Rapid Microbiological Monitoring Methods

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INTRODUCTION

Microbiological Growth

Control of biological growth is important in cooling water systems because of its impact on heat transfer and corrosion. Biofouling in industrial water systems adversely affects heat exchanger efficiency, corrosion resistance, ion exchange and RO (reverse osmosis) performance. The accumulation of slime masses may also result in MIC (microbiologically induced corrosion) related failures as well as producing undesirable odors and posing potential health concerns.

Traditionally, microbicides have been used to control microbiological activity, which in turn minimized slime-related problems. However, stricter governmental regulations combined with a general trend in industry toward "greener" environmental profiles has caused plants to establish a finer balance between the amount of microbicide used and the level of protection afforded in their systems. In the past, the amount of biocide used was dictated by the desire to have "no" problems; today this balance is struck by intensified microbial surveillance being used to identify the onset of undesirable microbial growth and matching the amount and type of microbicide against the assessed risk. The goal of this "balanced" approach is to obtain acceptable performance, concurrently with lowering the biocide use to the minimum amount that is feasible.

The direct physical and microbiological methods used to assess slime build-up include measurements of slime weight and thickness, viable and total cell counts, and ATP (Adenosine Triphosphate) bioassay among others. Significant effort and attention on the part of plant operators is required to assess biofouling by these means, and success can be hampered by the inaccessibility of critical fouling surfaces (e.g. deadlegs, valves, heat exchanger tubes). Nevertheless these methods are important in establishing biological trends in systems and when combined with system performance, provide an insight into what is happening.

An alternate approach has been to employ modeling methods that use plant water and simulate plant conditions, in order to predict trends in the cooling system. These can be either used alone or in conjunction with more traditional monitoring methods.

In this paper we will be discussing both approaches to microbiological monitoring and will present results on an on-line monitoring tool that address many of the concerns that have limited their application in the past.

DISCUSSION

Bulk Water Testing

Most microbiological testing is performed on the bulk water in the cooling water system (planktonic organisms). Tests for planktonic bacteria are easier to perform than tests for sessile bacteria simply because of sampling. Although planktonic bacteria results may not have a direct relationship to the amount of sessile organisms in a system, there generally does exist a correlation. This is why planktonic bacteria testing results cannot be taken as "one-time" measurements; rather they need to be evaluated over time so that trends in general microbiological activities are seen.

While a variety of different methods can measure planktonic bacteria, there are only three types that are widely used. Depending on the test used, the units will vary and the points at which different actions are necessary will vary, depending both on the test results and the past system history. For instance, the standard plate count-method (or variants) provides results in colony-forming units per milliliter of sample (CFU/mL). By contrast, Adenosine triphosphate (ATP), will give results in nanograms of ATP/mL of sample (ng/mL) or in a less specific way, Relative Light Units (RLU).

Dipslides

The most common type of test for planktonic organisms are dipslides (or paddle testers), which are a semi-quantitative method for total aerobic bacteria. Each dipslide unit consists of agar plates mounted on a sampling paddle which is permanently attached to the cap for ease of handling during use. Some testers have a dual medium type, with an agar for total aerobic bacteria on one side of the paddle, and a medium for yeast and mold on the other side. In effect, dipslides are a simplified field method for doing “spread plate counts”.

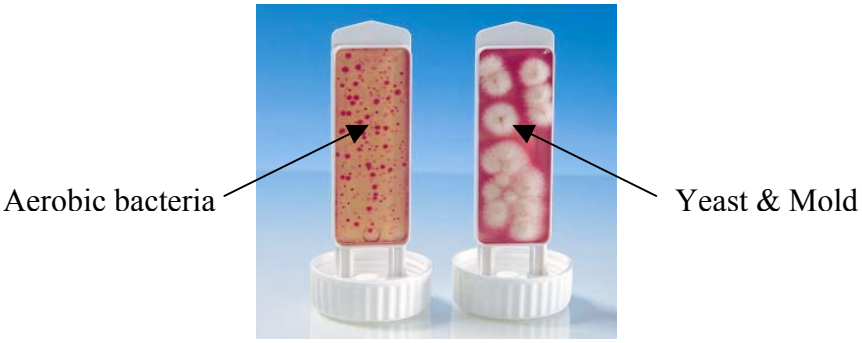
In the dipslide method, the estimate of the plate count is determined by comparing the density of the number of colonies on the slide with the chart provided. To make the comparison easier, triphenyltetrazolium chloride (TTC) salt is incorporated into the medium and turns red when reduced by most bacteria during their growth. However, dipslides are a screening test only, and more accurate results can only be obtained via other techniques.

