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SHORT COMMUNICATION

Eur J Biol 2020; 79(1): 62-66

Comparison of the Anti-Legionella Fill Material against Standard Polypropylene Fill Material in Model Cooling Tower Water System

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Please cite this article as: Turetgen I, Vatansever C. Comparison of the Anti-Legionella Fill Material against Standard Polypropylene Fill Material in Model Cooling Tower Water System. Eur J Biol 2020; 79(1): 62-66. DOI: 10.26650/EurJBiol.2020.0004

ABSTRACT

Objective: Cooling towers are heat exchangers which are utilized in specific industrial devices. They possess the potential to support Legionella bacteria. The objective was to evaluate the efficacy of biocide impregnated polymer against regular polypropylene polymer in terms of anti-Legionella features during a 120-day period.

Materials and Methods: To reduce the bacterial colonization in towers, anti-Legionella splash fill and regular polypropylene splash fill material were tested to compare anti-Legionella activity and biofilm formation potential within a 120-day period using a lab-scale recirculating cooling tower model system. The system was experimentally infected with Legionella suspension and operated continuously for 120 days.

Results: Legionella colonization occurred on both test material surfaces beginning at the first month. Legionella counts on surfaces were increased over time on standard polypropylene surfaces. The product with anti-Legionella activity showed significantly lower Legionella colonization in comparison to standard polypropylene fill.

Conclusion: The product with anti-Legionella activity has a significant biocidal effect against surface-associated Legionella under the above-mentioned conditions which mimics cooling tower water systems. Product seems to facilitate effective control program criteria against Legionella colonization in cooling towers.

Keywords: Legionella, cooling tower, fill material, biofilms, *Legionella pneumophila*

INTRODUCTION

Wet type cooling towers are heat exchangers that allow water and air to come in contact to decrease the temperature of the circulating water and they provide an ideal niche for bacteria to grow and colonize (1,2). One of the best known water pathogen in cooling systems is Legionella bacterium, which causes Legionnaires' disease. The ever first Legionella outbreak in 1976 led to the recognition of a new genus, responsible for both Pontiac fever and Legionnaires' disease. Legionella can flourish in cooling tower water and spread to humans when expelled aerosols containing the bacteria is inhaled (3,4). Mostly, Legionnaires' disease outbreaks were sourced from wet cooling towers. The recent deadly Legionnaires' disease outbreak occurred in Brescia, Italy in 2018, where 42 people contracted the bacteria, two of whom died (5-7). Infections caused by Legionella are the leading cause of waterborne disease outbreaks.

The formation of a robust biofilm on a man-made distribution system can be detrimental (8). It is generally accepted that biofilms have a crucial role in the survival of Legionella bacteria within man-made water systems (9). Biofilm layers are suitable environments for pathogens microorganisms and harbors bacteria that could damage the material (10-12). Recently, microbiological

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Submitted: 10.01.2020 • **Revision Requested:** 11.03.2020 • **Last Revision Received:** 06.05.2020 • **Accepted:** 11.05.2020

contamination in industrial and domestic water systems has been a growing problem for many years. From the water source to the industrial water systems, the bulk water passes through kilometers of pipes (13). At any point in this distribution system, the microorganisms involved in the water are transported together with the water flow and multiply by adhering to the surfaces of the man-made water systems (14,15). Biofilm layers in industrial cooling systems develop mostly on fill material, as temperatures there support the fast multiplication of the genus Legionella (16). The polymer surface is always at risk of becoming colonized with harmful bacteria and, therefore, it is a hazard to humans because of the potential for it to indirectly pass on these disease-causing microorganisms.

Ideally, preventing biofilm formation and Legionella colonization would be a more logical option than cleaning or treating it. The appearance of biofilm associated problems plainly shows that new anti-biofilm and Legionella control procedures are required. However, there is currently no better technique or procedure that is able to completely eradicate the unwanted biofilms (17,18). Several procedures to reduce the colonization of microbial biofilms have been studied over the years, with diverse degrees of success (19). In various industrial set-ups, toxic metals or a spectrum of biocides have been used for sanitizing purposes and biofilm reduction. However, a single strategy may not be enough for comprehensive prevention. Evidence has shown that environmental surfaces play an important role in the transmission of nosocomial pathogens like Legionella. Extensive research has focused on mitigating the development of biofilms at the water-solid interface (2,19-21).

