Global Journal of Medical Research: K
Interdisciplinary
Volume 15 Issue 4 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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Keywords: *legionella sp.; in-situ formed monochlorammine; industrial cooling tower.*

GJMR-K Classification: *NLMC Code: QW 50*

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Abstract- The aim of the study was to determine the effectiveness of in-situ-formed monochloramine for the control of *Legionella* growth in a real cooling tower system. The cooling tower utilizes an industrial water (makeup) made of by blending industrial raw water and decarbonated raw water. A generator device injecting in-situ-formed monochloramine into the cooling system was installed. The detection of *Legionella,* heterotrophic plate count (HPC - 22°C) and continuous monitoring of oxidation reduction potential (ORP) were carried out to check the efficacy of monochloramine. Water samples were monthly collected for 6 months from the makeup, two collecting basins and the blow-down. Monochloramine was injected at 4 mg/L for 60 days to achieve a mean residual concentration of 0.145 mg/L in the blow-down. Over time, at the same pumping rates, a greater monochloramine residual was achieved so the dosage was decreased at 2.5 mg/L. *L. pneumophila* (SG 7-14) decreased from an initial load >10.000 cfu/L to undetectable levels and remained stable till the end of the experimental period while HPC decreased slowly (mean count <10.000 cfu/ml) after the first 60 days. From an initial value of 300-400 mV during the first 60 days, the ORP dropped to around 150 mV, which rather explains why monochloramine residuals in the blow-down increased at the end of the experimental time. The results indicate that in-situ-formed monochloramine can control *Legionella* and HPC growth in industrial cooling towers using industrial raw water and decarbonated raw water. Moreover, the ORP can be useful for monitoring the effectiveness of monochloramine.

Keywords: legionella sp.; in-situ formed monochlorammine; industrial cooling tower.

I. Introduction

Cooling towers have been consistently attributed to community-acquired legionellosis outbreaks (Engelhart et al. 2007; Yu 2008) because *Legionella* emitted by contaminated cooling towers can be transported over several kilometers within respirable vesicles (Walser et al. 2013). Typical operating conditions maintained in a cooling system, such as temperature range, pH as well as continuous airflow, make it ideal for biological growth or biofouling. In cooling towers biofouling can interfere with normal system operation (for example, heat transfer resistance) and it can also enhance corrosion and scaling problems, which may be critical risk factors for *Legionella* growth together with stagnant water and nutrient availability in the makeup water (Ludensky 2005). Thus, in order to minimize the production of contaminated aerosols by *Legionella* it is necessary to minimize microbial multiplication.

Due to its low cost and high effectiveness, chlorine is the most used biocide as antifouling agent in cooling towers (Nebot et al. 2007; Rubio et al. 2015). However, it is highly reactive with natural organic matter contained in water - especially when the cooling tower utilizes wastewater as makeup water (Hsieh et al. 2010; Li et al. 2011a) - leading to the formation of by-products such as trihalomethanes or haloaceto-nitriles which can have adverse impact on human health and ecosystems (Schwarzenbach et al. 2006; Nieuwenhuijsen et al. 2000).

In cooling water bromine is less effective than chlorine against *L. pneumophila*. Nonetheless, with continuous or semi-continuous low level dosing it can reduce and control *Legionella* populations as long as the residual concentrations are maintained in the circulating water (Thomas et al. 1999). Bromine is also used for the oxidation of biofouling but unfortunately it is toxic for the environment, in which it is released via blow-down water (Meesters et al. 2003).

Among the oxidizing agents, monochloramine seems to be the most materials respectful and to produce the lowest concentration of by-products in drinking water (Melada et al. 2015). It is also more effective for decreasing *Legionella* within the biofilms in drinking water systems (Coniglio et al. 2015) and even at concentrations as low as 1 ppm it is able to penetrate complex biofilm matrixes like that in cooling towers (Turetgen 2004; van Schalkwyk et al. 2010). It has been demonstrated that in the presence of organic nitrogen compounds, like in cooling systems employing secondary-treated municipal wastewater as makeup water, adding pre-formed monochloramine to reduce biological growth of *Legionella* species is better than forming monochloramine in-situ (Chien et al. 2012). Moreover, pre-formed monochloramine was found to be

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less aggressive than free chlorine, while still being an effective biocide (Li et al. 2011b).