Pros:

Despite the fact that the dipslide method only measures a fraction of all colony forming units (cfu/mL), it continues to work, and has served the industry well. Most people realize that a dipslide reading represents an estimation of what is actually present in a system and that the traditional "clean" value of 1,000 cfu/mL (10^3) is far from being a sterile system. This easy-to-use test method allows for the monitoring of general bacterial levels, and can therefore give a good indication of what trends are taking place in a system and whether remedial action may be required.

The advantages of dipslides are that:

- At least one side of all industrial dipslides is coated with nutrient agar to which a small quantity of the dye 2,3,5- triphenyltetrazolium (TTC) is added. Aerobic bacteria species reduce TTC to a red colored complex rendering them easily visible even to untrained personnel.
- Some dipslides are double sided – enabling simultaneous assessment of two media and hence for different organisms, e.g. yeast and fungi on one side and aerobic bacteria on the other.
- No special equipment or training is necessary.
- The dipslide handle facilitates sampling and reduces the opportunity for contamination. In other words, manipulation is limited to opening the container and inserting the dipslide into the sample.
- The plastic vial serves as a lightweight, convenient incubator and transport container.
- The transparent container allows colonies to be viewed and counted safely, since there is no need to open the container.
- Simple color changes enable colonies to be easily recognized and counted.
- This is a good tool, for routine approximations of system cleanliness.



Dipslides provide a simple and easy-to-use alternative to the plate count technique for assessing microbial contamination and their ease of use has extended the instances where at least some monitoring takes place.

Cons:
The main drawback of dipslides is that the sampling technique itself, has a significant impact on the results. The results are particularly affected by the amount of time the slide remains in the water. Unlike plate counts where the amount of water in contact with the medium is controlled, dipslides rely on organism sticking to the medium. In effect the amount of water used to run the test is very small, on the order of only a few microlitres - which is the water that is in actual contact with the medium.

Furthermore, although many organisms will generate red colonies, this is not universal. Some type of bacteria form colonies that do not turn color or may form their own pigment; and for this reason it is important to inspect the dipslide carefully for such colonies before estimating the count. A final source of significant error can be the incubation itself. An incubation period is at least 24-48 hours at temperatures between 25°C (77°F) to 35°C (95°F). However, if room temperature is used, final counts should not be taken until for as long as 5 days. Earlier inspection of the slide may be helpful in highly contaminated waters since a fraction of the colonies may be visible within a day or two.

Traditionally, dipslides, like plate counts, suffer from several limitations:

- No one medium will detect all types of organisms and the results are only an approximation of what is present.
- Dipslides are applicable only to aerobic bacteria and fungi/mold. Algae and anaerobic microbes are not "seen".
- If bacteria are clumped together, they will still show up as only one colony, which will under-estimate the actual count. This can be significant in cases where biological deposits are liberating clumps of organisms.
- Only planktonic organisms are measured and this may not reflect what is present on surfaces in the system.

In spite of these limitations, field test methods, such as dipslides, are a useful way of getting reasonably accurate results at the plant level, if no alternative exists. Field tests should be viewed as a quick way to screen systems in the field, in lieu of other methods, that offer better accuracy.

Standard Aerobic Plate Counts

Standard Aerobic Plate Counts can be done by pour plate, spread plate, or a membrane filtration method. As for dipslides, plate counts provide an approximation of the total number of aerobic bacteria by measuring those organisms that can grow under the conditions provided. While the control limits can vary by plant, a typical value is 10^3 (1,000) CFU/mL. However, as pointed out before, this is a somewhat arbitrary figure based on "average" conditions. One of the simplest derivatives of plate counts are Petrifilms™, which will be discussed below.

Pros:

Petrifilm™ Plates are referred to in many plants as the "Post-it Notes" of the microbiology field. Petrifilm™ Plates are playing-card size, sample-ready plates for the direct enumeration of aerobic bacteria, yeasts, or molds. Their exclusive design features a film coated with nutrients and gelling agents as well as an indicator dye. This method offers a significant accuracy advantage over dipslides for three reasons:

- The use of serial dilutions allow a level of precision that rivals with laboratory methods, while at the same time retaining much of simplicity of the dipslide method.
- Unlike dipslides, this method relies on a specified quantity of sample (1 mL) being delivered to the media. Which means that it does not rely on the organisms having to "stick" to the media to be measured, but that they are “forced” onto the media, therefore, resulting in a lesser sampling error.

These easy-to-use plates offer a number of benefits to operating personnel:

- Fast - no media preparation or quality control required.
- Improved efficiency - no preparation is needed.
- Reduced labor costs - simply open the package and they are read to use.
- Standardization of methodology - the slides are the same each time.
- A built-in grid facilitates counting - which means virtually anyone can use them.
- Takes up little storage or disposal space.

