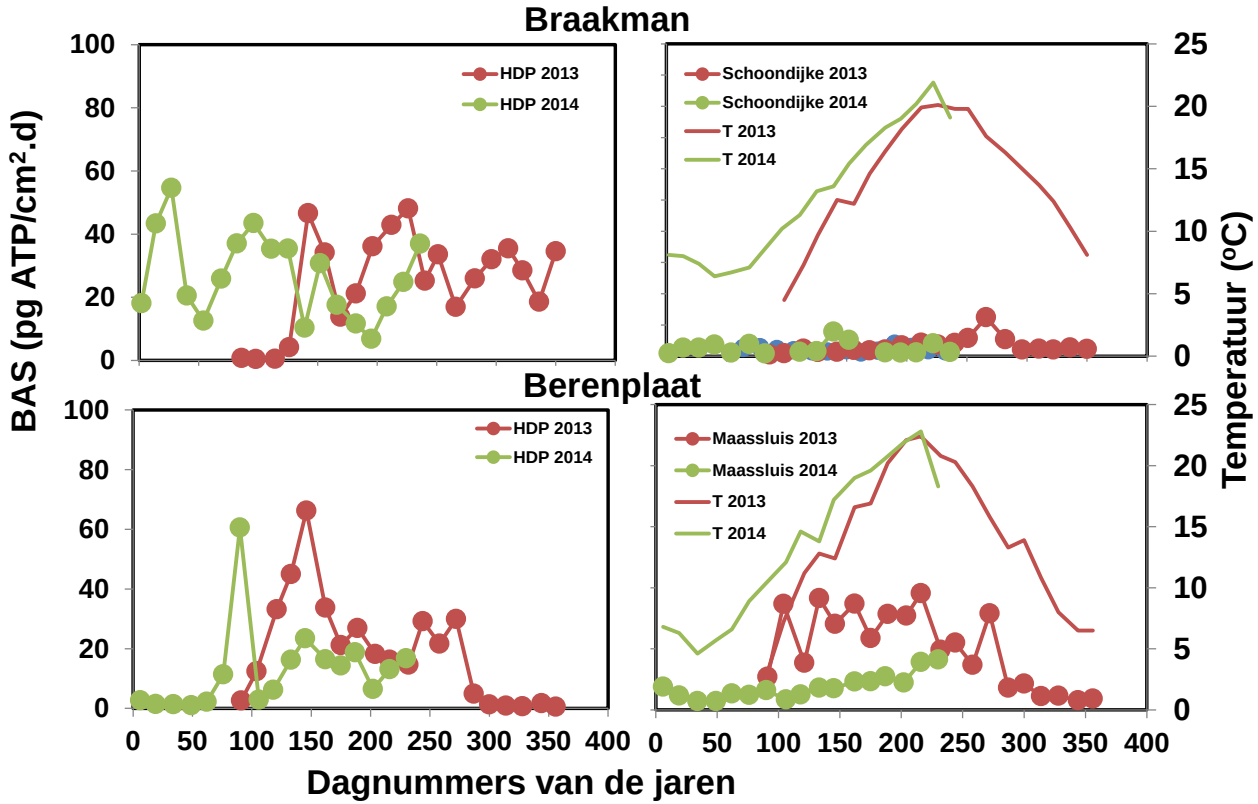
Continue biofouling monitor (CBM)

- Biomassa accumulatiesnelheid BAS: van der Kooij e.a. 2015
- Drinkwater uit eutroof oppervlaktewater: na AKF (GACF) en in het distributienet (D)
- BAS-toename distributie
- Variatie BAS: temperatuur, biologische stabiliteit, Fe …….



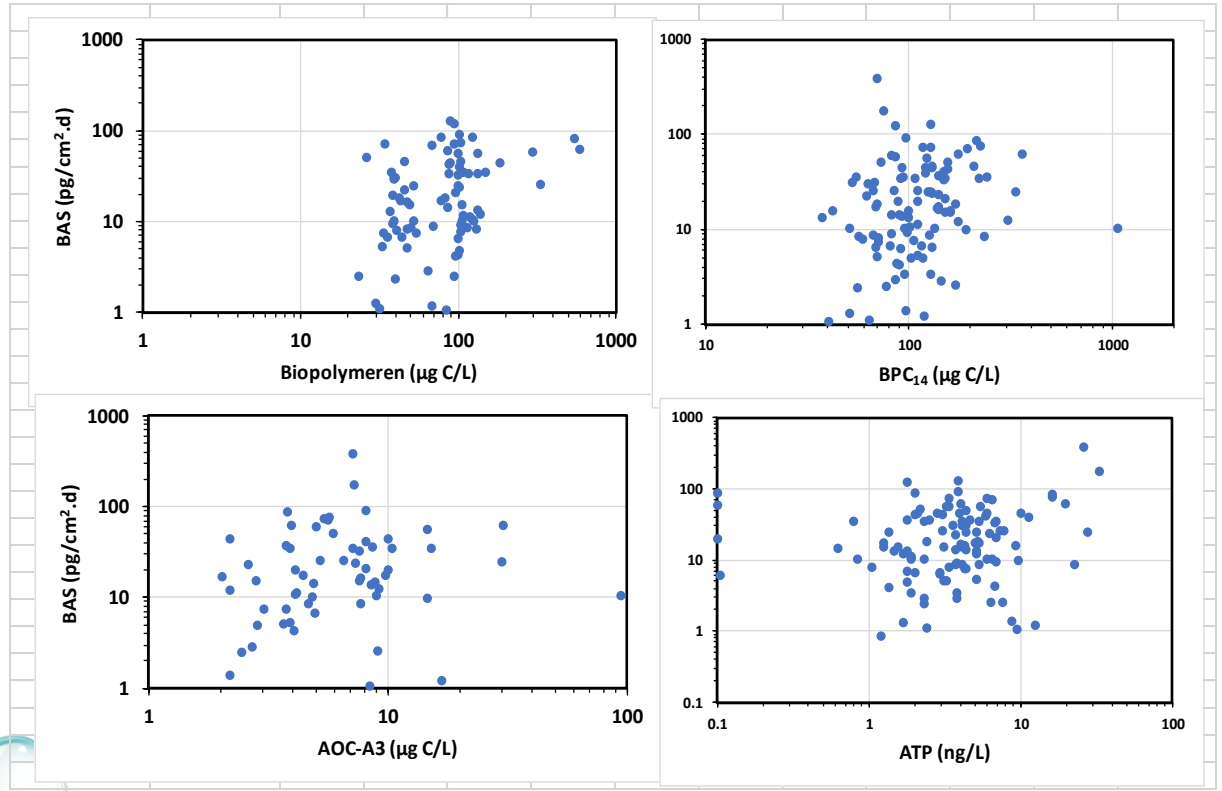
Continue biofouling monitor (CBM)

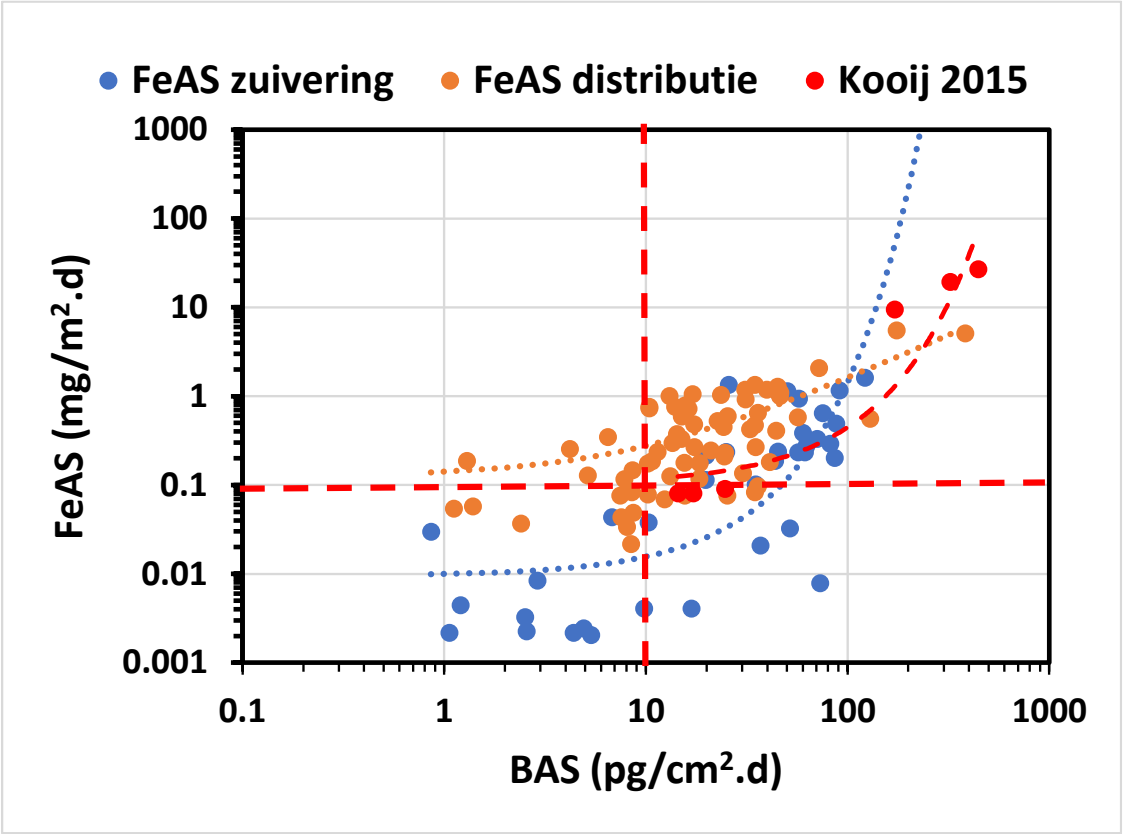
- Biomassa accumulatiesnelheid BAS Evides: Braakman – Berenplaat 2013-2014
- Berenplaat: seizoenseffect HDP ≤ leidingnet
- Braakman: geen seizoenseffect HDP, daling in leidingnet



