

EFFICACY OF MONOCHLORAMINE AGAINST SURFACE-ASSOCIATED *LEGIONELLA PNEUMOPHILA* IN COOLING TOWER MODEL SYSTEM

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ABSTRACT

Legionella pneumophila is commonly found in cooling towers and biofilm is a preferred site for growth of *L. pneumophila*. Previous studies have shown a number of Legionnaires' disease outbreaks that originate from cooling towers. Therefore, routine monitoring and disinfections for Legionella has proven to be an important strategy in prevention. Previous works indicate that the ability of monochloramine to penetrate the biofilm matrix is higher than that of free chlorine, even at high pH values. In this study, the efficacy of monochloramine was tested against surface-associated *L. pneumophila* on different surfaces. Three log reductions were recorded on PVC, PE, copper and stainless steel surfaces, whereas slight reduction of *L. pneumophila* was observed on PP and galvanized steel surfaces.

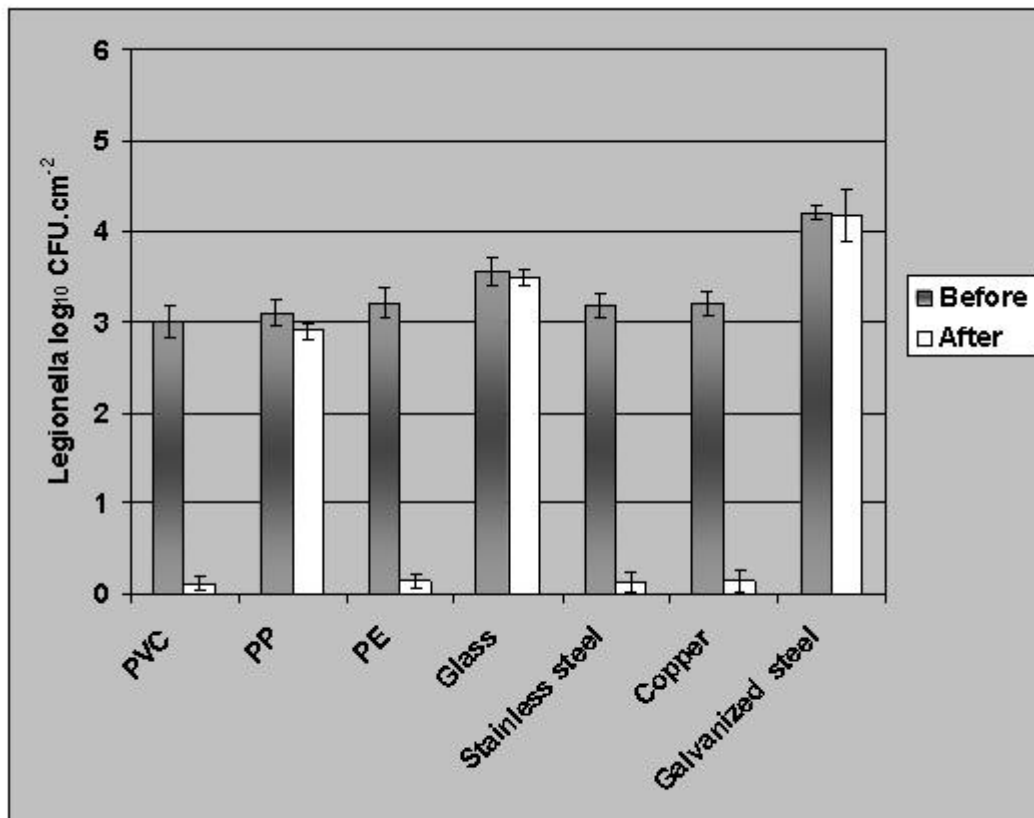
INTRODUCTION

Cooling towers have the potential to develop infectious concentrations of *Legionella pneumophila*, the bacteria responsible for causing Legionnaires' disease (2, 7, 8). Legionella counts increases where biofilm and warm water are present. Therefore, biocides are employed to reduce the potential for the development of biofilm on equipment surfaces (4, 6). Chlorine has been used as a popular disinfectant for controlling bacterial growth in drinking water and cooling towers (5), but monochloramine was found more stable than free chlorine, even at high temperatures and elevated pH values. Moreover, it exhibits greater biofilm penetration, lower disinfection by-products and is less corrosive than free chlorine (3). A further disadvantage of free chlorine is its reaction with natural organic matter to form trihalomethanes, which are considered to be carcinogens. Chloramines do not form these products to the same degree. The previous study of the authors showed that disinfection of biofilms with 1.5 ppm monochloramine for 180 min resulted in 3 log reductions of heterotrophic bacteria in cooling towers water (6). In this study, the efficacy of monochloramine against biofilms formed in model recirculating water system was compared on different surfaces under identical conditions.