The Total Aerobic Count Plates version utilizes TTC (Triphenyltetrazolium chloride), a chemical dye that reacts with the oxidation reactions that take place within the bacteria. As the TTC is oxidized, the growing colony becomes red which makes spotting and counting colonies easy. The Yeast and Mold Petrifilms operate under a similar mechanism with different colored dyes. Yeasts in the water sample form well-defined blue colonies on the Petrifilms™, while molds produce more diffuse colonies that may be one of several different colors. Both yeast and mold grow and are counted on the same Petrifilm™.

Cons:
The characteristic of any culturing method is that many aerobic organisms are unable to grow on the nutrients provided or at a particular incubation temperature. Additionally, flocculated groups of bacteria will count as a single Colony Forming Unit (CFU), instead of several colonies. Plate counts are more quantitative than dipslides, but require a higher skill level to perform, especially in the dilution series. The method is also not specific for any particular type of bacteria, although that is seldom an issue in industrial settings.

ATP Bio-activity

ATP technology is a bioluminescence method for determining levels of biological activity not based on culturing organisms, but rather on measuring a cellular component. ATP stands for Adenosine Triphosphate, which is the chemical in which biological energy is stored and transmitted. ATP has been found to be present in all living organisms, therefore is not limited to bacteria. This technique uses bioluminescence to measure living organisms with a photometer (referred as ATP Luminometer).

In contrast to culturing methods, the amount of ATP in cells (algae, fungi or bacteria) is proportional to the cell's mass. Therefore, the measurement of ATP provides information of the total mass of living cells, NOT the number of organisms present. This is of significant difference between this method and conventional culturing methods; and why it is difficult to develop a direct correlation between these two testing approaches.

The chemistry used to measure ATP levels is the same reaction a firefly uses to produce light. An enzyme, "Luciferase", acts upon a substrate, "Luciferin", to generate light. This reaction requires ATP. When the enzyme/substrate complex is in excess relative to ATP, the amount of light generated is proportional to the amount of ATP available to drive the reaction:

$$\mathbf{ATP + Luciferin + O_2} \xrightarrow{\textbf{Luciferase}} \mathbf{Luciferin + AMP + H_2O + Light}$$

The light produced by this reaction is measured with a photometer. The light is amplified by a photomultiplier and counted on a photocathode over a given period of time. The results are produced as Relative Light Units (RLU), whereas:

$$\mathbf{RLU \propto Light \propto ATP \propto Microbial\ activity}$$

The ATP testing protocols can be grouped into two general types:

- the "separate step approach" where the individual steps (isolation of the organisms, extraction of ATP, enzyme addition, ATP assay, etc.) are done individually. This way of measuring ATP is well suited to doing multiple samples, where reagent costs are significant.

- the "pen" methods. This approach is designed for handling small numbers of samples and the focus is on user convenience. This type of test measures ATP by employing a self-contained "sampling pen" that performs all the functions needed to determine ATP in cooling water or process waters.

ATP technology has been used for microbial detection for over 25 years. However, industrial usage was limited because the procedure was perceived as expensive, difficult to perform, and not portable. Recently, advances in instrument technology, extractant chemistry, and enzyme stability have made ATP detection a practical and economical field test method.

Pros:
The advantages of ATP analysis, is that it is a very rapid technique that produces results in a few minutes. Consequently, ATP monitoring eliminates the need for extended incubation times associated with traditional culturing procedures (dipslides, Petrifilm™, etc.), which reduces service time and comes close to providing "real-time" assessment of microbial loading in an industrial system.

In addition, it detects all microbiological species (often referred as the total biomass), including:

- aerobic bacteria
- anaerobic organisms
- algae
- fungi and mold

Since it is so inclusive, the ATP assay can provide a very complete assessment of overall microbiological status of a system. This is why it is a much more reliable method of assessing the degree of microbiological control than culturing methods.

Cons:
The ATP content decreases as the health of the cell lessens and will disappear when the cell dies. However, the dividing line between a cell near death and it being dead is ambiguous and this can influence the ATP value. In addition, in some circumstances ATP can persist for an appreciable amount of time after the cell has died, giving the impression that the level of biological activity is higher than it really is.

Since ATP in the cell decays rapidly when the organism dies, ATP is a measure of live organisms. It is not truly quantitative for actual bacteria populations because different microbial species (bacterial, algal or fungal species) contain different amounts of ATP. Depending on the time of year, or different systems, equivalent amounts of ATP can represent considerably different ratios of each class of microbes. This non-specificity is a virtue as well as being a drawback.

Due to the reasons already discussed, it is impossible to select a specific ATP reading that can be universally used as an indicator of whether microbiological activity is "low" or "high." Even the equipment used can influence the values, because each type of ATP measuring equipment (ATP luminometers) has varying sensitivities, depending on its construction.

Total ATP vs. Free ATP:
Although the term ATP is discussed as being one value, there are in fact two types in any system.