Verband BAS met biologische stabiliteit parameters

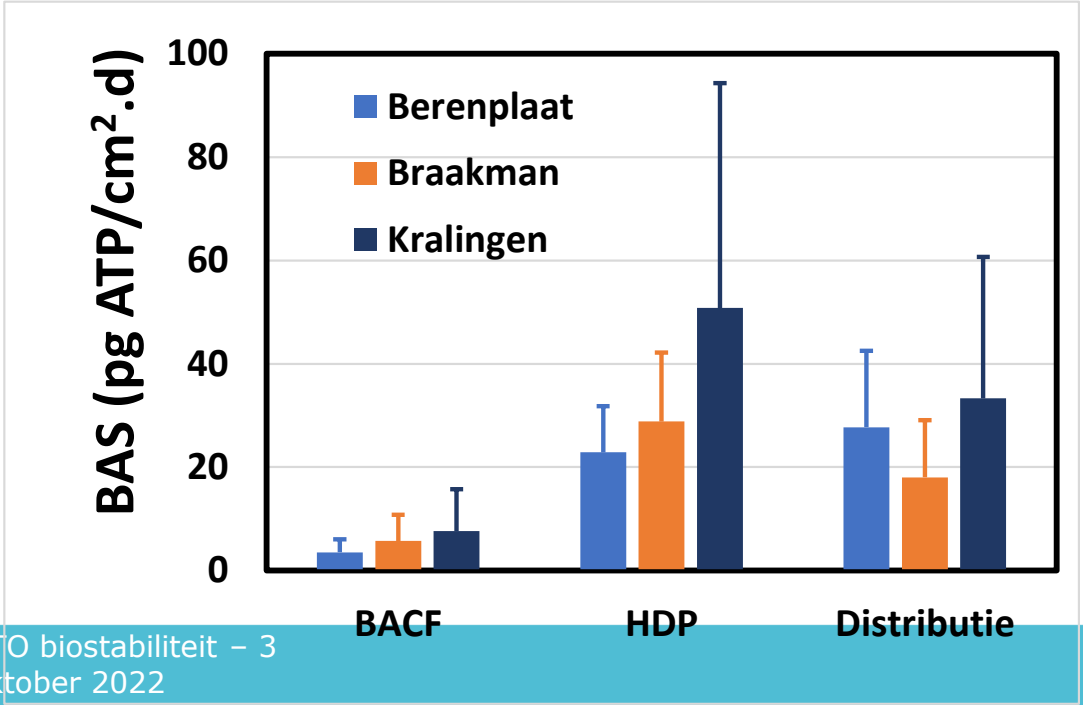
- Geen significante enkelvoudige correlaties met ATP, BP, CBP14, AOC A3
- Wel significante correlaties met FeAS in de CBM: BAS ≥ 10 en FeAS ≥ 0.1
- Biofilmvorming: multi-variabel proces ……wordt vervolgd





Biologische stabiliteit CBM in leidingnet

- Metingen: BACF – HDP (ClO_2 +RW) – Distributie (dichtbij-midden-ver)
- BAS:
  - Bij hogere waarden grotere variatie: seizoenspatroon
  - BACF – HDP = toename
  - HD – distributie: toename Berenplaat; afname bij Kralingen/Braakman
  - Distributie: Braakman < Berenplaat en Kralingen = gelijk aan verschil in CBP14
  - Geen duidelijke verklaringen voor dynamiek: biofouling = multi-variabel proces



Andere factoren dan biologische stabiliteit drinkwater

- Fe als chemische reactieve component: coagulatie/deeltjesvorming en oxidatief
- BPL/KRA Fe als coagulant en BRA aluminium als coagulant
- Leidingnet configuratie en verbruikspatronen:

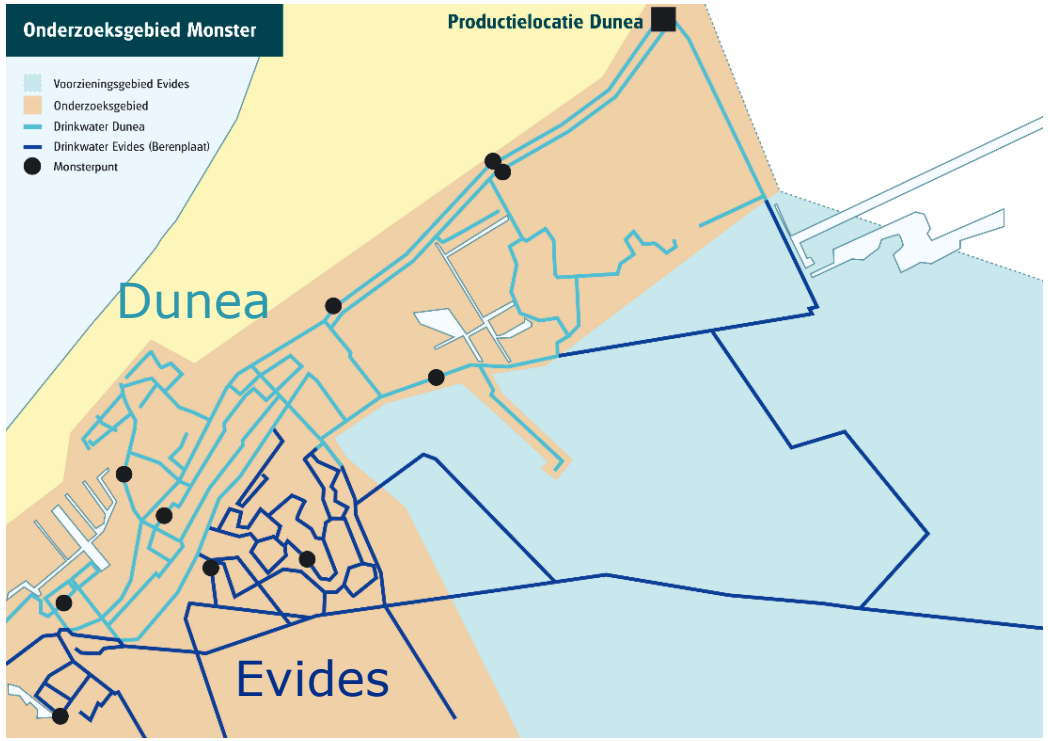
Gebiedseigenschap	Berenplaat/Kralingen	Braakman
Stedelijk/vermaasd/vertakt	x	-
Landelijk/minder vermaasd/eindstandig	-	X
Verschil zomer-winter verbruik door populatieverschil in de vakantieperidode	Braakman > Berenplaat/Kralingen	

Veldstudie 1: verhogen biologische stabiliteit

- Distributie biologisch stabiel drinkwater: 2017-2020
- Dunea: biologische stabieler duinwater na langzame zandfiltratie in Monster

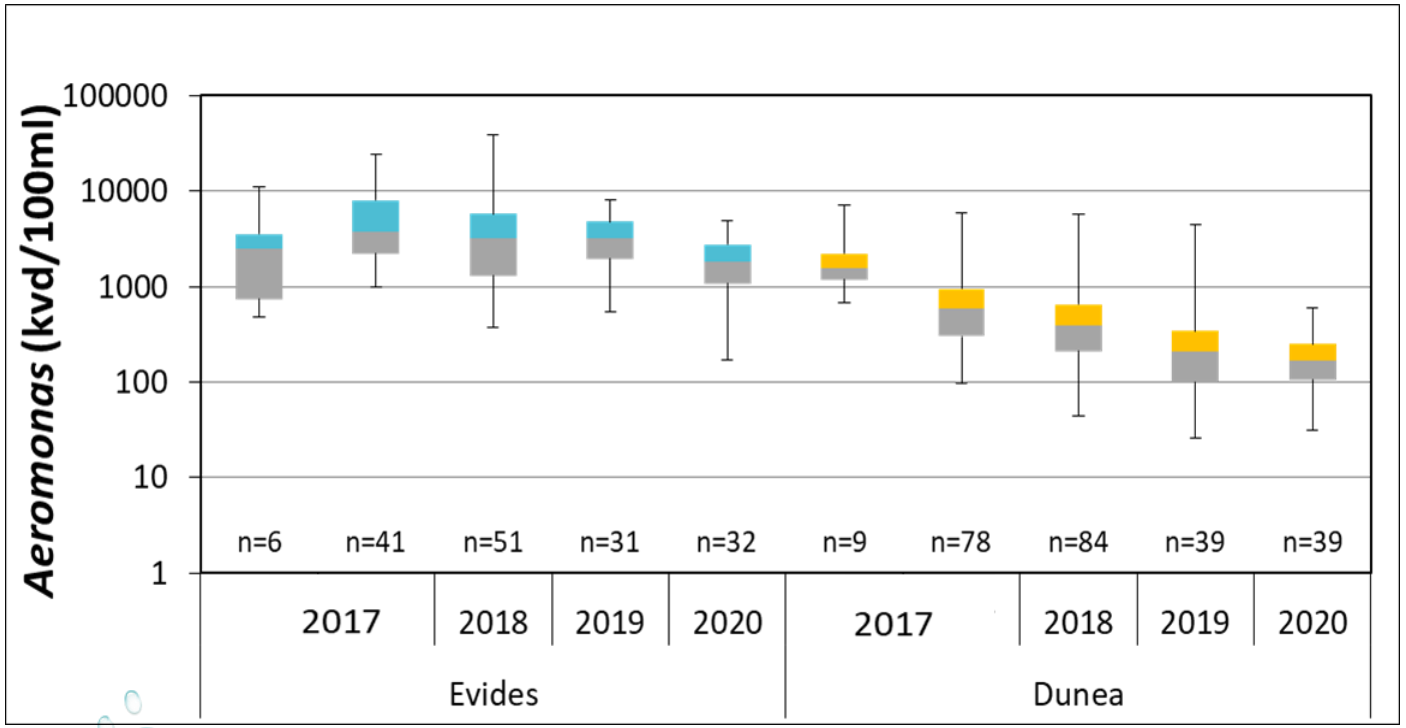
Parameter	Evides	Dunea
Biopolymeren	70	8
AOC A3	6	<1
CBP14	113	66

- Distributiegebied van Monster
- Evides versus Dunea water
- 4 jaar



Biologisch stabiel duinwater

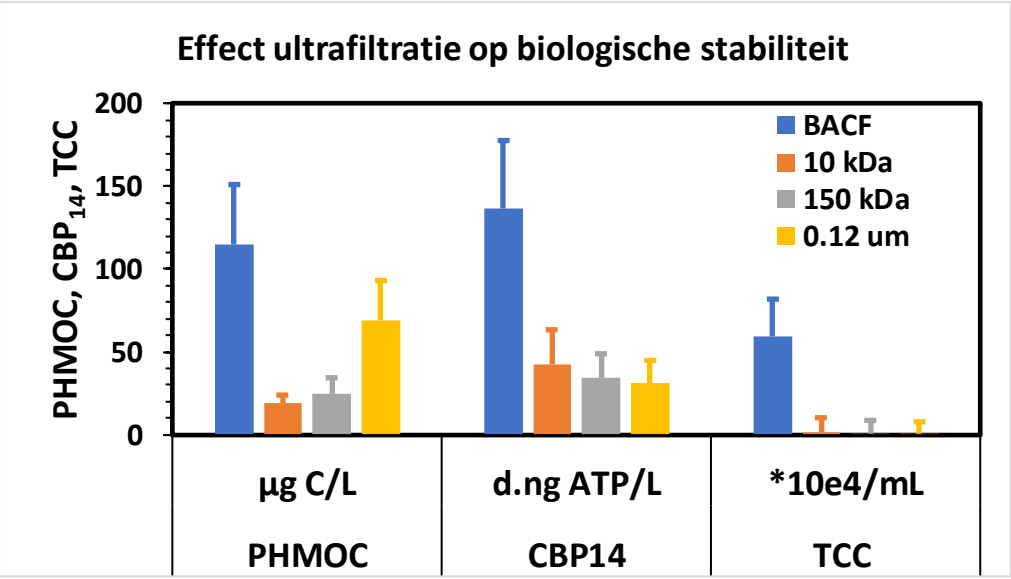
- Daling *Aeromonas* mediaan waarden juli-september
- Naast waterkwaliteit verbetering ook reductie transportleiding volume: invloed?

