Chapter 8

Adenosine Triphosphate (ATP) measurement technology

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8.1 INTRODUCTION

As water management continues to move to the forefront of worldwide resource concerns, better information is required to facilitate effective decision making. Not only does the information have to be more accurate, it also must be obtained faster and acquired from multiple locations. This challenge is common across all industries where water is a critical commodity.

Heterotrophic Plate Counts and other culture-based tests have traditionally been used to estimate microbiological content, but they must be completed in a laboratory, require two or more days for feedback, and do not truly measure total microorganisms (Bartram *et al*., 2003). Because of these limitations, municipal water contamination issues can go unnoticed until major deterioration has already occurred. By the time culture test results are realized on contaminated water samples, the potable water has already been distributed to and likely utilized by consumers.

Adenosine Triphosphate (ATP) is the primary energy carrier for all forms of life on Earth. Given its evolutionary importance, its quantification is seen as one of the fundamental tools in understanding biological processes. Since the 1960s,

The chapter is from the book *Microbiological Sensors for the Drinking Water Industry,* Torben Lund Skovhus and Bo Højris (Eds.).
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the principles of ATP measurement have been successfully applied to a variety of applications, from environmental (Cairns *et al.*, 1979) to marine monitoring (Holm-Hansen & Booth, 1960) and even in space (Birmele *et al.*, 2010), in addition to food hygiene monitoring. The latter application has been the most successful, in which the vast majority of food processing and distribution companies have adopted ATP methods as a quick test for total contamination under Hazard Analysis Critical Control Point (HACCP) strategies. These First Generation ATP test methods use basic principles and as such provided users with only a semi-quantitative measurement of ATP content. Due to their basic nature, these First Generation ATP kits have limited applicability.

The advantages of ATP testing over traditional culture tests are that the results are obtained in mere minutes (not days), that the results are complete (rather than the small fraction that will grow on culture media), and that the methods are generally field- and user-friendly. However, none of these advantages will provide significant value unless the results produced are reliable and accurate. On that vein, there were two main reasons that the technology did not achieve successful adoption in the field of water: for one, the techniques developed for hygiene monitoring were not designed to be fully quantitative – rather, they were designed to simply provide a clean/not-clean determination for microbiological attachment as well as food residue. Secondly, the industry operated under the assumption that success of such techniques would be based upon achieving direct correlations with incumbent methods (e.g. culture tests), which is quite simply an impossible objective since the tests measure different things.

Second Generation Adenosine Triphosphate (ATP) were introduced early in the 21st century and provided several advancements including enhanced resistance to interferences and high sensitivity, while maintaining the same fast response time. Being the primary energy transfer molecule for all living cells, successful measurement of ATP is a direct indication of the level of total microbiological contamination and serves as the ideal basis for risk assessment and disinfection program optimization. While being a rapid microbiological test makes it valuable, the real power comes from the fact that ATP testing is extremely sensitive to changes by measuring total biomass, which is not easily achieved using traditional methods.

8.2 ATP MEASUREMENT FUNDEMENTALS

ATP is the keystone of metabolic activity. Most of the energy for microbiological processes is stored and transmitted via ATP. ATP is produced as microbiological food is consumed and is subsequently utilized for cell maintenance as well as the synthesis of new cells and biochemicals (Knowles, 1980).

ATP provides energy for cellular operations by donating phosphate groups, resulting in the formation of either Adenosine Diphosphate (ADP) or Adenosine Monophosphate (AMP). ADP and AMP are subsequently recycled and regenerated back into ATP as the organism consumes food. This operation typically occurs many times per second (Goodsell, 1996). Therefore, the measurement of intracellular

ATP (i.e. ATP contained inside living cells) can be considered as the 'potential energy' contained within an active biomass population at any given time. ATP production is directly related to the growth rate of the cell and therefore higher ATP levels are indicative of greater mass and cell volume (Johnston *et al*., 2012). Additionally, when cells become weak and/or lyse, they release their ATP into their external environment. This is termed 'extra-cellular' ATP (i.e. ATP outside of living cells). The presence of a greater proportion of extra-cellular ATP (relative to intracellular ATP levels or compared to an established "baseline") is indicative of a less healthy population (Maeir *et al*., 2009).

