Legionella Bacteria Control in Cooling Tower Water Systems - An Independent Study Indicates that Current Acceptable Microbiological Control Methods are Ineffective and the Industry Standard Levels of Acceptable Bacteria Need to be Changed

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ABSTRACT

Recent Legionella *outbreaks in 2015 and 2016 due to cooling tower water systems has suggested that a closer look at current methods of microbiological control is needed for these systems. The failure of what was once considered adequate control methodologies for these systems has prompted an independent study of the current recommendations and monitoring methods implemented by end users. This study concentrated on typical HVAC cooling tower water systems not only those system types that were involved in recent out breaks but also in systems used in a variety of industries where no outbreak has occurred.*

The findings of this study strongly suggest that procedures for Legionella *control are ineffective in many cooling tower systems. This report discusses these findings in detail and the reasons why accepted control methods are likely ineffective. It also shows what needs to be done to improve the effectiveness of microbiological and* Legionella *control procedures using a system evaluation.*

The study suggests the current industry standard for acceptable levels of total bacteria is too high and should be reduced and that bio-film must be measured and eliminated. It also suggests that Legionella *control can be improved with the addition of more effective microbiological monitoring method.*

INTRODUCTION

Cooling tower water systems present an ideal environment for microbiological accumulation throughout the system including the cooling tower, the heat transfer equipment and piping. This includes essentially all types of microorganisms such as bacteria, algae, fungus, and pathogens such as *Legionella* bacteria. Cooling tower water systems have an ideal water temperature, and nutrients from the makeup water and the air support the growth of microorganisms including *Legionella* bacteria. A severe example is seen in Figure 1, which exhibits many varieties of microbiological organisms, including green algae, white slime and high levels of *Legionella* bacteria. This microbiological deposit is often called bio-film or biomass or just plain “slime”.

Figure 1. - Microbiological "Bio-mass" in Cooling Tower

A reduction of these microbiological organisms is necessary to prevent their adverse impact on water contacted surfaces from deposits, corrosion, and reduction of cooling system operating efficiency and to minimize levels of *Legionella* bacteria. The reduction of this bio-mass to an acceptable level to maintain the operating efficiency of the cooling water contacted equipment is accomplished by using both chemical and non-chemical methods**.** However total elimination of this bio- film is not achieved in many air conditioning cooling tower water systems as identified in a recent article (Puckorius, 2017). This is primarily due to the methods used to apply microbiocides to the cooling tower water compare against accepted industry levels of total bacteria counts in the cooling tower water. This may be one reason that cooling tower water systems in the HVAC&R industry have been the suspected primary source of Legionnaires Disease in recent outbreaks. This led the authors to initiate a study at an operating cooling tower system to evaluate microbiological monitoring methods.

CURRENT ACCEPTED MICROBIOLOGICAL MONITORING

To maintain an acceptable level of microbiological organisms in cooling tower water system it is common to measure the heterotrophic bacteria, commonly referred to as Heterotrophic Plate Counts (HPC) of the cooling water by various methods. This monitoring is performed on a cooling water grab sample that by its nature only measures the suspended total bacteria in that particular water sample. These suspended bacteria are referred to as planktonic microbiological organisms.

Legionellae bacteria is also measured in planktonic phase by testing grab samples of the cooling water even though these organism mainly thrive in the biofilm. (Murga, 2001)

An acceptable level of total bacteria found in cooling water that has been used for many years by most cooling tower water systems in all industries is 10^4 (10,000) or less colony forming units per milliliter (cfu/mL). This target is recommended by numerous government organizations and technical societies (CTI, 2008; AWT, 2009; PWGS, 2013). This quantitative measurement has been the standard used for many years across all industries and water treatment practitioners.

An acceptable level of *Legionell*a bacteria varies depending upon the organizational guidance. It is generally acknowledged that "a low level" is desirable (CTI, 2008). Differing qualitative references are often reported on *Legionella* sample analysis reports.

Yet it is well known that problematic bacteria are those causing corrosion. Bacteria species such as sulfate reducing bacteria, acid producing bacteria (nitrifying bacteria) and iron oxidizing bacteria. These species are not primarily suspended in the cooling water but prefer to adhere to the surfaces in the cooling system as a bio-film or biomass. This is where *Legionella* bacteria thrive. (Declerck, 2010) Those bacteria that adhere to the water contacted surfaces are referred to as "sessile." To date there are no organizational or industry-wide recommendations on acceptable total bacteria or *Legionella* bacteria levels as measured in a bio-film sample.

