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The application of flow cytrometric based monitoring technologies on full-scale treatment and distribution systems

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The application of flow cytrometric based monitoring technologies on full-scale treatment and distribution systems

Author(s)
Marius Vital
Karin Lautenschlager
Frederik Hammes

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Summary

During the course of the Techneau project, Eawag developed and optimised a several methods for the analysis of bacteria and nutrients which support bacterial growth in drinking water. The methods include flow cytometric enumeration of total and viable bacteria, adenosine tri-phosphate for the analysis of viable microbial biomass, the measurement of assimilable organic carbon and the measurement of pathogen growth potential in water. Within the Techneau project, these methods were applied in several full scale treatment and distribution systems across Europe and also on samples from the Windhoek treatment plant (Namibia). This report summarises some of the main results that were obtained, with references to Techneau reports and peer-reviewed publications where detailed information can be obtained.

Contents

1 Introduction

1.1 Background

During the course of the Techneau project, Eawag developed and optimised several analytical methods enabling the quantification of (1) the total and viable bacteria in water and (2) the available substrates for growth for both total bacteria as well as for specific pathogens. Detailed descriptions of the developed methods can be found in the individual deliverables within WP 3.3 and related publications (indicated in brackets below).

1.) Enumeration of total bacteria in water

- Enumeration of total bacterial cell concentration using flow cytometry (FCM) (D 3.3.7; Hammes et al., 2008; Hammes et al., 2010a)
- Enumeration of total "viable cell" concentration using FCM (D 3.3.8; Berney et al., 2008)
- Enumeration of cell-bound ATP as indicator of viable bacteria (D 3.3.8; Hammes et al., 2010b)

2.) Quantification of substrates for growth

- Quantification of assimilable organic carbon (AOC) (D 3.3.1; Hammes & Egli, 2005; Vital et al., 2008)
- Quantification of the pathogen growth potential (PGP) (D 3.3.14; Vital et al., 2010)

The developed methods are all cultivation independent and yield accurate and fast results with the possibility of high throughput. They provide an opportunity to describe and quantify microbiological processes during drinking water treatment and distribution with more detail, accuracy and speed than previously possible.

1.2 Goal of this deliverable

After lab-scale development and testing of methods (WP 3.3.) they were applied first on the treatment and distribution system of Zurich (Switzerland) and subsequently on several full-scale systems in Europe, e.g., Riga (Latvia) and Amsterdam (The Netherlands) (within WP 3.6 and WA 7). This deliverable provides an overview of the gained results.

2 Results & Discussion

2.1 **Treatment train** - Zürich (Switzerland)

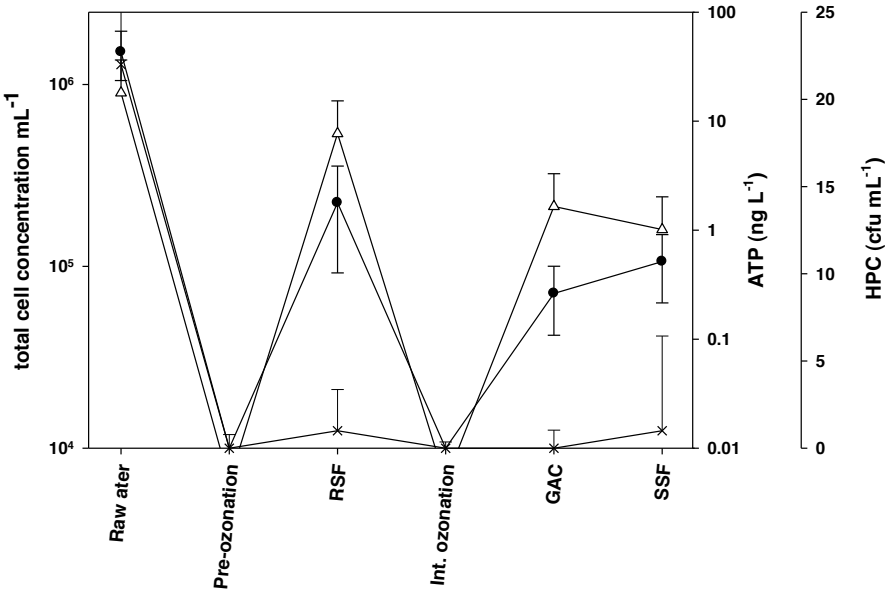

Figure 1. *Total bacterial cell concentration (●; n = 54), cell-bound ATP (Δ; n = 3) and heterotrophic plate counts (HPC; *; n = 54) during drinking water treatment in Zürich (Switzerland). RSF: rapid sand filtration; GAC: granular activated carbon filtration; SSF: slow sand filtration.*