ATP can be easily measured with high specificity via the firefly luciferase assay. Luciferase is a naturally occurring enzyme that is most commonly found in the tails of fireflies but can now be reliably sourced through several commercial sources thanks to recombinant DNA technology. The reaction between ATP and Luciferase is described in Equation (1):

$$ATP + O_2 + luciferin \xrightarrow{Mg^{++},\, luciferase} AMP + PPi + oxyluciferin + light \qquad (1)$$

where,

ATP = Adenosine Triphosphate
AMP = Adenosine Monophosphate
PPi = Pyrophosphate
Mg^{++} = Magnesium ion

The chemical energy produced from the breakdown of ATP is converted into light. Each molecule of ATP consumed in the reaction produces one photon of light. This light output can be quantified using a luminometer within a matter of seconds. A luminometer is a light-detecting instrument like a spectrophotometer; however, luminometers are generally much more sensitive and do not contain a light 'source'. It is the luminescent reaction that acts as the light source in luminometers.

The result produced by luminometers is typically expressed as a Relative Light Unit, or RLU. This value represents the instrument's interpretation of the amount of light detected. As such, RLU results from different instruments will tend to produce significantly different RLUs due to variation in instrument tuning, manufacturer calibration, and other design factors (Berthold *et al*., 2000). It is therefore important to run ATP standards with the reagent system and luminometer being used to account for these variables.

There is a wide variety of luminometer brands on the market with their designs depending on the intended application. For example, hygiene swab ATP tests often rely on photodiode-equipped luminometers, which, while not overly sensitive, are usually available at a low cost and are acceptable for surface analyses. For more sensitive applications, photomultiplier-tube-equipped luminometers are often used. Photomultiplier tubes, or PMTs, can typically achieve several orders of magnitude worth of enhanced sensitivity compared to photodiodes (Berthold *et al*., 2000). Luminometers are also available in both single-chamber format as well as 96-well

and even 384-well plate instruments (intended for research applications rather than practical field testing), which can sequentially perform many assays in an automated fashion.

8.3 FIRST VERSUS SECOND GENERATION ATP MEASUREMENT

As with any enzymatic reaction, the luciferase assay for ATP is susceptible to interferences that can be caused by various components in a sample. For this reason, ATP testing has not been traditionally applied in water, wastewater and industrial applications. In addition, ATP on its own is highly unstable and readily degrades to ADP (adenosine diphosphate) and AMP (adenosine monophosphate), unless it is complexed with other molecules or cell debris (Cairns *et al.*, 2005).

Developments in the reagents and methodology used have led to substantial improvements in the ATP test method. These new, Second Generation protocols and reagents were specifically designed to mitigate various types of interferences found in water applications including suspended solids, dissolved solids, biocides and organics. This is accomplished using chemical treatment and/or separation to isolate microorganisms from potential interference. These methods also ensure the necessary quantitativeness in ATP recovery, conversion to known levels of ATP concentration, and sufficient sensitivity to meet the needs of drinking water samples.

8.3.1 Overcoming interferences

Second Generation ATP testing methods were originally designed for use in biological wastewater treatment plants. To provide accurate test results in this situation, significant interferences needed to be overcome which were considerably more challenging than what is typically encountered in most other situations where microbiological quantification is carried out. Therefore, the robustness of the reagents developed for wastewater analyses was more than sufficient for application to less contaminated sample types such as potable water, cooling water, oilfield water (e.g. produced water, fracturing fluids, etc.), industrial process streams, petroleum products, and chemical products (e.g. paints, coatings, adhesives, admixtures, emulsions, etc.). The focus was then shifted to establish protocols that provide enhanced sensitivity for applications that deal with smaller levels of bioburden.

The optimum test protocol choice is driven by the properties of the sample to be tested. These tests can be divided into two main classes:

(1) *Filtration-based:* The sensitivity of ATP test protocols can be significantly improved by concentrating microbiological cells on a filter sufficient to capture intact microbiological cells (e.g. 0.2 μm) and directly extracting the ATP from the captured cells. This allows ATP tests to be performed in several “clean” applications for which first generation ATP products are not applicable such as drinking water and purified water.

(2) *Dilution-based:* Many industrial samples such as wastewater or industrial process streams cannot be filtered due to high amounts of suspended solids or high viscosities. The sample is therefore added directly to extraction reagent to release ATP from living cells and stabilize extracellular ATP. Extracellular ATP can be measured separately through an additional dilution-based test that does not harm intact cells; rather, it releases bound extracellular ATP and stabilizes soluble ATP prior to measurement (Cairns *et al.*, 2005).

