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# Microbiological control agents for cooling systems in hydroelectric power plants

**Authors:**
**Paulo RD Marangoni[1*], Carolina Gracia Poitevin[2], Patricia R Dalzoto[2], Marcos AC Berton[1] and Ida C Pimentel[2].**

**Institution:**
1. Serviço Nacional de Aprendizagem Industrial – SENAI, Instituto Senai de Inovação em Eletroquímica, CEP 80215-090, Av. Comendador Franco 1341, Jardim Botânico, Curitba (PR).

2. Universidade Federal do Paraná, Setor de Ciências Biológicas, Laboratório de Microbiologia e Biologia Molecular, CEP: 81530-900, Av.Cel Francisco H dos Santos s/n – Jardim das américas, Curitba (PR) – Brasil.

**Corresponding author:**
**Paulo RD Marangoni.**

**Email Id:**
paulo.marangoni@pr.senai.br

**Web Address:**
http://jresearchbiology.com/documents/RA0451.pdf.

**ABSTRACT:**

Many hydroelectric power plants and industries use chemicals to minimize problems caused by clogging and corrosion consequence from accumulated organic material in cooling systems. The chemicals used to avoid these processes must be strictly controlled, especially those based on chlorinated compounds, potential precursors of trihalomethanes, which are carcinogenic to humans and other animals. This study compared the sensitivity of potential alternatives to the use of chlorinated compounds in cooling systems, releasing free chlorine in the riverbed downstream of hydroelectric plants, besides the evaluation of the efficiency of these compounds in the control of bacteria that are surface colonizers and potential biofilms formers. Considering microbiological aspects, the results indicated three options for replacing Calcium Hypochlorite. Such products are MXD-100®, analyte of water electrolysis system of Radical Waters®, and application of NaOH for changing the pH in cooling systems. The use of efficient methods to control the adhesion of microorganisms in cooling systems assists the power plants in reducing unscheduled maintenance of equipment that are exposed to corrosion processes influenced by microorganisms and consequently suffer mechanical failures, which interferes in the duration and frequency of electricity production interruptions.

