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

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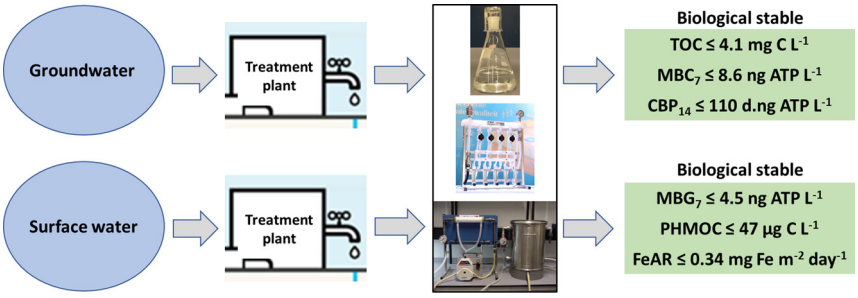
HIGHLIGHTS

- Guidance values for novel biostability parameters for drinking water were deduced.
- Treated water of 34 different treatment plants in the Netherlands were analysed.
- Guidance values for TOC, MBC_7 and CPB_{14} define biostable water from groundwater.
- Guidance values for MBG_7, PHMOC and FeAR define biostable water from surface water.
- Multiple parameters are needed to reliable define biostability of drinking water.

GRAPHICAL ABSTRACT



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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. The maximum biomass concentration (MBC_7), cumulative biomass potential (CBP_{14}) from the biomass production potential test (BPP-W) and the total organic carbon 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}, 110 d·ng ATP L^{-1} and 4.1 mg C L^{-1} were deduced for these parameters, under which the HPC22 and *Aeromonas* numbers remain at regulatory level. The maximum biomass growth (MBG_7) from the BPP-W test, the particulate and/or high molecular organic carbon and the iron accumulation rate in treated water from surface water 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

Abbreviations: $Aeromonas_{90P}$, yearly 90-percentile for *Aeromonas*; AOC-P17/Nox, assimilable organic carbon determined with strain P17 and Nox; AOC-A3, assimilable organic carbon determined with strain A3; ATP, adenosine triphosphate; BAR, biomass accumulation rate determined with the CBM; BFR, biofilm formation rate; BPP-W, biomass production potential for water; BPP-M, biomass production potential for materials in contact with drinking water; CBM, continuous biofilm monitor; CBP_{14}, cumulative biomass production during 14 days of incubation in the BPP-W test; DOC, dissolved organic carbon; FeAR, iron accumulation rate determined with the CBM; HPC22, heterotrophic plate counts at 22 °C; $HPC22_{gm}$, geometric yearly mean for HPC22; MBC_7, maximal biomass concentration during the first seven days of incubation in the BPP-W test; MBG_7, maximum biomass growth during the first seven days of incubation in the BPP-W test; PFe, particulate iron; PHMCHC, particulate and/or high molecular carbohydrates carbon; PHMOC, particulate and/or high molecular organic carbon; TOC, total organic carbon.

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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.

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 and van der Kooij, 2013; 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).

There are different factors that influence growth of microorganisms in drinking water systems that distribute unchlorinated drinking water, e.g. the nutrient concentration in the water, the biomass production potential of materials in contact with drinking water (BBP-M), temperature, hydraulics, distribution and premises plumbing configuration (van der Kooij and van der Wielen, 2014; Prest et al., 2016a). However, the pivotal factor that determines the biological stability of a drinking water system is the nutrient concentration in the drinking water system, as microbial growth will be limited when nutrient concentrations are low enough, irrespective whether the other factors are favourable for microbial growth (van der Kooij and van der Wielen, 2014; Prest et al., 2016a). The biological stability of drinking water describes this nutrient concentration in drinking water without a disinfectant residual (van der Kooij, 2003; Prest et al., 2016b). It has also been observed in previous studies that the biological stability of unchlorinated drinking water in the Netherlands is determined by carbon limitation and not by limitation of other nutrients such as phosphate or nitrate, as addition of BDOC to drinking water enhance microbial growth and biofilm formation (van der Kooij et al., 1995a, 1995b; Sack et al., 2014). Consequently, to be able to comply to the working definition for biological stability, predictive parameters that focus on carbon limitation must be available to monitor the biological stability of drinking water. 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). These 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 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.

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). 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).

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). These methods have helped to identify components that are responsible for the lower biological stability of drinking water at specific production locations that treat surface water (Hijnen et al., 2018; Schurer et al., 2022; van der Kooij et al., 2015). However, it remains unknown whether the same parameters determine biological stability in drinking water treated from groundwater, as the quality of both sources are different. It is currently also unknown 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.