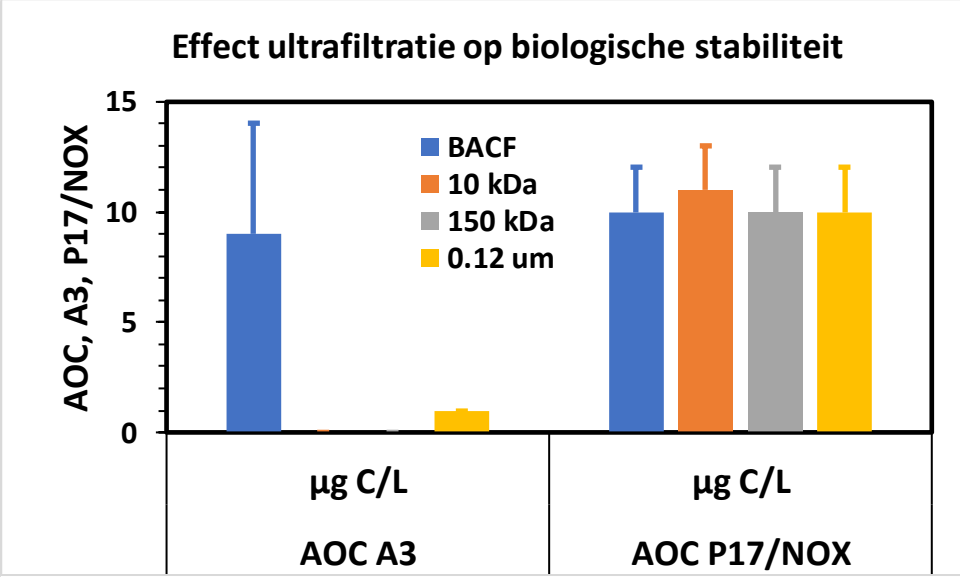


Mediaan Kvd/100 ml in:	Evides	Dunea
2017	5350	495
2018	3400	340
2019	3200	210
2020	1800	170

Verhogen biologische stabiliteit: ultrafiltratie

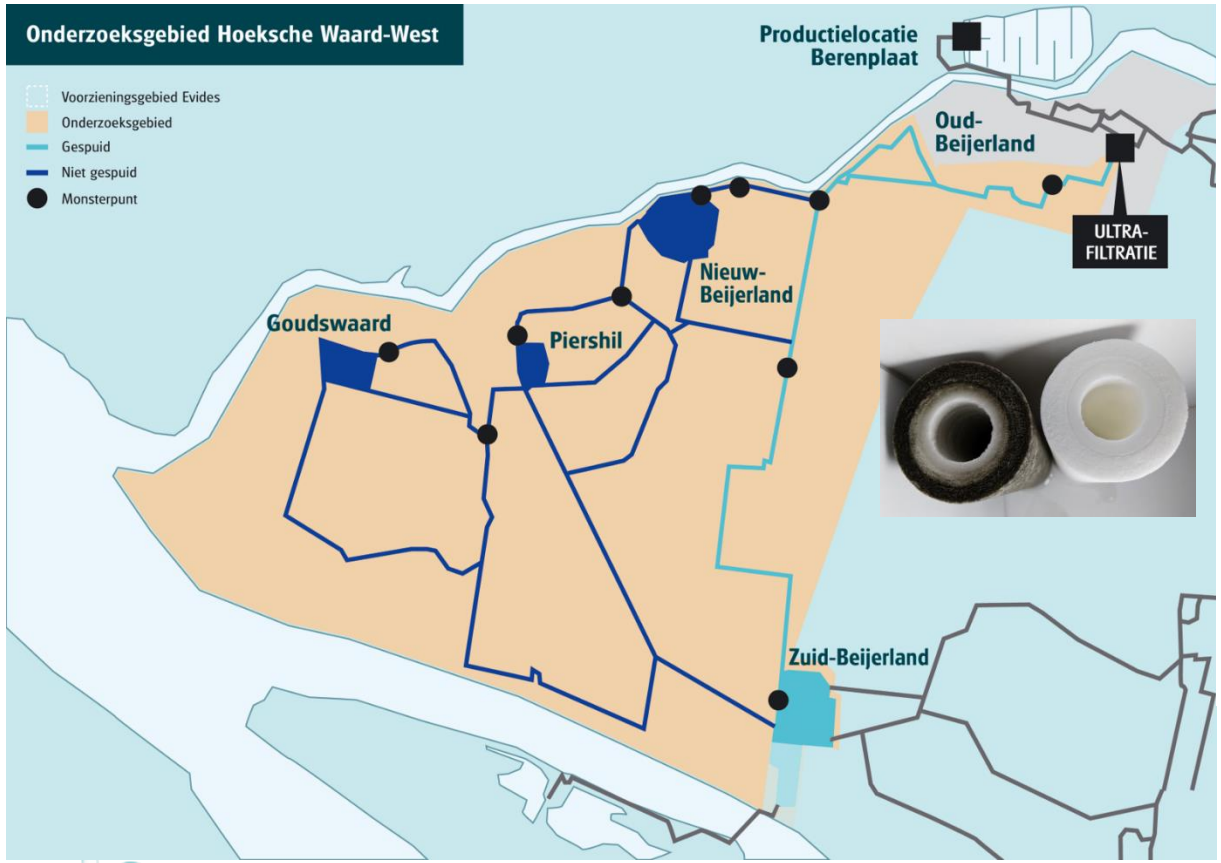
- Ultrafiltratie: Schurer e.a., 2019
- PHMOC biopolymeren, CBP14 en biomassa (cellen, TCC)
- AOC moeilijk en makkelijk afbreekbare OC

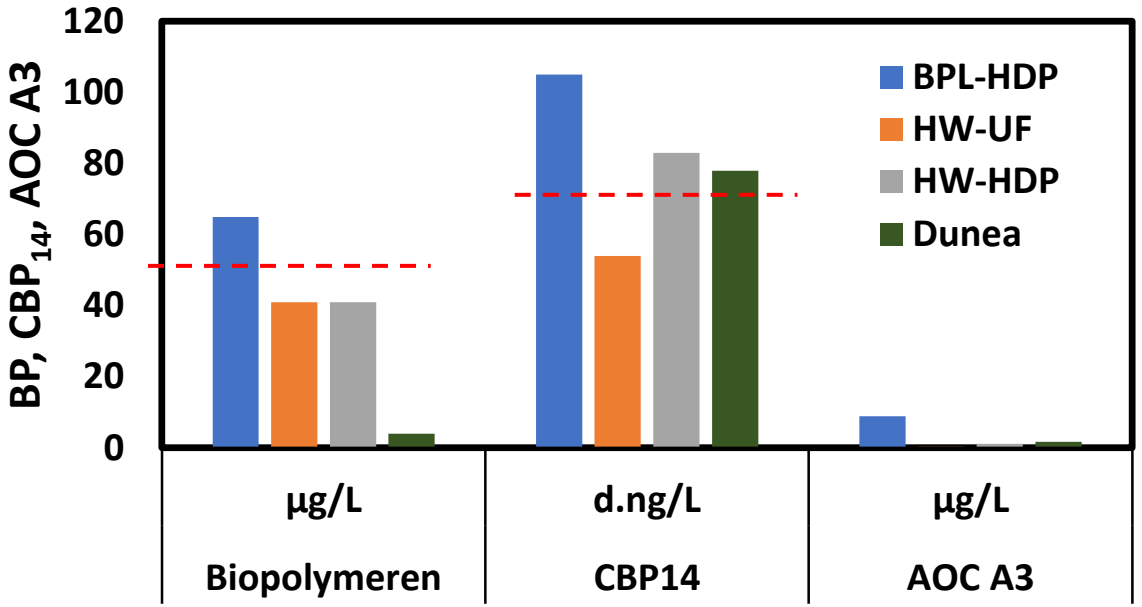




Ultrafiltratie 150 kDa

Berenplaat – Hoeksche Waard: verbetering biologische stabiliteit met en zonder reinigen



