

Fireflies shed light on safe water treatment processes

Monitoring of microbial load in industrial and environmental water analysis

Biological contamination of water represents a major risk to plant, equipment and human health in applications such as raw water intake, cooling water systems, reverse osmosis demineralisation and drinking water distribution. Water utilities, oil & gas facilities, chemical manufacturers, desalination plants, data centers, bottling plants and power stations therefore measure microbial load to help minimise corrosion, optimise plant performance and prevent outbreaks from pathogens such as Legionella. Traditionally, this has involved sampling for laboratory analysis, but a new online technology (based on the chemical process that fireflies use to attract mates) is dramatically improving the effectiveness, speed and value of monitoring.

In the following article we outline the reasons for monitoring, how the new technology works, and its advantages over traditional methods. This will be followed by three brief case studies where users have exploited the benefits of continuous monitoring of microbial load with Hach®'s EZ7300 monitor – the world's first microbiology analyser using the ATP firefly assay.

Reasons for monitoring microbial load

The presence of microorganisms in water represents a problem for a wide variety of industries where water purity is a priority. This is because the proliferation of microorganisms can affect plant efficiency, and when released into the air affect human health.

Water handling systems, particularly those involving recirculation, provide a favorable environment for the growth of microorganisms, resulting in the develop-



Standard testing for total microbial count requires lengthy incubation. When the results are finally in it is too late to act upon them.

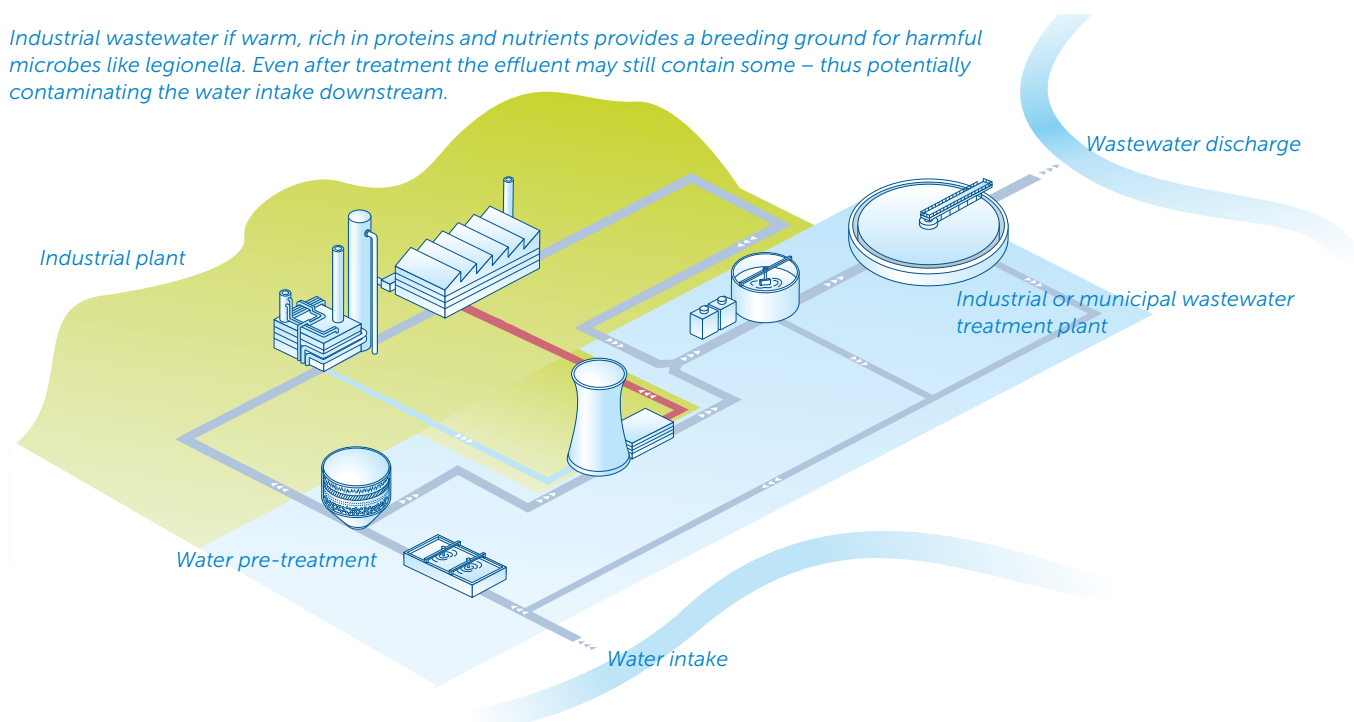
ment of biofilms and slime. A biofilm is the collection, on a surface, of organic and inorganic, living and non-living material. These biofilms can lower the levels of disinfectant residuals, increase bacteria levels, lower dissolved oxygen, and cause taste and odor problems where the water is destined for human consumption. Biofilms can include bacteria, fungi, and higher organisms such as nematodes, larvae, and even crustacea.

