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Whitepaper: BactiQuant®

Attain Microbiological Quality and Biological Stability in Water: Detect, Assess and Respond.

Content

Introduction

Mycometer is a world leader in developing rapid and robust microbiological methods, to detect and quantify microbiological contamination, based on a well proven highly sensitive fluorescence technology. No other technology allows quantification of bacteria and fungi in air, surface, bulk and liquid samples.

This paper briefly discusses Mycometer's new method BactiQuant®, how it works, how it is applied, and how it solves problems in Water Works.

Current Technology and the Consequences

Currently, Heterotrophic Plate Counts (HPC) is used for monitoring overall water quality. In both disinfected and non-disinfected systems, sudden increases in HPC above normal baseline levels can indicate a change in raw water quality or a problem such as bacterial re-growth in the distribution system. Measuring HPC levels in water upon leaving the treatment plant or during treatment is used by plant operators to monitor plant operation. High HPC measurements indicate low biological stability of the water. Bacterial re-growth can promote corrosion, foul-tasting or discoloured water and harbour secondary respiratory pathogens as well as increase the demand for disinfectant (Health Canada, 2006).

The existing conventional testing methods have several drawbacks:

- The process is labour intensive.
- The test methods are sensitive to errors during performance of the tests
- The results are generally not available for two to four days.

Among these undesirable attributes, the consequences of time delayed feedback of results are generally the most significant and include processes (sampling and analysis) that are to episodic and slow for rapid detection and action.

Mycometer's Technology and the Advantages

Mycometer's technology has been developed to mitigate these consequences for the professional consultants and the industry. Specifically, the method facilitates detection and quantification of microbial contaminants in near real time. The methods are based on measuring microbial enzyme activities by use of a highly sensitive fluorescence technique. The use of this technology for environmental sampling and analysis is well documented in the scientific literature (Miller et al., 1998; Krause and Hammad 2002; Reeslev et al, 2003; Krause, 2003; Corfitzen et al., 2006).

There are several advantages of targeting microbial enzyme activities:

- Method is culture-independent
- High versatility – many enzyme targets
- Easy adjustment of sensitivity.

The advantage of using fluorescent enzyme substrates:

- No alteration of bacterial cell necessary
- Simple chemistry
- Low detection limits

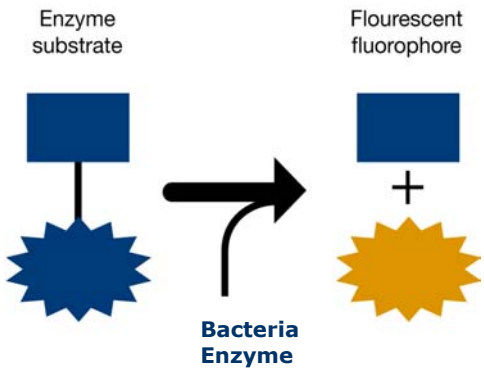
Mycometer's BactiQuant® method detects and quantifies enzyme activity in bacteria. The fluorescence signal produced is directly proportional to the amount of bacteria present in a given water sample. With the BactiQuant® (BQ) system, bacterial contaminants can be concentrated from large volumes of water allowing low analysis time (10 – 30 minutes) even at relatively low bacterial concentrations.

As an additional benefit, the method can also be used to detect bacteria on surfaces and in air samples.

Mycometer Technology

Mycometer methods are based on a fluorescence technique that makes use of methylumbelliferyl (4-MU) labelled enzyme substrates. 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 (Figure 1). 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 fluorescence detection techniques.

Figure 1. Schematic representation of chemical reaction



In practice, a sample containing bacteria is saturated with enzyme substrate. The bacterial enzyme reacts with the enzyme substrates and 4-MU is released. The 4-MU is excited by photons of wavelength 365 nm. The 4-MU absorbs the photons and enters an excited state S_1'(higher level of energy). This excited state is unstable and only exists for a very short interval (nanoseconds). When the excited state returns to the more stable ground state (S_0'), a photon is emitted. Due to energy dissipation during the brief excited-state lifetime, the energy of the emission photon is lower and therefore, of longer wavelength than the excitation photon (Richard P. Haugland, 2002). The photons (fluorescence) of 4-MU are measured at 445 nm.