2. Materials and methods

2.1. 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.

2.2. Predictive biological stability parameters

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

2.2.1. Growth potential tests

2.2.1.1. 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}). The way these parameters were exactly calculated is explained in the supplemental information.

2.2.1.2. 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 min 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 $\leq 1.5 \times 10^5$ cfu mL^{-1} and 0.98×10^7 cfu μg^{-1} when the N_{max} of strain A3 is $>1.5 \times 10^5$ cfu mL^{-1}; Sack et al., 2011) were used to calculate the AOC-A3 concentrations.

2.2.2. Growth and biofouling potential tests

2.2.2.1. 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.

2.2.2.2. 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.

2.2.3. 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.

2.3. Analytical analyses

2.3.1. 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}.

2.3.2. 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).

2.3.3. 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.

2.3.4. 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 s 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 h. *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.

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 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.

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 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. Results

3.1. 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 Figs. 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 (Figs. 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 8.1 ± 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 (Fig. 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.

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} (Figs. 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 (Figs. 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 and 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. S4). The lowest and highest concentrations for

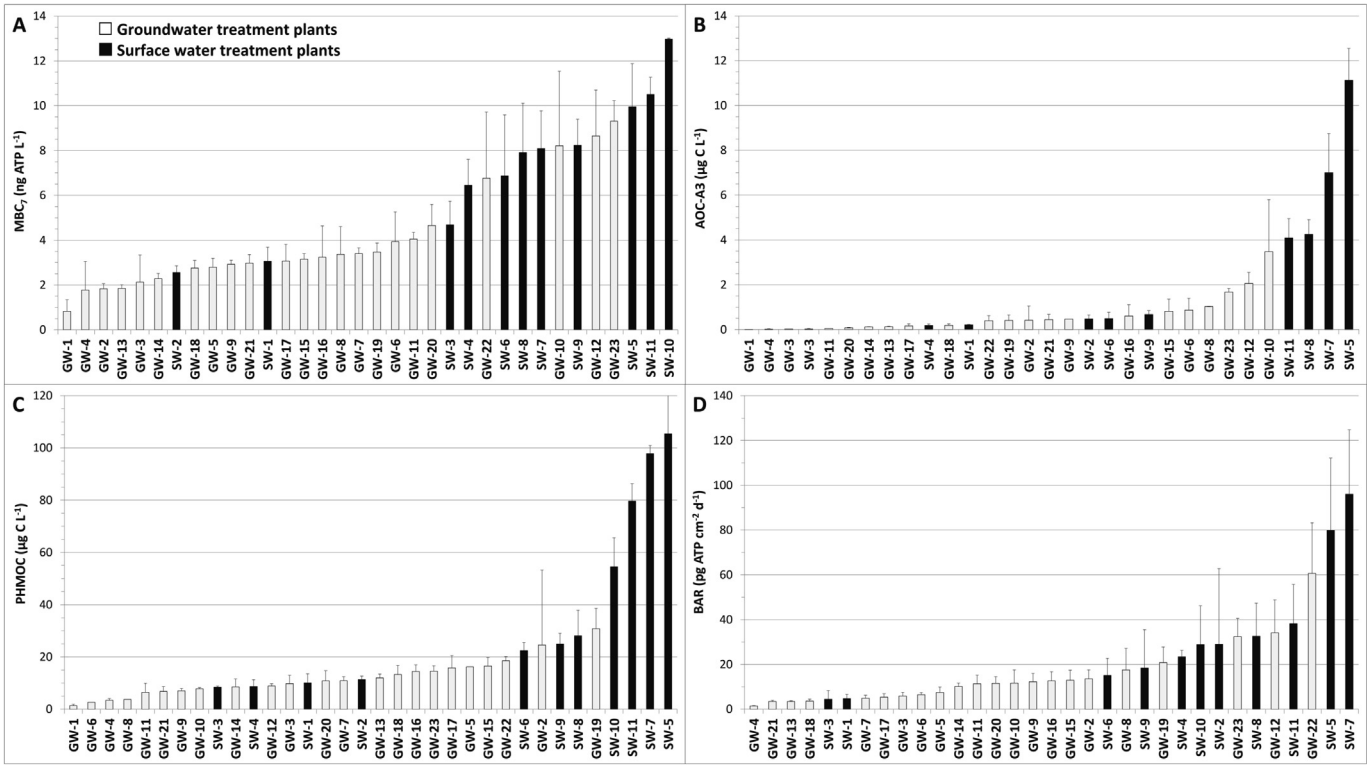

Fig. 1. The mean MBC_7 values (A), AOC-A3 concentrations (B), PHMOC concentrations (C) and BAR values (D) ± standard deviation of treated water from 34 different drinking water treatment plants that process groundwater (open bars) or surface water (closed bars). Standard deviations are only visualized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

both parameters were observed in drinking water produced from groundwater.