Thus, as far as we know, at the moment there are few studies on the effectiveness against *Legionella* of in-situ-formed monochloramine in cooling systems. For this reason, in the present study the effectiveness of in-situ formed monochloramine was evaluated for its ability to control biological growth in a cooling system.

II. Materials and Methods

A cooling tower system of an Italian industrial plant was considered. The cooling tower system studied has a flow rate of 270-300 m^3/h of recirculating cooling water, an average of 6-10 m^3/h of makeup water and a water evaporation of about 4 m^3/h. The water lost by evaporation is reintegrated with an industrial water (makeup water) made of by blending industrial raw water and decarbonated raw water. A drawing of the cooling system is depicted in Figure 1.

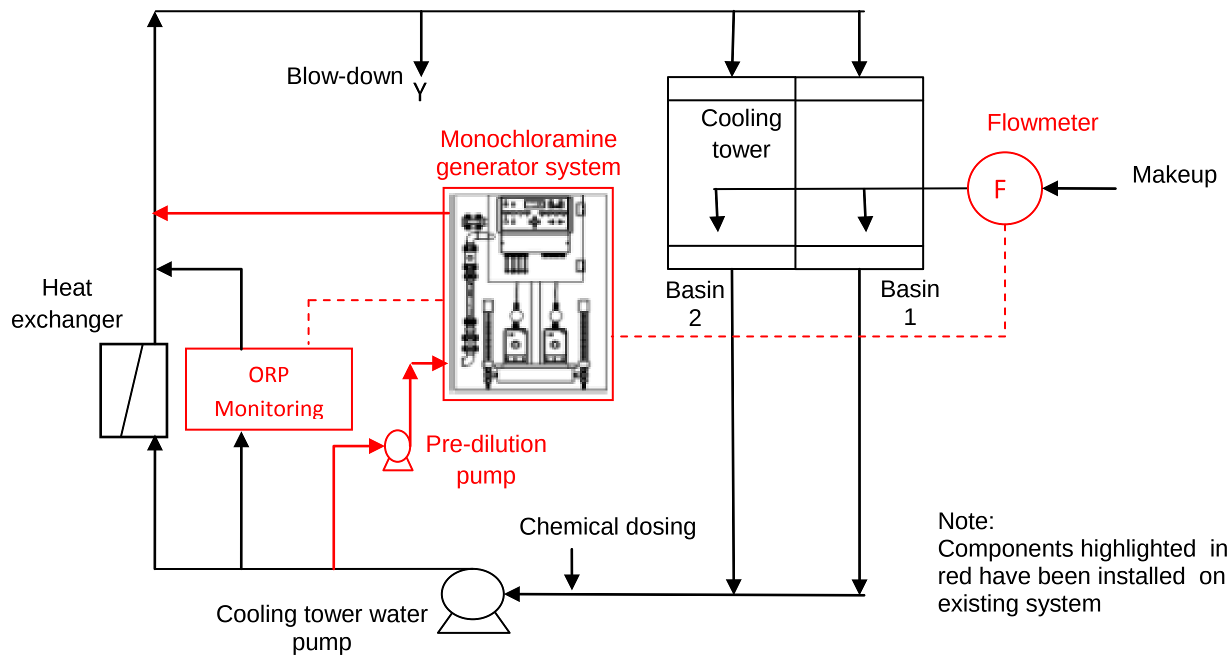

Figure 1: A drawing of the cooling system

Prior to the application of monochloramine, to minimize biofouling, the water of the cooling circuit was successfully decontaminated via continuous disinfection with bromine at low levels (assuring a residual concentration of free chlorine of ~ 0.1 ppm) added with a scale and corrosion inhibitor. To prevent legionellae growth additional periodic chlorine shock was made with 5 ppm free chlorine for 5h.

