Deliverable 1.2.2 (2)

Pilot study of the application of biofilm monitoring to treated drinking water in Berlin

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Contents

Colophon iii

Acknowledgements 1

Contents 2

1 Introduction 3

1.1 Background 3

1.2 Demonstration site 4

1.3 Demonstration objectives 5

2 Methods 6

2.1 ATP measurement 6

2.2 Flow cytometry 6

2.3 Sampling procedure and sample treatment 7

3 Results 8

3.1 ATP Measurements 8

3.2 Flow cytometry 9

3.3 CFU results 10

4 Conclusions 11

5 References 12

1 Introduction

1.1 Background

Any drinking-water supply system is also a habitat for microorganisms. In groundwater, microorganisms are typically adapted to high flow and low nutrient conditions and their number is low compared to surface water. Although the treatment steps during drinking water production usually have a high removal capacity, it is not possible to achieve full removal of all microorganisms. They might survive in biofilms attached to surfaces as well as in the water phase In addition, the microbial quality of water normally changes in a piped network [AINSWORTH 2004], and changes in the treatment or operation scheme may affect the performance of the system, too. As excessive microbial activity can lead to quality deterioration (odour, taste, discolouration), monitoring of the biofouling potential, and adapted treatment schemes, operation, and maintenance of the whole treatment and distribution system are therefore inevitable.

Climate change involving higher temperatures is believed to enhance the re-growth potential of (health-relevant) bacteria in groundwater [BLOKKER et al. 2013], and thus in the raw water for drinking water production, and in distribution systems.

The microbiological quality of water samples (presence of bacteria) is typically described by the number of colony-forming units (cfu) in the water phase. The German drinking water ordinance limits colony-forming units at the waterworks outlet to 100 cfu/100mL at 22 and 36°C in drinking water without disinfection and to 20 cfu/mL after treatment at 22°C and 100 cfu/mL at 36°C in drinking water with previous disinfection [TRINKWV 2011].

Within PREPARED, in Oslo a biofilm monitor was applied after changing the local treatment scheme from microstraining and chlorination to NOM removal by Actiflo/ dual-sand-filtralite filtration and UV-disinfection (D 1.2.8 of PREPARED Project). The measurements involved BDOC analysis, biofilm formation analysis by ATP measurement, moulds analysis by cultural methods and bacteria analyses in cultures and by applying fluorescent in-situ hybridisation (FISH) from biofilm samples grown in raw water, filtered water, treated water at the waterworks outlet and at sampling points after 2.5 hours and 24 hours residence time in the distribution system. Coupons were analyzed every 14 days over a period of 160 days to follow the establishment of a biofilm in three different water types.

In Berlin, drinking water supply relies mainly on groundwater sources within the city's limits and involves a natural treatment scheme. The raw water abstracted from the wells is aerated and iron-removal is achieved by rapid sand filters. Disinfection is generally not provided, only in case of contamination events.

Previous work in Berlin focussed on bacteria in drinking water treatment (e.g. Biosens (KWB 2004-2005, [MITTENZWEY et al. 2006] and [SZEWZYK et al. 2008]). There, carrier materials were, for example, installed in rapid sand filters, raw water pipes and distribution network pipes and the surface biofilm formation potential was investigated for various pipe materials and water types. The free water phase, in contrast, has not been subject to such extended assessment involving culture-independent microbiological analysis technologies, so far.

Advances in laser and optical technologies provided the so-called flow cytometry as a new opportunity to detect and quantify micro-organisms in free water [DUFOUR et al. 2003]. In contrast to ATP-measurements, which give the sum of microbial activities of all microorganisms in the sample, flow cytometry measures particles and distinguishes bacteria by DNA staining. The objective for the Berlin pilot study in WP 1.2 was therefore to evaluate the suitability of both methods under full-scale conditions for quantifying micro-organisms in the free water as basis for further biofilm studies.

1.2 Demonstration site

The waterworks Tegel is one of nine drinking water treatment plants of the Berliner Wasserbetriebe. With a maximum capacity of 260.000 m³ per day, it is the largest waterworks of Berlin.

