ENHANCED AND ACCELERATED BIOLOGICAL MONITORING FOR MEMBRANE TREATMENT OPTIMIZATION: MEASURING THE BENEFITS OF AN OPTIMIZED MONITORING PROGRAM

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ABSTRACT

The groundwater that St. John The Baptist Parish in southern Louisiana supplies to residents has traditionally carried a high amount of organic material and color. In the past, organics were oxidized and broken down by chlorination before discharging to water customers but this practice had gone out of favor due to production of disinfection by-products (DBPs) such as Trihalomethanes (THMs) and Haloacidic Acids (HAAs). The utility therefore sought to remove pre-chlorination and operate a membrane filtration process for water treatment instead. However, it was observed that complete removal of pre-oxidation resulted in severe membrane fouling and eventual failure. It became clear that enhanced monitoring capabilities were needed to optimize the pre-oxidation and pre-filtration prior to the membrane banks to avoid fouling as well as disinfection by-product formation.

In this study, it was shown that proactive biological monitoring using advanced ATP (Adenosine Triphosphate) was able to guide mitigation activities and optimize several design modifications to provide improved plant operation and product water quality. ATP is the primary energy transfer molecule for all living cells on Earth and therefore its measurement is a directly tied to the microbial population. In this situation, ATP monitoring quickly identified elevated microbial content not only in the raw water, permeate, and reject, but also within the membranes themselves. This enabled personnel to assess the effects of decreased pre-chlorination, diagnose the fouling issue as a biological problem, and optimize of the membrane cleaning process – all within minutes of sample collection.

One year later, the design changes that were implemented based on this enhanced monitoring scheme have proven to be very effective. This included re-piping the reverse osmosis skids to operate in a 6 x 1 (six units in parallel) configuration rather than 3 x 2 (three primary and two secondary RO units). The results of this modification included: far lower microbial content (as indicated by ATP monitoring) in the permeate streams, enhanced biostability resulting in

the ability to maintain chlorine residual for a longer period, and thousands of dollars per month in electricity savings due to reduced fouling and the head loss that occurs across fouled membranes.

Real-time assessment of membrane fouling is achieved through conducting a mass balance around each membrane. This is done by comparing the bioburden in the membrane to sum of permeate and reject bioburden levels. Should the bioburden out be greater than that which is entering, it immediately indicates that growth is occurring within the membrane, thereby initiating preventative maintenance. This operational strategy maximizes membrane life while optimizing produced water quality and overall process efficiency.

INTRODUCTION

Adenosine Triphosphate (ATP) – The Key to Life

ATP is the keystone of metabolic activity (Trefil, 1992). Most of the energy for microbial processes is stored and transmitted via ATP. ATP is produced as microbial food is consumed and is subsequently utilized for cell maintenance as well as the synthesis of new cells and biochemicals.

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 'intra-cellular' 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). In addition, when cells become weak 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 is indicative of a less healthy population (Maier et al, 2009).

BACKGROUND

Measuring Adenosine Triphosphate

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. 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 not unlike a spectrophotometer; however luminometers are generally much more sensitive and do not contain a light 'source'. Rather, a luminescent reaction 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, 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 than photodiodes provide (Berthold, 2000). Luminometers are also available in both single-chamber format as well as 96-well and even 384-well plate instruments (usually intended for research applications rather than practical field testing), which can sequentially perform a large number of assays in an automated fashion.

Methods for Detection of Total Microorganisms

There are several methods presently used to quantify microorganisms in water. Table 1 compares each method according to four categories:

- Applicability (i.e. the specificity with which the quantity of total microorganisms is measured);

- Speed (i.e. the speed with which the result is obtained);
- Degree of Difficulty (i.e. the relative expertise required to complete an analysis);
- Relative Cost (i.e. the capital investment required to obtain equipment and recurring consumables required to complete the analysis).

Table 1: Comparison of Microbial Quantification Methods

Method	Measures	Applicability	Speed	Degree of Difficulty	Relative Cost
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.

As can be seen in Table 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 benefit of being portable.

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 Dipslide and Biological Activity Reaction Test (BART).

The most widely accepted 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. 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 a number of factors that affect this correlation, including:

1. Population Specificity
   ATP tests provide an indication of the total amount of microbial content, while a culture test does not. Because all living cells contain ATP, 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 microbial population is detected by HPCs. In fact, when considering all known species of microbe, only 0.01% of waterborne microorganisms are considered to be heterotrophic bacteria (Bartram et al, 2003).