8.3.2 Extraction and recovery

To get an accurate ATP reading, it is critical that ATP from all cells in a sample is recovered. It is especially difficult to extract ATP from biofilm clumps, microbiological floc, yeasts and moulds. As such, having a weak extraction reagent can lead to incomplete cell lysis and thus only partial ATP recovery yielding lower than actual results. Therefore, it is important to ensure that the extraction reagent used is strong enough to completely lyse all microorganisms present in a sample for maximum ATP recovery, while not being so strong that it inhibits the luciferase enzyme.

Additionally, the high-energy bonds in an ATP molecule make it an unstable molecule and when not stabilized, ATP readily hydrolyses to ADP and phosphate. Most extra-cellular ATP is complexed to various molecules and cell debris and is therefore unavailable to react with the luciferase enzyme. When intra-cellular ATP is extracted, it can readily complex or hydrolyse, making it too unavailable to react with the luciferase enzyme. If left unbuffered, the instability of an ATP extract can lead to lower than actual results since most ATP would have either degraded or complexed before the luciferase assay is performed. It is therefore important to ensure that all complexed extra-cellular ATP and all extracted intra-cellular ATP is stabilized for maximum recovery.

8.3.3 Importance of calibration

As mentioned previously, while many First Generation ATP tests rely on RLU values alone, a low result from an ATP test can be due to low levels of contamination or because the enzyme has lost a significant amount of its activity. Without a way to gauge the activity of the enzyme, it is impossible to tell for what reason a low RLU is observed. Other factors such as the temperature of the reagents, samples, or the surrounding environment as well as the make, model, and condition of the luminometer used can significantly affect the RLU produced. As such, an RLU value on its own is essentially an uncalibrated result.

For this reason, sample RLU's should be converted to ATP concentrations by standardizing the result to a known amount of ATP. The RLU produced by a standard ATP solution can normalize the sample RLU to determine the amount of ATP in the sample. This also provides a direct measure of enzyme activity and thus the ability to determine the lower detection limit and enzyme reagent shelf life. If the sample volume and dilution factor for a given kit are fixed, the sensitivity of the

ATP assay is solely decided by the activity level of the enzyme formulation used. Fresh reagent will produce more light than an older batch when used to analyse the same sample, and for this reason, samples that may have been below the detection limit when using an older batch become within reach of quantification.

8.3.4 Limit of detection

One of the biggest benefits of Second Generation ATP monitoring is the enhanced sensitivity that is made possible through optimized reagents and test protocols. In general, Equation (2) indicates how the ATP concentration is calculated for all test protocols:

$$[\mathrm{ATP}] = \left(\frac{\mathrm{RLU}_{\mathrm{sample}}}{\mathrm{RLU}_{\mathrm{ATP\ Standard}}}\right) \times \left(\frac{\text{Dilution Factor} \times \text{ATP Standard Conc.}}{\text{Sample Volume}}\right) \qquad (2)$$

Aside from the RLU value produced by the sample assay, modifying any of the other three variables in the above equation will affect the dilution factor of the test. The practical limit of detection of the ATP assay is around 0.1 pg ATP/mL, though higher sensitivity may be achieved through filtration of large sample volumes and special care in reagent and test component decontamination.

While high-solids samples such as wastewater must be analysed using a dilution-based method, sample types that can be filtered allow the user to process larger sample volumes, and in doing so, significantly lower the detection limit of the test. When dealing with samples containing very low microbiological concentrations, it is important to keep in mind that sampling error becomes a bigger issue. It also becomes more important to perform replicate analyses to ensure that the results are accurate.

8.4 METHODS FOR DETECTION OF TOTAL MICROORGANISMS

There are several methods presently used to quantify microorganisms in drinking water processes (Ashbolt *et al.*, 2001). Table 8.1 compares each method according to four categories for measuring total microorganisms:

(1) Applicability (i.e. the specificity with which the quantity of total microorganisms is measured);
(2) Speed (i.e. the speed with which the result is obtained);
(3) Degree of Difficulty (i.e. the relative expertise required to complete an analysis);
(4) Relative Cost (i.e. the capital investment required to obtain equipment and recurring consumables required to complete the analysis).

As observed in Table 8.1, successful ATP measurements provide relevant results in a short period of time and at a moderate cost. For quantifying the total

microbiological activity in a water sample, ATP provides the greatest cost and labour efficiency, and has the additional benefit of being portable.

Table 8.1 Comparison of microbiological quantification methods.