3.2. 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.

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-W parameters MBC_7, MBG_7 and CBP_{14} were significantly lower in drinking water produced from groundwater than

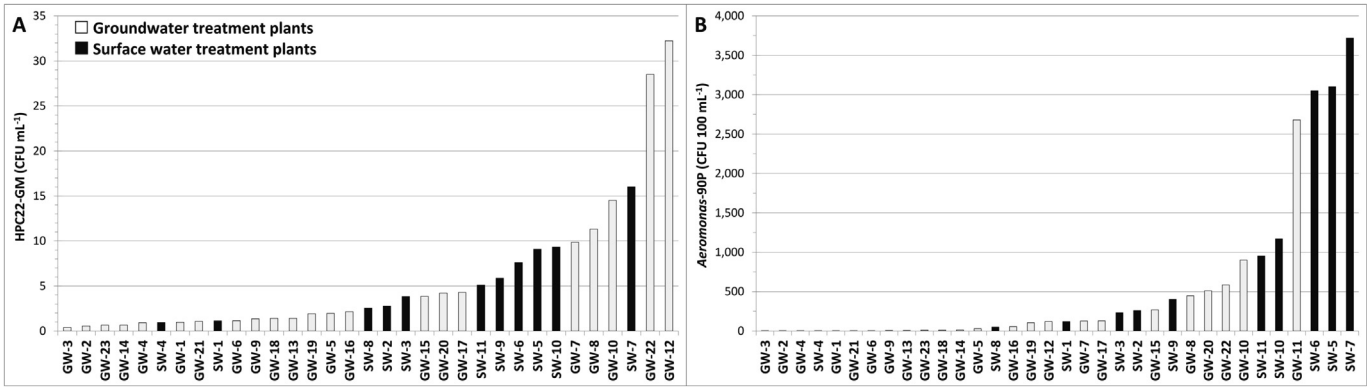

Fig. 2. The geometric mean of HPC22 (A) and 90-percentile of *Aeromonas* (B) in drinking water samples from the distribution system of different treatment plants that treat groundwater (open bars) or surface water (closed bars).

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

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 (Figs. 1 & 2 and Figs. 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.

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 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 <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 Fig. 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 off 0.88. 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.

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.