2. Particle Association
   Culture tests tend to underestimate the number of microorganisms because a clump of many organisms produces only one countable colony (Todar, 2008). ATP measurements will count all of the organisms in a clump or filament separately.

3. 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. 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. 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 a number of factors add up. 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 and 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 microbial contamination and to assess disinfectant performance for many years. Although plate counts have been the standard to assess the amount microbial 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.

CASE STUDY: MEMBRANE TREATMENT OF ORGANICS-LADEN GROUNDWATER

The groundwater that a southern Louisiana water utility supplies to local residents has traditionally carried a high amount of organic material and color. In the past, the organics were oxidized and broken down by chlorination, but this practice had gone out of favor due to production of disinfection by-products (DBPs) such as Trihalomethanes (THMs) and Haloacidic Acids (HAAs).

The utility therefore decided to construct a water purification plant to remove organics and microbial content rather than rely on pre-chlorination at the storage tank. A process flow diagram of the plant is shown in Figure 1:

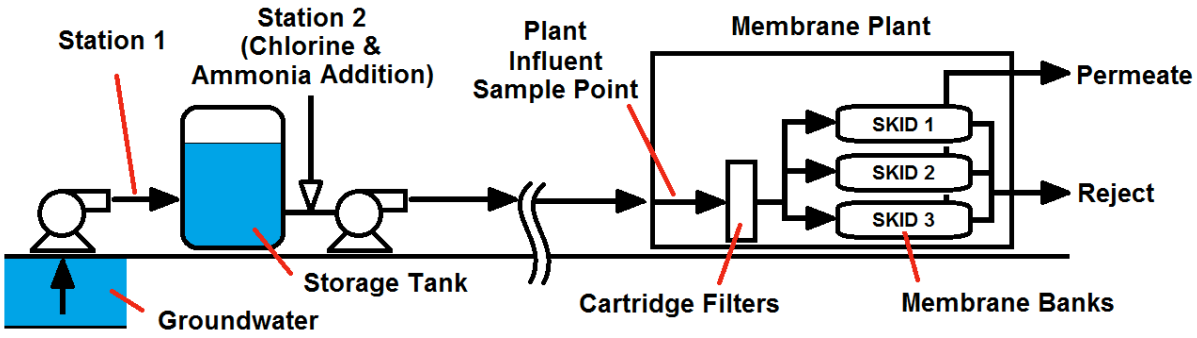


Figure 1: Groundwater Treatment PFD

While the plant performed well initially, significant problems developed once pre-chlorination was shut off. The pre-filters and membranes became severely fouled with a thick film and had considerable odor. To troubleshoot this problem, the utility enlisted the services of Thornton, Musso, & Bellemin, a local water and wastewater service company. TMB uses LuminUltra's 2nd Generation ATP monitoring technology as their primary tool for rapid assessment of microbiological content in municipal water systems and aimed to use it in this situation to characterize the nature of the treatment problems. If the issue was found to be biological, ATP tests would be used to help monitor the efficacy of both disinfection and membrane treatment throughout this optimization study.

Results

The pre-chlorination dosage was set to a reduced rate compared to the previous dosage during the mid-summer months in hopes of establishing a middle ground where DBP formation was reduced while still cutting down the organics loading and color in the raw water. After several days, the following results in Table 2 were seen compared to before the change in chlorine dosing:

Table 2: Effects of Pre-Chlorination on Microbial Loading (< 10pg/mL = Acceptable, > 10pg/mL = Contaminated)

Sample Point	[ATP] (pg/mL)	
	Historical Pre-Chlorination Strategy (2ppm Total, 0.15ppm Free)	Without Pre-Chlorination (< 1ppm Total, 0ppm Free)
Station 1 Effluent	6.3	2.8
Plant Influent	5.3	33
Skid 1 Permeate	3.2	-
Skid 2 Permeate	2.8	-
Skid 3 Permeate	6.9	8.3
Skid 3 Reject	-	210

While the reduced chlorination prior to membrane filtration resulted in less DBP formation than in the past, the microbial loading at the plant inlet became significantly higher. The treated water quality also suffered, as was seen from the Skid 3 Permeate test. Following this, plant personnel decided to allow the experiment to continue for the time being since permeate quality was still deemed to be acceptable.