METHODS

A lab-scale cooling tower was designed to simulate a wet evaporative cooling system, which was experimentally seeded with *L. pneumophila* (Fig. 3). At the beginning of the experiment, recirculating system was experimentally infected with standard strain (ATCC 33152) suspension (1 ml *L. pneumophila* inoculum of 10⁵ cell.ml⁻¹) and operated continuously until all experiments had been completed. Throughout the experiment, the water temperature was kept constant at 30°C. Rather low final inoculum was provided to the model system to mimic the natural entry of LP from supply water. Blowdown optimization was conducted by measuring total dissolved solids of recirculating water. Coupons were representing the fill material, which cooling water is generally distributed on them. All the materials are in rigid form, certified and commercially available. Biofilm had been allowed to grow on coupons and model system was disinfected after 180-day period with

monochloramine. A residual of 1.5 ppm was maintained for 180 minutes, and then neutralized with sodium thiosulphate. Copper, stainless steel, galvanized steel, polyvinyl chloride, polyethylene (PE), polypropylene (PP) and glass (as control) were tested regarding heterotrophic bacteria and *L. pneumophila* counts on their surfaces. For heterotrophic plate count (HPC), 10-fold diluted biofilm homogenates and bulk water were spread-plated (0.1 ml) onto R2A agar plates and incubated at 28°C for 10 days. HPC determinations were performed by triplicate analyses. For the enumeration of *L. pneumophila*, biofilm homogenate was treated with acid solution for 15 min (KCl-HCl solution, pH 2.2) to reduce overgrowth of commensal flora. Pre-treated and untreated samples were inoculated (0.1 ml) onto alpha ketoglutarate-supplemented buffered charcoal-yeast extract. Analyses were performed in triplicate. Phenol-sulfuric acid method was used to quantify the extracellular polymeric substances on substrata. To detect the entry of wild *L. pneumophila* bacterium from supply water, several isolates were taken from different sampling times and compared using RAPD-PCR fingerprinting analysis (1).



The *L. pneumophila* counts on surfaces before and after disinfection.

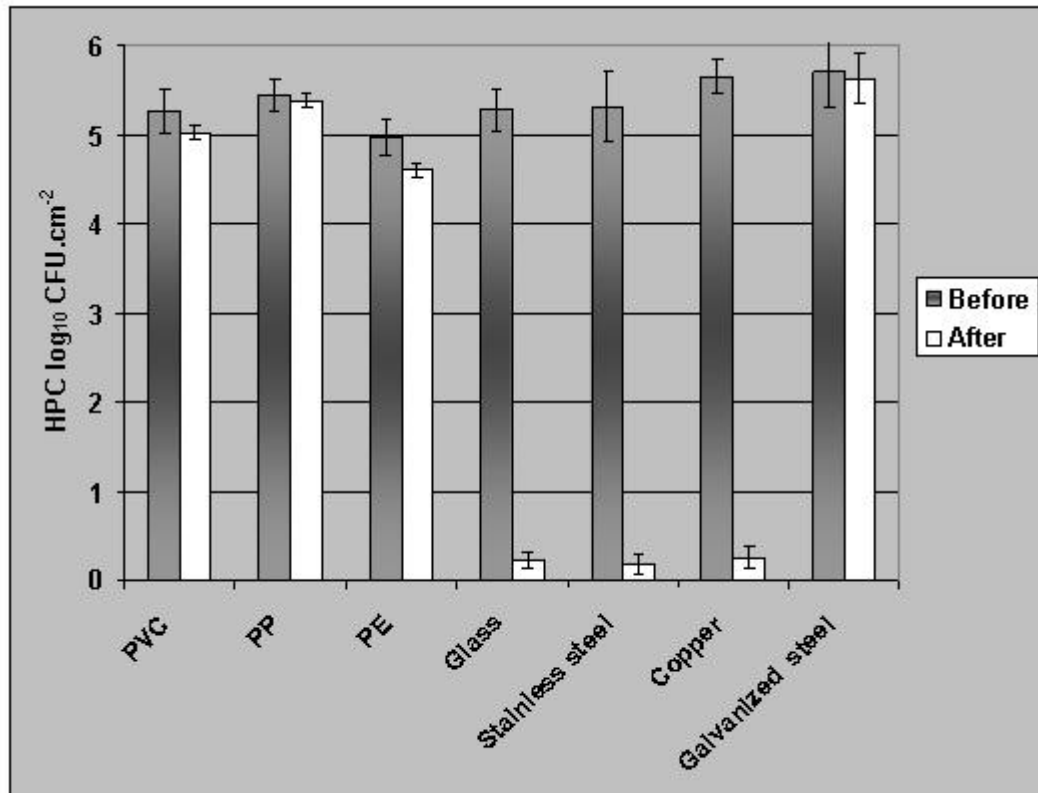


Fig. 2. The HPC counts on surfaces before and after disinfection.