Total ATP is a measurement of **all** ATP in the sample. This includes background ATP (from dead microbes, whose cell contents have leaked into the water) as well as ATP from live microorganisms. Since it is only the ATP related to viable organisms that interests us (from a fouling control perspective), there is a need to eliminate ATP from microbes that are already dead.

The way this is done is by measuring Free ATP, in other words, ATP not associated with cells and which is not a true indicator of microbiological activity. Free ATP, also called background or extracellular ATP, exist outside of the microbial cell. A certain amount of Free ATP exists in all samples but it is particularly prevalent in cooling water systems where oxidizing or quat-based biocides are used.

When the Free ATP is subtracted from the Total ATP, the difference is the Cellular ATP (ATP inside active cells). Normally it is not necessary to measure both types but, there are times when it allows for a more accurate assessment of what is happening. This is particularly the case when it appears that the biocide program is not providing the expected test results and yet the system remains clean. Frequently the reason is that the biocide program is in fact working as designed but that there is enough Free ATP, to give a false impression that there is a problem.

If both the Free and Total ATP tests are to be determined on the same water sample, it is important to run the Free ATP first. The Total ATP Pen contains a lysing agent, which will convert Cellular ATP in the water sample to Free ATP and cause and artificially high Free ATP reading.

ATP Pens:
Currently, field ATP tests are performed with the use of a self-contained "pen", that allows for rapid measurement of either Total or Free ATP, depending on the type of "pen" used. A third type is the ATP Swabs that are use on surfaces. Although this feature was designed primarily for food hygiene purposes, it can also be adapted to monitor trends in ATP on surfaces in a cooling system, for instance, corrosion coupons, heat exchanger tubes and tower fill. This easily extends the application of ATP from being strictly a way to track planktonic organisms to one that is also applicable to trending sessile populations.

ATP luminometers will provide a read-out in RLU (Relative Light Units), and are an arbitrary measure of the amount of ATP present. Using RLUs as an indicator of microbiological activity is flawed since:

- Variations exist between the brands of luminometers (and to some extent even within a company with different models) and as different luminometers are used, the values obtained can vary.

- The pens rely on an enzyme that can change over time and storage conditions, both of which can have a dramatic effect on the results, giving a false indication of good system control.

- Different manufacturer's pens will have differing concentrations of enzyme and components, leading to different light output from the devices even if the ATP levels are the same.

The solution is to convert the RLU readings to standard units (ng/mL of ATP), that allow them to be transferable between plants, meters/pen batches and over time. This permits the data to be generally applicable, rather than being limited to only one site and one instrument.

The principle of running an ATP Standard is based on the measurement of a standard concentration of Adenosine Triphosphate (typically 1 nanogram of ATP per ml of water), used as an assurance of reagent and instrument function.

The selection of 1ng/mL of ATP is based on the quantity of ATP expected from an ideal pure culture population of 1×10^6 bacteria/mL. In turn, this number of bacteria is generally viewed as being the maximum amount that can be tolerated in a cooling system. For these reasons, 1 ng/mL is a convenient concentration to use to standardize equipment.

Standardization of a meter (or pens) is done by substituting the ATP Standard for the water sample. The procedure is no different than what is normally done, i.e. one would take a representative pen (for Total ATP) and dip it in the vial containing the ATP Standard and

measure the bioluminescence (RLU) from the ATP meter. Knowing that the vial in question represents 1 ng of ATP/ml, then the measured RLU from the meter is equal to 1 ng of ATP/ml.

For instance if the particular meter gave a reading of 2500 RLUs with a 1 ng/mL ATP standard and the cooling water showed 1250 RLUs, then the ATP concentration in the cooling was is:

$$(1250/2500)*1 = 0.5 \text{ ng/mL}.$$

This calibration permits readings to have a common basis, regardless of the instrument used or the batch of pens.

ATP Results Interpretation:
Interpreting ATP results can be aided by establishing a relationship between ng of ATP/ml and CFU (colonies forming units) for a given water sample. This is needed because ATP testing detects a much broader range of microbes than simple plating. Additionally, factors other than cell number affect ATP levels such as microbe type, cell size and metabolic rate.

Note however that the ng/ml:CFU relationship will vary from system to system and within the same system as microbial populations change over time, i.e. the ratio of different types of organisms. If there is a need to develop a correlation, it is best to establish this relationship doing plate counts (i.e. dipslides or Petrifilm) and ATP determinations side by side for a period of time. In the long term, ATP (ng/ml) alone can be used to track system cleanliness.