Biofilms can accumulate and cause the clogging of filters and other systems. However, the presence of a biofilm changes the environment at the fouled surface causing anaerobic and anodic conditions. This creates differences between colonised and uncolonised sites, which promotes an electrochemical differential that can cause corrosion. Biofilms often create local areas of low oxygen in which fermentative microbes produce organic acids and lower pH. Nevertheless, sulfide by-products may be corrosive or may contribute further to the electrochemical differential between fouled and unfouled areas.

High levels of microbial load can result in the dissipation of tiny water droplets (aerosols) containing harmful bacteria, such as Legionella. Legionella bacteria are widespread in natural water systems but rarely cause disease. However, outbreaks of Legionellosis occur from exposure to legionella caused by poorly managed cooling towers and evaporative condensers. Legionellosis is a potentially fatal disease and

Monitoring of Microbial Load

Industrial wastewater if warm, rich in proteins and nutrients provides a breeding ground for harmful microbes like legionella. Even after treatment the effluent may still contain some – thus potentially contaminating the water intake downstream.



in most countries regulations require employers to make a suitable and sufficient assessment of the risks from any work liable to expose employees to any substance hazardous to health, and for other people not in their employment who may be affected by the work activity.

In many applications, biocides are added to water to prevent the build-up of microorganisms, and the effectiveness of this process has to be monitored – not just to ensure that it is having the desired effect, but also to optimise the frequency and concentration of dosing.

Early detection of microbiological contamination of a water source allows further investigation to identify the source of the contamination by specific detection methods so that timely corrective actions can be taken.

Perhaps the most important reason for monitoring is the risk that no monitoring would cause. Microbial proliferation can cause damage and even plant shutdown, resulting in significant financial loss. However, it also represents a threat to human health with potentially enormous reputational risk.

Continuous monitoring of microbial load – how it works

The ASTM D4012-81 'firefly method' was developed as a faster, more effective alternative to traditional microbial tests such as cell tagging, plate counting and turbidity. It involves a rapid, sensitive determination of viable biomass of bacteria in water and wastewater, cleaning and hygiene applications, by monitoring levels of Adenosine triphosphate (ATP).

ATP is the energy currency of life, and as such provides a reliable indicator of a living organism. The measurement of ATP mimics the chemical reaction undertaken by fireflies,

whereby ATP and luciferin generate light in a reaction catalysed by the firefly luciferase enzyme. This light generated is proportional to the amount of ATP in the sample.

The EZ7300 continually pulls samples with an analysis cycle of 10 - 15 minutes, and up to 8 sample streams are possible per analyser. In the first stage of the measurement process the level of ATP in the 'raw' sample is measured – this represents extra-cellular (or 'non-living') ATP. In the second stage, an ultrasonic (non-chemical) method is used to lyse the cells in the sample and free the 'live' ATP. A second measurement is then taken, giving the 'total ATP' in the sample. The difference between these two measurements represents the 'live' ATP and is proportional to the amount of live microorganisms in the sample.

With a detection limit of 0.05 picograms of ATP per mL, the EZ7300 is able to measure at very low levels (0.05 pg \approx 50 *E. coli* sized bacteria), with a wide measurement range extending to over 200 pg/mL*.

Portable instruments have been developed to take advantage of the ATP method, but the Hach EZ7300 is the first instrument to successfully deliver this technique in a continuous monitor.

The advantages of continuous monitoring

Before discussing the obvious advantages of continuous monitoring, it is important to be aware of the differences between the ATP method and traditional techniques which generally involve the cultivation of target or indicator organisms. These methods generally cultivate less than 1% of bacteria in a sample, so do not provide a good indication of bacterial load. They also rely on the skill of the operator and assume no effects from human error or variability.