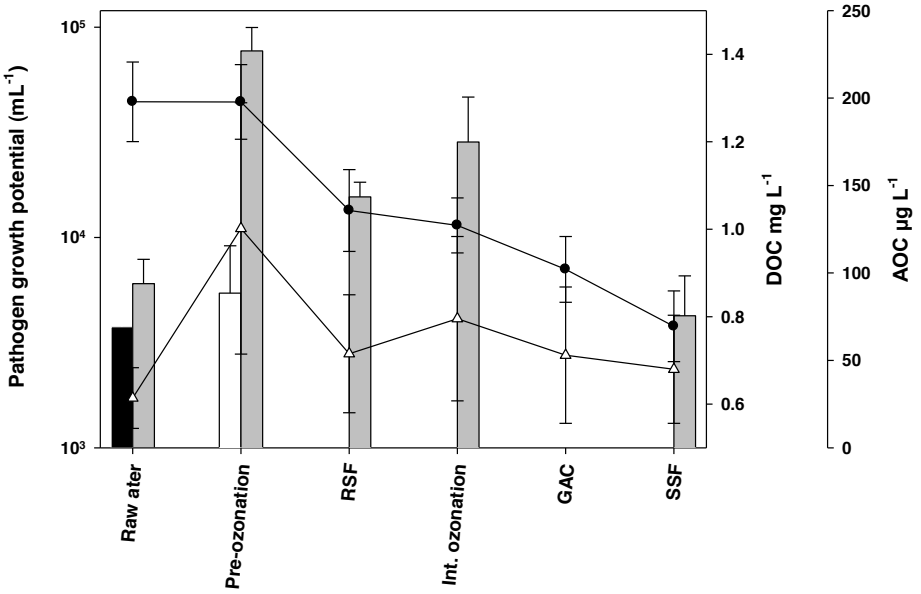

Figure 2. *Dissolved and assimilable organic carbon (DOC (●; n = 54) and AOC (Δ; n = 54)) and the pathogen growth potential of Escherichia coli O157 (white bar; n = 3), Vibrio cholerae O1 (black bar; n = 3) and Pseudomonas aeruginosa (grey bar; n = 3) during drinking water treatment at Lengg (Zürich, Switzerland).*

2.2 **Treatment train** - Amsterdam (the Netherlands)

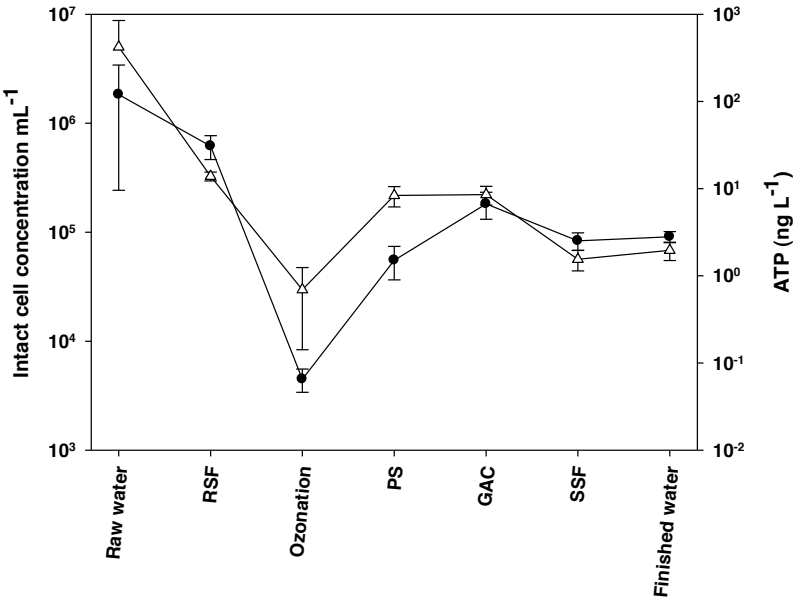

Figure 3. *Intact bacterial cell concentration (●; n = 3) and cell bound ATP (Δ; n = 3) during drinking water treatment at Amsterdam (the Netherlands). For key to abbreviations see Figure 1; PS = Pellet Softening reactor.*