The absorption and fluorescence spectra of the unhydrolysed enzyme substrate and the 4-MU are shown below in Figures 2 and 3 (adapted from Wilson and Goulding, 1986). The figures indicate that if the assay system is irradiated with 350 to 400 nm light virtually all of the fluorescence measured around 450-500 nm is due to the 4-MU product of the enzyme reaction.

Figure 2. Absorption spectra of enzyme substrate and 4-MU.

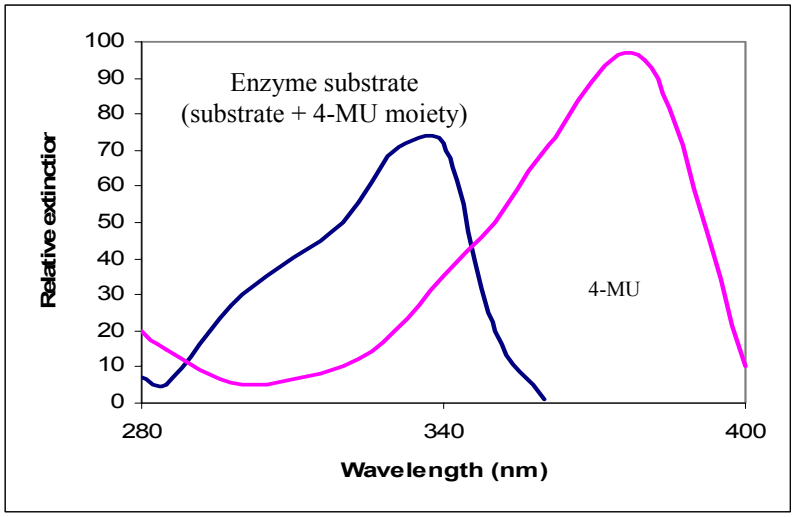
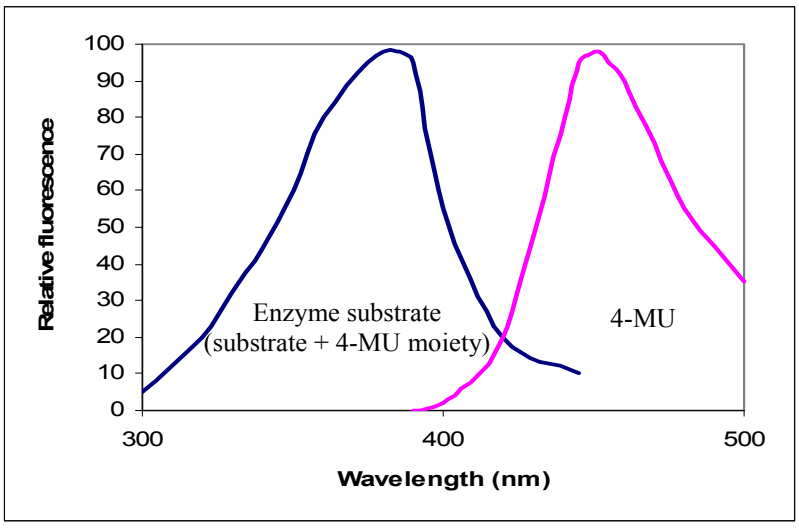


Figure 3. Fluorescence spectra of enzyme substrate and 4-MU



For over ten years, Mycometer has been collaborating with the US manufacturer of fluorescence instruments, Turner Biosystems (TBS, Sunnyvale, California). The high performance instrumentation technology TBS provides for the fluorescence detection in the Mycometer methods consists of:

1) Excitation source which emit light at a wavelength that excites the 4-MU (wavelength 365 nm)
2) Wavelength filters to isolate emission photons (445 nm) from excitation photons and
3) Detector that registers emission photons and produces a recordable output.

The handheld instrumentation is light weight and battery operated for use on location. It has a menu driven keypad for easy use and LCD display. TBS instruments and Mycometer technology have met the demanding specifications of the US military for mold detection in buildings.

Other Mycometer products include microbial contamination monitoring by air sampling, using generic air sampling equipment and the new air sample protocol for fungi and bacteria. Mycometer technology can also be used for surface tests for bacteria and for fungi as well as in bulk material samples.