In order to verify the effectiveness of in-situ formed monochloramine for the control of *Legionella* growth in the cooling tower considered for the study, on July 2014 the injection of bromine was stopped and water samples were collected every 15 days till the detection of *Legionella* (> 100 cfu/L). When, on September 2014, *L. pneumophila* SG7-14 was recovered in the cooling tower (makeup water, the two collecting basins and the blow-down) at high concentrations (>10.000 cfu/L), a patented generator device continuously injecting in-situ-formed monochloramine into the cooling system was installed. A regular testing program for the detection of *Legionella* and heterotrophic plate count (HPC) at 22°C was carried out to check the efficacy of the water treatment with in-situ formed monochloramine.

The makeup water and water samples taken from the cooling tower collecting basins and the blow-down were collected weekly from September 2014 to March 2015. At the time of each sampling, the cooling system was operating. As shown on Table 1, for the makeup water and the blow-down physical and chemical parameters (DPD Free and Monochlor-F methods, Nitriver and Nitraver, HACH Company, USA) were determined. For *Legionella* determinations, 1L water samples were collected, taking care to gently resuspend the fluffy sediment at the bottom of the collecting basins. Isolation of *Legionella* was performed in accordance with standards procedures ISO 11731 (International Standard Organization 1988). Colonies suggestive for *Legionella* were confirmed on the basis of cultural testing and serogrouped by slide agglutination using commercial antisera (Oxoid and Biogenetics). Results were expressed in cfu/L and the counts referred to water samples concentrated 100 times (1L in 10 ml of the water sample). The detection limit of the culture procedure was 10 cfu/L. The HPC at 22°C was detected in accordance with ISO 6222 (International Standard Organization 1988) to provide information on the disinfection process with monochloramine. For HPC, 100 ml of water were sampled. Sampling bottles for *Legionella* and HPC were steam sterilized and contained sufficient sodium thiosulfate to neutralize chlorine. All samples were stored at 4°C and analyzed within 12 h.

III. Results and Discussion

After the start of the cooling water in-situ monochloramination, a total of 32 water samples were monthly collected for 6 months from the same sites of the cooling tower: the makeup, the two collecting basins (named BASIN 1 and BASIN 2, respectively) and the blow-down.

An interesting phenomenon was observed when the response of monochloramine residuals was tracked over time. As shown on Table 1, at the beginning of the experimental period monochloramine had to be injected into the cooling water system at an average concentration of 4 mg/L for about 60 days in order to achieve a mean residual concentration of 0.145 mg/L in the blow-down. Over time, at the same pumping rates, a greater monochloramine residual could be achieved. For this reason, subsequently the dosage was regulated to obtain a continuous concentration of 2.5 mg/L monochloramine. We attributed this increase in residual to at least two main reasons: *i.* the monochloramine biocide effect towards *Legionella*, and *ii.* the monochloramine reaction with biofilm constituents that probably bromine could not get to.

Table 1: Physical and chemical parameters of the makeup water and the blow-down

	Makeup water	Blow-down				
	Nov 2014	Nov 14	Dec 14	Jan 15	Feb 15	Mar 15
Free chlorine (mg/l)	0.02	0.04	0.04	0.02	0.03	0.09
Total residual chlorine (mg/l)	0.03	0.33	0.11			
Monochloramine (mg/l)	0.00	0.35	0.23	0.17	0.11	0.38
Ammomium ion (mg/l)	0.00	0.08	0.13	0.09	0.06	0.08
Nitrite (mg/l)	0.011	0.121	0.234	0.066	0.138	0.217
Nitrate (mg/l)	15.4	26.6	100.8	21.3	26.8	31
Chloride (mg/l)				390	320	
pH	7.4	7.5		7.8	7.7	7.8
Conductivity (µS)	890	1727		1870	1790	
ORP* (mV)			174	180	160	152

**oxidation reduction potential*

The biocide effect of monochloramine towards *Legionella* and HPC is shown on Figure 2. At the beginning of the disinfection program, one week after the continuous injection of monochloramine, the load of *L. pneumophila* SG7-14 decreased at undetectable levels, with the exception of the makeup water, where *Legionella* was recovered at a concentration of 200 cfu/L.