Groundwater is abstracted from altogether 130 vertical filter wells and one horizontal filter well. Groundwater replenishment involves bank filtration from lake Tegel and artificial recharge from three infiltration ponds with about 30.000 m² surface area and about 50.000 m³ infiltration capacity (average per day). The average raw water composition shows a DOC of 4.5 to 5.0 mg/L, dissolved iron of 0.1 to 0.5 mg/L and manganese of 0.3 to 0.6 mg/L [SHARMA et al. 2011]. Drinking water treatment involves aeration in up to three aeration towers followed by rapid sand filtration (iron and manganese removal) via open single-bed filters at a filtration rate of 2.5 to 6 m/h. Afterwards, the water is distributed without chlorination (Figure 1).

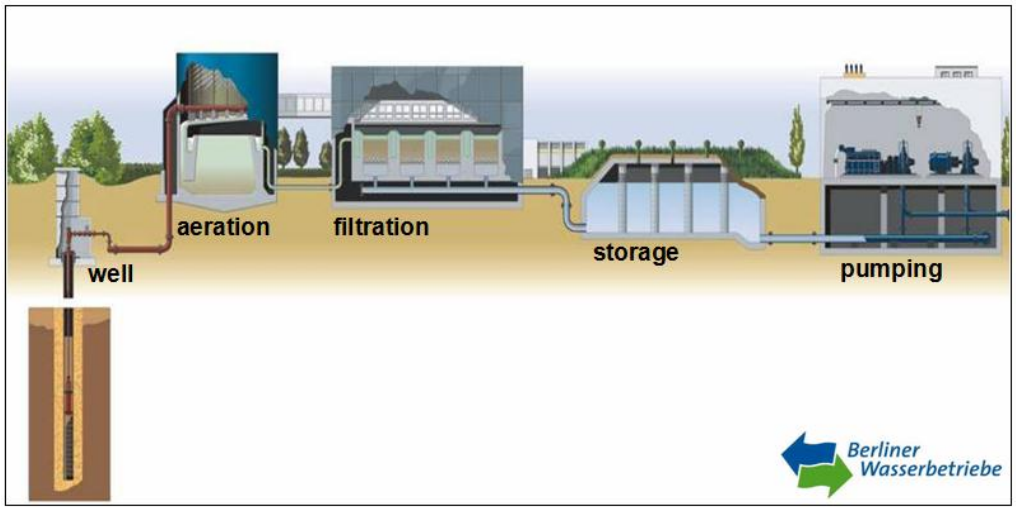

Figure 1:Principle of post-treatment in the Berlin waterworks [translated after BWB.de, access date 09.12.2013]

At the Tegel waterworks, 20 rapid sand filters are operated in parallel. Beside the operation hours, the residence time of the water and the filter resistance are monitored. Threshold values for these parameters determine the need for backwash.

On average, the filters are operated for 10 to 14 days between two backwashs. The latter is done by a three-step process involving air (break-up), air and water mixture (cleaning) and water (flushing).

Regular monitoring of the water quality involves continuous pH, temperature, redox, electric conductivity, dissolved oxygen and turbidity measurements and samplings twice

a week to determine amongst others the number of colony-forming units. The investigations summarized in this report were undertaken independent of routine monitoring, but the colony-forming units obtained from routine sampling on 09th and 19th December 2013 were included in the results discussion (see chapter 3.3).

1.3 Demonstration objectives

At Berliner Wasserbetriebe (BWB), currently two advanced treatment options are being investigated in the scope of research projects. These are

1) the addition of granular activated carbon for trace organic removal, and
2) the replacement of stand-by chlorination by UV-disinfection (no repository effect).

The objective of this pilot study was therefore to determine a reference status with regard to the presence and number of bacteria after the single treatment steps of the current scheme. At the same time, it was intended to assess the applicability of flow cytometry to evaluate the biofilm formation potential, and to compare flow cytometry results to the usual cultivation-independent state-of-the-art method of ATP measurement used by the Oslo utility in PREPARED.

Samples were taken at the raw water inlet before aeration, at the sand filter outlets of various filters with different filter resistivities, and at the waterworks outlet. The sampling campaign was repeated once to additionally observe changes in bacteria numbers and potentially relate them to different operational parameters of the rapid sand filters.

2 Methods

ATP measurement and flow cytometry are both rapid and cultivation-independent methods to assess the general microbiological quality of groundwater or drinking water samples [VITAL et al. 2012]. While ATP represents a bulk parameter for the presence of bacterial and higher organisms, flow cytometry provides information on single cell level.