**Keywords:**
Antimicrobial agents, biofilm, water treatment, biocides, biocorrosion, hydroelectric power station

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## INTRODUCTION

Microbial corrosion, or biocorrosion, is the electrochemical process of metal dissolution initiated or accelerated by microorganisms. The formation of deposits on the surface of equipment is generically denominate fouling or accumulation. The negative effect of such deposit is a significant decrease in the equipment´s efficiency and life cycle (Videla, 2003). According to the medium and material, two main corrosive mechanisms may occur, electrochemical and chemical. In the electrochemical mechanism, chemical reactions involving charge or electrons transfer occur through the interface or electrolyte, while in the chemical mechanism direct reactions between the material and the corrosive media occur, with no electric current generation (Gentil, 2011). In accordance with FHWA and NACE International reports (FHWA and NACE 2002) about costs of corrosion and prevention strategies in the United States, the direct cost of corrosion represents 3.1% of national GDP, that is US$ 276 billion, according to data collected in 1998 from across the North American industrial chain. The same report shows that the cost of corrosion prevention represents 1.38% of GDP resulting in US$ 121 billion. If control techniques, such as paints and coatings, cathodic protection, application of corrosion inhibitors and biological control agents, were adopted, it is estimated that between 25% to 30% of the total cost of corrosion could be saved annually, which means between 69 and 82 billion (1% of U.S. GDP). The direct cost of corrosion in the U.S. electricity sector (generation, transmission and distribution) is US$ 6.9 billion, shared by the major sources of American energy as follow: US$ 4.2 billion for electricity generated by nuclear power, US$ 1.9 billion for corrosion in sources of energy generated by fossil fuels, US$0.15 billion for corrosion in hydroelectric power plants and other sources and US$ 0.6 billion for transmission and distribution. According EPRI, Electrical Power Research Institute (Gorman, *et al.,* 2001), the total cost (corrective, direct and indirect) with corrosion in the electricity sector was $ 17.3 billion, which is 2.5 times the value presented in FHWA and NACE International report (FHWA and NACE, 2002). In Brazil, this scenario presents itself differently due to the level of industrialization and especially the differential energy matrix, as the main source is hydroelectric, with 69% of the whole production, instead of nuclear thermoelectric and fossil fuel (Agência Nacional de Energia Elétrica, 2014). The indices presented above are used by the Brazilian Association of Corrosion - ABRACO *Associação Brasileira de Corrosão* - as parameters for sizing corrosion costs in Brazil (Associação Brasileira de Corrosão, 2012). Several sectors are affected by biocorrosion: industries in general (naval, petrochemical, bioprocess, chemical, refineries, etc.), buried pipelines, sealing fuel tanks in aircraft and vessels, power generation plants (thermoelectric, hydroelectric, nuclear, etc.), but it is estimated that 20% of the deterioration of metallic surfaces are derived from biological processes related to electrochemical factors inherent to corrosion (Beech and Gaylarde 1999). Effective control of biofilms can be achieved by understanding the type and nature of contamination on the surface (carbohydrates, fats, proteins, minerals) and microorganisms involved in that colonization. The selection of sanitizers and microbial biocontrol agents depend on their effectiveness and safety to the applicator and to the environment, therefore the corrosive nature of the product should be observed so that it does not amplify the deterioration of the surface colonized by microorganisms (Simões *et al.,* 2010). The compounds to be used can be disinfectants, preservatives, sterilizing or antimicrobial agents (Sondossi, 2004). The development of antimicrobial resistance mechanisms is not entirely clear, but recently several studies have shown a wide variety of models and explanations for the factors that influence the resistance of microorganisms to antimicrobial agents (Simões *et al.,* 2010; Bauer and Robinson 2002; Sondossi 2004; Mah and O'Toole 2001). There are multiple mechanisms involved in resistance presented by biofilms, each of them contributing to the group's survival and better adaptation to the environment where they live. The factors vary depending on the bacteria on the biofilm, type of control agent to be used, product concentration, equipment design and operating conditions (Mah and O'Toole 2001; Simões *et al.,* 2010; Eguía *et al.,* 2008; Héquet *et al.,* 2011). Each of these mechanisms form physical and chemical barriers to the penetration of the biofilm control agent that, due to the combination of these factors, shall have an "own phenotype", which should be evaluated so that the best strategy may be used. As each microbiological control agent may be applied to a particular type of biofilm, preliminary studies are necessary in order to assess the type of "contamination" (adhesion) occurring on the surface to be treated (Simões *et al.,* 2010). To control biofilm formation and biocorrosion, several hydroelectric power plants in Brazil use chemical compounds to avoid clogging in cooling systems and hence interruption of electric power generation. For that, most use chlorine compounds for treatment of natural water used in the cooling system. Currently, it is meant to replace these compounds, since it is known that in contact with organic material they can influence the formation of trihalomethanes, a group of organic compounds derived from methane in which three molecules of hydrogen are substituted by an equal number of atoms of halogen elements such as chlorine, bromine and iodine (Hong *et al.,* 2007; Xue *et al.,* 2009; Hong *et al.,* 2013; Palacios *et al.,* 2000). These compounds are known for their toxic action and carcinogenic potential (Liu *et al.,* 2011; Takanashi *et al.,* 2001), therefore it is important to monitor the use of a microbial control agent to ensure the efficiency of cooling systems (Morato *et al.,* 2003; LeChevallier *et al.,* 1988) and maintain the quality of waters from the rivers that are normally used by people who live around power plants. In the present study, synthetics and natural products were tested for their efficiency in the control of colonizing surface bacteria and potential biofilms formers. Some industrial biocides applied to water treatment, cooling towers and cooling water systems are presented by Sondossi (Sondossi 2004), who presents some compounds used in the present work, such as Hypochlorite, Sodium Hydroxide and Glutaraldehyde. However, the present work proposes the replacement of chlorine compounds by substances that reduce the concentration of free chlorine in natural water treatment systems that are used in industrial cooling systems, because it should meet quality requirements in order to keep equipment in operation and not to harm the environment when used in open circuits.

## MATERIALS AND METHODS

The microbial biocontrol agents used for disinfecting surfaces were: glutaraldehyde (1 ppm), bleach (1 and 3 ppm), calcium hypochlorite (1 and 3 ppm), sodium hydroxide (pH 9 and 12), dichloro (1 and 3 ppm), ECA anolyte (RW) (1, 3, 5 and 10 ppm), MXD-100® (1 and 3 ppm), azadirachtin (1 and 3 ppm), extract of neem (1 and 3 ppm), neem Oil (1 and 3 ppm), orobor (1 and 3 ppm), copaiba oil (1 and 3 ppm), clove oil (1 and 3 ppm).

### Antimicrobial Activity in Solid Medium

To evaluate the sensitivity of microbiological control agents disc diffusion method on agar (NCCLS and CLSI 2003; NCCLS and CLSI 2005) was used. The microorganisms employed for this study were: *Enterococcus faecalis* ATCC® 29212, *Escherichia coli* ATCC® 25922, *Escherichia coli* ATCC® 35218, *Haemophilus influenzae* ATCC® 49247, *Haemophilus influenzae* ATCC® 49766, *Klebsiella pneumoniae* ATCC® 700603, *Neisseria gonorrhoeae* ATCC® 49226, *Pseudomonas aeruginosa* ATCC® 27853, *Staphylococcus aureus* ATCC® 25923 and *Streptococcus pneumoniae* ATCC® 49619.

The strains were inoculated into tryptic soy broth (TSB) and incubated at 35 °C in an incubator for 18h. After culture, turbidity was measured using a spectrophotometer UV/VIS in a wave length of 600nm, equivalent to McFarland 0.5 (approximately 1,5 x $10^8$ UFC/mL of *E. coli* ATCC® 25922).

Holes were done in tryptic soy agar plate using sterile metallic punch (8 mm in diameter and 4mm in depth) aiming deposition of biocidal test solutions for the bacteria of interest. This step was performed prior to the inoculation of microorganism with swab to ensure that no contamination and differential growth occurred due to the time of preparation of these plates with holes. The plates were incubated (non-inverted) in an incubator, at 35°C, up to 15 min after the application of inoculum and Biocide with swab and were incubated for 24 h (Gelinski *et al*., 2007; Siqueira Jr *et al*., 2000).