Method	Measures	Applicability	Speed	Degree of Difficulty	Relative Cost
2nd Generation ATP	Total Active Organisms	High	Fast	Low	Moderate
Culture (i.e. Plate Counts)	Specific Viable Organisms	Low	Slow	Moderate	Low
DNA methods	Specific Organisms	High to Low*	Moderate	High	High
Microscopic observation	Physical Cell Count	High	Moderate	High	High

*Depends on primer selection.

8.4.1 Why measure total microorganisms?

The most widely accepted method for quantifying microorganisms in water systems is the culture test. Of these analyses, the Heterotrophic Plate Count (HPC) is most commonly associated with indication of total microorganisms. This analysis involves addition of a sample to a culture medium that is subsequently incubated until sufficient growth is apparent via visual inspection. Colonies are then physically counted and the concentration of microorganisms in the sample is estimated. More user-friendly adaptations of these analyses have been developed and widely utilized in industry, including the dip slide (Vanderzwaag *et al.*, 2009).

The most widely recognized deficiency of culture tests is slow feedback. Days or even weeks can be required to obtain results due to slow growth rates of certain species. A more recently recognized drawback is that it is impossible to quantify total microorganisms without performing hundreds or thousands of culture tests on a given sample (Laupland & Valiquette, 2013). As such, many researchers view ATP technology as a potential rapid estimator of microorganisms, much in the same way as turbidity is used throughout industry to rapidly estimate total suspended solids. Although ATP test results will strongly correlate with culture test results under most conditions, there are several factors that affect this correlation (Sloan *et al.*, 2008).

8.4.2 Population specificity

ATP tests effectively provide a superior indication of the total amount of microbiological content compared to culture tests since all living cells contain ATP. As such, all living microorganisms in a sample will contribute to the ATP

measurement. Conversely, a heterotrophic plate count only recovers a small portion of metabolically active organisms and results will vary a great deal according to the method used. Results have indicated that in drinking water systems, only 0.1–1% of the total microbiological population is detected by HPCs. In fact, when considering all known species of microorganisms, it is estimated that only 0.01% of waterborne microorganisms are heterotrophic bacteria (Bartram *et al.*, 2003).

8.4.3 Particle association and agglomeration

Culture tests tend to underestimate the number of microorganisms because a clump of many organisms produces only one countable colony (Todar, 2008). As with other biochemical and molecular tests, ATP measurements will count all the organisms in a clump separately. It is worth noting however that as with all cellular components, the quantity of ATP will change depending on the size and metabolic state of the cell. All these factors must be taken into consideration by the operator when using and comparing microbiological tests in different circumstances.

8.4.4 Disinfection efficacy

Exclusive use of culture tests can pose disadvantages in the context of disinfection monitoring. In addition to slow feedback, culture tests provide no information about the effectiveness of the biocide treatment on organisms that they do not measure. Furthermore, they can be misleading if a biocide fails to penetrate a clump of microorganisms or, alternatively, disperses the clump. DNA-based methods also struggle to provide relevant information of disinfection efficacy, primarily since there are as of yet no universally accepted mechanisms to distinguish live from dead DNA when using genomic methods (Cangleosi & Meschke, 2014).

Conversely, ATP measurements alone can underestimate the efficiency of a biocide kill since they will detect ATP from cells that are still alive but are rendered unable to reproduce. However, they will quantify the effect of the biocide on the entire population, meaning that it provides a more complete indication of true kill efficacy than do culture tests (Corrin *et al.*, 2009). As such, it would be most effective to use ATP together with conventional culture-based methods, especially when the culture tests used are for nuisance organisms or specific pathogens (e.g. *E. coli* in drinking water).

ATP measurements will therefore only correlate strongly with plate counts if several conditions are met. Many attempts in the past at establishing a correlation between culture-based analyses and ATP analyses have not been routinely successful. Although correlations are typically not as strong as researchers hope, they attribute this to some shortcomings in the plate count methodology since many species will not be detected nor will cells that have been injured to the point where they are incapable of growing in the time permitted (Stopa & Orahovec, 2002).

The interest in conducting comparison studies between culture tests and ATP results is understandable, as culture-based techniques have served as the benchmark

to assess the degree of microbiological contamination and to assess disinfectant performance for many years (Bartram *et al*., 2003). Although plate counts have been the standard to assess the amount of microbiological contamination at a site, any lack of correlation with ATP is not a suitable basis to reject the use of either method. Rather than attempting to completely replace plate counts with ATP tests, ATP monitoring can serve as a screening and routine monitoring tool for detecting the total quantity of active microorganisms where the total population is measured with a routine frequency. Culture-based tests can then be used to troubleshoot revealed issues that become evident through rapid screening.