2.1 ATP measurement

Adenosine triphosphate (ATP) is a coenzyme involved in the intracellular energy transfer of all living cells. It can thus be used to quantify active biomass. Measurement is based on luminescence detection from the reaction of ATP with the naturally occurring enzyme luciferase. Luciferase converts luciferin into the corresponding enzyme-bound luciferil adenylate in presence of ATP and Magnesium firefly D-. The luciferil adenylate complex is then the substrate of the subsequent oxidative reaction leading to oxyluciferin. The light emission at a wavelngth of 560-562 nm is a consequence of a rapid loss of energy of the oxyluciferine molecule from an excited state to a stable one [CHOLLET et al. 2012]. The amount of light, measured as "relative light unit" (RLU), is directly proportional to the amount of living organisms in the sample.

Within a water sample, two types of ATP are related to the microorganism's content: (1) *intracellular ATP* (cell-bound) from within the living cells and (2) *extracellular ATP* (free) that has been released from dead or stressed organisms. Their accurate differentiation is complex and thus a major drawback of the method. A critical step is thus sample preparation, i.e. extraction of ATP from the cells. In order to distinguish cell-bound from free ATP, samples are usually filtered before ATP extraction. Advantages of ATP measurements are (1) a simple measurement procedure, (2) the ability to detect very low amounts of biomass and (3) that the analysis is conducted within a few minutes [Van der Kooij 1999].

Within the PREPARED pilot study in Berlin, a BioFix® Lumi-10 luminometer (Macherey-Nagel) with a spectral wavelength of 380-630 nm was used. For sample preparation, the BioFix Lumi ATP for liquid samples (Macherey-Nagel) was used. Sample preparation involved the following steps:

(1) addition of 0.1 mL sample to a cuvette
(2) addition of extraction reagent
(3) mix and wait for 60 seconds
(4) addition of bioluminescent reagent
(5) mix and wait for 30 seconds

For each sample, measurement was repeated five times. Subsequently, average values and standard deviation were calculated. A blank value was obtained by measuring with an empty cuvette prior to each set of measurements. Results (total ATP) are given in relative light units (RLU).

2.2 Flow cytometry

Flow cytometry is a method to detect and count particles and bio-marked cells in fluids via scattered light, light extinction and fluorescent measurement. Particles and cells in a size range of 0.5 to 40 µm are suspended in a stream of fluid and passed through a focal laser beam one-by-one.

Electronic detection units measure backscattered light and fluorescence emission. Thus, the method allows simultaneous and real-time analysis of several physical and chemical characteristics of up to thousands of particles per second.

Sample preparation involves staining, e.g. fluorescent staining to bind to DNA in order to identify cells of living organisms in the particle stream. Other bio-markers could be propidium iodide binding only to dead cells, or specific anti genes allowing separation of subpopulations. From the scattered light patterns of forward scatter and 90° (side) scatter, size and complexity of cells can be differentiated and cells can be assorted to groups of organisms (bacteria, spores, ...), too.

Within the PREPARED pilot study in Berlin, a CyFlow space (Partec) was used. Sample preparation involved

(1) 1:10 dilution with sterile-filtered bottled water (bought on the day of measurement) and staining of DNA: per 1 mL cuvette 100 µL sample + 890 µL bottled water + 10 µl SYBR-Green (unspecific DNA marker)
(2) incubation of sample mix for 15 minutes
(3) measurement
(4) data interpretation (gating)

For each sample, measurement was repeated five times and subsequently average value and standard deviation were calculated. Between each measurement, the flow cytometer was flushed with a special cleaning fluid and 1% sodium hypochloride solution. A blank value was determined by measuring sterile-filtered bottled water with SYBR Green. Results (number and size ranges of cells) are given as total cell count per mL.

2.3 Sampling procedure and sample treatment

For the PREPARED pilot study, two sampling campaigns were carried out; a first one on December 10th 2013 and a second one on December 19th 2013. Both involved:

(1) one sample of raw water before aeration;

(2) six, and seven samples taken from the outlet of different rapid sand filters. The filters were chosen after their current filter resistance on the day of sampling covering the whole range of low (0 mbar, directly after backwash) to high (129 mbar, directly before backwash) resistivities;

(3) one sample of drinking water at the outlet of the waterworks.

At each sampling point, two sterile falcon tubes were filled with 100 mL sample volume.