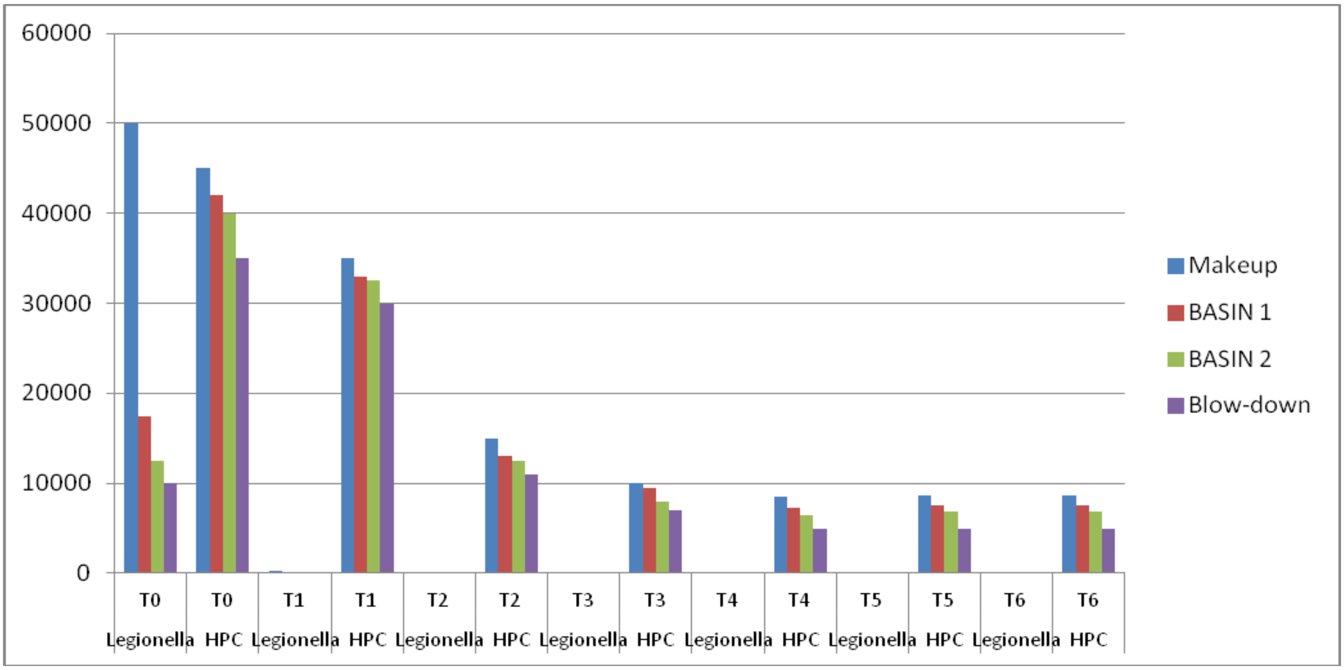

Figure 2 : The biocide effect of monochloramine towards *Legionella* and HPC

Further analyses showed that in the makeup water entering the cooling system *Legionella* was absent, while the bacterium was isolated by means of swabs in the tap from where the makeup water had been collected, showing that it was a local contamination instead of makeup water contamination. In fact, two weeks after the sanitization of the tap and the monochloramination of the entire cooling system, the load of *Legionella* decreased at undetectable levels also in the sampling site of the makeup water and remained stable in all the sampling sites till the end of the experimental period. On the contrary, during the entire experimental period the HPC load decreased slowly, with a mean count ranging lower than 10.000 cfu/ml after the first 60 days.

The interaction between monochloramine and the biofilm is probably confirmed by the minimal algae growth on the partially wetted structure of the cooling tower at the end of the experimental time. In fact, to get an idea of how well algae growth was being controlled, the cooling tower was visually inspected three times during the experimental time. As shown on Photographs 2 and 3, two months after the start of the disinfection program (Photograph 2) with monochloramine and at the end of the experimental period (Photograph 3) small amount of green algae growth was observed on collecting basins, much less than when bromine was used (Photograph 1).