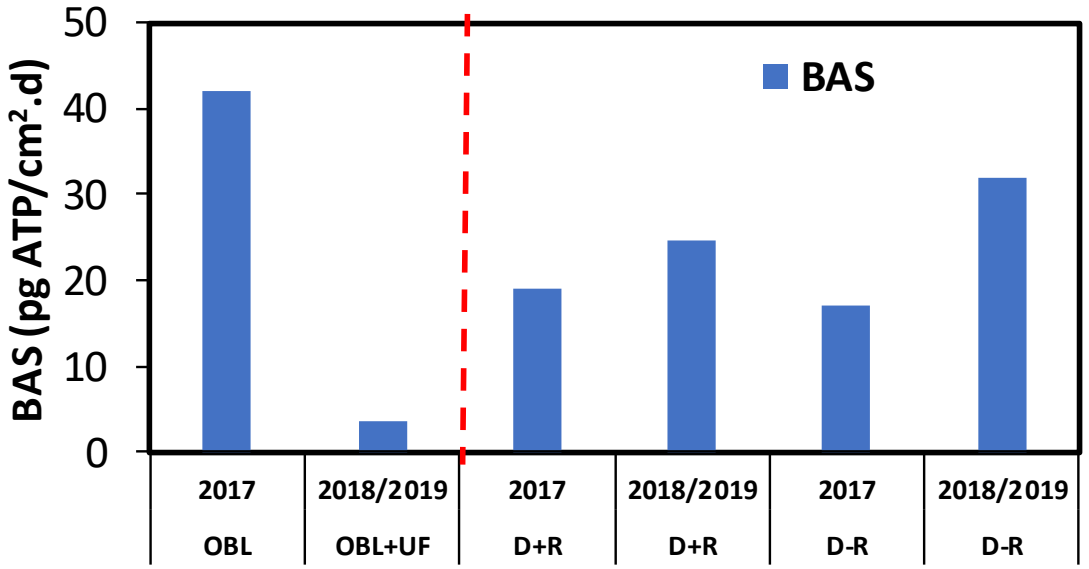


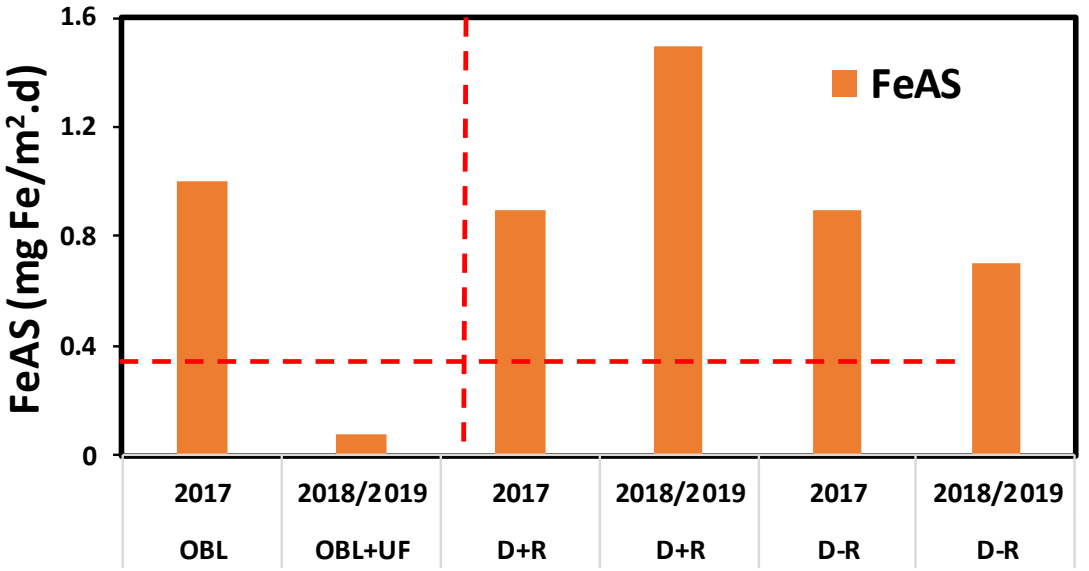
Richtwaarden biologische stabiliteit:

- BP = 47
- MBG_7 = 4.5
- [CBP_{14} = 75 ranking methode]

Ultrafiltratie 150 kDa Oud-Beijerland (OBL)

Berenplaat – Hoeksche Waard: verbetering biologische stabiliteit met en zonder reinigen





Richtwaarden biologische stabiliteit:

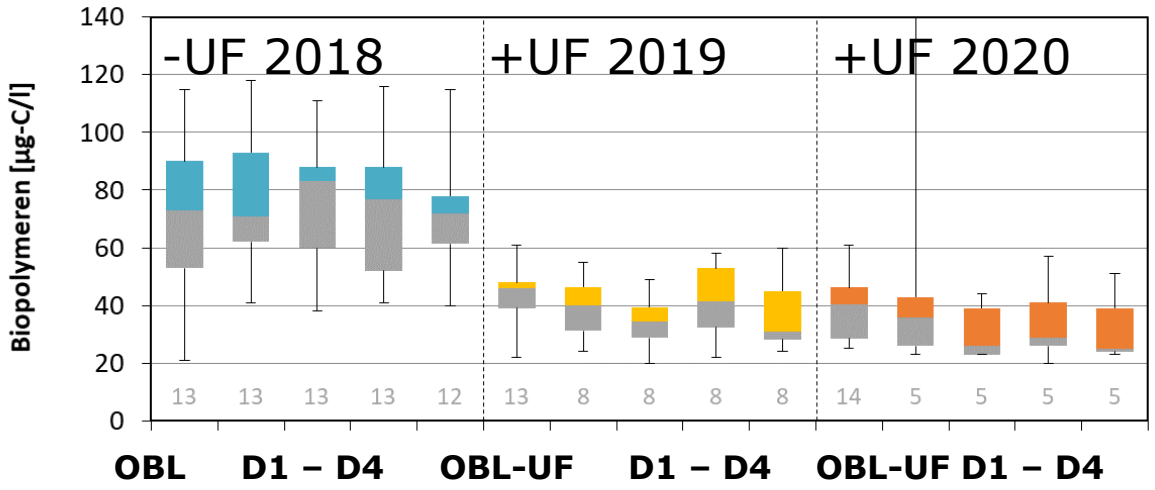
- BAS geen aandachtswaarde gevonden
- FeAS = 0.34 mg Fe/m².d

Distributiegebied

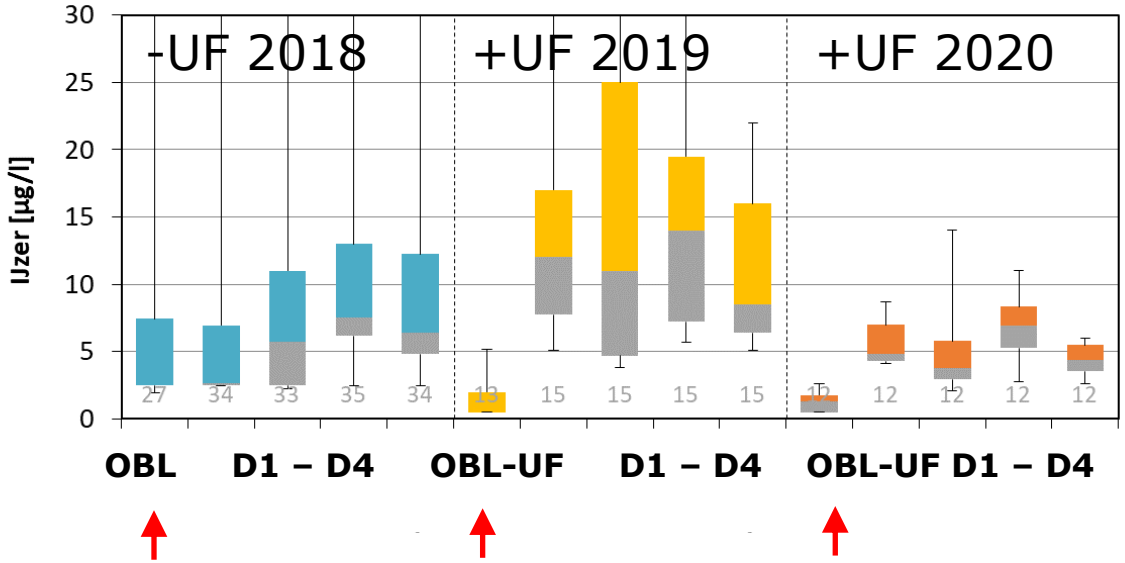
Oud-Beijerland OBL zonder (2018) en met UF (2019-2020)

- D1 – D4 = 4 woonkernen op toenemende verblijftijd (max. ca. 3 dagen)
- Aangeboorde monsterkasten
- Verlaging biopolymeren en ijzerdeeltjes
- Toename Fe tijdens distributie 2018 en 2019 > 2020 (biofilm verlaging door lagere AOC?)

Biopolymeren



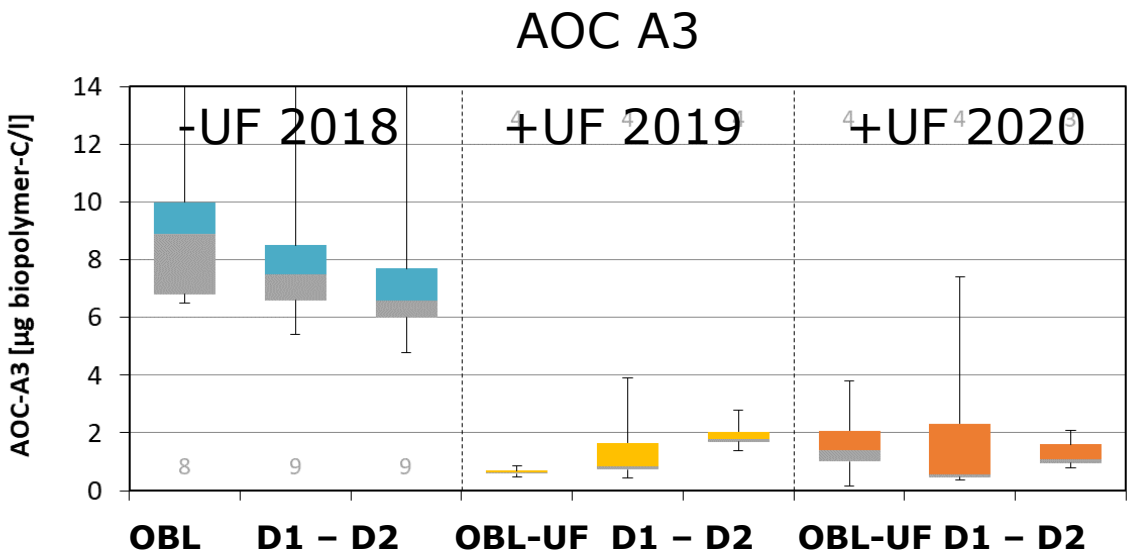
Ijzer

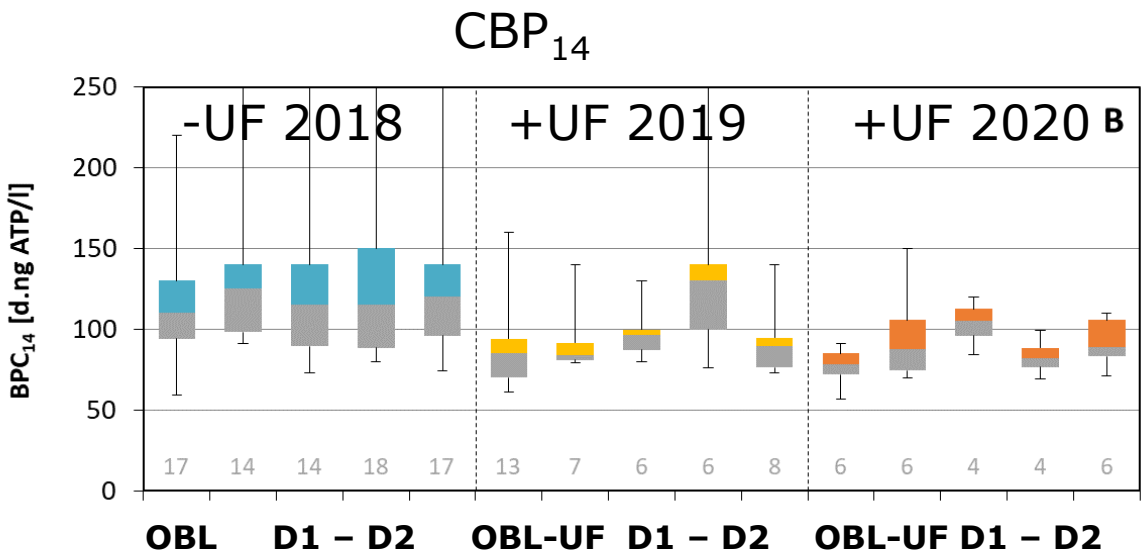


Distributiegebied

Oud-Beijerland OBL zonder (2018) en met UF (2019-2020)