The general technical performances of the cooling towers are mainly influenced by the functional characteristics of the cooling tower fill used in the cooling systems. The objective of the recent study was to evaluate the efficacy of biocide impregnated polymer against regular polypropylene polymer in terms of anti-Legionella features during a 120-day period. Targets have achieved enhanced cooling performance, lengthened the material life, reduced clogging and limited the growth of biofilm associated bacteria. The study was implemented using a recirculating water system under persistent hydraulic shear stress, which corresponds with the conditions in real life man-made water systems.

MATERIALS AND METHODS

The experiment was completed using a laboratory scale recirculating water system (150 l water capacity). Distributed municipality water was added to complete the water lost by evaporation and blowdown. Control and test surfaces were not immersed; they were only in contact with spray water. All test surfaces were secured with suspenders over the water surface without any touch to each other (Figure 1). During the study, the temperature of the water was set stable at 37°C, an average of general cooling tower bulk water temperature. Constant temperature also eliminates the probable effects on biofilm formation.

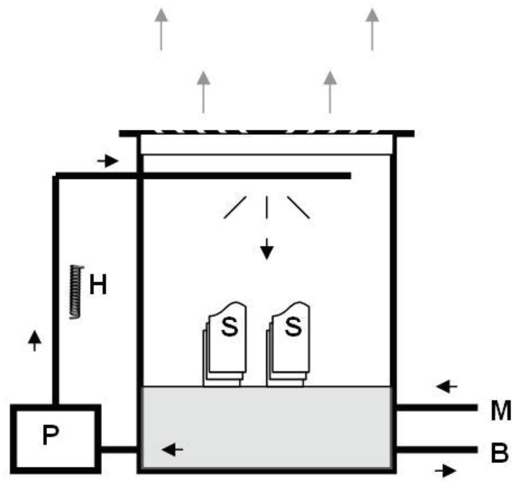

Figure 1. Schematic view of the lab-scale water system, black arrows indicate water flow, gray arrows indicate evaporation. S: surfaces, P: pump, B: blowdown outlet, M: make-up water inlet, H: heater.

At the beginning of the setup, bulk water was seeded with standard strain suspension containing *Legionella pneumophila* ATCC 33152 (1 ml of *L. pneumophila* inoculum at 10^5 cells/ml) and operated for 120-days. No chemicals were supplemented to the water, to exclude their adverse effects on natural biofilm formation and bacteria.

The test objects were a hybrid (mix) type splash fill, adapted to the technological requirements of any wet cooling tower (Figure 2). Anti-Legionella fills were factory produced and the anti-Legionella feature of the chlorine based agents was immobilized during production. Unlike classic sheet fill, the splash fill is very efficient, practically invulnerable at impurity clogging, resistant to the influence of physical and chemical factors. The splash fill consists of individual elements with network appearance from injected polypropylene with optimized apertures and plies which generate both drops and films in its volume.

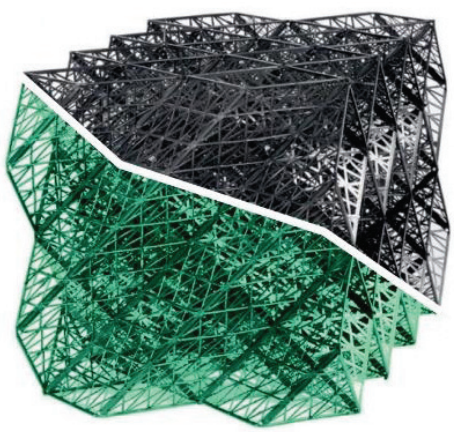

Figure 2. Cooling tower splash fill. Green one is the product with anti-Legionella features.

