

# Determining Biological Regrowth Potential in Drinking Water

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**Abstract.** The problems of decreased biological stability of treated drinking water are the main reason for residual disinfection in water treatment. Further the problems of THM (Tri Halo Methane) formation and disinfection by products both tend to align in the long chain of problems involved in biological stability of treated drinking water. As established by scientists in 2004, the levels of Assimilative Organic Carbon (AOC) are not negligible in Danish drinking water. Thus, problems of reduced biological stability are not only a problem observed when treating surface water, but may also be a potential problem in treating ground water. The analytical procedure for determining AOC level in drinking water is complicated, time consuming and not free of bias. This paper introduces a new method for establishing regrowth potential in drinking water. The method is based on the Mycometer Bactiquant test and utilises the variation of response as a function of time. The Bactiquant test is a generic test for bacteria with a duration of the analysis of only 30 minutes. The test can be preformed by non-technical personal. We used the variation of the Bactiquant signal in water samples stored for up to 96 hours, to predict/evaluate regrowth potential. We found that treatments with O<sub>3</sub>, UV and HClO, gave significant increase in regrowth potential, while GAC filtration gave a significant reduction in regrowth potential.

## Methods

*Measurement principle.* Controlling production processes is the prerequisite for producing high quality drinking water from surface waters. Daily process and quality control is dependent on technologies that allow for rapid and reliable monitoring of raw water quality changes and water treatment processes. The Bactiquant method is a culture-independent technique for detection and quantification of bacteria in water samples (Corfitzen et al., 2006). The method is based on fluorometric detection and makes use of methylumbelliferyl (4-MU) labelled enzyme substrates measuring a specific enzyme activity present in viable and non viable bacteria.

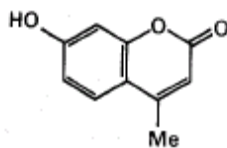


Figure 1. Molecular structure of 4-methylumbelliferone used in the Bactiquant method.

These enzyme substrates consist of a derivative of an enzyme specific moiety and a fluorophore in this case 4-MU. When the enzyme specific moiety is recognized by the enzyme, the chemical bond between the enzyme specific moiety and 4-MU is cleaved, thereby releasing free 4-MU. When a fluorophore is excited by monochromatic light it emits energy in the form of photons. The fact that a single fluorophore can

generate many thousands of detectable photons is fundamental to the high sensitivity of the fluorescence detection techniques. The principle of the assay is illustrated in figure 2.

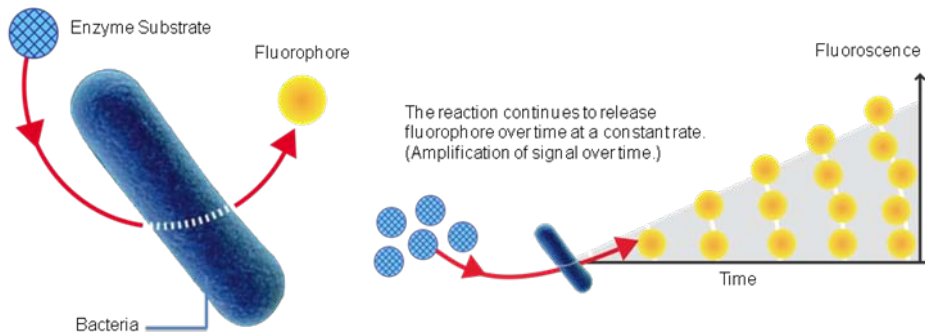


Figure 2. The method principle.

*Sample preparation and analysis.* A 250 ml water sample is filtrated through a 0.22  $\mu\text{m}$  filter retaining the bacteria on the filter surface (figure 3) The 4-MU labelled enzyme substrate is added to the filter for a given reaction time (e.g. 30 minutes). A subsample of the reaction mixture is then added to a cuvette and the fluorescence is measured at an excitation wavelength of 365 nm and emission wavelength of 445 nm. The method is patented by Mycometer AS, Copenhagen.



Figure 3. Bacteria retained on the surface of the filter membrane.

In this study, the treatment processes and water biostability was monitored in a Surface Water Treatment Plant during three periods in May, August and December 2009. All steps in the water treatment processes were monitored, including raw water intake, flocculation, sand filtration, ozonization, GAC filtration, UV treatment and chlorination. In the study the Bactiquant method was used to determine water biostability by monitoring bacterial regrowth potential in water samples over a period of five days.

## Results

Nonlinear regression analysis of growth data allowed a categorization of biostability at different stages during the treatment process.