ATP measurements were carried out directly on the day of sampling. The remaining sample volume was fixed with glutaraldehyde and stored for up to two weeks until flow cytometer measurements were performed. Glutaraldehyde is a chemical conservation agent preventing further growth of microorganisms by denaturing their proteine contents and fixing their outer shapes and structures.

In order to relate total cell counts obtained from the two tested methods, total numbers of colony forming units (CFU) from routine sampling of the Berliner Wasserbetriebe laboratory on 9th and 19th December 2013 were considered for results interpretation as well.

Other parameters were neither analyzed nor considered for further data interpretation.

3 Results

3.1 ATP Measurements

For the ATP measurement, values ranged between 74 and 163 RLU for the first, and 46 to 163 RLU for the second sampling campaign. All samples showed high variations between the repeated measurements with coefficients of variation (COV) between 7 and 54%. Blank values ranged between 23 and 74 RLU (COV of 33%) and in one measurement, the blank value lay even above the sample value. Figure 2 summarizes the ranges of measured values together with median and outliers in boxplots and Figure 3 shows the median value out of the five measurements for the different sample locations.

ATP measurements show high variations for each of the samples and no correlation between the amount of ATP at the rapid sand filter outlets and their filter resistivities. The two sampling campaigns further show different patterns for the sampled water types. While in the first sampling campaign, the potable water at the waterworks outlet showed the highest content of ATP and raw water and filtrate were in a similar range, in the second campaign the median ATP content of the filtrate of the sampled rapid sand filters was higher than in raw and potable water.

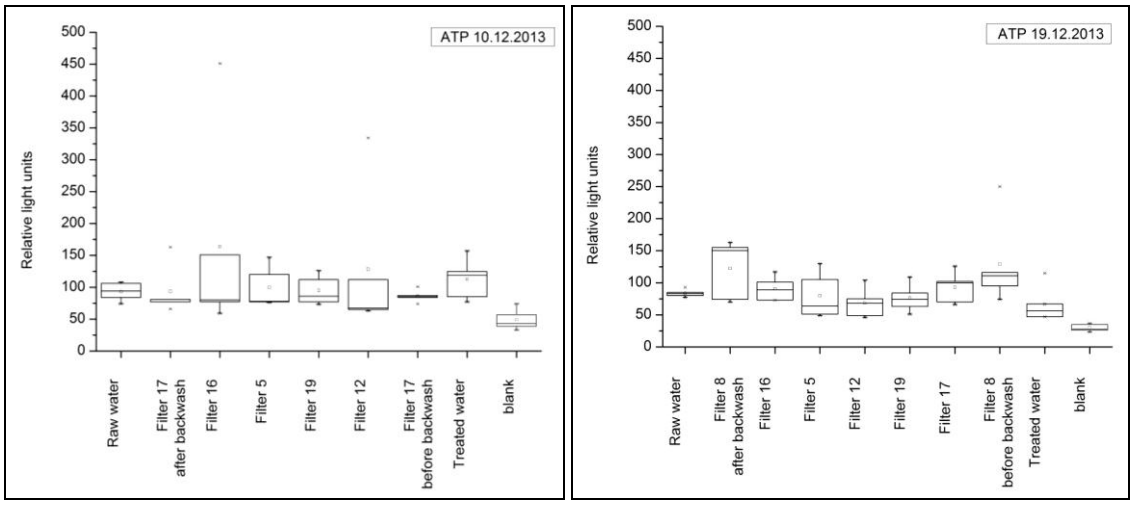

Figure 2: Range of measured ATP values (as RLU); left: sampling of 10th December 2013; rigth: sampling of 19th December 2013; samples from rapid sand filters assorted with increasing filter resistance from left to right