Surface sections of the two test materials were removed monthly from the model system, gently washed by sterile tap water to remove planktonic cells. Samples on preset locations were removed by sterile lancet, suspended in phosphate buffer saline and vortexed slowly for 1 min. For heterotrophic bacterial plate count (HPC) determination, 10-fold diluted biofilm and bulk water samples were spreaded (0.1 ml) onto R2A agar (OXOID, UK) containing Petri dishes and kept at 28°C for 10 days. As an ideal tool to cultivate heterotrophic bacterial numbers in oligotrophic waters, R2A plates incubated at 28°C for 10 days were recommended by Reasoner and Geldreich (22). All platings were done by triplicate analyses. To culture Legionella bacteria, biofilm samples were pre-treated with acid solution for 15 minutes (KCl-HCl solution, pH 2.2) to decrease the growth of accompanying co-flora. Pre-treated and untreated samples (0.1 ml) were inoculated onto BCYE agar (OXOID, UK) containing selective supplement and incubated at 37°C for 10 days. Colonies similar to Legionella morphology were subcultured to blood agar and BCYE agar plates. Final identification was completed using Legionella Latex Agglutination Kit (OXOID, UK) (Dennis, 1988).

Bacterial numbers were $\log_{10}$ converted and standard errors of the means were calculated by software. Differences between numbers were tested for significance using a t-test; differences were accepted significantly different at $P < 0.05$. Statistical analyses were performed using SPSS 21.0 software.

RESULTS AND DISCUSSION

Following successful seeding of Legionella bacteria, they were rapidly adhered to surfaces, colonized and increased up to 400 CFU/cm^2 (CFU: Colony Forming Unit) on surfaces at the end of the first 30-days. Legionella bacteria have maintained their cultivation and viability throughout the test period on all surfaces and also in bulk water. The heterotrophic bacterial numbers showed a similar growing trend during the test period. The heterotrophic bacteria in bulk water were also adhered to the test surfaces and colonized rapidly until day 120.

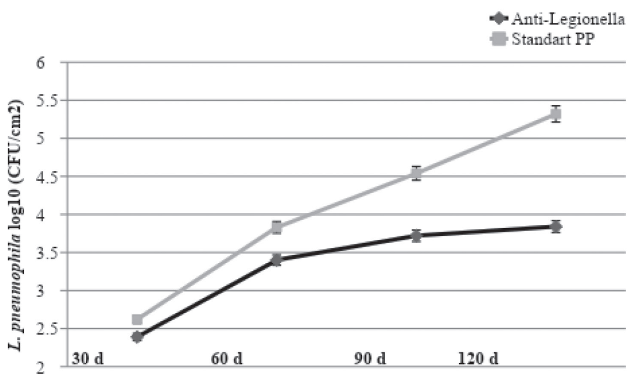

Figure 3. *L. pneumophila* numbers on surfaces during 120 days. Error bars represent standard error. *$P < 0.05$ compared with Anti-Legionella group.

Depending on the *L. pneumophila* culture results, Legionella colonization was monitored on both test material surfaces beginning at the first month of the experimental period (Figure 3). Legionella counts on surfaces were increased gradually over time. Statistically, a significant difference was found between two test materials in terms of Legionella growth on surfaces. The green colored product with anti-Legionella activity showed significantly lower Legionella colonization in comparison to standard polypropylene splash fill after completion of the 120-day test period ($P < 0.05$). Legionella counts on standard polypropylene surfaces increased gradually from 416 CFU/cm^2 to 208.929 CFU/cm^2 within the 120-day period. The highest Legionella count on anti-Legionella surfaces was recorded as 7.244 CFU/cm^2. The 2 log reduction on anti-Legionella test surfaces was statistically significant.

Furthermore, the fill material with anti-Legionella activity showed also significantly lower heterotrophic bacterial colonization in comparison to standard control group polypropylene fill after 120-day test period ($P < 0.05$) (Figure 4). Heterotrophic bacterial counts on standard PP surfaces increased gradually from 107.151 CFU/cm^2 to 2.754.228 CFU/cm^2 within the 4-month period. The highest heterotrophic bacterial count on anti-Legionella surfaces was recorded as 218.776 CFU/cm^2, where the value was one log smaller than on standard polypropylene fill surfaces.