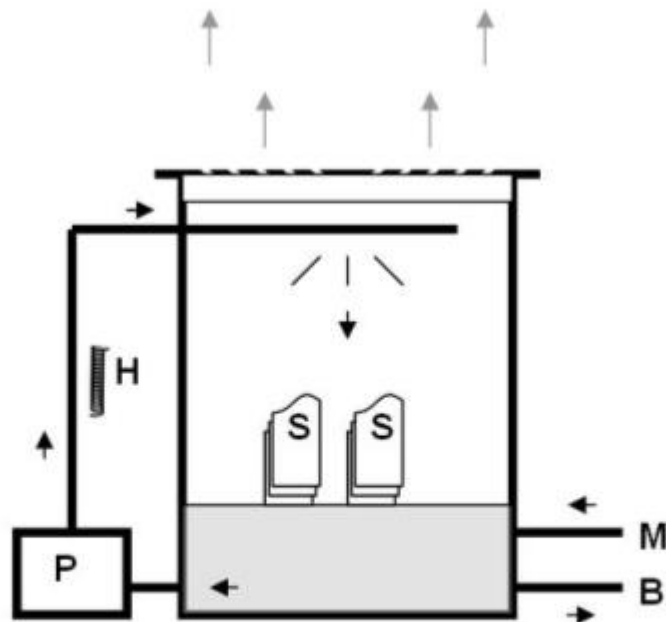


Fig. 3. Schematic diagram of model recirculating water system, arrows indicate the flow direction. P: pump, S: surfaces, H: heater, M: make-up water inlet, B: blowdown outlet.

RESULTS

Before disinfection, significantly high heterotrophic bacteria were observed on galvanized steel (Fig. 2). Low numbers of *L. pneumophila* were present on PVC and PP. The bacterial densities in biofilms from different materials after disinfection (180 min - 1.5 ppm dose) suggested a material-dependent activity of monochloramine. Four log reductions of heterotrophic bacteria were recorded on glass, copper and stainless steel surfaces (Fig. 2). Three log reductions were recorded on PVC, PE, copper and stainless steel surfaces, whereas slight reduction of *L. pneumophila* was observed on PP, glass and galvanized steel surfaces (Fig. 1). Monochloramine was found ineffective against microorganisms on PP surfaces. Four log reductions were recorded against planktonic heterotrophs and *L. pneumophila* in bulk water. The 180-d period was run in duplicate, no significant differences found between two periods in regard to bacterial accumulation on surfaces and disinfection efficacies. No entry of wild *L. pneumophila* from make-up water was detected.

CONCLUSIONS

Results indicated that monochloramine shows material-dependent activity in cooling tower model system and it has long residual activity in high temperature and pH levels, leading to improved performance in recirculating water. Material-dependent activity could be explained by the formation of biofilms on different surfaces, which affects the architecture of biofilm matrix. Choosing the appropriate material will reduce populations of pathogens thereby reducing the risk of associated illnesses. Chemical disinfection is strongly recommended from the beginning of the tower operation to prevent or reduce the occurrence of Legionnaires' disease.

REFERENCES

1. BANSAL, N.S., MCDONELL, F., 1997, Identification and DNA fingerprinting of Legionella strains by randomly amplified polymorphic DNA analysis, **J Clin Microbiol**, 35, 2310-2314.
2. BENTHAM, R. H., 2000, Routine sampling and the control of *Legionella* spp. in cooling tower water systems, **Curr Microbiol**, 41, 271-275.
3. CAMPOS, C., LORET, J.F., COOPER, A.J., KELLY, R.F., 2003, Disinfection of domestic water systems for *Legionella pneumophila*. **J Wat Sup** 52, 341-354.
4. DONLAN, R., MURGA, R., CARPENTER, J., BROWN, E., BESSER, R., FIELDS, B., 2002, Monochloramine disinfection of biofilm-associated *Legionella pneumophila* in a potable water model system. In : Marre R, Kwai Y A, Fields B (eds) **Legionella** ASM Press, Washington DC, pp 406-410.
5. FLIERMANS, C.B., BETTINGER, G.E., FYNSK, A.W., 1982, Treatment of cooling tower systems containing high levels of *Legionella pneumophila*, **Wat Res**, 16, 903-909.
6. TÜRETGEN, I., 2004, Comparison of free residual chlorine and monochloramine for efficacy against biofilms in model and full scale cooling towers, **Biofouling**, 20, 81-85.
7. TÜRETGEN, I., SUNGUR, E.İ., ÇOTUK, A., 2005, Enumeration of *Legionella pneumophila* in cooling tower water systems, **Environ Monitor Assess**, 100 (1-3) 53-58.
8. YAMAMOTO, H., SUGIURA, M., KUSUNOKI, E., EZAKI, T., IKEDO, M. YABUUCHI, E., 1992, Factors stimulating propagation of legionellae in cooling tower water, **Appl Environ Microbiol**, 58, 1394-1397.