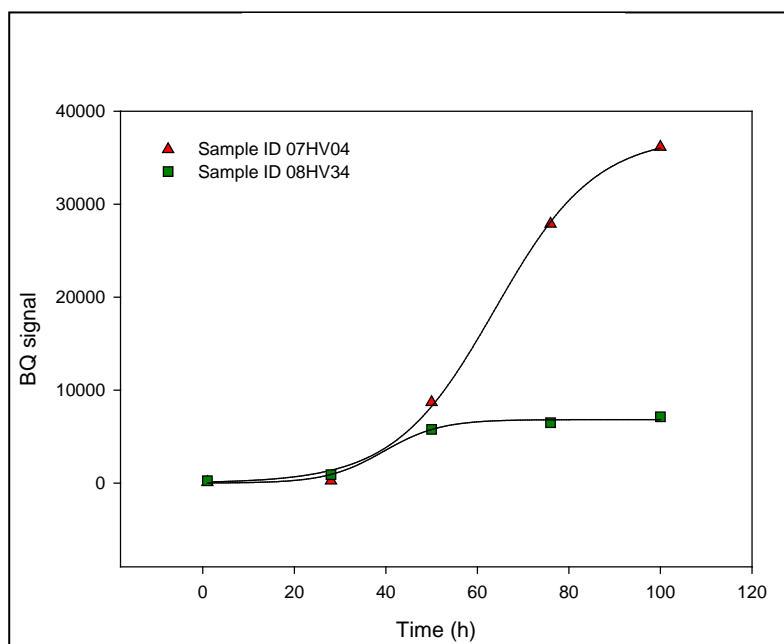


Figure 4. Nonlinear regression analysis of data after Ozone treatment (triangles) and after GAC treatment (squares).

The data showed that the Bactiquant method could be used to describe significant differences ( $p < 0,001$ ) in regrowth potential depending on the water treatment. The ozone treatment in particular had a significant decreasing effect on biological stability as determined by an increase in regrowth potential. We speculate, supported by the literature (Hammesa 2007 & 2006), that the oxidation power of Ozone ( $E^{\circ} = 2,07 \text{ V}$ ), initiates a production of assimilable organic carbon, that can initiate and sustain microbial growth. This hypothesis and our observation of a significant decrease in regrowth potential ( $p < 0,001$ ) following the GAC treatment, is consistent with the findings of Hu et al., 1999, who observed an increase in AOC following Ozone treatment and an 80 % removal efficiency of AOC following GAC treatment.

The slope in the mathematical fit of regrowth data can be expressed as:

$$f'(x) = \frac{1}{b} \times \frac{\exp^{-(x-x_0)}}{\sqrt{(1 + \exp^{-(x-x_0)})}}$$

We used this expression to rate the regrowth potential as shown in table 1.

Treatment	Parameters			Quality	Rating
	a	b	X <sub>0</sub>	R <sup>2</sup>	(a/b)
Ozone	37372,1	11,0	63,8	0,999	3405
GAC	6831,3	6,2	39,5	0,994	1097
Clean Water	114,2	1,5	33,2	0,869	75

Table 1. Rating of regrowth potential based on nonlinear regression analysis of Bactiquant data.

The determination of parameters in the nonlinear regression analysis of the regrowth data showed a clear categorization of the Ozone treated and GAC treated water when compared to the regrowth potential in the treated clean water ( $p < 0,001$ ). Our findings emphasize the importance of considering the biological stability of water as an important parameter in evaluating water treatment processes in the utilities.

## References

- Corfitzen, Charlotte B., Bettina Ø. Andersen, Morten Miller, Christian Ursin, Erik Arvin, Hans-Jørgen Albrechtsen. (2006). Rapid methods for detection of bacteria. Nordic Drinking Water Conference, Reykjavik, Iceland.
- Hammesa F, Meylan S, Salhi E, Köster O, Egli T, von Gunten U. (2007). Formation of assimilable organic carbon (AOC) and specific natural organic matter (NOM) fractions during ozonation of phytoplankton. *Water Apr*; 41(7), 1447-54.
- Hammesa F, Salhi E, Köster O, Egli T, von Gunten U. (2006). Mechanistic and kinetic evaluation of organic disinfection by-product and assimilable organic carbon (AOC) formation during the ozonation of drinking water *Water Research*, 40/12, 2275-2286.
- Hu, J.Y., Z.S. Wang, W.J. NG, S.L. Ong. (1999). The effect of water treatment processes on the biological stability of potable water. *Water Research* . 33. (11), p. 2587-2592.
- Iriarte, U, Inaki J, Alvarez-Uriarte J.I, Ruben Lopez-Fonseca, R., Gonzalez-Velasco, J.R.(2003). Trihalomethane formation in ozonated and chlorinated surface water. *Environ Chem Lett*, 1, 57–61.

Plummer J.D,\* and James K. Edzwald (2001).Effect of Ozone on Algae as Precursors for Trihalomethane and Haloacetic Acid Production. *Environ. Sci. Technol.*, (35/18), 3661–3668