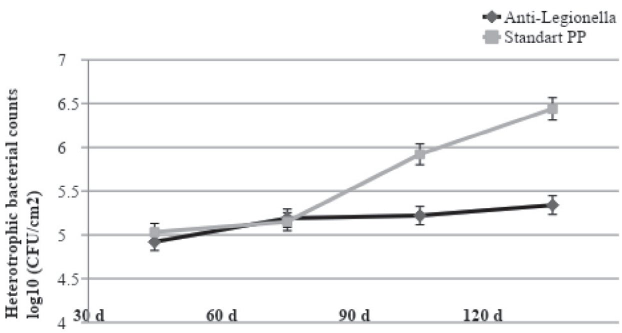

Figure 4. Heterotrophic bacterial numbers on surfaces during 120 days. Error bars represent standard error. *$P < 0.05$ compared with Anti-Legionella group.

The dissolved oxygen amount of the water circulation system was measured monthly with the oximeter (WTW-Oxi 330, Germany). The device has been calibrated before each use. Also the bulk water pH value was measured (KCP Handheld Digital pHmeter, China) and recorded every month before samples were taken (16). Despite the water blowdown regime (added daily ~3 liters of fresh network water), the dissolved oxygen amount and the pH values in the water phase were slightly increased throughout the experiment. Blowdown (make-up) was regularly done to avoid concentration of total dissolved solids in the system water. The dissolved oxygen amount and pH value raised gradually from 6.55 mg/l to 7.98 mg/l and from 6.52 to 6.96, respectively. Some selected make-up water parameters at the local water company are given in Table 1.

Table 1. Average water quality parameters at the municipal water company (units in mg/l except of turbidity and pH values).

Parameter	Value
pH	6.67
Turbidity (NTU)	0.66
Hardness - $CaCO_3$	73
Na	18.1
Ca	27
Mg	4.44
K	2.77
Fe	0.044
Mn	0.010
SO_4	41
F	0.06
NO_2	2.92
Zn	0.011
NH_4	0.05

Antimicrobial polymers are designed to inhibit the growth of bacterial biofilms on a variety of surfaces and the industry shows the greatest growth and growing demand (23). Our objective was to observe and evaluate the anti-Legionella property of the fill material in biofilm within mixed flora in the presence of Legionella bacteria. Data suggest that the anti-Legionella splash fill has an effective surface agent that limits Legionella colonization on its surface. A similar study also revealed significantly low counts of microorganisms colonized on the SANIPACKING® cooling tower sheet fill (24), which contains impregnated chlorine based antimicrobial agents. Damian and Paţachia (25) tested polypropylene polymers containing antimicrobial agents. They emphasize the advantages of using antimicrobial pipes in comparison to standard pipe material. On the other hand, results clearly depict that *L. pneumophila* proliferated in a very short time during the study within the bulk water system, which proves that cooling towers are ideal incubators for that bacteria and therefore regular control programs are crucial. The efficacy of the fill material against these organisms provides promise for future applications in reducing the transmission of Legionnaires' disease outbreaks sourced from cooling towers and reducing the numbers of pathogens in public spaces.

Due to increasing constraints on environmental discharge of disinfecting agents, as well as a demand to reduce costs, the water treatment industry has been looking for alternative ways to reduce biofilm formation and microbial counts in industrial systems. There is still scope for further work in terms of controlling biofilm formation in man-made water systems (26). The results also demonstrate that one way approaches could not control the bacterial colonization efficiently. It could be concluded that polymers impregnated with antimicrobial agents might decrease biofouling on water associated surfaces. A combination of protective measurements should be routinely applied and monitored in man-made water systems for individual and environmental protection.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of study: I.T., C.V.; Data Acquisition: I.T., C.V.; Data Analysis/InterpretationI.T.; Drafting Manuscript: I.T., C.V.; Critical Revision of Manuscript: I.T.; Final Approval and Accountability: I.T., C.V.

Conflict of Interest: The authors declare that they have no conflicts of interest to disclose.

Financial Disclosure: This work was supported by Research Fund of the Istanbul University. Project number: 26088.

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