- Verlaging AOC A3 en CBP_{14}
- Leidingnet waarden stabiel

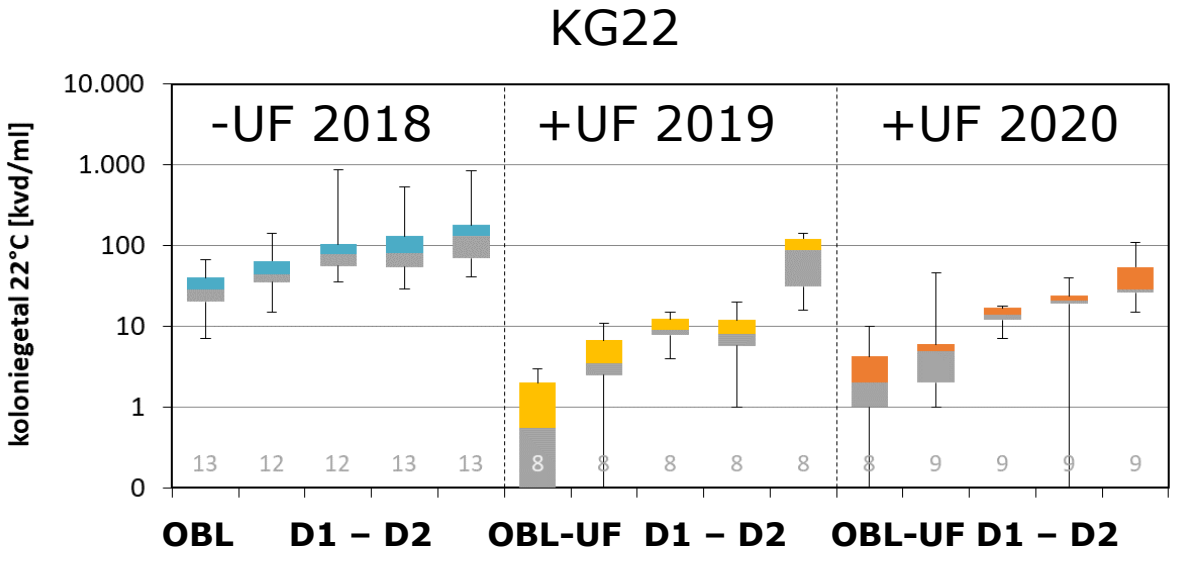


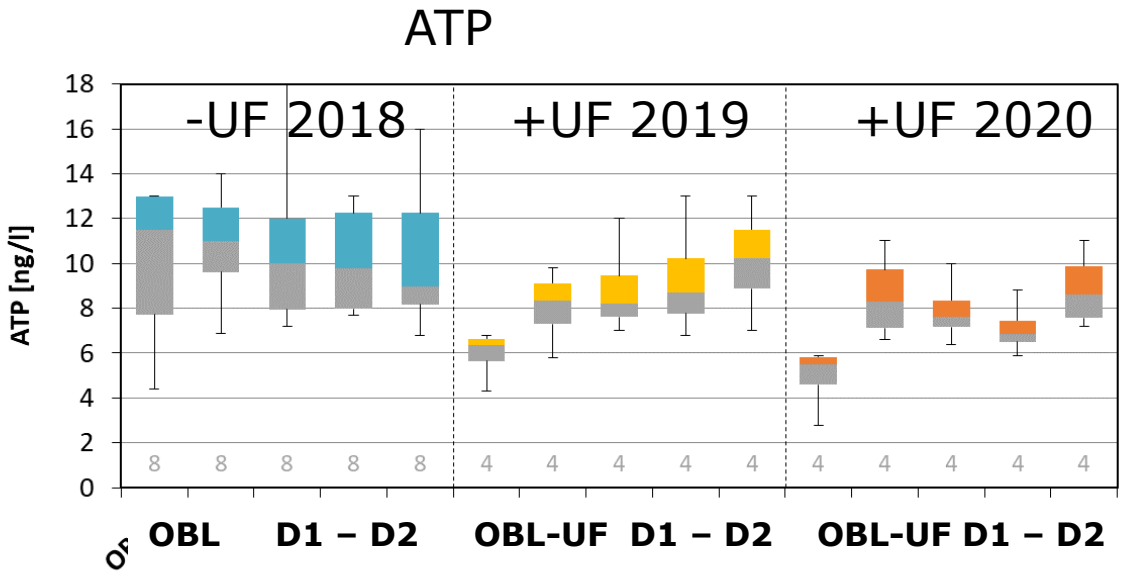


Distributiegebied

Oud-Beijerland OBL zonder (2018) en met UF (2019-2020)

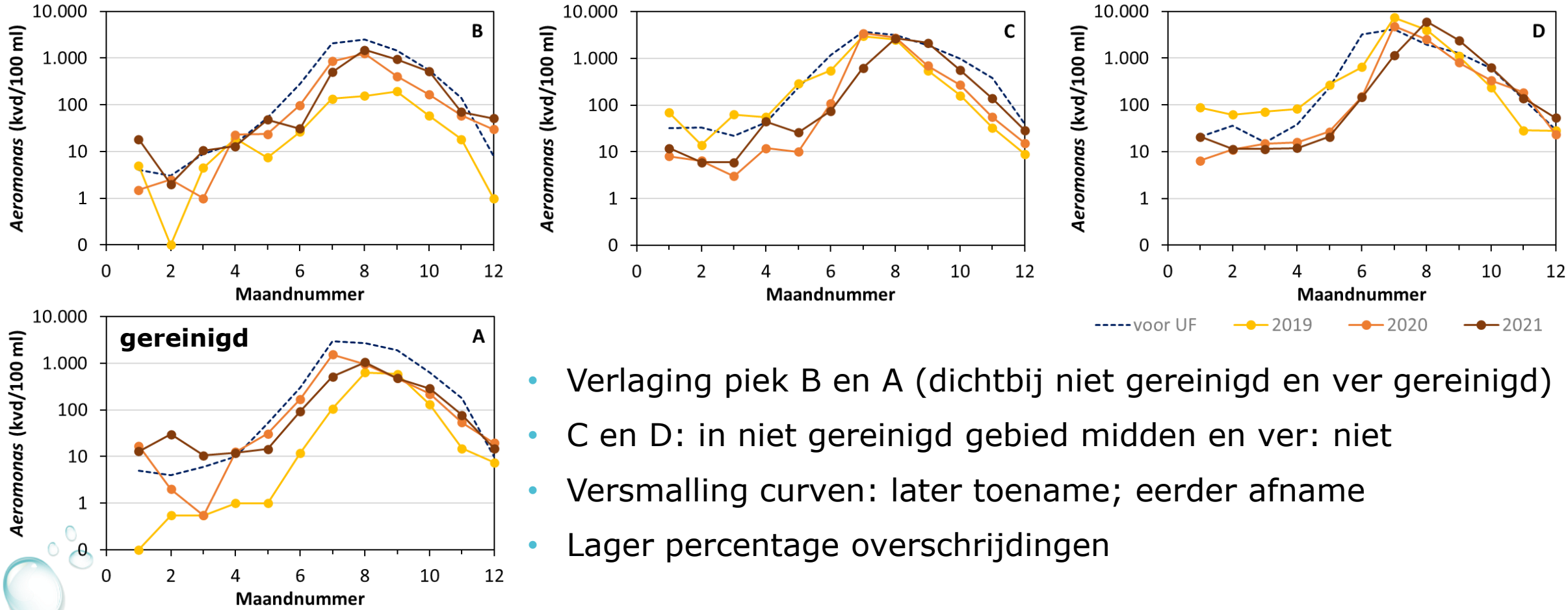
- Verlaging KG22 en ATP maar …
- Leidingnet toename: nagroei 2019 > 2020





Aeromonas maand mediaanwaarden woonkernen

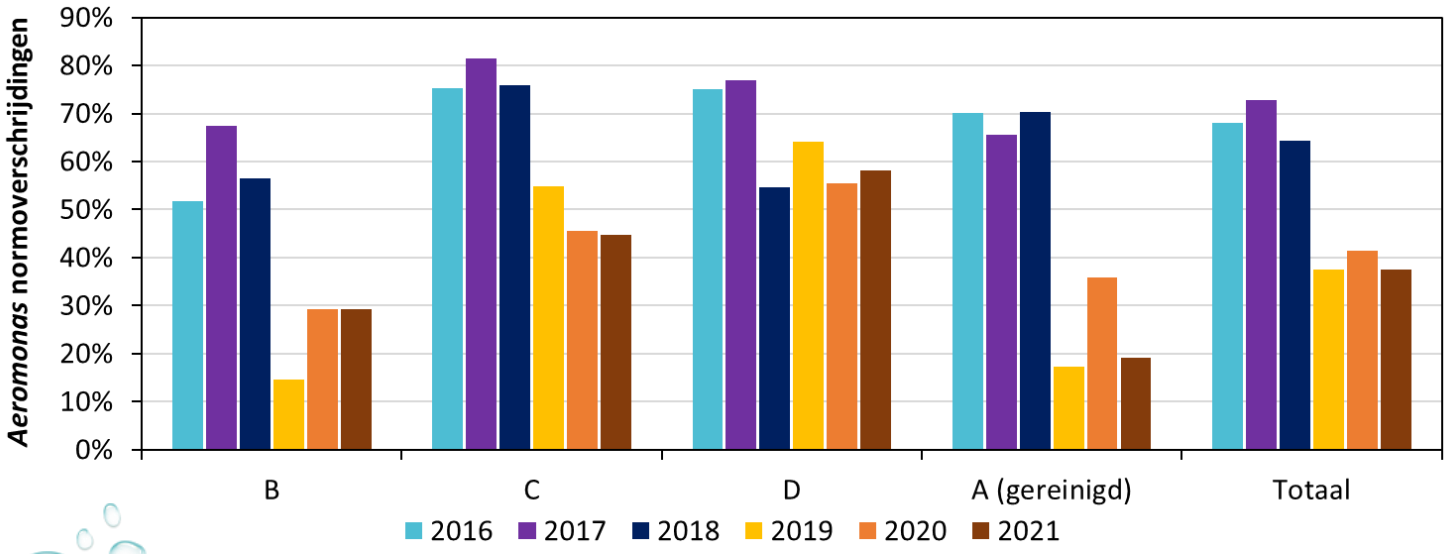
- Distributielocaties zonder reinigen: dichtbij UF – verder - verst = B – C – D
- Distributie met reinigen: verst = A