3.5. 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

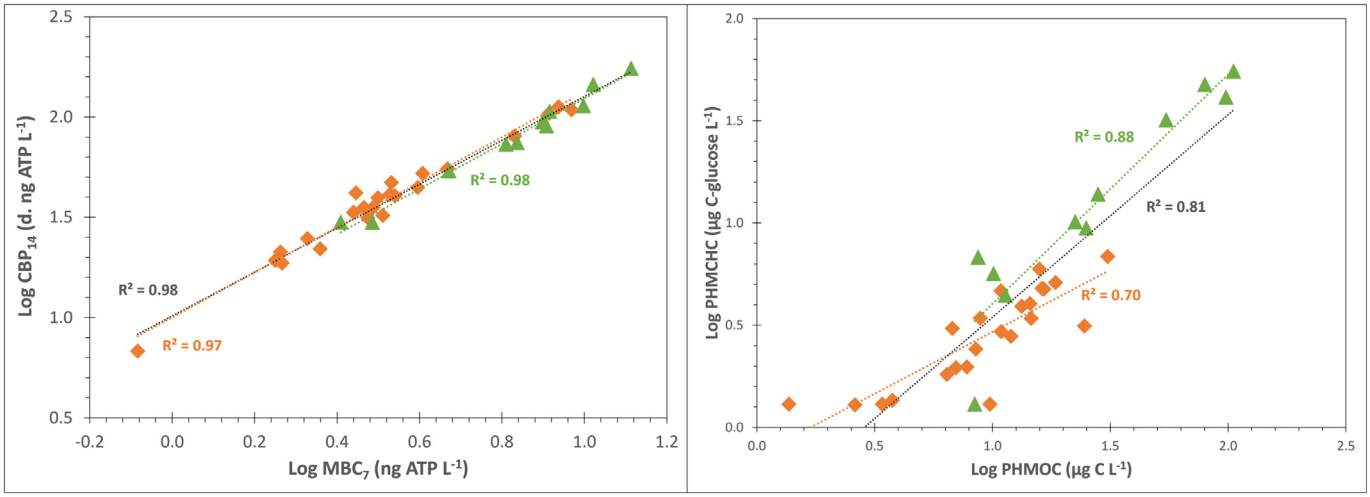

Fig. 3. The correlation between the log transformed BPP-W parameters MBC_7 and CBP_{14} (A) and the log transformed hemoflow parameters PHMOC and PHMCHC (B) in treated water from 34 different treatment plants. Orange diamonds and line: data for treated water produced from groundwater; green triangles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

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 (Tables 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 (Tables 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).

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 9.3 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}.

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

4. Discussion

4.1. Growth potential and biofouling potential of drinking water

4.1.1. Growth potential of drinking water

The BPP-W 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} were very strongly correlated. Such a correlation was already reported for drinking water produced from surface water that had slow sand filtration as final step in treatment (van der Kooij et al., 2017). 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.

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., 2017). 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, probably because AOC-A3 is a specific indicator for slowly biodegradable biopolymers (Sack et al., 2010, 2011), whereas the BPP parameters are determined by biopolymers and other biodegradable compounds. 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.