Regardless of whether a culturing method or ATP is used as the primary means of monitoring biological activity in a system, the important thing is to ensure that acceptable standards are met in order to maintain a well operating cooling system. While it is difficult to provide definitive values, experience at numerous sites has given the following recommended guidelines for cooling water systems:

Method	Organisms	Maximum	Preferred Value
Dipslides/Plate Count	Aerobic	$<10^6$ CFU	$<10^3$ CFU
Agar Count	SRB/Anaerobic	non detectable	non detectable
ATP	All Types	<1.0 ng/mL	<0.5 ng/mL

Sessile Bacteria Monitoring Techniques

Following the levels of planktonic organisms is well developed and in most cases widely practiced. However, the same cannot be said regarding sessile microbes. In a large part the problem has been developing a technique that adequately tracks the changes and can provide useful operating data.

The interest in sessile bacteria is because they accumulate on surfaces (while planktonic bacteria are those that are “free floating” in the water) within the cooling system. The sessile bacteria are of more concern than the planktonic bacteria, because they:

- Directly influence corrosion rates on the system metal due to the environment they create beneath the biofilm.

- The biofilm can either interfere with flow and/or heat transfer or it can act as a "trap" for other debris and initiate deposition.

- Act as a reservoir for biological contamination and it is more difficult to kill the microbes within the matrix.

There are several techniques for doing this, such as visual inspections; stainless steel corrosion coupons, where the biofilm is removed from the coupon and the organisms cultured or tested for ATP (biomass/cm^2); the Robbins Device and several others. While each of these devices offers specific benefits, we would like to discuss a Dual-Channel Biofouling Monitor that provides real-time tracking of the biofouling tendencies of system waters.

Dual-Channel Biofouling Monitor

Several indirect methods to measure slime build-up in side-stream devices have been used in attempts to mimic fouling in cooling water systems and to provide a real-time indication of biofouling. Current methods all share the drawback that fouling due to causes other than biofilm growth cannot be positively distinguished from microbial growth. Fouling caused by dirt or leaves (or other debris) can impair a system's performance but it cannot be corrected by changes to the biocide program and if they influence the test data, the utility of the data is reduced.

Cooling water by its very use contains suspended solids that can be readily incorporated into deposits and regardless of whether these deposits are primarily biological or not. If the monitor uses pressure differential as a monitoring parameter, fouling due to suspended solids can easily obscure the effects of biological activity. This type of interference limits the utility of monitors that use pressure differential.

This would be a major limitation of the Dual-Channel Biofouling monitor since it to can be described as a differential pressure biofouling monitor, in which a packed column of metal beads provides a large surface area for fouling. The difference is in how the instrument deals with this type of interference that permits it to retain the simplicity of more common tube type differential pressure fouling monitors and at the same time eliminating the need for high flow velocities.

A challenge in using packed column technology to monitor biofouling is the tendency for non-biological material and debris to clog the column and interfere with the biological signal. This is particularly true in cooling systems. The ability to handle high levels of suspended solids is achieved by using a recirculation pump to generate flow across the column while using a separate make-up pump to introduce fresh cooling water into the system at a relatively slow rate. This approach maintains a continuous supply of cooling water without the need to pump large volumes of fresh solution (and suspended solids). The small volume of make-up water can be

readily filtered upstream of the biofouling monitor permitting high solids sources water to be used.

This provides continuous inoculation of the media in the instrument as well as exposing any biofilms that develops to the same conditions as the cooling loop, including any biocides or organic dispersants that may be used. The retention time of water in the monitoring system can be adjusted using the make-up pump, which provides one of several means to modify the sensitivity of the instrument.

Instrument sensitivity is of key importance in correlating the response of a biofouling monitor with the performance of the water being monitored. In almost all cases, the purpose of the monitor is to detect slime deposits before they reach a level that degrades heat exchanger performance or corrosion protection. In order to accomplish this, the monitor must generate a measurable signal in advance of a significant build-up. The Dual-Channel Biofouling monitor incorporates several means to control the rate of biofouling in the instrument enabling the instrument response to be readily correlated with process quality.

The device allows the rate of cooling (or a process) water feed to be adjusted to increase or decrease the supply of fresh water to the monitor. This in turn influences the microbial growth rate by increasing or decreasing the supply of nutrients available for growth and also alters that exposure to changes to the microbial population in the cooling water.

A second means to control instrument sensitivity is by adjusting the recirculation flow rate. Higher flow rates amplify the pressure changes caused by changes in media roughness, fluid viscosity, density, and porosity of the packed bed. It is even possible to alter the size and shape of the material used to pack the columns to further adjust sensitivity. As an example, columns packed with small diameter bead will respond quickly to biofouling since little deposit is needed to plug the voids between the beads.

The above adjustments can all be used to modify the sensitivity of the monitor. However, experience has shown that long periods of slime free systems operation are often followed by abrupt increases in fouling that develops as little as a few days. The goal of having on-line monitoring is to provide an insight has to when fouling is likely to occur – before it takes place. This allows the biological control program to be "tuned" to where the minimum effective amount of biocide is used and still avoid any problems.