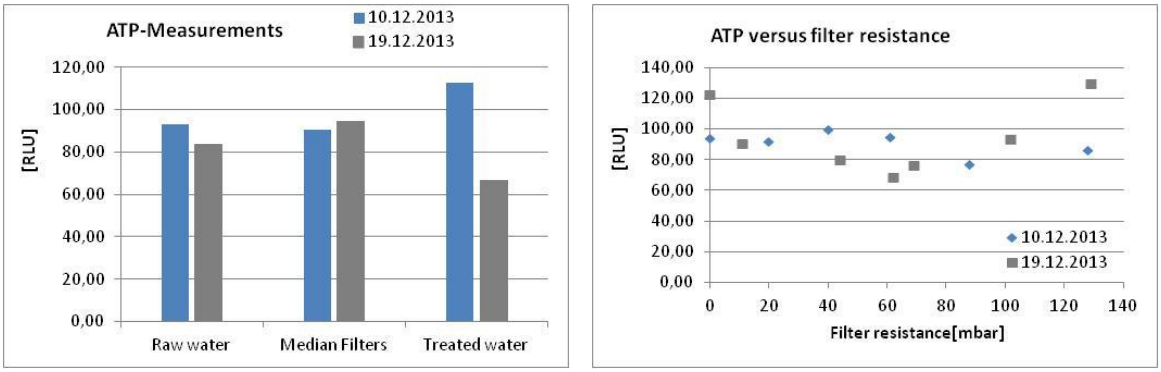

Figure 3: Median (of five measurements) of ATP measurements for the different sample locations; left: by water type (raw water - after rapid sand filters - at waterworks outlet); right: sampled rapid sand filters versus filter resistance

The reason for the observed high variation remained unclear with the limited set of sample repetitions. As even blank values (empty cuvettes) had a coefficient of variation of 33% for the first and 22% for the second sampling campaign, they were most probably caused by the luminometer itself,. Influences of other water constituents are unlikely as the variations occurred for all sampled water types (raw and filtered water).

3.2 Flow cytometry

Flow cytometry returned values between 34x10^5 and 51 x10^5 cells per mL for the first, and 34x10^5 to 46x10^5 cells per mL for the second sampling campaign. With about 10^{05} cells per mL, values are comparable to references from other authors for groundwater and drinking water samples [EGLI et al. 2010; KÖTZSCH et al. 2012].

Compared to ATP measurements, variations of flow cytometry were lower having COVs between 1 and 9% (Figure 4). Furthermore, the two sampling campaigns showed similar patterns for the different water types with raw water having the highest number of cells per mL and filtrate of the rapid sand filters and waterworks outlet being in the same range (Figure 5 left). As in ATP measurements, the number of cells did not correlate with the filter resistance (Figure 4 and Figure 5 right).

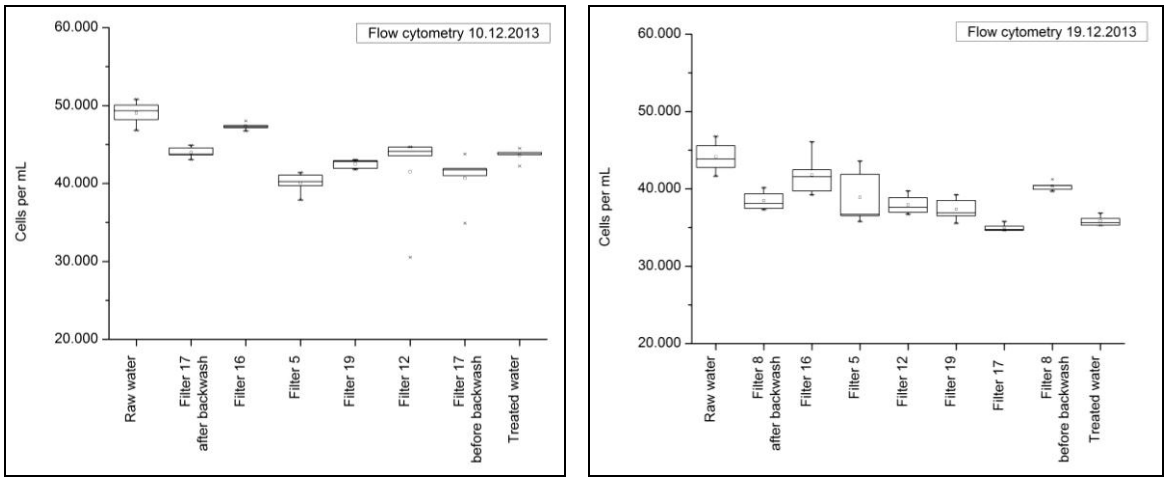

Figure 4: Range of Flow cytometry values (as cells per mL); left: sampling of 10th December 2013; rigth: sampling of 19th December 2013; samples from rapid sand filters assorted with increasing filter resistance from left to right