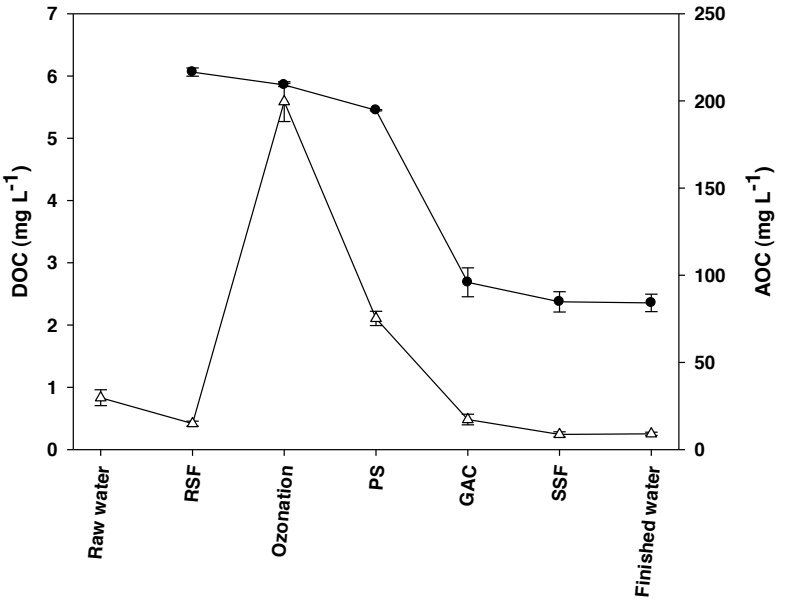

Figure 4. *Dissolved (DOC (●; n = 3)) and assimilable organic carbon (AOC (Δ; n = 3)) during drinking water treatment at Amsterdam (the Netherlands). For key to abbreviations see Figure 1.*

Discussion – Treatment train analyses

- Nearly all steps in a drinking water treatment train have a profound impact on the general microbial quality of the water. The developed/optimised methods (ATP & FCM) for enumeration of total/viable bacteria in water could accurately describe the major microbial changes (die-off during disinfection and growth during biofiltration) during water treatment. More details are discussed in Hammes et al. (2010a).
- A good correlation was found between ATP and FCM data, but not with conventional HPC data. This correlation is further discussed in (Hammes et al. 2010b).
- In fact, conventional plating severely underestimated the concentrations of bacteria in the water (also discussed in Hammes et al., 2008) and did, therefore, not allow describing microbial changes during water treatment adequately.
- Both ATP and FCM use transportable instrumentation that can easily be set up in a location different to the user's laboratory, thus allowing broad application potential for these technologies.
- Together with DOC and AOC measurements the developed methods enable a better understanding of microbial processes and events occurring during water treatment. A clear example is the ozonation process, which leads to a dramatic decrease in (viable) bacterial numbers (i.e. disinfection) and a dramatic increase in AOC (through breakdown of complex organic carbon molecules).
- While AOC detects only a small fraction of the organic carbon in drinking water, it represents an important fraction with regards to microbial growth, and is generally seen as an important parameter for biological stability of drinking water.
- PGP is complimentary to AOC measurements, and the importance of this parameter is further discussed in Vital et al. (2010). However, the PGP assay could not be applied in other countries during the Techneau project because of strict S2 laboratory requirements for this assay.

2.3 **Distribution system** - Zürich (Switzerland)