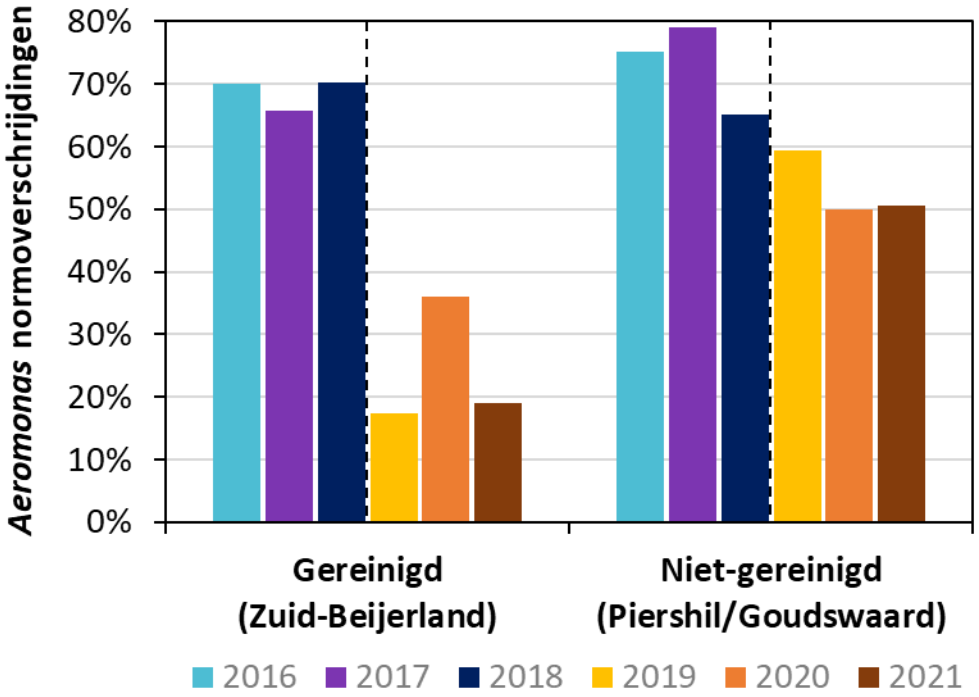


- Verlaging piek B en A (dichtbij niet gereinigd en ver gereinigd)
- C en D: in niet gereinigd gebied midden en ver: niet
- Versmalling curven: later toename; eerder afname
- Lager percentage overschrijdingen

Normoverschrijdingen

- 2016-2018 voor en 2019-2021 na UF
- Verlaging van de normoverschrijdingen
- Grootste daling in gereinigd gebied
- 2021: na 3 jaar geen verdere daling?
- Geen coliformen, afname sediment





Conclusies 1

- Evides ervaart *Aeromonas* als gevoelige indicator voor ‘nagoei’ of ‘ biologische instabiliteit’
- Beperkte coliformen en *Legionella* overschrijdingen in distributieleidingen
- Beperkte technische/esthetisch problemen: reuk/smaak, deeltjes/bruinwater, watermeter verstopping

- Meer nagroei in oppervlaktewater- dan grondwaterlocaties
- Traditionele BS parameters AOCP17/NOX en BVS: niet onderscheidend
- Nieuwe BS parameters met verbreding (A)OC: wel onderscheidend
- Grondwater biologisch stabieler, humuszuren-fractie OC als factor van belang?!
- PHMOC (biopolymeren) en CBP_{14} (BP_7)
- CBM: waarde van de parameter nog niet geheel duidelijk

Conclusies 2

- Verbetering BS betekent niet momentaan verbetering *Aeromonas*: lange duur
- Biologisch stabiel duinwater: positief effect met infrastructurele kanttekening
- Minder biologisch stabiel UF permeaat:
  - Verlaging coliformen, KG22, Fe, verder nog geen ervaringen …….
  - Effect: breder kijken dan alleen *Aeromonas* en Geduld
- Aandachtswaarde BS nog niet duidelijk: lager dan landelijke aandachtswaarde biopolymeren (gebaseerd op Braakman)!
- Twee andere factoren die een rol spelen in het leidingnet verder onderzoeken:
  - IJzer
  - configuratie/verbruikspatroon
- Vervolgonderzoek: van 150 naar 10 kDa membranen voor verdere verlaging biopoymeren

Bedankt voor de aandacht

VIII Presentation Study of Biological Stability at PWN – Emmanuelle Prest

STUDY OF BIOLOGICAL STABILITY AT PWN:

A combination of laboratory and on-site measurements

- BTO workshop, 3 october 2022

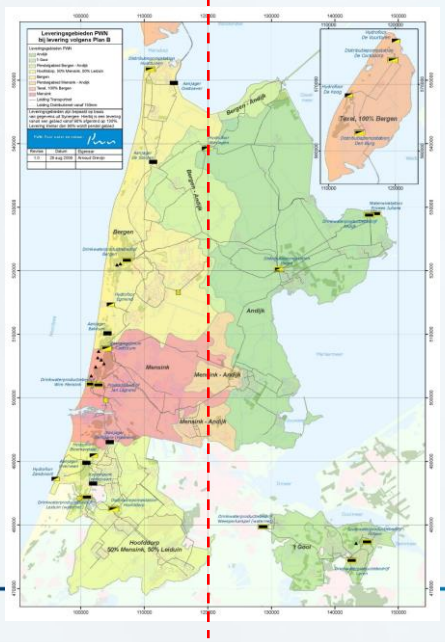
Emmanuelle Prest

1

BIOLOGICAL (IN)STABILITY AT PWN

BERGEN

Dune infiltration



ANDIJK

Direct treatment

- Customer complaints for brown or turbid water or presence of visible invertebrates
- *Aeromonas* guidelines exceeded (>30% every summer)
- Legionella (non-pneumophila) growth in buildings
  (Van der Lught et al., 2019, Water Research 161)

Biological **STABLE** water

Biological **UNSTABLE** water

2

BIOLOGICAL STABILITY STUDIES AT PWN

Main goal / focus

Understand mechanism leading to sediment formation in distribution system of Andijk

Approach:

1. Description of spatial and temporal dynamics in the system
2. Mechanistic understanding of processes leading to biological instability

This presentation:

Examples of different methodologies / analytical strategies for the study of biological stability

Advantages and drawbacks

3

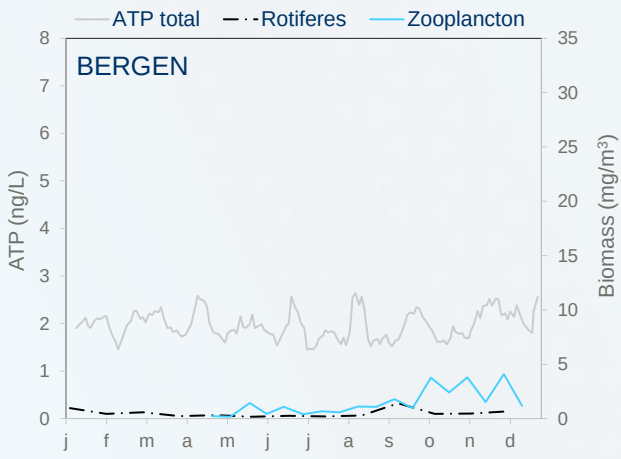
SYSTEM DESCRIPTION

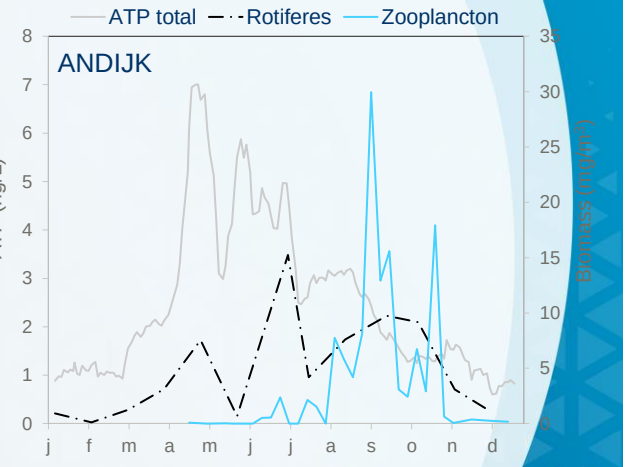
- Use of large-scale monitoring data

4

BIOMASS RELEASED BY THE TWO TREATMENT PLANTS

Measurements of ATP, rotifers and zooplankton > 30 µm





Impact of dune infiltration:
stable water quality over the seasons

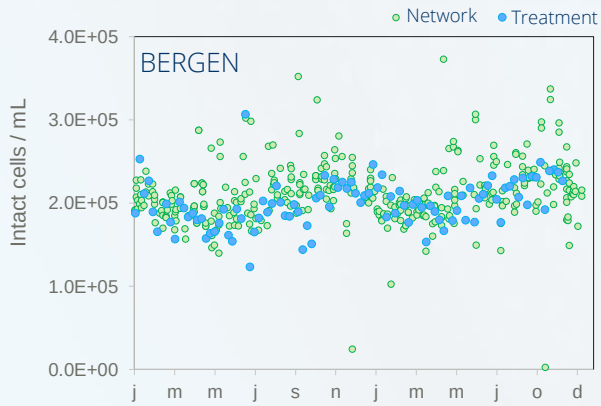
Impact direct treatment:
high variations over the seasons

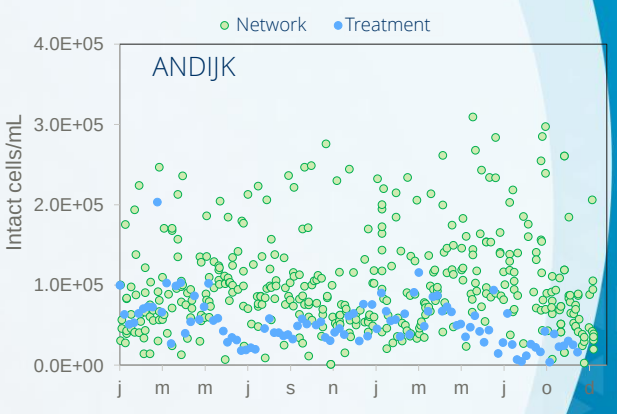
Seasonal variations essential aspect

5

INTACT CELL CONCENTRATION IN THE TWO DISTRIBUTION SYSTEMS

Flow cytometry measurements at treatment effluent and throughout the distribution system





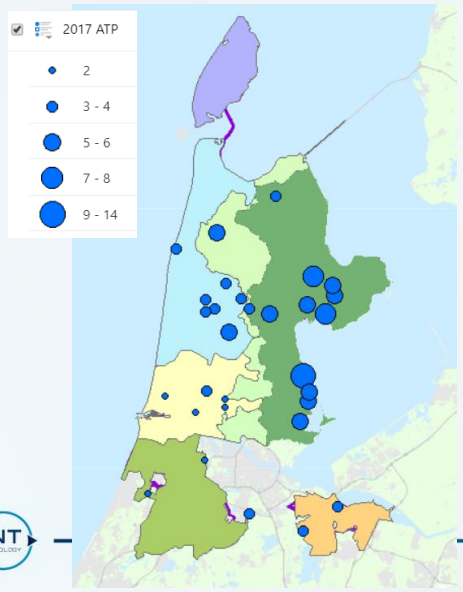
Limited bacterial growth

Lower intact cells counts from treatment
but Higher bacterial growth

Lower values at treatment effluent does not mean better quality / stability!