After an additional week under these conditions, though, the treatment plant was shut down due to excessively high pressure differentials across the membranes. The ATP results at the time of shut-down were as follows in Table 3:

Table 3: Effects of Long-term Removal of Pre-Chlorination

Sample Point	[ATP] (pg/mL)
Station 1 Effluent	2.4
Plant Influent	87
Skid 1 Permeate	17
Skid 1 Reject	280
Skid 2 Permeate	620
Skid 2 Reject	790
Skid 3 Permeate	680
Skid 3 Reject	240

The effects of reduced pre-chlorination are clearly seen here by the significant increase in microbial loading to the plant between the Station 1 Effluent and the Plant Influent. At the time of this measurement, the raw water carried no chlorine residual (neither free nor total) so there were no barriers to the proliferation that occurred in the pipeline. This increased loading resulted in significant fouling of the pre-filters and eventual microbial breakthrough and significant fouling of the membranes downstream. Membrane fouling was confirmed by testing the surfaces of the end cap of a membrane in addition to a deposit that was removed from the membrane surface as shown in Table 4:

Table 4: Results of Membrane Biofilm Analyses

Sample Point	[ATP] (pg/in^2)
Deposit from membrane surface	5380
End cap sample	1260

These values for surface buildup were 10-100 times higher than what would be considered tolerable, although they were not surprising considering the magnitude of the bioburden in the water feeding the membranes. Upon seeing this, a series of membrane cleanings took place to remove the fouling that had developed. Table 5 illustrates the efficacy of this cleaning:

Table 5: Effects of Membrane Cleaning

Sample Point	[ATP] (pg/mL)
Skid 1 Permeate (1^{st} stage)	0.25
Skid 1 Permeate (2^{nd} stage)	0.55
Skid 1 Combined permeate	0.41

According to these results, it is clear that the cleaning cycle did an excellent job to clean the fouled membranes. The product water quality is now in the acceptable range (< 10pg/mL) and therefore carries a lower risk for microbial proliferation downstream assuming that an acceptable disinfectant residual is maintained. It was now clear that a certain degree of disinfection was necessary to minimize fouling of the pre-filters and membranes in the plant, so after the cleaning process, the following changes were instituted to the operating procedure:

1. Chlorine was discontinued prior to the storage tanks.
2. Chlorine and Ammonia (Chloramine) was instead fed to the water as it left Station 1 to inhibit microbiological growth in the transmission line from Station 1 to the Plant Influent.
3. De-chlorination was moved from before the prefilters to behind them.

After three weeks of running under these new conditions, another set of samples collected and analyzed with the results shown in Table 6:

Table 6: Baseline Data Following New Operating Conditions

Sample Point	[ATP] (pg/mL)
Station 1 Effluent	5.71
Plant Influent	6.29
Prefilter 1 Outlet	4.17
Prefilter 2 Outlet	6.40
Prefilter 3 Outlet	5.32
Skid 1 Permeate	10.5
Skid 1 Reject	3.90
Skid 2 Permeate	1.80
Skid 2 Reject	4.77
Skid 3 Permeate	2.12
Skid 3 Reject	6.29
Blended Permeate	4.86

While the overall cleanliness of the product water has risen slightly, the overall picture is significantly better than when the membranes had become fouled earlier in the summer. The plant's strategy was then to perform semi-routine tests on the water downstream of the pre-filters as well as the combined membrane product water to detect deviations from baseline conditions to take a more pro-active stance. The following graph shows a summary of results from the beginning compared to the optimized conditions (expressed as Log(pg ATP/mL)):