Laboratory analysis incurs a significant delay between the taking of a sample and the delivery of a result. In contrast, the EZ7300 is able to conduct an analysis in less than 15 minutes; 24 hours a day; every day of the year. This continuity of data opens a new world of possibility in process control. The provision of almost real-time data means that process operators are able to respond immediately to any change in conditions. This fast response is often more effective and much less costly than that which was possible after receipt of a grab sample result.

One of the most important advantages of continuous monitoring is its ability to record the timing and severity of spikes in the data. In combination with other process data, this helps to identify the causes of such spikes and thereby inform appropriate mitigation measures. Microbial load can increase very quickly under certain conditions (such as failure of biocide dosing equipment), and the time delay from laboratory samples imposes a high level of risk in comparison with the almost immediate response of a continuous monitor.

With the ability to take samples as frequently as six times per hour, the EZ7300 is able to generate large data sets, which dramatically improves the statistical significance of results and thereby radically improves R&D activity and the analysis of trends. As utilities begin to extract real value from 'Big Data' this will be of great value in the development of predictive algorithms, smart systems and artificial intelligence.

Microbiologists are generally highly qualified so their deployment in traditional routine laboratory tests is a poor use of their time. Continuous monitors therefore free these staff for more important work – such as process optimization, R&D, and the analysis of cause and effect in the data provided by the monitors. Also, the amount of labor and materials involved with laboratory analysis means that traditional forms of analysis are several times more costly per sample than the method employed by the EZ7300.

Case Study: Power Generation, Indiana, USA

There are two major factors affecting biocide dosing strategy in power station cooling towers. Firstly, the discharge permit may restrict the rate or timing of dosing, and secondly, the dosing strategy needs to meet the microbial load in the water, which may vary, depending on the source and whether recycling is employed.

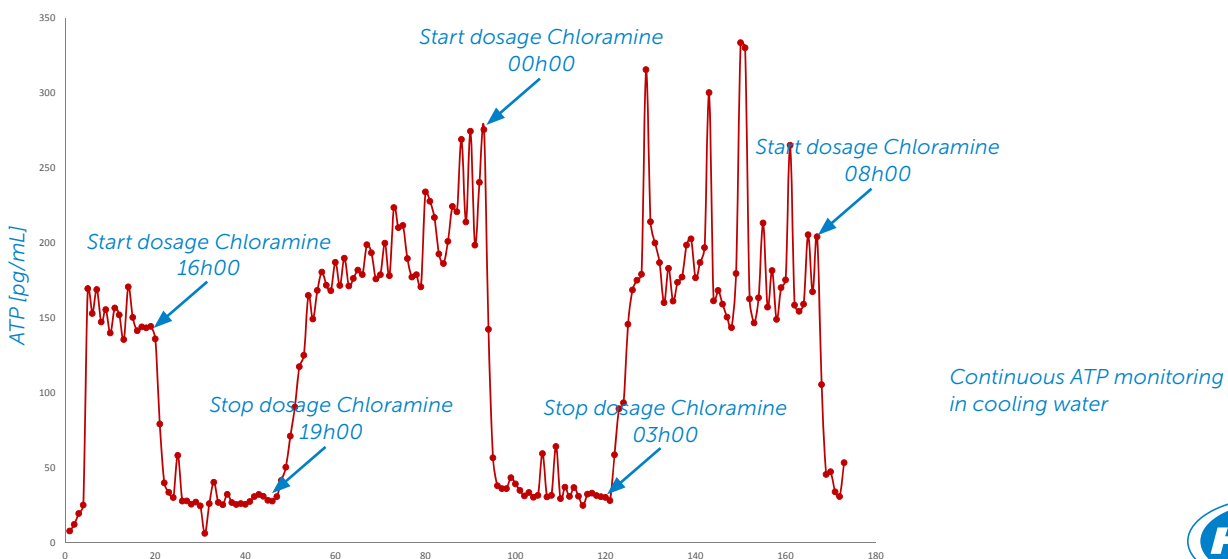
The operators of a power station in Indiana needed real-time information to optimise the biocide treatment protocol. This data was required to determine whether intermittent dosing or continuous dosing (with a lower concentration of chloramines) was more efficient and cost-effective. It was also necessary to reduce the overall microbial load in the cooling water circuit and cooling towers; to reduce biofilm formation and the associated risk of a Legionella outbreak from the large cooling tower.