8.5 CASE STUDIES

8.5.1 Direct comparison of second generation ATP to culture-based methods

One of the reasons why comparisons between ATP and HPC methods have not always shown a strong correlation is because culturing methods tend to vary significantly based on the culture media used. To investigate this, an independent laboratory performed a study using two different types of HPC media alongside the Quench-Gone Aqueous (QGA, LuminUltra Technologies Ltd., Fredericton, NB, Canada) test protocol to examine the difference between the methods for 12 unique sample types including clean samples (e.g. ultra-purified water), dirty samples (e.g. untreated surface water), and a selection of others of varying cleanliness with each test done in duplicate. One low-nutrient media (TGYA) and one high-nutrient media (NWRI) were selected as comparative culture tests. The variation of media was a necessary component of the experiment due to their tendency to encourage the growth of different species of microorganism even though both media are intended to provide a measure of the total microbiological population. The correlations of each method to each other are shown in Table 8.2.

Table 8.2 Correlation (Pearson R^2) between microbiological enumeration methods (L'Abbee & Ritchie, 2008).

Method	Quench-Gone Aqueous	High-Nutrient HPC NWRI	Low-Nutrient HPC TGYA
Quench-Gone Aqueous	1.00	0.88	0.78
High-nutrient HPC (NWRI)	0.88	1.00	0.79
Low-nutrient HPC (TGYA)	0.78	0.79	1.00

The results shown in Table 8.2 indicate a strongly positive correlation between ATP tests and the culture methods chosen. While the R^2 values are strong, the reason that they are not higher lies with the fundamental differences between the methods as it pertains to the fact that ATP measurements detect all species

under all different metabolic states as opposed to those that are detected through conventional culturing techniques under very specific conditions.

In addition to comparison tests on discrete samples, analyses using the aforementioned methods were also done before and after chlorination of a selection of samples from the previous sample set, as shown below in Table 8.3.

In addition to illustrating how Second Generation ATP monitoring can provide a good early indication of disinfection efficacy on the entire population; this experiment shows the significant difference that can be encountered when utilizing different HPC media in terms of the magnitude of the microbiological population measured. This comparison highlights the reliance of different culture media on detection of cultivable organisms, which typically represent a small proportion of total microorganisms. Conversely, ATP results revealed the presence of microbiological activity, albeit in small quantities, in several cases where the HPC methods did not. The results of ATP tests were above what would be considered "noise" and therefore indicates the presence of non-culturable species or unhealthy cells which could not be grown in the media, time, or incubation conditions chosen.

The laboratory performing this experiment deemed it to be successful in demonstrating that, while the media chosen significantly affects the outcome of the comparison, HPC and ATP tests generally correlate quite well under the proper circumstances. They stated that method selection should be consistent with the goal of the testing program. That is, if measuring for specific organisms to meet regulatory requirements is the goal of a test, culture-based analyses are required. However, ATP tests may be used as a suitable alternative to HPC tests with certain benefits for operational purposes including total detection and near-real-time feedback.

It is important to point out, though, that while this study showed good agreement between the ATP and HPC tests, this correlation tends to become weak in situations where non-culturable species (i.e. nitrifiers, sulphate-reducers, iron bacteria, etc.) are present. Regrowth within distribution systems due to nitrification is a prime example of when ATP monitoring provides the extra insight required to quickly and properly diagnose problems that conventional methods such as HPC tests would otherwise miss.

8.5.2 Using second generation ATP testing to optimize biologically active filters

The concept of biological filtration has become a common means to remove organics prior to disinfection to minimize formation of disinfection by-products. However, the tools available to assess biological growth tendencies in biological filters are limited. Heterotrophic Plate Counts and other culture-based methods take days to obtain results, only measure very small portions of the true microbiological population, and struggle with the ability of quantifying cells attached to media. By contrast, the ATP measurement has been cited as one of the most reliable tool for monitoring of biological filtration processes (Evans *et al.*, 2013).

Table 8.3 Method comparison in response to shock chlorination (L'Abbee & Ritchie, 2008).