To address this issue, the device incorporates two channels, one of which could employ a supplemental nutrient feed pump to introduce additional nutrients, with the goal of accelerating microbial growth.

Using this approach, the nutrient enriched channel gives an advance warning of biofouling compared to the channel supplied with process water only. In the case where the biocide program is providing acceptable control, organism in the cooling water will not readily reproduce, even when afforded increased nutrient levels. However, if sudden spurt in growth is recorded, it is confirming that the health of microbes in the cooling system are improving and that the potential exists for fouling to get out of control.

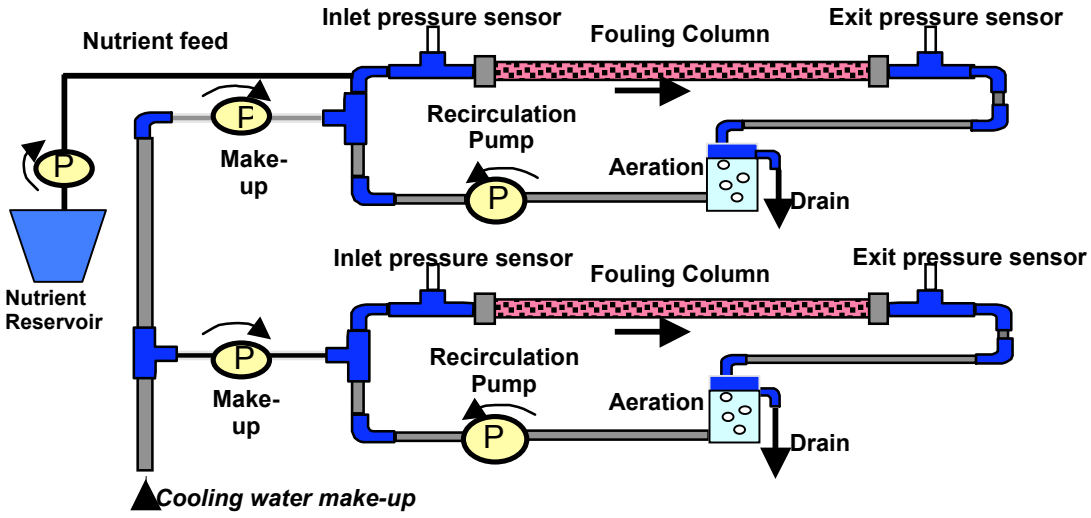
Furthermore, it is an advantage of this design that acceleration of the fouling signal by nutrient addition confirms the at least partly biological nature of the fouling, since deposition due to non-microbial material would not respond nutrients.

System Description

The Dual-Channel Biofouling monitor is a stand-alone system designed to monitor slime and deposit build-up in industrial water circuits. The monitor operates on the principle of pressure-drop, utilizing water circulated through a pair of packed bed columns to generate a ΔP related to biofouling. The system comprises multiple pumps, pressure transducers, temperature controllers, and data loggers housed in a metal cabinet. Auxiliary filtration equipment can be used in cases where the system water has excessive amounts of suspended solids.

Cooling or process water is drawn into the monitor via a dual-channel pump and circulates the water through a pair of long PVC columns. The columns are filled with stainless steel beads. A separate pump recirculates water through the columns to generate a suitable pressure drop. In the case of the second column, supplemental glucose is fed to stimulate microbial growth.

The bead columns are attached with quick connect unions for easy replacement and the entire biofouling monitor liquid flow path can be cleaned by pumping cleaning solution through the system. The bead columns are heated by electric mantles to maintain a desirable temperature for microbial growth. Differential pressure gauges are used to measure pressure drop across the bead columns and to transmit the signal for display on digital and bar graph meters located on the cabinet front panel. Data loggers collect data from both channels at user selected intervals for analysis.



Signal Analysis

The Dual-Channel Biofouling can provide semi-quantitative results of trends within a cooling system. Dual fouling columns within the instrument enable simultaneous comparison of treatment (microbicide/bio-penetrant) or fouling environment (nutrient) versus control protocols under identical test conditions. It is possible to develop comparisons of build-up rates under