The bio-film needs to be eliminated since this is the source of problematic microbiological organisms including *Legionella* bacteria (Flemming, 2002). Culturing only the bulk water and reporting the planktonic bacteria is not only misleading but does not eliminate the source of these problem-causing bacteria (T.C. Soli, 2015). The bulk water is continuously re-inoculated from the biofilm (Watnick and Kolter, 2010). This is perhaps the main reason that Legionnaires" Disease outbreaks occur even while maintaining planktonic levels of 10^4 or less cfu/mL HPC (Thomas, 2004). The presence of planktonic bacteria at 10^4 means there is always a bio-film present; and one which likely contains *Legionella* bacteria. The industry-accepted level of total bacteria at 10^4 or less cfu/mL in a water sample that here-to-fore indicates good results is wrong. In the absence of measuring the biofilm the industry accepted level of 10^4 HPC of planktonic bacteria is not an adequate prediction for keeping *Legionella* bacteria at a safe level. Industry standards should be changed to levels that reflect the elimination of a biofilm.

The authors maintain that a procedure is required to measure the biofilm in which the vast majority of microorganisms exist in cooling water systems. In the absence of a methodology, the authors developed a field-friendly biofilm testing method that needs to be in common use and gain acceptance by the cooling tower water industry. An independent study was conducted to demonstrate the importance elimination of biofilm in cooling water systems.

INDEPENDENT STUDY

An independent microbiological monitoring study has been undertaken over the period of six months from June through November 2016 in a California facility. An existing cooling tower and water treatment system was identified. This cooling tower water system operates 24/7 for air conditioning and was selected since no chemicals are being used for cooling water treatment. If chemicals were used, we believe that it would be more difficult to evaluate steady-state microbiological conditions accurately due to the effect of biocide feed frequency and fluctuating HPC. This cooling tower applied an existing physical water treatment system employing electro-dynamic pulsed electric fields to control scale, corrosion and microbiological deposits (Puckorius, 2014; Puckorius 2015; Patton, 2009).

City water was used as cooling tower makeup and the cycles of concentration were maintained in the range of 20 to 24 as determined by chloride ratio (as Cl^-). Make-up water averaged pH of 8.2, conductivity at 100 micro-Siemens/cm, calcium hardness at 15 ppm (as $CaCO_3$), total alkalinity of 29 ppm (as $CaCO_3$) and chloride at 5.9 ppm (as Cl^-)

System bulk water ranged at pH of 8.2 to 8.4, conductivity at 1000 to 1200 micro-Siemens/cm, calcium hardness at 140-160 ppm (as $CaCO_3$) and a total alkalinity of 210 to 230 ppm (as $CaCO_3$) and chloride at 125 ppm (as Cl^-).

Figure 2 - Cooling Tower used in the Study

Figure 3 – Physical Water Treatment System

The condenser water flow rate was 1820 gallons per minute (GPM) flow, with a maximum water temperature of 70^o to 90^{o}F. The average load of the system is 600 tons during the evaluation.

Microbiological testing was performed by three CDC ELITE certified labs for *Legionella* bacteria. On state certified lab was used to perform heterotrophic bacteria and sessile (biofilm) bacteria. In addition, an independent contractor sampled and cultured dip slides for total bacteria field tests. All samples were taken at the same time and the results compared.

The bio-film or sessile bacteria tests were determined using a procedure developed by Mr. John Dresty (Dresty, 2016a) by modifying and expanding Dr. Lui procedure (Liu, 2000). This procedure exposes a flat 316L alloy stainless steel flat metal coupon and a 316L alloy stainless steel wire mesh coupon to the cooling tower water. The coupons utilized are illustrated in Figure 4 with a close-up of the weave pattern in the mesh coupon illustrated in Figure 5. Biofilm results are reported as colony forming units per square centimeter (cfu/cm^2) after relative coupon area calculations are determined. (Dresty, 2016b)