Source	Chlorine Added (mg/L)	Cellular ATP (pg cATP/mL)			HPC – NWRI (CFU/mL)			HPC – TGYA (CFU/mL)		
		Before Chlorination	After Chlorination	% Reduction of ATP	Before Chlorination	After Chlorination	% Reduction of CFU	Before Chlorination	After Chlorination	% Reduction of CFU
River water	10	94.5	0.902	99%	2930	1	100%	1650	3	99.8%
Ground water	10	113	ND	100%	39.0	ND	100%	779	ND	100%
Potable water	10	0.116	ND	100%	ND	ND	100%	3.00	ND	100%
Potable water storage tank	10	1.83	ND	100%	873	ND	100%	778	ND	100%
Reverse osmosis permeate	10	0.223	ND	100%	ND	ND	—	ND	ND	—

ND = not detectable.

The following process monitoring and control opportunities are made possible via analysis of ATP results (LuminUltra, 2017a):

- Assess filter capacity in terms of biomass population size as it relates to organics removal.
- Quantify the biological population at the beginning and end of filter runs to determine the magnitude of growth that occurred during the cycle.
- Investigate the amount of biomass purged from the filter during backwash cycles. Once a baseline has been established for normal operation at the beginning and end of filter runs, targets can be established to optimize the duration of backwash cycles to minimize downtime while avoiding the release of too much or too little biomass.

The above example shown in Figure 8.1 illustrates the following findings via ATP results (as determined using the Deposit & Surface Analysis (DSA) test kit from LuminUltra Technologies):

- Enhanced biological growth capacity is seen in granular activated carbon compared to anthracite.
- The greatest concentration of biomass is found at the top of the filter where there is an abundance of food and nutrients.
- Biomass concentrations decrease proportionally according to bed depth.

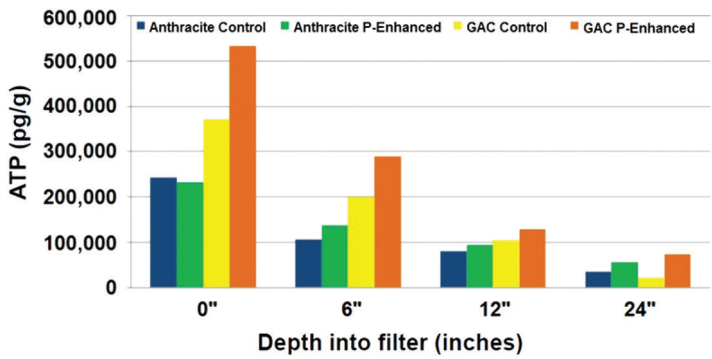

Figure 8.1 Comparison of biomass response to process modifications (Stoddart, 2017).

8.5.3 The Membrane Biofouling Index

Membrane filtration units are widely used in the water treatment industry for applications ranging from groundwater treatment and desalination to industrial water treatment and wastewater processes. While they can produce extremely high-quality water, they require a significant investment to acquire and maintain

therefore it is important to protect the membranes from excessive wear to maximize the lifespan of the modules (LuminUltra, 2017c).

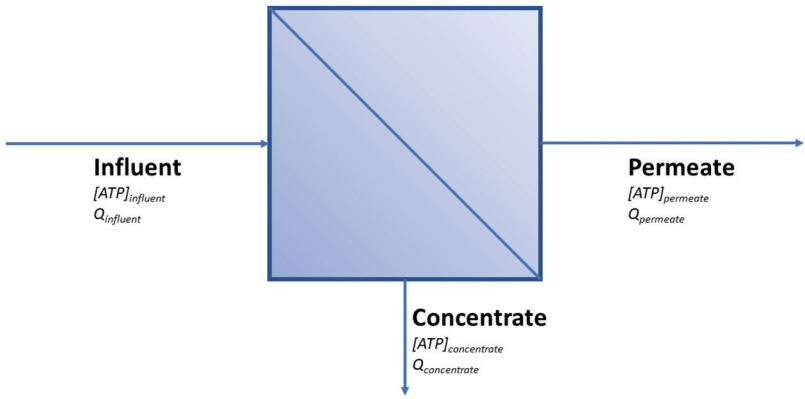

Figure 8.2 A typical 3-flow membrane.

Due to the porous nature of the membranes, they are susceptible to both chemical scaling and biological fouling which can increase energy requirements and operating costs and shorten their useful lifespan. Treatments differ depending on the nature of the fouling and each cleaning cycle imposes extra stress on the membranes, so cleaning chemicals should be targeted to the specific type of fouling that is present and cleaning frequency should be optimized.