Application of the BactiQuant® Method to Water Treatment Operations

The BQ method is a flexible tool that enhances the ability in the Water Works to detect, assess and respond to changes in the biological stability of water.

The BactiQuant® delivers a robust system to monitor source water quality at intake, treatment efficiency, distribution system integrity and as a quality assurance following renovation of water systems. The BactiQuant® system offers significant advantages to the waterworks:

- Low supply and human resource demand
- Applicable in chlorinated water
- Parameter is directly linked to biological stability of water

High frequency monitoring with the BactiQuant® allows for timely trending to spot problems early and lowers the chances of contamination. As opposed to on-line monitoring systems that are installed at fixed control points, BactiQuant® can be used in emergencies by rapid response teams for flexible and rapid surveys of the whole water system. The multiple sampling modes allow sampling in areas with difficult access. The flexibility in sampling facilitates a rapid sampling strategy in case of contamination and the ability to rapidly determine where the leading edge of the contamination is located as well as delineate the extent of contamination.

Practical uses of the BactiQuant® in US water works.

1) Monitoring the efficiency of the water treatment process, including disinfection

2) Assessing changes in finished water quality during distribution and storage

3) Assessing microbial growth on materials used in the construction of potable water treatment and distributions systems

4) Measuring bacterial regrowth or aftergrowth potential in treated drinking-water

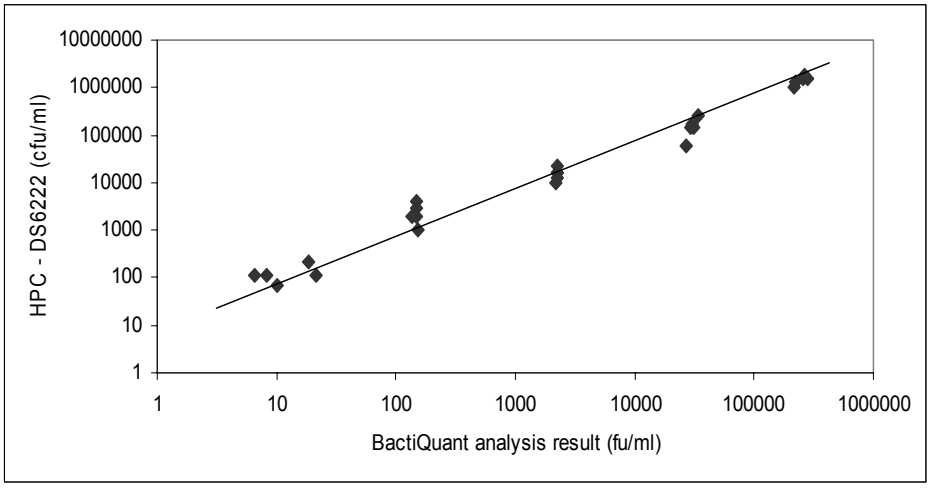
5) Monitoring bacterial growth changes following treatment modifications, such as a change in the type of disinfectant used.

Laboratory Validation Studies with Mycometer's BactiQuant®

The BactiQuant® system has been evaluated in laboratory experiments. Figure 4 shows a scatter plot of HPC counts and results of the BactiQuant® analysis from an experiment simulating organic pollution in drinking water. The drinking water was diluted 10^1,10^2,10^3,10^4 and $5x10^5$. The scatter

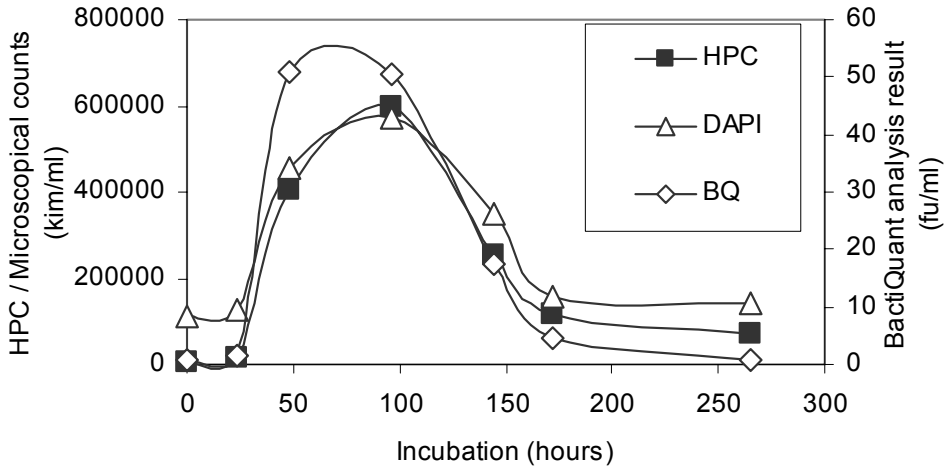
plot shows a very strong correlation between HPC counts and the results of the BactiQuant® analysis.