6

DETECTION OF PRIORITY AREAS



Highest ATP values in area supplied by WTP Andijk

⇒ Confirms known problems at priority areas: sediments, past customer complaints on turbid water, need for regular cleaning / flushing program

7

USE OF LARGE-SCALE MONITORING DATA: ADDED VALUE

- Good understanding of the system / dynamics in the system
- Detection of hotspots

8

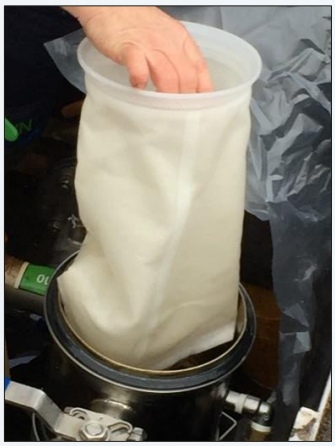
SYSTEM DESCRIPTION

- Use of large-scale monitoring data
- Continuous collection of sediments from distribution system

9

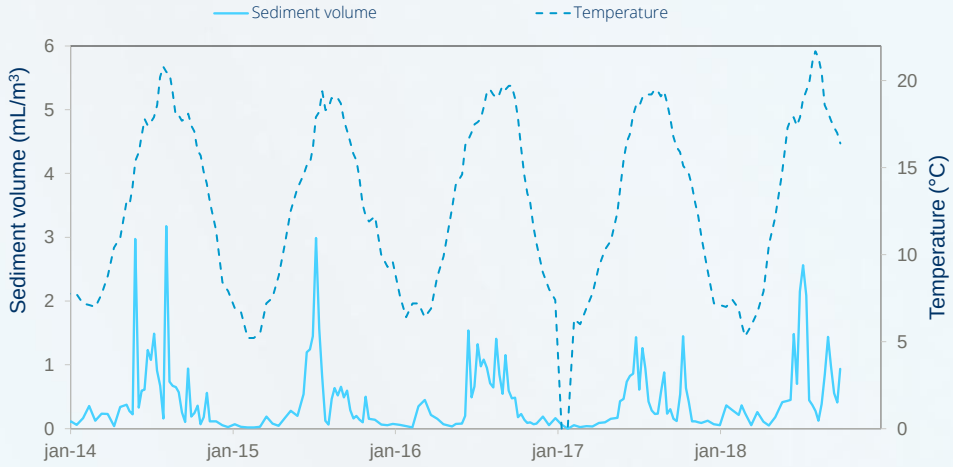
SEDIMENT FORMATION IN THE TWO DISTRIBUTION SYSTEMS

Methodology: collection of sediments > 25 µm with "zakfilters"



10

SEDIMENT FORMATION IN THE TWO DISTRIBUTION SYSTEMS



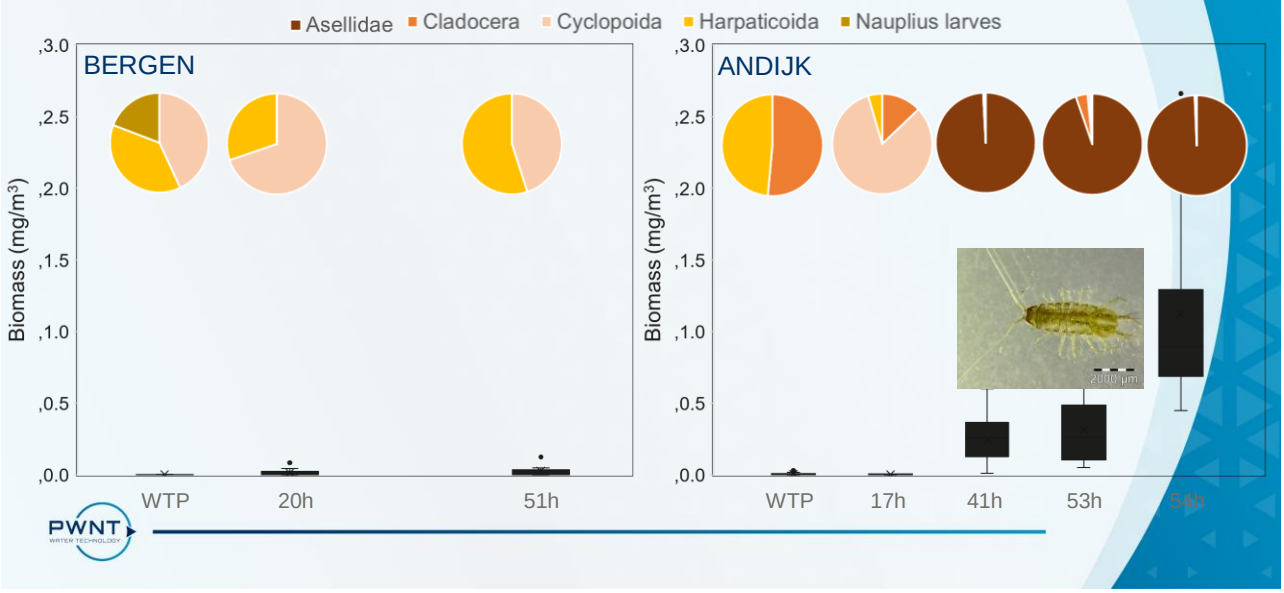
Seasonal formation of sediments; same variations as Aeromonas and zooplankton

Continuous sampling enables large dataset to describe seasonal variations

11

SEDIMENT FORMATION IN THE TWO DISTRIBUTION SYSTEMS

Biomass along known water trajectories



12

CONTINUOUS SAMPLING: ADDED VALUE

- Continuous sampling methods: e.g. “zakfilters” or biofilm monitors
- Provide insights on temporal dymanics
- All events during sampling period are taken into account, even if infrequent sampling (≠ grab sampling)
- BUT: Only possible to apply to a limited number of locations

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SYSTEM DESCRIPTION

- Use of large-scale monitoring data
- Continuous collection of sediments from distribution system

MECHANISTIC UNDERSTANDING

- Growth potential tests in combination with other methods

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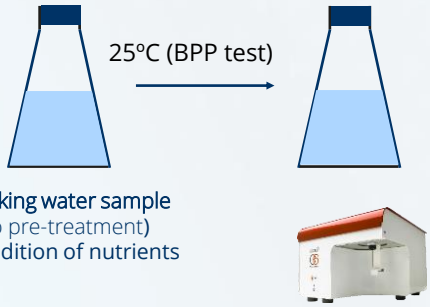
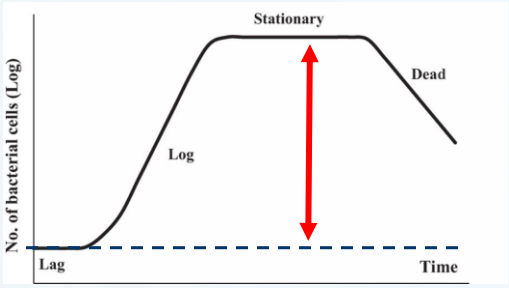
GROWTH POTENTIAL TESTS – METHODOLOGY AT PWN(T)

Similar BPP but <u>without</u> nutrients addition, monitoring with FCM next to ATP



Drinking water sample
(no pre-treatment)
No addition of nutrients

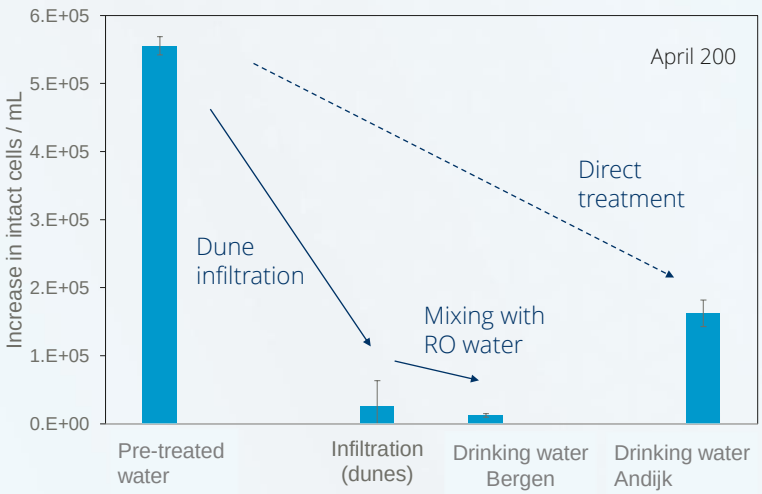
Counting until stationary phase
With **flow cytometry and ATP**



Results expressed as increase in intact cells or ATP between day 0 and maximum value

15

IMPACT WATER TREATMENT ON WATER GROWTH POTENTIAL



Growth potential is influenced by treatment

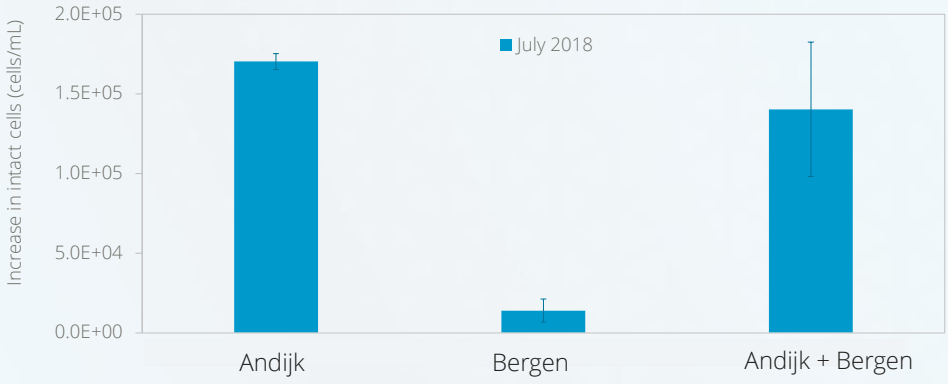
Higher growth potential in Andijk than in Bergen

Dune infiltration enables production of stable drinking water

16

IMPACT MIXING ZONES?