Through Second Generation ATP testing, influent water quality can be monitored; permeate water quality can be assessed to reveal contamination and microbiological breakthrough; and membrane fouling can be assessed through a mass balance of total biomass across the membrane (Figure 8.2) according to Equation (3):

$$\text{Membrane Biofouling Index (MBI)} = [(Q_{\text{permeate}} \times \text{ATP}_{\text{permeate}}) + (Q_{\text{concentrate}} \times \text{ATP}_{\text{concentrate}})]/[(Q_{\text{influent}} \times \text{ATP}_{\text{influent}})] \quad (3)$$

By monitoring membrane fouling on a regular basis, the appropriate treatment can be applied, and cleaning cycles can be optimized to reduce excess wear on the membranes (Travis & Tracey, 2015).

8.5.4 Using second generation ATP testing to address biological hotspots

A Southern US municipality experiencing taste and odour issues in a certain neighbourhood was also having difficulty maintaining chlorine residual levels in the area. Biological growth was suspected; however, water leaving the treatment

plant met and exceeded all water quality requirements. After several investigations, the source of contamination in the distribution system could still not be identified.

The utility was successfully using Second Generation ATP testing in their water treatment plant to monitor biological fouling in the membrane filtration unit. Since they were already equipped to perform water analyses, it was decided that the kit would be taken into the field to audit the distribution system and determine whether the cause of the taste and odour complaints was biological contamination (LuminUltra, 2017b).

Testing began at the water treatment plant where it was confirmed that biological content was very low (<1pg ATP/mL) and therefore well within the acceptable range. Operators then travelled to the affected neighbourhood and began to audit the system, tracing back to the water treatment plant (Figure 8.3).

The results confirmed that biological growth was significantly higher in the affected neighbourhood with ATP levels in the high-risk range of >10pg ATP/mL. The line was traced back toward the treatment plant and water samples were tested at several major junction nodes. ATP results remained high near the neighbourhood until suddenly, between sample point B and A, there was a significant drop in ATP levels back into the good control range of <1pg ATP/mL (Figure 8.4).

Figure 8.3 Map of distribution system.

Upon further investigation, operators discovered a leaking valve that was not identified on the design drawings. Significant biofilm growth had built up on the valve and was entering the distribution system causing the disinfectant residual loss and water quality issues for consumers. Operators repaired the valve immediately and flushed the lines. Residual chlorine levels were quickly restored, and follow-up ATP testing confirmed that biological growth in the system had dropped back into the good control range.

Having a field-ready rapid microbiological test allowed water distribution system operators to quickly audit their system and pinpoint the source of water quality issues. Routine distribution system monitoring was implemented to proactively

monitor for biological regrowth and prevent similar water quality issues from affecting consumers in the future.

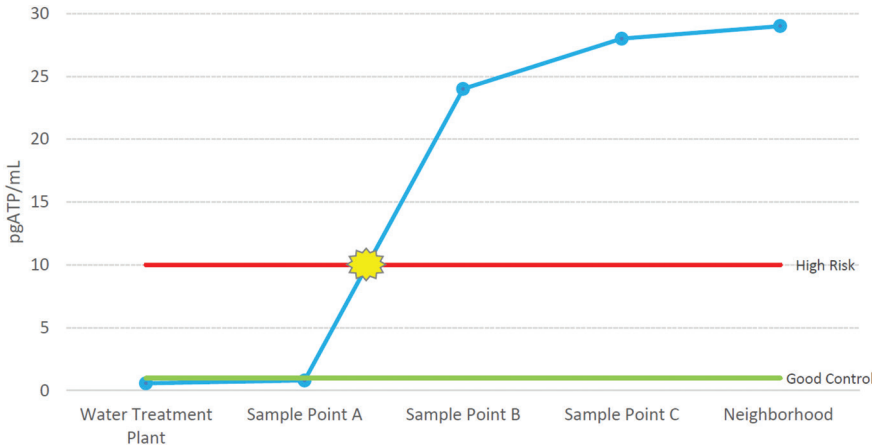

Figure 8.4 Results across distribution system.

8.5.5 Audit of public building water distribution system

A French spa utilized untreated mineral water (from ground wells) in its daily operation (LuminUltra, 2017d). The source water is distributed to 40+ stations for therapeutic purposes. One week prior to the site audit, two stations had tested positive for pathogenic organisms in the form of *Pseudomonas*. Second Generation ATP testing was utilized to locate the source and nature of the contamination using a HACCP-style audit, on the fresh water and various water distribution branches. Samples were taken from the cold and hot water of each bank (Figure 8.5).