A Hach EZ7300 analyzer was installed and during a two month period provided a clear demonstration of the advantages of continuous monitoring. For example, the data from intermittent biocide dosing showed dramatic effects on ATP levels, and therefore microbial load, comparing the dosing with no-dosing periods (see graph).

Based on their results the plant now continuously monitors two sample streams, helping to optimize biocide dosing and mitigate any potential risks. A second analyser has also been purchased to monitor a sister power plant's low head service water system.

Case Study: Drinking Water Treatment Plants, Nevada, USA

Source waters may contain microorganisms such as bacteria, viruses and protozoa which present a risk to human health if not effectively treated and disinfected. Successful water treatment involves the removal and/or inactivation of pathogenic microorganisms to prevent the spread of water-borne disease. The removal of pathogenic organisms is



Continuous ATP monitoring in cooling water

effected by coagulation followed by sedimentation and filtration and by other filtration processes such as membrane filtration. In contrast, the inactivation of pathogens relates to the effect of a disinfectant in destroying the cellular structure of the microorganisms or in disrupting their metabolism, biosynthesis or ability to grow and reproduce. For bacteria, this inactivation is measured by an inability to divide and form colonies – traditional tests therefore measure ‘colony forming units.’ For viruses, inactivation means the inability to form plaques in host cells. For protozoan *Cryptosporidium* oocysts, inactivation means the inability to multiply, thereby preventing infection of a host by *Cryptosporidium*.

Standard laboratory tests are required for compliance with drinking water regulations. However, it is clear that the success of disinfection relies on the inactivation of all microorganisms; not just those that are tested as indicative organisms. Consequently, with the ability to measure all ‘live’ organisms, the continuous monitoring of ATP provides a useful tool for the measurement of disinfection efficiency.

In 2017, a large water treatment facility (2.3 million cubic meters per day) installed the Hach EZ7300 analyzer on an ozone disinfection facility treating lake-fed raw water. The operators were delighted with the results because continuous data gave them much greater insight into the process, and enabled them to optimize the disinfection process; ensuring maximum disinfection whilst minimizing costs.

The installation was so successful that another instrument was then purchased for a chlorine gas disinfection process on finished drinking water. This EZ7300 is now providing ongoing disinfection optimization, whilst also demonstrating the successful inactivation of microorganisms.

Case Study: Biological Filter Optimization – Drinking Water, Minnesota, USA

Biological filters remove contaminants in three ways: biodegradation, adsorption of micropollutants, and filtration. The microorganisms on the filter media consume organic matter producing end products including carbon dioxide, water, biomass, and simpler organic molecules.

Biological treatment removes a wide variety of contaminants, eliminating the need for chemical treatment prior to filtration or settling, without the issues associated with by-products. In comparison with other drinking water treatment technologies that sequester contaminants for removal, biological treatment destroys and removes multiple contaminants simultaneously and reduces sludge production.

Biological filters lower chemical costs, but can only function efficiently with a healthy population of microorganisms on the filter media. The functioning of a biological filter can be estimated with indirect measurements of parameters such as pH and dissolved oxygen, but a direct measurement of microbes in the influent and effluent is far more useful.

In November 2015, a prototype of the EZ7300 was deployed to monitor a biological filter at an award winning water treatment plant in Minnesota. By monitoring both the influent and the effluent concurrently, the instrument was able to measure the filter’s biomass removal efficiency under a range of different conditions. Importantly, by measuring both free and intracellular ATP, the analyser was also able to demonstrate the efficiency of removal of viable microorganisms.

The plant operators were impressed with the capabilities of the analyzer, and a new unit was installed in May 2017 and has been in continuous operation since that time, monitoring biological filter performance. In addition, staff at the plant have utilized the instrument’s grab sample capability, which enables them to take samples from other parts of the plant for analysis on the same instrument. This activity has enabled them, for example, to investigate microbial induced corrosion.

Summary

The feedback from users of the Hach EZ7300 has been extremely positive, with most commenting on the new world of opportunity that continuous monitoring provides. The ATP monitors are providing much greater insight into process conditions than has ever been possible before; helping to optimise process control and mitigate the risks presented by microbial load... all thanks to the humble firefly.