Growth test of Andijk water, Bergen water and a mix of Andijk + Bergen water (lab mixing)



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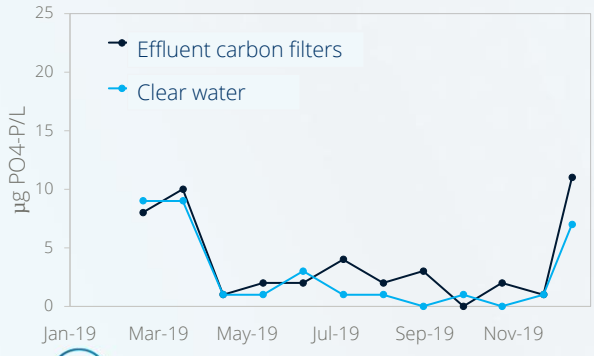
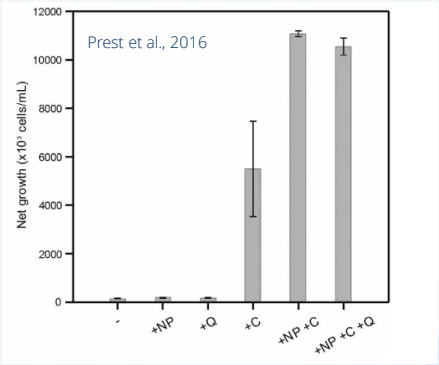
GROWTH LIMITING COMPOUND?

Not studied yet for PWN. But...

Extremely low P concentrations in May - Nov at PWN



Possibility to identify growth limiting compound using growth potential test with series of nutrient addition



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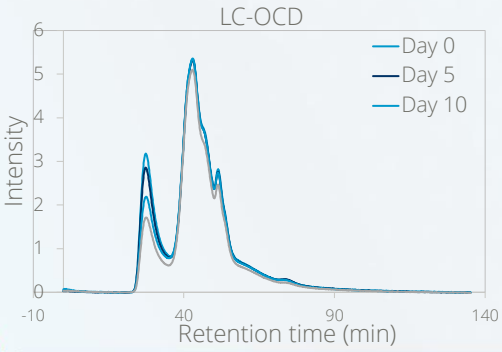
WHICH ORGANIC COMPOUNDS IMPACT GROWTH IN WATER?

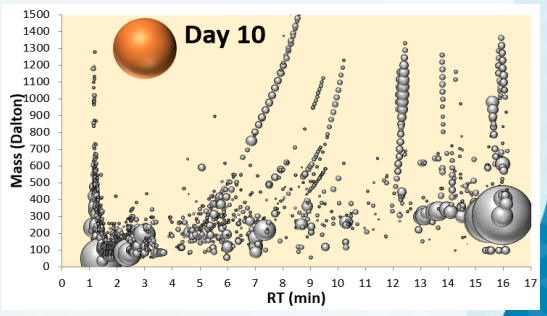
AOC free glassware

Incubation in the dark at 30 °C

Daily monitoring: ATP, FEEM
Start / End: LC-OCD, LC-QTOF

Data Cléa Barbier 2022
(student at IHE-Delft)





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GROWTH POTENTIAL TESTS: ADDED VALUE

- Enables to test research hypothesis in controlled conditions
- Can be combined with other analysis
- BUT: limited to processes in water phase

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SYSTEM DESCRIPTION

- Use of large-scale monitoring data
- Continuous collection of sediments from distribution system

MECHANISTIC UNDERSTANDING

- Growth potential tests
- Towards other approaches / technologies / analytical tools

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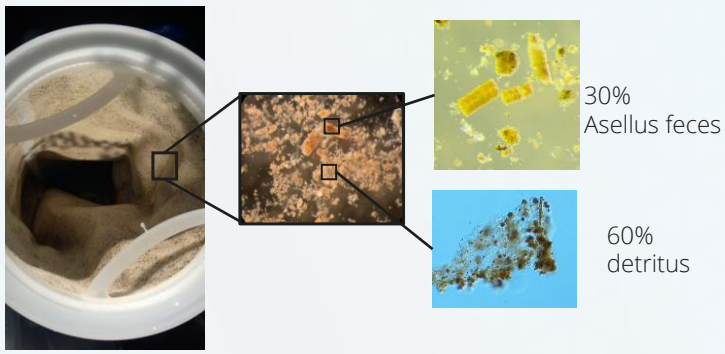
MECHANISM SEDIMENT BUILD-UP?

Microscopic observation sediment at one location



Sediments of biological origin

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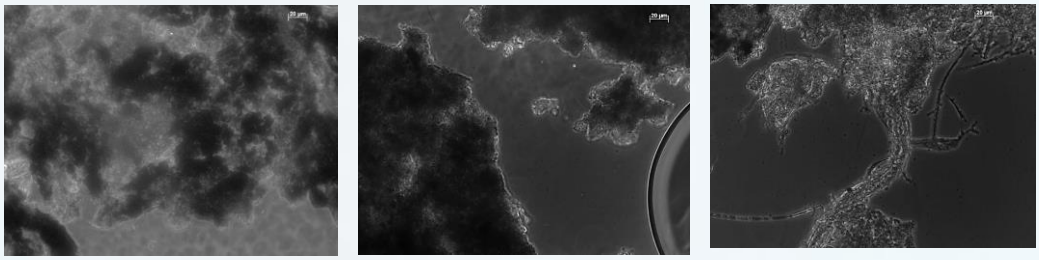
SEDIMENT ANALYSIS



WTP 41h 51h

Formation of larger aggregates along distribution

Filaments
(potentially filamentous bacteria)

FTIR : All sediment samples contain proteins and calcium carbonate
Calcium carbonate in a poorly-ordered amorphous form -> connected to biomineralization

XRD: presence of calcium carbonate, iron precipitates, and sodium chloride

Information used for hypothesis sediment formation mechanism

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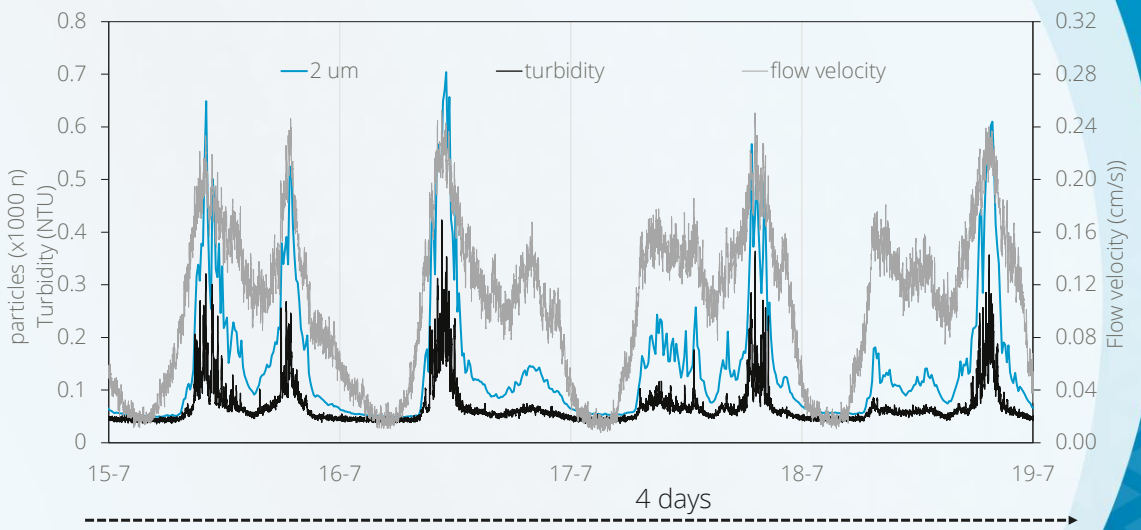
IMPACT HYDRAULICS?



Frederik Hammes | WQTC 2018

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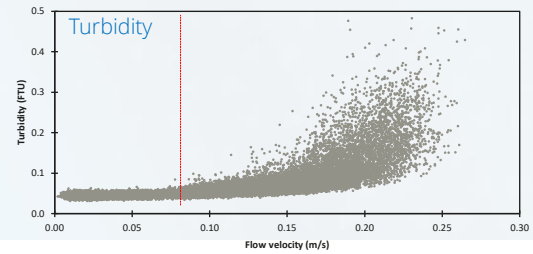
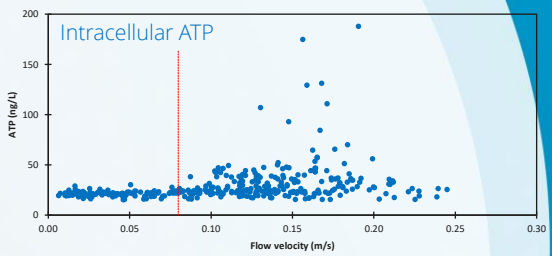
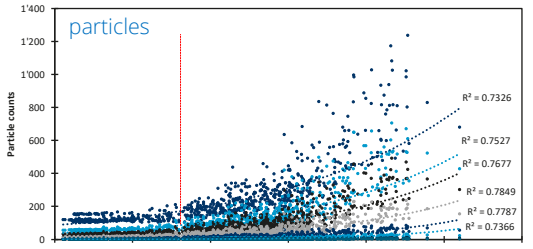
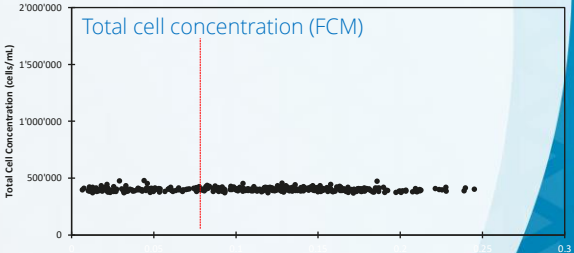
HYDRAULIC FLUCTUATIONS DO NOT AFFECT ALL ONLINE VARIABLES









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MECHANISTIC UNDERSTANDING REQUIRES OTHER TYPES OF ANALYSIS

- Not only predictive parameters at treatment effluent are needed to understand mechanism in distribution system

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SYSTEM DESCRIPTION

- Use of large-scale monitoring data
- Continuous collection of sediments from distribution system

MECHANISTIC UNDERSTANDING

- Growth potential tests
- Towards other approaches / technologies / analytical tools

CONCLUSIONS

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CONCLUSIONS

"Biological stability methods" often used as predictive methods (e.g. BPP).

BUT need to be complemented by other approaches:

Essential to have good knowledge of system to define appropriate research questions:

- Large scale / long term monitoring is absolutely necessary
- Continuous sampling systems are useful for description of time variations of sediment / biofilm phases

Essential to have mechanistic understanding:

- Growth potential tests, often in combination with other methodologies, enable controlled studies of processes in water phase
- Other approaches are needed for understanding specific mechanisms (e.g. sediment analysis, asellus life cycle and living conditions, impact hydraulic conditions, etc.)

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