Once testing was complete at each location in the network, the results immediately indicated that the feed water was not the source of contamination. Further inspection showed that elevated microbiological content originates in branch #2L and carries over to branch #3 in the hot water system only (Figure 8.6). Therefore, rather than disinfecting the entire water network, additional treatment was necessary in the hot water lines of Bank #2L and #3 to eliminate the biological contamination. This remediation was undertaken and in a matter of hours it was confirmed that clean-up had been achieved.

The results obtained clearly indicated the source (microbiological build-up, or biofilm) and nature (localized) of the water contamination in less than two hours. Using this methodology, any operator of any water system can detect contamination in the same manner and act to correct the problem on the same day. Routine testing of critical control points (for example, feed water, Bank #2L) facilitates preventative maintenance to maintain compliance.

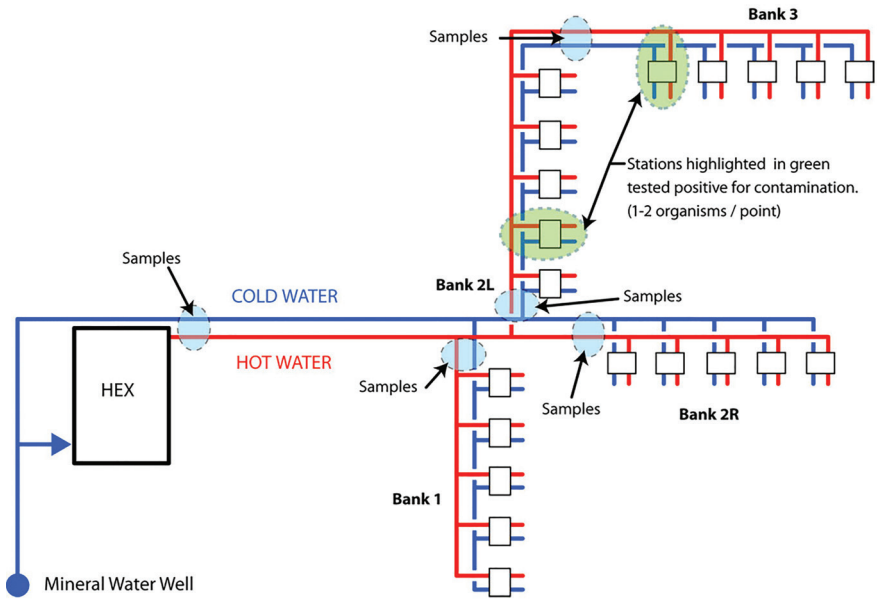

Figure 8.5 Location of sampling & contamination in water system (LuminUltra, 2017d).

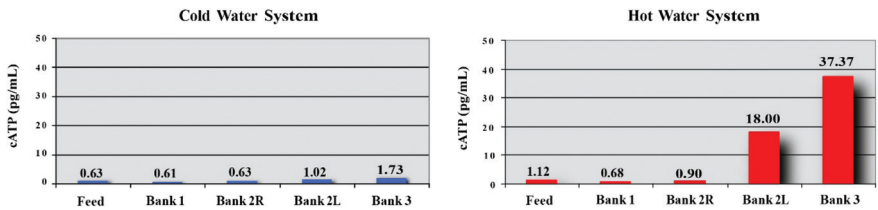

Figure 8.6 Audit results for water system (LuminUltra, 2017d).

8.6 CONCLUSIONS

Because of its speed, ease-of-use, and specificity to total living organisms, ATP monitoring serves as a valuable compliment to conventional water quality tests. The advancements made as part of Second Generation ATP technology enable rapid and accurate measurements in a significantly wider range of applications than what ATP tests have traditionally been applied to, both in terms of challenging sample matrices and sensitivity requirements. When dealing with potable water systems, it not only facilitates routine maintenance and troubleshooting but also helps maintain water quality by detecting microbiological contamination at the earliest signs so that they can be dealt with as quickly as possible. Similar concepts can

be extended to other microbiological growth control applications such as product quality control, oil and gas, fuel systems, and manufacturing to immediately assess the level of microbiological contamination, determine if action is necessary, and assess the efficacy of mitigation activities in near-real-time.

8.7 REFERENCES

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