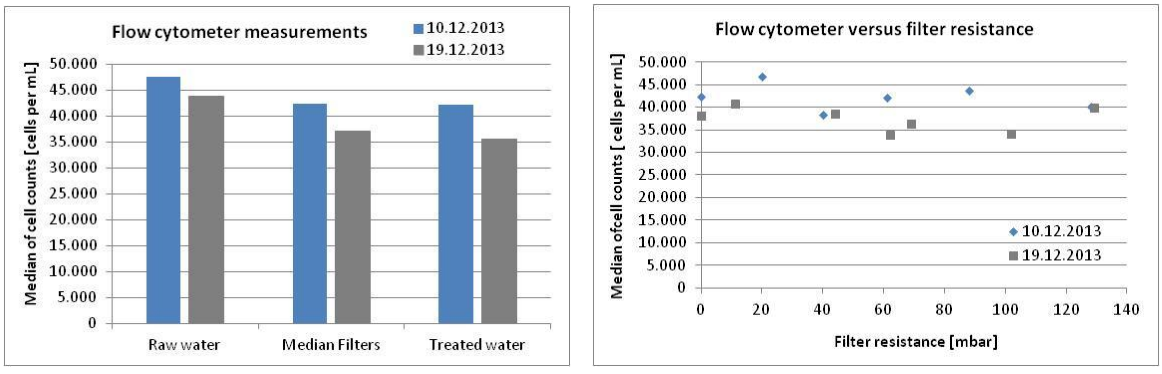

Figure 5: Median (of five measurements) of flow cytometer measurements for the different sample locations; left: by water type (raw water - after rapid sand filters - at waterworks outlet); right: sampled rapid sand filters versus filter resistance

The observed variation of measurement results could be caused by the age of reactants or the dilution, but the bottled water was fresh. A contamination of the device is unlikely, too, because of flushing prior to each single measurement. As such variations were also observed for other kinds of samples, currently matrix effects of the sheath-fluid are the most probable reason.

3.3 CFU results

Routine sampling of Berliner Wasserbetriebe was performed on 9th and 19th December (after PREPARED sampling campaign). There, all samples were in line with the drinking water directive thresholds. ATP results of the first sampling campaign however correspond to CFU results at 22°C with 1 cfu/100 mL in the raw water and 3 cfu/ 100 mL at the waterworks outlet. All other values considered for comparison were 0 cfu/ 100 mL.

4 Conclusions

Despite the limited number of samples and series of repetitions, both ATP and flow cytometer measurement proved their applicability for rapid, cultivation-independent assessment of microbial activity in water samples. The methods worked for raw water (waterworks inlet) and filtered water (waterworks outlet) in a waterworks of Berlin with its near-natural drinking water treatment scheme (without disinfection). Results were obtained within a few hours after sampling.

Because ATP measurement results have not been converted to gram ATP per sample volume so far (ongoing work), results cannot be compared to ranges from literature, but flow cytometry showed the expected order of magnitude of 10^5 cells per mL highlighting the presence of bacteria although not detected with cultivation-based standard methods (cfu were below thresholds for all samples).

The obtained values represent a first reference for both, ATP and flow cytometry measurements for the groundwater/ raw water type and drinking water treatment scheme of Berlin. Further investigations of the obtained samples in the scope of a Bachelor thesis at the Technical University Berlin will include (1) calibration of ATP measurement against a standard to convert "relative light units" to "gram ATP per cell" and (2) DGGE fingerprinting to analyse the bacterial community and to identify shifts of populations due to rapid sand filtration. The latter will also set a baseline, against which future investigations of climate change impacts can be compared.

For future pilot tests and investigations of changes in microbial activity due to changed treatment schemes in the waterworks, however, both methods are recommended to be used in parallel, because most information can be obtained from combining the results of the two methods relating e.g. cell sizes and ATP-per-cell concentrations. For ATP measurements, cell-bound and free ATP should be distinguished to evaluate the impacts of the treatment steps and potential changes in subsequent distribution. For flow cytometry, intact and dead cells should be distinguished via the corresponding bio-markers. Investigations by VITAL et al. (2012) for example had shown that the concentration of intact cells decreased significantly during rapid sand filtration, accompanied by an increase in membrane-damaged cells and a shift in the community observed from the distribution of low and high nucleic acid bacteria.

Thus, ATP and flow cytometer measurements, being rapid, culture-independent methods to assess the presence and activity of microorganisms in water samples represent well-applicable bio-monitoring methods for future investigations. They are recommended to being used in parallel to already well-established chemical and biological monitoring techniques e.g. to assess the impacts of adaptation and/or optimization measures in waterworks treatment schemes to microbial activity.

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