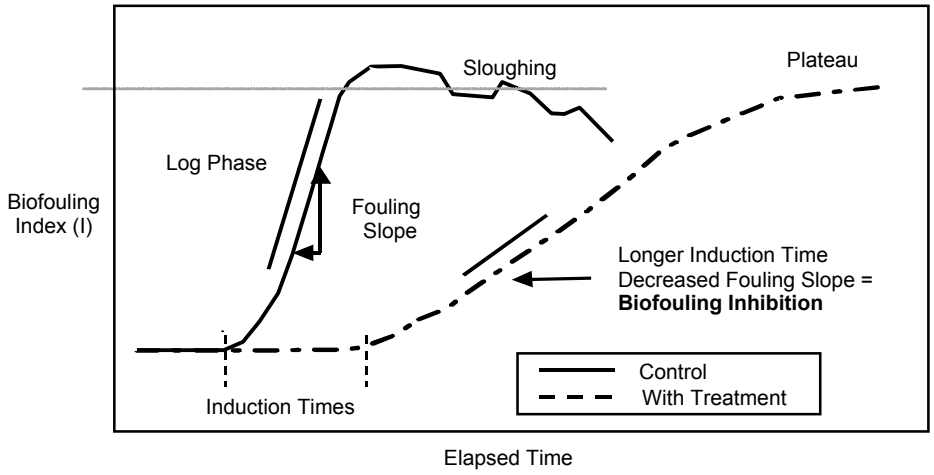
varying treatment conditions, using the induction time and the exponential accumulation phase (log phase) as critical parameters.

A general description of biofouling encompasses three-phases of development[5]:

1) the induction phase during which initial surface colonization evolves but little slime production takes place;
2) a log phase characterized by exponential biomass accumulation; and
3) a plateau period during which biomass production and removal rates are roughly equal.

A fourth phase may also develop during which biofilm sloughing causes a net decrease in attached biomass.

The induction time is measured simply as the duration of baseline signal preceding the exponential accumulation. By examining the induction time and the rate of accumulation under different treatments, their effectiveness can be compared. Although biofouling may be mitigated at any stage of development, prevention is generally preferred over efforts to remediate existing deposits. In practice, prevention means prolonging the induction phase. Microbicide application during early colonization causes chemical damage to cellular processes that retards microbial growth and interferes with extracellular polymer production. Once biofouling reaches the log phase, microbicide treatments become less effective due to transport and reaction losses associated with microbicide penetration into the biofilm. At this stage, organic dispersants and penetrants may be required to enhance treatment efficacy. Advanced stages of biofouling will often require aggressive treatment to remove attached biomass and suppress further fouling.



Schematic progression of biofouling, illustrating stages of fouling and the effect of inhibiting treatment

By having an understanding of the factors that can lead to a loss of microbiological control in a particular system, provides insight into how this can be avoided. The key is the supplemental

nutrients feed and its acceleration of microbial growth as a predictor of future potential trends. This results in a several day decrease in the induction time and enables the instrument to show a strong fouling response well in advance of fouling that could occur in the system. When the accelerated channel fouls (or begins to foul more rapidly) the alarm gives the operator time to supplement the microbicide and/or correct the cause of fouling, before it becomes a problem in the system.

PLANT TRIAL

The biofouling monitor was tested to evaluate the instrument performance and to examine the effect of supplemental nutrients on the fouling response. Slime formation at the plant was kept under good control using a combination of halogen and organo-bromine microbicides. At the same time the plant wished to decrease microbicide dosage, which made the plant an excellent site for the trial. During the initial days of the trial, a baseline fouling response was established against which future efforts to reduce microbicide usage were compared.

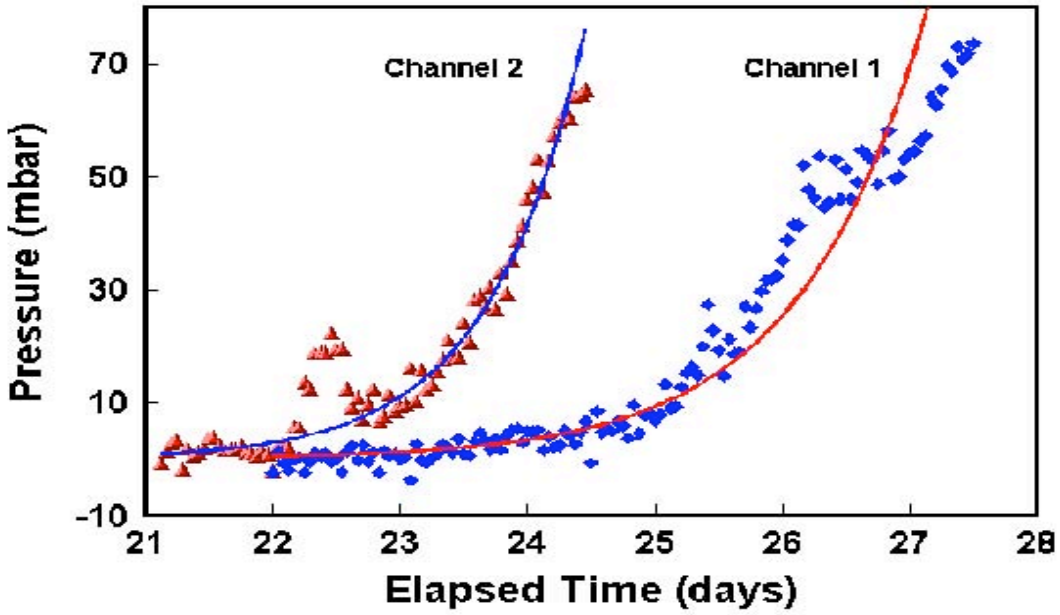
The Dual-Channel Biofouling monitor was fed with water from the tower basin. The water was filtered to lower suspended solids prior to entering the biofouling monitor. Make-up to the biofouling unit was continuous at 2-3 mL/min giving a residence time of approximately two hours within the recirculation loop. Temperature of the influent solution was 120-130°F. Supplemental glucose was fed to the second of the two biofouling channels to give a final concentration of 600 ppm.

