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Assessment of the Effectiveness of Different Biocides for Biofilm Eradication

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In the current study, it was aimed to determine the efficacy of the different dosages of sodium hypochlorite, benzisothiazol/isothiazolin-ones and chloramine T trihydrate compounds against 12 months old mature biofilm. Biofilm samples which grown in model recirculating system were exposed to biocides for 1 and 24 hours. At the end of the contact time, the numbers of heterotrophic bacteria in samples exposed to biocides were compared with the control samples.

It has been determined that all concentrations of tested compounds provided >1 log reductions in heterotrophic cultivable bacteria at 1 hour contact time. After 24 hour contact time 20 and 50 mg/L sodium hypochlorite provided >3 log; 15, 100 and 150 mg/L benzisothiazol/isothiazolin-ones compound provided >2 log; 1000, 2000, 3000 mg/L chloramine T trihydrate compound provided >3 log reductions.

There was no statistically significant difference in efficacy between doses. Different dosages of the same biocide provided similar log reductions in heterotrophic cultivable bacteria. Study results demonstrated that similar antibacterial effect can be achieved with applying lower dosages of tested compounds. Thus, by determining the minimum amount of biocide which is sufficient for biofouling control, equipment and environment can be protected by preventing excessive biocide usage; additionally the development of drug-resistant microorganisms would be prevented.

Index Terms: Biofilm, disinfection, water recirculating system, heterotrophic bacteria, stainless steel

I. INTRODUCTION

Microorganisms, the result of their natural tendency in aquatic environments, form a biofilm layer by attaching to the surfaces, multiplying and embedding themselves in a slimy polymeric matrix [1]. This layer, act as protective cover for microorganisms against adverse conditions such as biocides and nourishment [2, 3]. On the other hand, biofilms can lead hygiene problems by hosting pathogens [4, 5] and serious economic losses by causing microbiological corrosion [6, 7]. Complete eradication of the biofilm is not possible in the industrial water systems, the only option is prevention and minimising the risk by conducting periodic disinfection program using specific chemicals.

Conventional approach is that testing the biocides in laboratory conditions according to the universal standards. Determining the antibacterial activity by pure cultures is a sterile approach, and it is not representative of the natural environment of mixed cultures.

Each biofilm is unique due to factors such as surface type, the presence of nutrients and oxygen, microbial species. Moreover, from early colonization to the mature biofilm, physiological and metabolical changes seen in the biofilms. Because of the diversity of biofilms, and the differences in antimicrobial sensitivity of the mature and young biofilms, direct implementation of laboratory results to the field treatments would be unrealistic [8]. The best biocide selection for a specific biofilm should be determined by modeling the closest of the natural conditions, before field applications.

In the current study, it was aimed to determine the efficacy of the different dosages of sodium hypochlorite, benzisothiazol/isothiazolin-ones and chloramine T trihydrate compounds against 12 months old mature biofilm on the surface of stainless steel.

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II. METHODS

The experimental study was performed using a 100-liter polypropylene laboratory scale recirculating model water system under constant hydraulic conditions. Model system was equipped with a recirculation pump (550 W, 40 $l.min^{-1}$, Pedrollo, Italy) in the basin and a heater (AT–100, 100 W, Atman, Germany) to provide accurate temperature control (Fig. 1).
No chemicals (disinfectant, pH regulators or anti-scaling agents) were added to the system in order to exclude their possible negative effects (such as their disinfecting effect) on the microorganisms and biofilm formation.

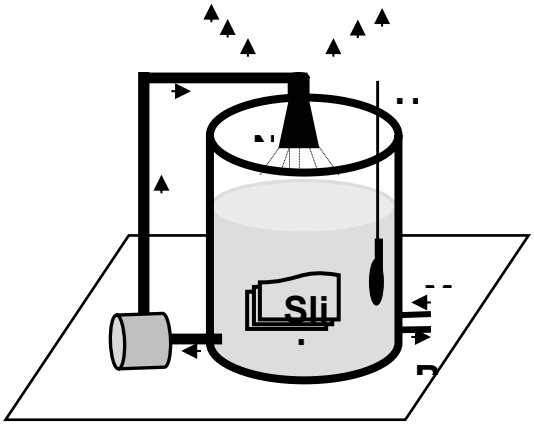

Figure 1. The schematic diagram of recirculating cooling tower model system, arrows indicating the flow direction.

Stainless steel slides (304) (SS) were prepared in 25x60x0.8 mm, dimensions by laser cutting. Before placed to the model system all slides cleaned using a neutral detergent (1% Triton) and after rinsing them with tap water, degreasing was carried out in acetone for 3 min by ultrasonicator. Then, rinsed sterile distilled water, dried at 60ºC both slide surfaces was disinfected by transilluminator (TI–100, Tomy Seiko Co. Ltd, Japan) for 12 hours [9].
Different dosages of tested compounds were prepared in sterile demineralized water.
12 months old biofilm samples which grown in model recirculating system were exposed to different dosages of biocides 1 and 24 hours. At the end of the contact time, the numbers of heterotrophic bacteria in samples exposed to biocides were compared with the control samples.