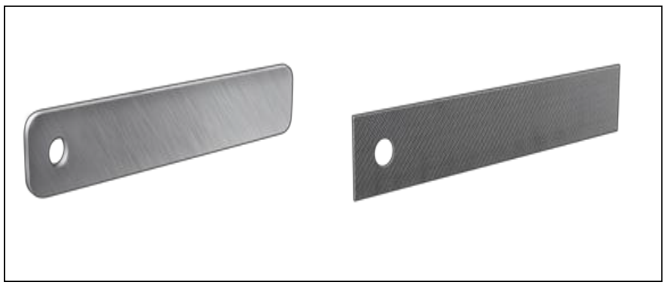

Figure 4- Flat and Wire Mesh Stainless Steel Coupons

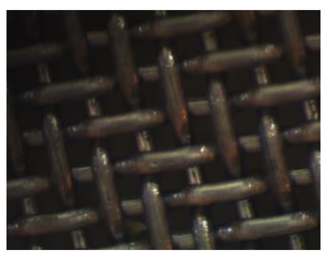

Figure 5- Stainless Steel Wire 80 Mesh Coupon- expanded view

Both coupons are the same length-width dimensions as standard flat corrosion coupons. The coupons are size 3 inches long and 0.5 inches wide. The flat coupon width is 0.0625 inches thick. The coupons were inserted in a typical corrosion coupon rack and exposed to the cooling water for 30 days. The cooling water flow past the coupons was 3 to 5 feet per second (fps).

The use of two coupon types was intentional. The wire mesh coupon is believed to be a more severe test for biofilm versus the flat coupon. The wire mesh coupon was used to provide an ideal complex surface upon which biofilm would accumulate while the flat coupon might exhibit reduced bacteria adhesion or biofilm shear.

Coupons were carefully removed and each was placed in separate 10mL volume of sterile Butterfield's Buffer Solution and subjected to ultrasonic cleaning. The ultrasonic cleaning removes the attached sessile bacteria and disperses the bacteria into the solution. The samples were delivered to an independent certified lab within two to three hours.

This study compared the results of a dip slide taken on site against results by an independent state certified labs for heterotrophic bacteria. The same lab cultured HPC from both the flat and wire mesh coupons.

Three independent CDC ELITE certified labs utilizing the CDC Method (CDC, 2005) completed Legionella bacteria testing.

Samples were taken monthly. All grab samples were taken by a third party at the same time and location and were delivered to the selected labs within hours of being drawn. Results in Table 1 show the difference between the various test methods.

Table 1. Culture Results of Cooling Water

2016	Dip Slides CFU/mL	HPC CFU/mL[1]	Stainless Bio-Coupon CFU/cm²	Mesh Bio-Coupon CFU/cm²	*Legionella*[2]
June	100	80	---	----	ND
July	10	6	0	----	ND
August	100	114	0	0.8	ND
September	100	108	0	1.4	ND
October	100	79	0.36	0.12	ND
November	100	150 Avg. - 79	0.73	1.3	ND

[1]Heterotrophic Plate Count SMEWW 9215b Plate Count Agar
[2]Legionella Culture by CDC Method, all three independent labs reported Non-Detectable (ND) *Legionella*

Dip Slides and HPC

Dip slides to measure planktonic bacteria are sampled on-site by inserting the paddles into a grab sample, and then cultured according to manufacturer's specifications. In this case, grab samples were obtained form the cooling tower basin furthest form the make-up water valve. Culture analysis of dip slides reports order of magnitude. The dip slides results confirmed the enumerated HPC (Heterotrophic Plate Count) results reported by the labs. While the overall average is slightly less for the enumerated HPC values both the dip slide and HPC tests indicate that results were well below the standard used by the water treatment industry of 10^4 (10,000) or less cfu/mL. The resultant values of 10^2 or less CFU/ml indicate excellent results by both of these test methods.

Stainless Steel Flat and Mesh Bio Coupons

The total bacteria levels recovered from both the stainless steel flat and wire mesh coupons were far below that found by dip slides and planktonic HPC, roughly thee orders of magnitude for the flat coupons, and two orders of magnitude for the mesh. Again, there are no standards for acceptable biofilm levels. The results indicate and the authors strongly maintain that sessile counts should not exceed 10 cfu/cm² in cooling water systems for a 30-day exposure.

The mesh coupon shown in Figure 6 is as removed from the coupon rack before ultrasonic cleaning. There is no visible biofilm present. This represents the end of 30 days exposure during November.