Figure 4. Comparison of BactiQuant® to HPC results



BactiQuant® results will differ with growth conditions and bacterial species. Therefore these results should be interpreted with caution when applied to more complex environmental samples. Figure 5 shows data from a similar experiment conducted in collaboration with the Technical University of Denmark. The figure shows the dynamic response pattern of HPC counts, DAPI microscopic counts and the results of BactiQuant® analysis, in response to a simulated organic pollution of drinking water.

Figure 5. Results of HPC, DAPI, BactiQuant® in complex environmental samples



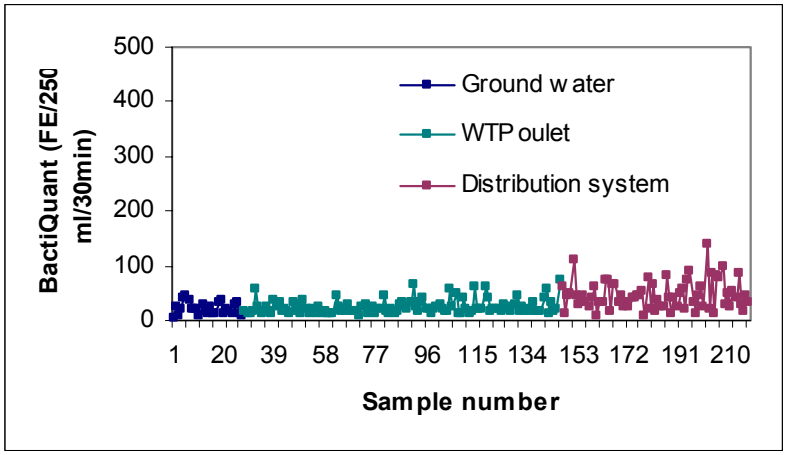
The results are consistent with a strong correlation between bacterial presence and analysis results obtained with the BactiQuant® method.

Field Validation Studies with Mycometer's BactiQuant®

The BactiQuant® system was evaluated at the Copenhagen Water Works (KE – Water) in 2005 and 2006. Water samples (n = 974) were collected from more than 200 sampling sites. Parallel analysis was conducted on all water samples. Parallel analysis included the traditional standard method (DS/EN ISO 6222) for enumeration of culturable micro-organisms at 22^{O}C and 36^{O}C on yeast extract agar and the BactiQuant® analysis conducted according to the standard protocol for drinking water analysis described in the BactiQuant® user manual. The water samples were collected from the source (ground) water, treatment plant (WTP) outlet (after aeration, precipitation and sand filtration), before and after water pipe renovations and from water taps in private and public buildings. Results of parallel analysis of HPC and BactiQuant® showed a good correlation (r = 0,75, n =975).

Excerpts of the baseline data are shown in Figure 6. The figure shows an increase in enzyme activity from source ground water (with expected low bacterial numbers) to the outlet at WTP and further downstream in the water distribution system where increasing HPC counts were expected.

Figure 6. Excerpts of baseline data



The extensive data set allowed operative threshold values to be established based on the empirical distribution of analysis data. The threshold values were organized with a level indicating a normal and acceptable condition, an intermediary level between normal and critical and finally an unacceptable level of bacteria in the water samples. An example of how categories were defined at the Copenhagen Waterworks (KE-Water) is shown below:

Best quality – no activation of control measures.
BQ values ≤ 40

Between best quality and unacceptable quality – control measures may be activated.
40 < BQ values ≤ 200

Unacceptable quality – high risk of exceeding tolerance limits for total bacteria in water – control measures activated.
BQ values > 200