Photograph 1 : Visual inspection of the collecting basin - September 2014

Photograph 2 : Visual inspection of the collecting basin - November 2014

Photograph 3 : Visual inspection of the collecting basin - January 2015

Finally, during the entire experimental period, makeup water flowmeter was used to control monochloramine dosage whereas continuous monitoring of oxidation reduction potential (ORP) was used to check it. The plot in Figure 3 shows the response of ORP with time during the entire experimental period. The monochloramine field trial and the continuous monitoring of ORP started on September 18th, 2014. On November 13rd, the probe was found incorrectly calibrated but it was recalibrated on November 20th. Anyway, from an initial value of 300-400 mV during the first 60 days, the ORP dropped to around 150 mV, which rather explains why monochloramine residuals in the blow-down increased at the end of the experimental time.

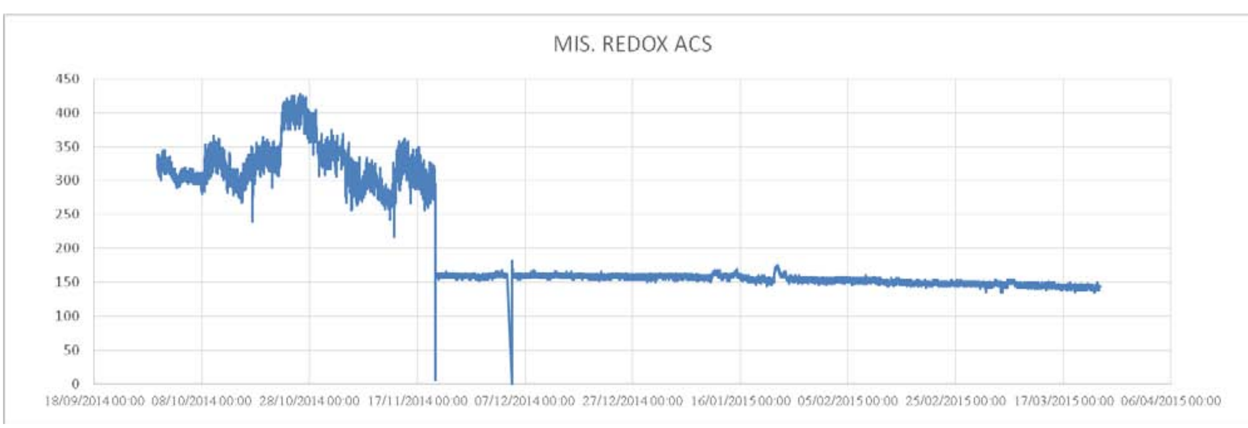

Figure 3 : The response of ORP with time during the entire experimental period

IV. Conclusions

The primary objective of this study was to investigate the effectiveness of in-situ-formed monochloramine as a biocide to control *Legionella* and HPC growth in a cooling tower system employing treated industrial water as makeup water. In particular, the HPC and continuous monitoring of ORP were determined to provide information on the disinfection process with monochloramine.

One week after the continuous injection of monochloramine, the load of *L. pneumophila* SG7-14 decreased at undetectable levels from an initial value >10.000 cfu/L and remained stable in all the sampling sites till the end of the experimental period. This data probably show that in-situ formed monochloramine can adequately control *Legionella* growth in industrial cooling towers using blended industrial raw water as makeup water. Nonetheless, the high load of HPC observed during the first 60 days, when residual concentrations of monochloramine were low and in turn ORP was high, probably suggests that at the beginning of the disinfection program some bacteria may require higher concentrations of the disinfectant to be completely killed. Anyway, our data suggest that ORP values and HPC measurements can play an important role in verification of treatment cooling tower procedures with monochloramine.

Finally, our data show that monochloramine was effective also at controlling algae growth. In fact, comparison of visual inspections of the collecting basins (Photographs 1-3) showed a decrease in green algae growth at the end of the experimental period. This could also explain why as time passed the residual monochloramine concentration that could be achieved in the blow-down increased.

Conflict of interest statement

Dr Stefano Melada, responsible for the Research & Development department of Sanipur Srl, contributed with a scientific and technical support necessary for the development of the management plan. He did not participate to the samplings of the water, isolation and identification of legionellae. No financial support was given to the authors.

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