III. RESULTS AND DISCUSSION

In biocide efficacy tests, ASTM E645−13 standard test method for evaluation of microbicides used in cooling water systems was taken into consideration, for the interpretation of results [10]. According to this standard, microbicides can be evaluated using simulated or real cooling tower water against (1) microbes from cooling water, (2) microbes in microbiological deposits (biofilms) from operating cooling systems, or (3) microorganisms known to contaminate cooling water systems, or a combination thereof.

It has been determined that all concentrations provided >1 log reductions in heterotrophic cultivable bacteria at the end of 1 hour contact time.

90% kill or 1 log reduction would be the minimum level of performance considered to show efficacy of a microbicide according to this guidelines. The reduction in the number of microorganisms at each

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biocide concentration was calculated relative to the count of the control sample. The control samples (without biocide) showed a stable population with no more than a 1-1.5 log increase or 0.5 log decrease in growth, during the test period for a 24 hours contact time period.

After 24 hours contact time, 20 and 50 mg/L sodium hypochlorite provided >3 log reduction, whereas 2 mg/L did not achieve reduction in cultivable heterotrophic biofilm bacteria, at the same contact time (Fig. 2)

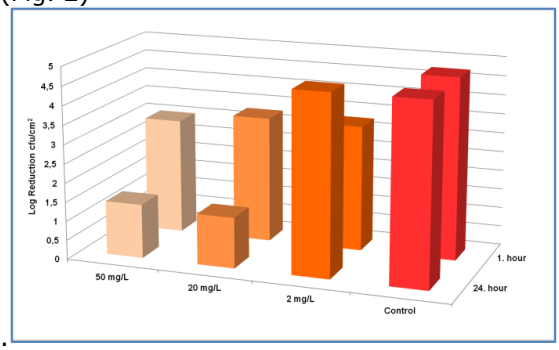

Figure 2. The efficacy of different dosages of sodium hypochlorite against cultivable heterotrophic biofilm bacteria

15, 100 and 150 mg/L benzisothiazol/ isothiazolin-ones compound dosages achieved >2 log reduction after 24 h contact time (Fig. 3).

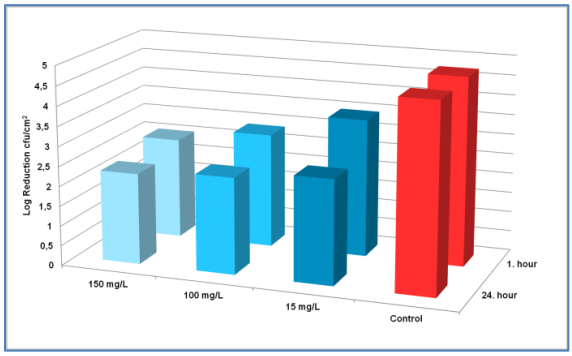

Figure 3. The efficacy of different dosages of benzisothiazol/isothiazolin–ones compound against cultivable heterotrophic biofilm bacteria

Treatment with 1000, 2000, 3000 mg/L chloramine T trihydrate compound for 24 h resulted in >3 log reductions (Fig. 4).

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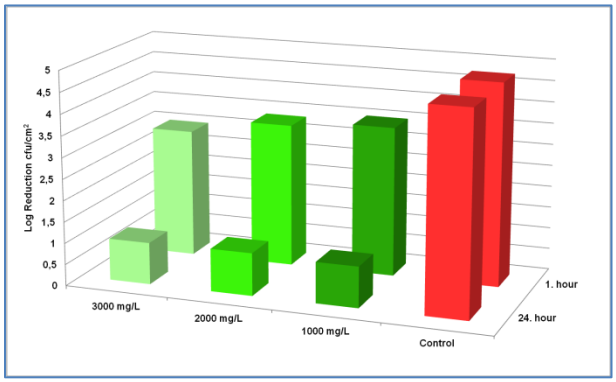

Figure 3. The efficacy of different dosages of chloramine T trihydrate compound against cultivable heterotrophic biofilm bacteria

There was no statistically significant difference in efficacy between doses. Different dosages of the same biocide provided similar log reductions in heterotrophic cultivable bacteria.

Conventional plate or colony forming units (cfu) count is commonly used in determination of bacteria number after biocidal treatment. However, as a result of inhibition and injury of metabolism due to the biocidal treatment, bacteria may become viable but non-culturable (VBNC). It should be noted that the presented data in this study is only based on culture results. Since <1% of the bacterial population from oligotrophic environments can be cultured, in order to obtain accurate assessment of biocide efficacy the viability of microorganisms which can not be detected by traditional plate method should be determined by more advanced techniques, also.

IV. CONCLUSION

The results showed that the activity of biocide is not proportional with the concentration of biocide. The strength of a concentration of the biocide depends on its relations with the organism. Similar antibacterial activity can be achieved with the lower dosages of tested compounds. Thus, by predetermination of efficient biocide concentrations, equipment and environment can be protected by avoiding excessive biocide usage and the development of resistance in microorganisms would be prevented.

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