Sensitivity and Specificity of the Method

The sensitivity of the method can be adjusted to fit the needs of the user. Protocols are available for drinking water and ultra pure water. The target enzyme activity in the assay is a hydrolytic enzyme belonging to the enzyme group of hydrolases. The enzyme activity that is measured with the method is not unique to bacteria, however has been shown to be preferential to bacteria. The target enzyme activity, used in the BactiQuant® method, has been shown to have utility as an indicator of microbial population density and biomass in freshwater sediments (Saylor et al., 1979); Utility water systems are dominated by bacteria hence in utility water systems the activity is of a predominantly bacterial origin. The good correlation with HPC in water samples shows that the target enzyme activity is a good indicator of bacterial presence and activity in water utility systems. The enzyme activity is widely distributed in bacteria. The table below shows examples of bacteria that posses the target enzyme. The bacteria in the table represent a wide range of bacterial phylogenetic groups including both gram negative and gram positive.

Organism	Gram stain	Phyllum/ class
Athrobacter sp.	Negative	Actinobacteria / grp. 20
Bacillus cereus	Positive	Firmicutes / grp. 18
Bacillus subtilis	Positive	Firmicutes / grp. 18
Bacillus thuringiensis	Positive	Firmicutes / grp. 18
Clostridium tetani	Positive	Firmicutes / clostridia
Desulfovibrio desulfouricans	Positive	Proteobacteria / δ
Enterobacter sp.	Negative	Proteobacteria / γ
Enterococcous faecalis	Positive	Firmicutes / bacilli
Eschericia coli	Negative	Proteobacteria / γ
Escherichia fergusonii	Negative	Proteobacteria / γ
Flavobacteria bacterium	Negative	Bacteroidetes / flavobacteria
Flavobacterium johnsoniae	Negative	Bacteroidetes / flavobacteria
Klebsiella pneumonia	Negative	Proteobacteria / γ
Lactobacillus acidophilus	Positive	Firmicutes / bacilli
Lactobacillus reuteri	Positive	Firmicutes / bacilli
Methanococcus aeolicus	Negative	Euryarchaeota / methanococci
Micrococcous sodonensis	Positive	Actinobacteria / actinobacteria
Pseudomonas aeroginosa	Negative	Proteobacteria / γ
Rhodobacter sphaeroides	Negative	Proteobacteria / α
Serratia marcescens	Positive	Proteobacteria / γ
Staphylococcous aureus	Positive	Firmicutes / bacilli
Sphingomonas Wittichii	Negative	Proteobacteria / α
Vibrio sp	Negative	Proteobacteria / γ
Xanthomonas campestris	Positive	Proteobacteria / γ
Yersinia pestis	Positive	Proteobacteria / γ

Source: Enzyme database Brenda and ExPASy (Expert Protein Analysis System) Proteomic Server.

Summary

BactiQuant® provides the professional a rapid, robust method to mitigate the consequences of current technology. With rapid detection onsite in less than 30 minutes, data collected is near real-time for fast response to biological instability in the system. The contamination can be isolated and delineated quickly to focus response resources effectively. The robustness of the method, based on scientific research and documentation, provides assurance that the data is repeatable and reliable. With BactiQuant®, water treatment operations can improve their performance, minimize contamination related maintenance, and exceed the expectations of the end user.

Comparison of Current Method vs. Mycometer BactiQuant®

The table below compares BactiQuant® against the current HPC method.

Feature	BactiQuant®	HPC	Consequence
Time from measurement to results.	**10 – 30 minutes**	**Typically from two to four days**	**Plate count processes are slow, not compatible with rapid reaction. Gives a historic recording of water quality. Rapid response teams cannot use plate counts for rapid surveys and delineation of contamination.**
Mode of detection	**Culture-independent, fluorescence**	**Culture-dependent – visual**	**The conventional method only detects culturable bacteria. BactiQuant detects bacteria in UV and MCA treated water. This provides the end users with a more comprehensive evaluation of the biological stability of drinking water.**
Time to set up sample	**5 minutes**	**Can be significant**	**The conventional method requires high labour costs and advanced lab facilities.**
Human intervention	**Minimal**	**High**	**Human intervention creates more possibilities for inaccuracies and contamination of sample**

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