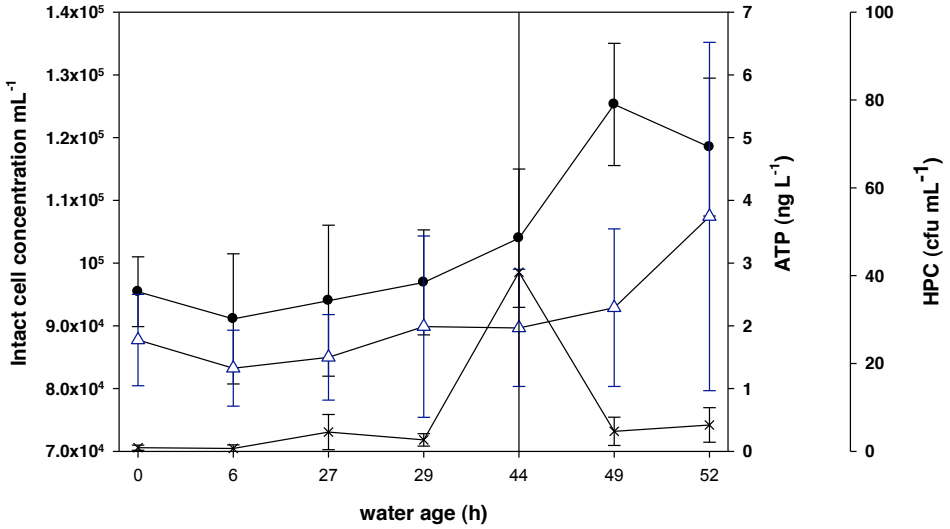

Figure 5. *Intact bacterial cell concentration (●), cell-bound ATP (Δ) and heterotrophic plate counts (HPC; *) during drinking water distribution in Zürich (Switzerland; data from Figure 4). Error bars indicate standard deviation on triplicate samples.*

2.4 **Distribution system** - Amsterdam (NL), Riga (LV) and Zürich (CH)

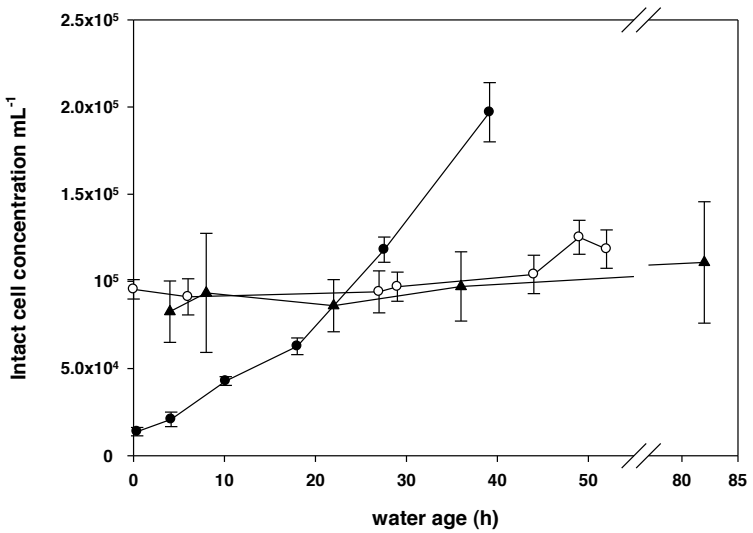

Figure 6. *Intact bacterial cell concentration during drinking water distribution in Amsterdam (◢; the Netherlands) and Riga (●; Latvia) and Zürich (○; Switzerland). Error bars indicate standard deviation on triplicate samples.*

Discussion – Distribution system analyses

- A major goal in drinking water treatment is the production of biologically stable water. The meaning of the latter is water which does not change with respect to microbial quality (cell concentration, viability and composition) from the treatment plant to the consumer's tap. Until now, biological stability was usually assessed by measuring the biodegradable organic matter in the water, using either AOC assays or so-called BDOC assays.
- During the Techneau project, a different approach was promoted to study biological stability in drinking water. This approach was based on the measurement of general microbial parameters at several locations in the network, for which approximate residence times were known.
- The developed/optimised methods (FCM and ATP) enabled the monitoring of microbial changes (or the absence thereof) during drinking water distribution. The results suggested that the treated water from Zurich and Amsterdam were essentially stable, while the treated water from Riga showed clear evidence of instability. It should be noted that a fundamental difference between these systems are the use of chlorine in Riga as final disinfectant prior to distribution.
- Hence, it can be concluded that the ATP and FCM methods allow a discrimination between biological "stable" and "unstable" water. However, minor changes in water quality (e.g., growth of a specific organism at concentrations lower than 1000 cells/mL) would go unnoticed with bulk measurement approaches.

3 Conclusions

Conventional cultivation based methods, namely HPC, which were developed 100 years ago by Robert Koch and colleagues, are till today the standard tool to monitor the general microbiology during water treatment and distribution. However, there are several disadvantages connected to them. They are not accurate (only a small percentage of total bacteria are also able to grow on an agar plate) and very time consuming. The newly developed/optimised methods presented here allow a description microbial processes during drinking water treatment and distribution in a fast, cultivation independent and robust manner.

4 References

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