The stainless steel flat and wire mesh biofilm coupon results showed extremely low levels or zero levels of total bacteria, with the wire mesh showing counts while the flat recorded zero. This was expected because the configuration of the mesh coupon supplied a greater variation of surfaces and angles allowing some microorganisms to collect in the crevices despite the constant flow of water, while the flat coupon may have allowed any biofilm to wash away.

The use of a flat stainless-steel coupon for biofilm attachment is useful. However the results show that at times there were no bacteria detected on the flat coupon while the mesh coupon still shows the presence of very low levels of sessile bacteria. It is possible that the smooth surface of the flat coupon allowed for biofilm shear that essentially washed the biofilm off at the specified flow rate of 3 to 5 feet per second through the coupon rack.

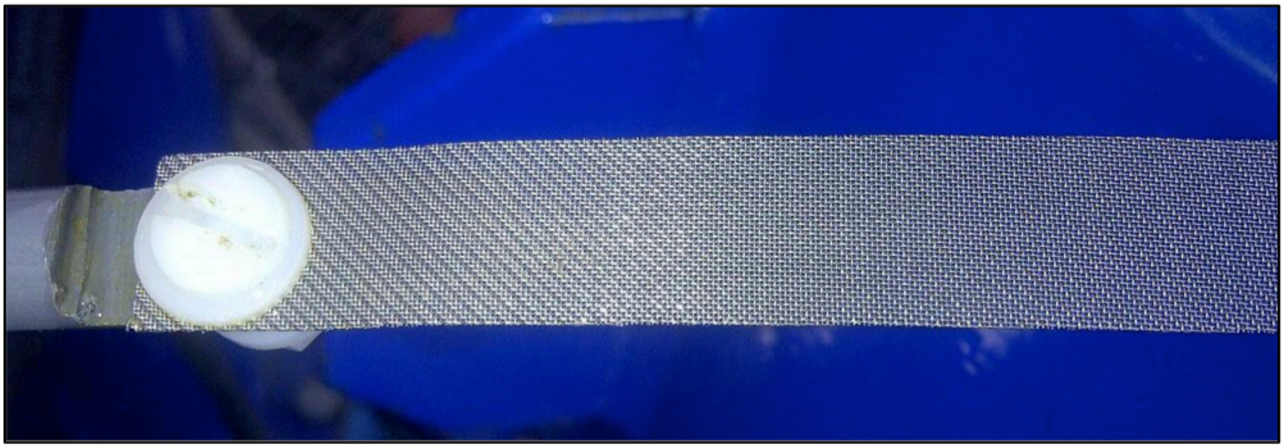

Figure 6. Exposed stainless steel wire mesh biofilm coupon before ultrasonic cleaning (3x magnification)

The cfu/cm^2 levels found with both flat and mesh coupons were extremely low after exposure for 30 days. This would mean that there was hardly any biofilm in the cooling water equipment and thus would indicate that this system is under excellent microbiological control.

All of the stainless-steel coupon results showed no detectable *Legionella* bacteria indicating that this cooling tower water system was safe and very acceptable.

CONCLUSION

This study has brought us to the conclusion that the industry standard measurement of 10^4 or less cfu/ml for planktonic monitoring is too high and should be lowered. This level of total bacteria present in the water certainly indicates that a biofilm that could contain Legionella is present in the system. This study indicates that a field-friendly procedure such as that provided in this study should be adopted for the measurement of biofilm.

Perhaps the use of the industry standard for acceptable HPC levels has given the operator and owner of the cooling tower water system a false sense of security. The current standard of 10^4 infer that microbiological problems will not occur or even that *Legionella* bacteria is absent or at low levels when the opposite is occurring.

Changing total planktonic bacteria monitoring to include the detection of a biofilm could help reduce the number of Legionnaires Disease outbreaks from cooling tower water systems.

Biofilm is where the *Legionella* bacteria and other corrosion causing bacteria exist. Biofilm measurement is a much better indication of their presence. The use of a stainless-steel wire mesh coupon is an excellent monitoring method to determine if a biofilm is present. Qualitative evaluation is better applied to the biofilm; if it is sufficiently low that bio-control is acceptable, or if it is too high and the microbiological control needs to be improved. Biofilm monitoring should be adopted to provide a more accurate measurement of microbiological organisms that are present in cooling tower systems. Adoption and consistent application of this monitoring method would be of great benefit to the cooling water industry and would help prevent Legionnaires' Disease from cooling tower water systems.

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