The monitor, outfitted with clean fouling columns, was placed on-line. After 31 days, the fouling columns for both channels were replaced and 44 days later the column for channel two (nutrient supplement portion) was again replaced. The fouled columns removed at day 31 were visually examined for the presence of slime by removing the stainless steel beads and examining the beads at 10X. The beads were also analyzed for microbiological activity by extracting the deposits with surfactant and analyzing the extract for the ATP level.

Results

The data record shows an initial fourteen day period during which time the signals for both channels remained stable at 1-2 kPa (10-20 mbar). Between days fourteen and twenty, an excursion to 3 to 4 kPa (30-40 mbar) and a subsequent return to baseline occurred for both channels. At day twenty one, the signal for the glucose channel (channel 2) began to increase exponentially to a limiting value of 8 kPa (80 mbar) corresponding to the maximum signal. Two to three days later, the signal for other channel showed a similar exponential increase in pressure. This showed that the glucose channel gives a two to three day advance warning of biofouling and confirmed laboratory testing which displayed the same response pattern.

New fouling columns were installed at day 31 causing the signals to return to their baseline level. The following graph shows the results from this plant trial.



Exponential curves fit to regions of sharp pressure increase for channels 1 and 2.

The next fouling event for channel two occurred three to four days after the new columns were installed, and was again followed approximately three days later by what appears to be the start of an exponential shift for channel one. Installation of new fouling columns in channel two (on day 31) resulted in an exponential increase similar to the one described above but which began much sooner. The more rapid increase started 3 - 4 days after the columns were installed and may have been promoted by an inoculum of microorganisms within the monitor that was not cleaned out when the fouling columns were replaced.

Visual examination of the deposits on the stainless steel beads was made at day 31 prior to installing new columns. Pale yellow to amber slime streamers within the columns were visible by eye and examination of the deposits at 10X showed a web-like matrix of biofilm spanning the voids between beads. ATP analysis showed the presence of heavy microbial activity in material extracted from the beads.

This trial demonstrated that microbial activity and slime formation in cooling water systems can be measured using the on-line monitor. ATP and elemental analysis confirmed that the fouling deposits contained both microbial and inorganic materials which is typical of the slime and related debris trapped by the biofilm.

The major point was that the response to microbial activity was accelerated by supplemental nutrient addition which in turn provides an advanced warning of the biofouling. Under normal operation, the signal on the nutrient augmented channel can be used to predict that fouling will occur in a few days time, giving plant operators the opportunity to take corrective action before a problem becomes evident.

SUMMARY

With increased focus to reduce our impact on the environment and to develop an optimal balance between microbicide feed and the risks to system performance, new methods to aid us in developing treatment regimes needs additional investigation. Industry trends towards greener profiles and reduced microbicide use are placing renewed demands on microbial surveillance.

In this paper, we have reviewed a variety of monitoring procedures and their limitations. Plate counts, ATP testing and other traditional methods used to measure microbiological activity, give a good indication of potential biofouling developing in a cooling water system, but often are not representative of sessile growth. Because ATP monitoring eliminates the need for extended incubation times associated with traditional culturing procedures, it has the advantage of being a very rapid technique that produces results in a few minutes, therefore providing "real-time" assessment of microbial loading in an industrial system.

On-line monitors that yield a real-time indication of the extent of biofouling and from which critical fouling levels can be predicted are an attractive addition or alternative, because they can be used in a predictive model. The Dual-Channel Biofouling monitor discussed in this paper utilizes supplemental nutrient addition to give a clear indication of microbial growth and which further provides an advance warning of biofouling. Evaluation of the system at a plant location showed that nutrient addition gave a two to three day advance warning compared to measurements without added nutrients. Data from the instrument yields a relative measure for slime formation that can be used to evaluate the effect of reduced microbicide dosage. This gives one, a way to determine the optimal balance between microbicide feed and the risks to system performance.

In closing, consideration should be given to the utilization of multiple methods of measurements and biological growth simulation to aid plants in determining the optimal balance between microbicide treatment regimes and the risks to system performance without using your system as a laboratory instrument.

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