4.1.2. 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

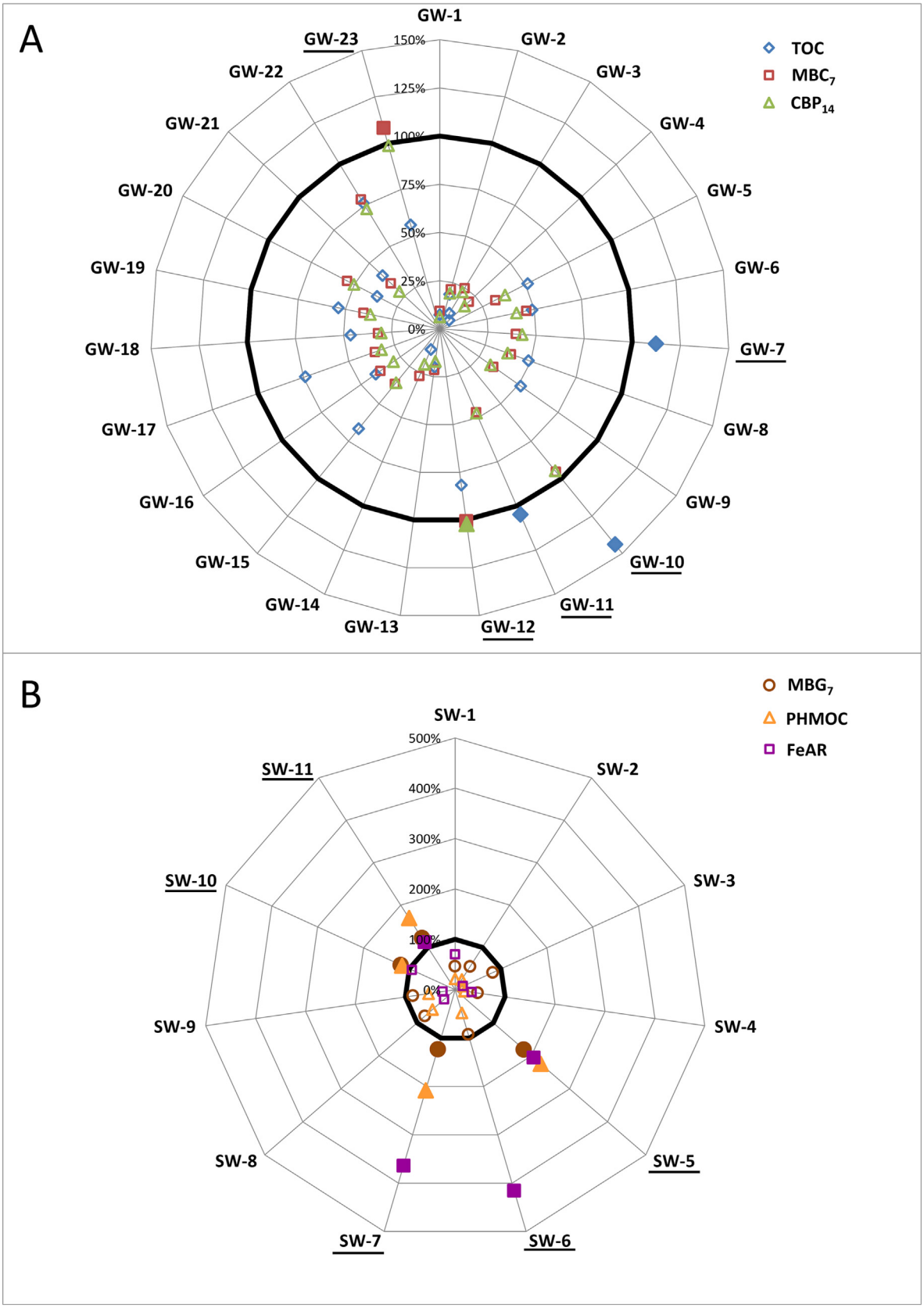

Fig. 4. Radar plots for the selected biological stability parameters in treated water that predict regrowth parameters in the distribution system of groundwater treatment plants (A) and surface water treatment plants (B). The biological stability parameters were expressed relative to the guidance value, 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.

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.

4.2. Biological stability

4.2.1. Multiple parameters determine biological stability of drinking water

We estimated the biological stability of treated unchlorinated drinking water with a suite of biological stability parameters to a wide range of treatment plants (n = 34) that vary in the level of regrowth in the distribution system. The results demonstrated that 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., 2016b; Sousi et al., 2018). It was suggested in several studies from different countries 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., 2016b; 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., 2017). Our observation that most biological stability parameters are not strongly correlated with each other confirm this last conclusion and encourage research groups across the world 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 assays to determine the biological stability of drinking water in different countries.

The lack of correlation we observed between ATP concentrations in treated drinking water and HPC or *Aeromonas* regrowth in the distribution system confirm the results from previous studies (Roeselers et al., 2015; van der Wielen et al., 2016). In addition, the lack of correlations between ATP in treated water and any of the biological stability parameters measured demonstrate that biomass concentrations in treated water cannot be used to predict the biological stability of drinking water.

The same 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 reported by van der Kooij et al. (2015) for the biological stability parameters in drinking water of one of these treatment plants 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. Season, thus, has an influence on the water temperature and biological stability of the treated water from surface water. To prevent an influence of sampling in different seasons on the outcome of our study, we decided to plan the sampling campaigns for surface water treatment plants in the warm season. The warm season was chosen, because regrowth in the distribution system occurs mainly in that season in the Netherlands. In contrast, the temperature and biological stability of treated water from groundwater is stable during all seasons (van der Kooij, 1992) and, therefore, groundwater treatment plants were sampled irrespective of season.

4.2.2. 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 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). 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 >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, resulting 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.

4.3. Predictive biological stability parameters for nuisance growth

4.3.1. 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 and biological stability tools are needed to predict this nuisance growth. 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., 2016b; 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., 1995a, b, 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). The CBM used in our study 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 that we applied as well contain organic carbon and iron attached to particles and high molecular weight organic carbon (Schurer et al., 2022). Overall, the results from our study confirm that the combination of these five parameters can be applied to monitor the biofouling potential of drinking water.

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. 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. Based on the results of our study it is, however, recommended to use the same guidance values in other countries to distribute unchlorinated drinking water with an impeccable quality.

4.3.2. 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 ten 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 the 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).

4.3.3. 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. More importantly, in some regions the public tends to push drinking water companies to switch to distribution of unchlorinated drinking water, because of sustainability goals, consumer complaints and prevention of toxic byproduct formation. The guidance values obtained in our study for the different biological stability parameters can directly be used by drinking water companies that want to switch from distributing drinking water with secondary disinfection to drinking water without a disinfection residual. Furthermore, 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.

5. 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.
- Drinking water produced from groundwater 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 distribute unchlorinated drinking water with a low regrowth potential and which is produced from 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 distribute unchlorinated drinking water with a low regrowth potential and which is produced from 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.
- These guidance values can be directly implemented in countries where drinking water without a disinfectant is distributed, or they can be used to guide countries that want to change to distribution of drinking water without a disinfectant residual.
- 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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CRediT authorship contribution statement

Paul W.J.J. van der Wielen: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Supervision. **Anke Brouwer-Hanzens:** Methodology, Validation, Investigation, Writing – review & editing. **Ronald Italiaander:** Methodology, Validation, Investigation, Writing – review & editing, Project administration. **Wim A.M. Hijnen:** Conceptualization, Investigation, Writing – review & editing.

Data availability

The authors do not have permission to share data.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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