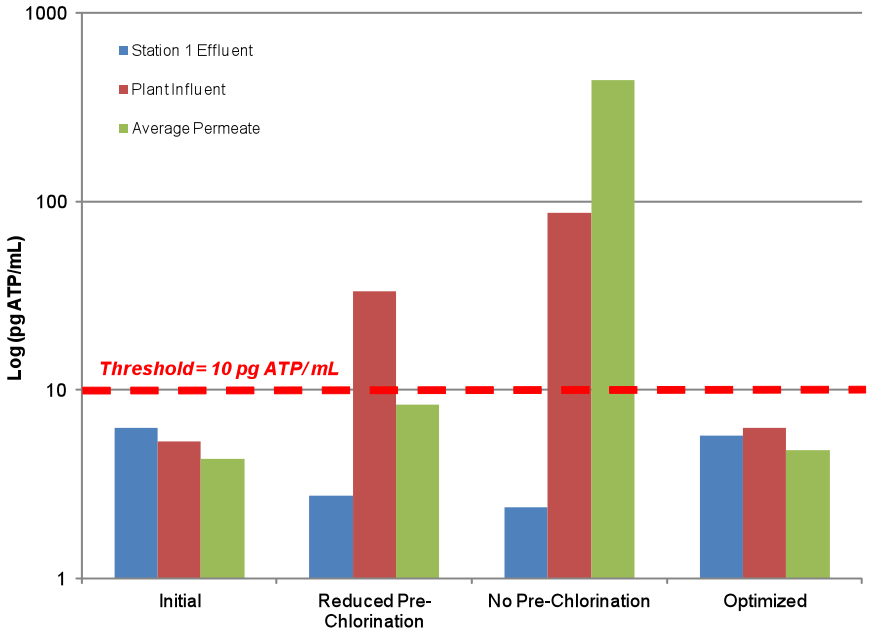


Figure 2: Summary of Results Under Each Control Scheme

Several months later, the site was revisited to verify that the operating conditions that were established at the conclusion of this study were still effective. A summary of these findings compared to previous levels are shown in Table 7:

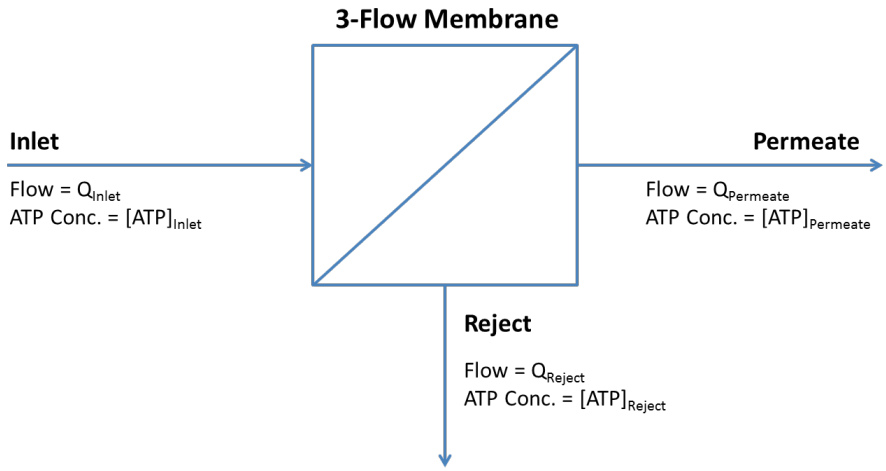
Table 7: Long-term summary

Sample Point	Historical Pre-Chlorination Strategy (2ppm Total, 0.15ppm Free)	Without Pre-Chlorination (< 1ppm Total, 0ppm Free)	Optimized Configuration with Monochloramine
Station 1 Effluent	6.3	2.8	2.3
Plant Influent	5.3	33	2.1
Skid 1 Permeate	3.2	-	1.6
Skid 2 Permeate	2.8	-	2.0
Skid 3 Permeate	6.9	8.3	2.5
Skid 3 Reject	-	205	10

The results shown in table 7 clearly indicate that the treatment strategy and configuration established at the conclusion of the initial investigation have been sustainable. In addition to the good quality of the treated water, the loading on each unit operation is minimal compared to previous operating conditions.

UPDATE: Design Modifications and Resulting Membrane Plant Performance

Following the modifications made in the spring of 2012, the plant continued to run much more effectively than under previous conditions but other opportunities for improvement became apparent. This was determined by establishing a monitoring strategy involving a mass balance using flow rates and ATP concentrations around each membrane. Essentially, ATP load in must be approximately equal to that which exits. If more ATP exits than what enters, it is indicative of a fouled membrane in which biological growth and breakthrough occurs. This concept is illustrated below in Figure 3:



Mass Balance: $(Q_{Inlet} \times [ATP]_{Inlet}) = (Q_{Permeate} \times [ATP]_{Permeate}) + (Q_{Reject} \times [ATP]_{Reject})$

Fouling Index: $FI = ((Q_{Permeate} \times [ATP]_{Permeate}) + (Q_{Reject} \times [ATP]_{Reject})) / (Q_{Inlet} \times [ATP]_{Inlet})$

If FI ≤ 1, membrane is not bio-fouled.
*If 1 < FI ≤ **X**, membrane is becoming biofouled.*
*If **X** ≤ FI, membrane is heavily biofouled.*

Figure 3: Membrane Mass Balance Concept Overview

The data collected to date was loaded into this model to assess the degree of fouling around the membranes historically compared to following the movement of disinfectant point and switch to Monochloramine. Results are shown in Figure 4:

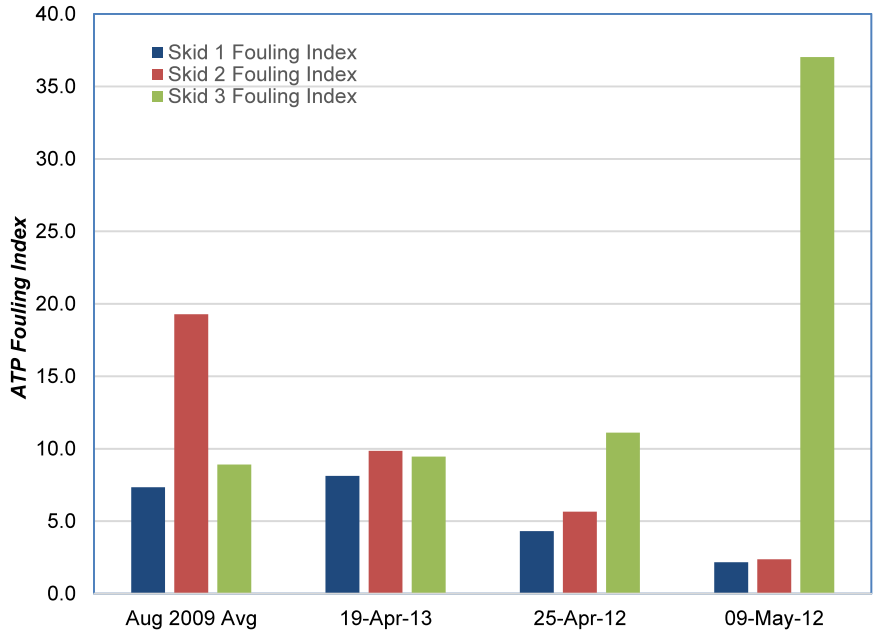


Figure 4: ATP Fouling Index on membranes throughout initial observation period

The "X" term listed in the biofouling assessment table can be considered to be a baseline measurement for a given site. That is, a membrane could operate effectively while maintaining a small degree of fouling. In the case above, a fouling index of 5 could be considered the point at which fouling becomes accelerated.

While membrane performance clearly improved and fouling reduced due to the initial design and operational modifications, fouling was clearly not completely eliminated as was evident from the results from Skid 3. This initiated discussion involving the loading applied on the membranes and whether it was too much for the three primary membranes to handle.

With this in mind, it was decided that rather than having three skids of two membrane in parallel, they should be operated as six parallel membranes all operating as single-stage units. This change was implemented in the fall of 2012, and after a routine cleaning, the membranes were operated in this arrangements as a long-term solution. The results of this are shown in Figure 5:

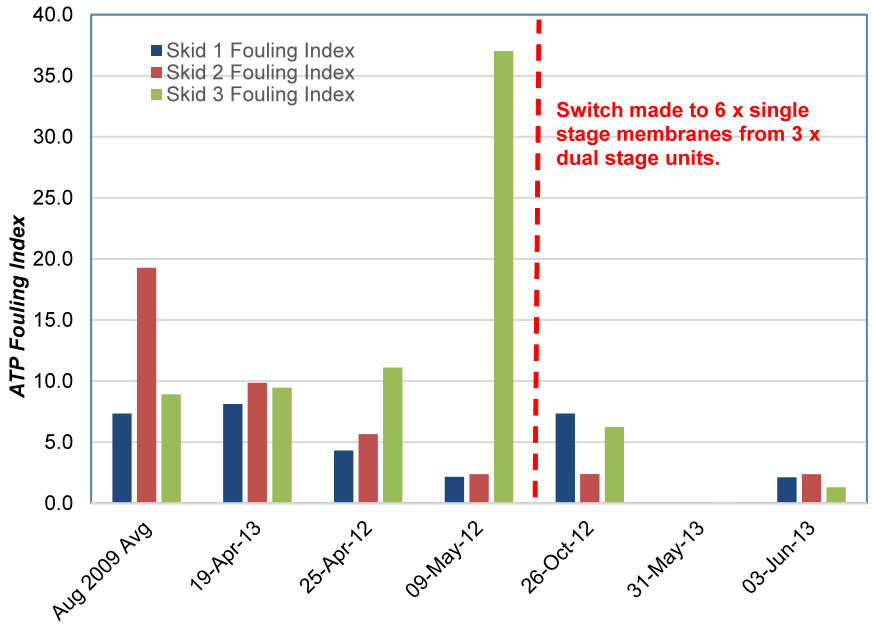


Figure 5: Updated fouling index data after switch from 3x2 to 6x1 arrangement

The results in Figure 5 clearly show that spreading the membrane feed stream over six parallel units eases the burden on each and results in reduced fouling tendencies. As such, it provides even more sustainable operating conditions. The resulting filtered water purities are shown below in Figure 6:

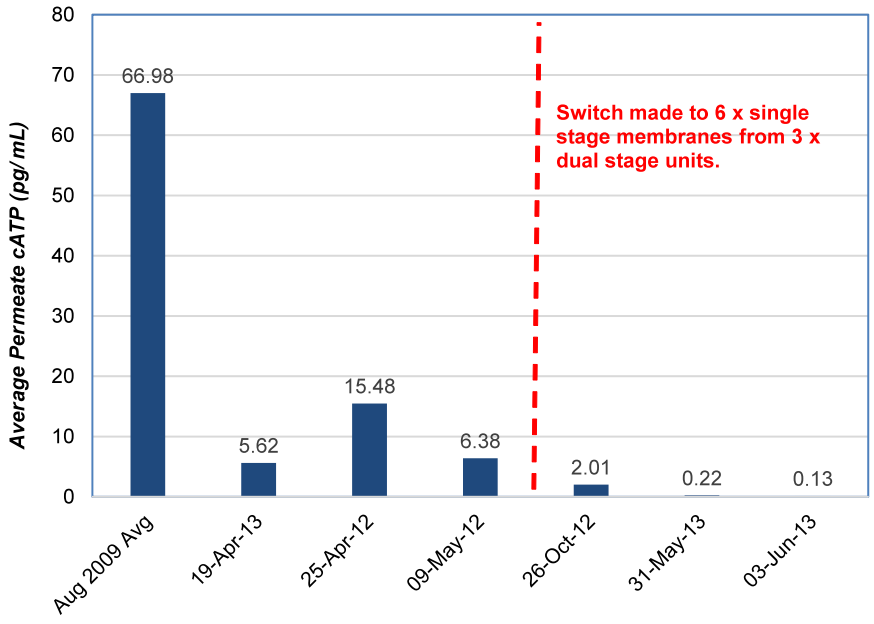


Figure 6: Final product water quality over the entire observation period

As expected, reduced fouling in the membranes as achieved through the switch to six single-stage membranes produced a significantly better quality product water. This was seen almost immediately after the switch and has actually gotten better over time. During this period, the electricity usage at the plant had also dropped so significantly due to the reduced pumping back pressure that plant personnel have observed reductions exceeding $1000 on a month-to-month basis following the switch.

UPDATE: Long-term Results

In January of 2014, a complete survey of water quality from source to plant discharge was conducted to determine the long-term feasibility of the design and operational changes that were implemented, with the results shown in Table 8:

Table 8: Assessment of long-term operating conditions

Sample Point	[ATP] (pg/mL)
Station 1 (well)	0.47
Plant Influent	0.15
Pre-filter Effluent	0.21
Skid 1 Reject	1.9
Skid 1 Permeate	0.32
Skid 2 Reject	0.74
Skid 2 Permeate	0.63
Skid 3 Reject	0.36
Skid 3 Permeate	0.16
Finished Water	0.92

The results clearly show that water quality continues to be excellent, meaning that the solutions that were put in place during the previous year continue to be effective and represents a sustainable solution.

CONCLUSIONS

Because of its speed, ease-of-use, and specificity to total living organisms, ATP monitoring serves as a very valuable method for rapid water quality assessment. The advancements made as part of 2nd 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 microbial contamination at the earliest signs so that they can be dealt with as quickly as possible.

The results of ATP monitoring as it applied to membrane treatment process was able to quickly identify elevated microbial content not only in the raw and treated water, but also

within the membranes themselves. This enabled personnel to assess the effects of decreased pre-chlorination, diagnose the fouling issue as a biological problem, and assess the efficacy of the membrane cleaning process – all within minutes of sample collection.

Upon further review of the modified arrangement, the process was further modified by switching to six parallel single-stage units as opposed to three sets of two in series as a result of fouling investigations using ATP test results in the feed, permeate, and reject streams of each membrane. Once the feed was spread over a larger number of units, fouling decreased even further and product water has been consistently of a very high quality ever since. This proved to be a sustainable solution after conducting a follow-up long-term audit of the system.

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