The diameters of the total inhibition halos were measured in millimeters using a pachymeter, including the diameter of orifice.

**Antimicrobial activity in liquid medium**

For the test in tubes with liquid medium, only one microorganism of each family mentioned below was used: *Pseudomonas aeruginosa* ATCC 27853 (Pseudomonadaceae), *Escherichia coli* ATCC 25922 (Enterobacteriaceae), *Staphylococcus aureus* ATCC 25923 (Micrococcaceae). The media used were: M9 Minimum Salts (M9), Peptone Water (PW) and Tryptic Soy Broth (TSB).

The strains were inoculated into tryptic soy broth (TSB) and incubated at 35°C in an incubator for 18 h. After culture, turbidity was measured using a spectrophotometer UV/VIS wave length of 600 nm, equivalent to McFarland 0.5 (approximately 1,5 x $10^8$ UFC/mL of *E. coli* ATCC® 25922). In each of the test tubes, $10^6$ UFC/mL was inoculated (concentration obtained by diluting the standardized inoculum). Concentration less than $10^8$ CFU/ml was used because at the end of cultivation in liquid medium this concentration was reached in the positive control so it was possible to compare the results between samples with microbial biocontrol agents and the positive control. The antimicrobial activity was evaluated using the methodology of test tubes with liquid medium and the turbidimetric analysis using a spectrophotometer UV/ VIS, in the range of 600 nm. Transmittance was measured and compared to a standard curve established through the spectrophotometer measures of MacFarland scale patterns.

**Experimental design**

The experiment was completely randomized and data were analyzed by Factorial Experiments followed by Tukey test for the comparison of means ($p<0.01$) using software ASSISTAT v7.6 (Silva and Azevedo 2009).

**RESULTS AND DISCUSSION**

Through disc diffusion test and turbidimetry, which tested the different microbial biocontrol agents against bacteria precursor of biofilm formation, it was possible to identify potential substitutes for chlorinated compounds, which results are shown in Figure 1 and Figure 2. It is possible to identify biological control agents with antimicrobial power similar to commercial compounds such as Sodium Hypochlorite and Calcium, that are currently used for the disinfection of natural waters in cooling systems (Netto and Samuel, 2011; Giordani *et al.,* 2005), and consequently used to control the corrosion influenced by microorganisms.

The product MXD-100 was effective in controlling the growth of microorganisms by diffusion in solid and liquid medium in the two concentrations tested, 1 ppm and 3 ppm (Figure 1 and Figure 2). It is also possible to verify that there are other options such as using NaOH (Figure 1 and Figure 2) to change the pH of the cooling system for pH 9 and pH 12. These compounds were as effective as commercial bleach at 3 ppm (Figure 1 and Figure 2), which is used as surface sanitizer.

Usually, in hydroelectric power plants such products are applied: calcium hypochlorite, sodium hypochlorite, dichloro, gaseous chlorine, sodium dichloroisocyanurate, and other chemical compounds such as Sodium Hydroxide, Ozone, Copper Sulfate and antifouling paints (Giordani *etal.,* 2005). The chlorine-based compounds follow environmental laws, establishing a limit of 0.01 mg/L for total residual chlorine (free + combined) downstream the power plants, when the receiving water body is classified as Class I freshwater, that meets the standards of human consumable supplies with primary treatment and protection of aquatic communities. The same law provides a limit of 250 mg/L Chloride for Class II rivers that meet the former requirements and are also destined to human and animal consumption after primary treatment, balneability, and use in agricultural activities (Conselho Nacional do Meio Ambiente 2005).

In the tests performed in this study, the calcium hypochlorite to 3 ppm showed 68.95% of efficiency in controlling the growth of bacteria in the disc diffusion test (Figure 1), and 75.57% in the liquid medium test (Figure 2) compared to positive and negative controls, respectively. But currently new molecules are being studied to avoid the use of this compound in open systems (Liu *et al*., 2011; Hong *et al.,* 2007).

The prevention and treatment of biocorrosion have as main feature the reduction and control of biofilm development. Chemical treatments applied to control biofilms involve the use of microbial control agents (Biocides), other penetrant and adsorbent substances among others to aid penetration and dispersion of these compounds to increase efficiency in the treatment (Guiamet and Saravia, 2005).

The criteria for selecting a good agent for microbiological control are summarized as follows: 1) effective control of microorganisms in general, 2) penetration and destabilization of mature biofilms, 3) physicochemical stability with other products, eg. corrosion inhibitors and environmental factors (pH, temperature, etc.), 4) low risk of handling and easy storage, 5) biodegradable, 6) low cost (Gaylarde and Videla, 1992).

Currently, three criteria are very important: efficiency, toxicity and biodegradability. Due to this, new sources, mainly of natural compounds for the control of microorganisms in water pipes and cooling systems are studied (Guiamet and Saravia, 2005). There are numerous plants, oils and alcoholic and aqueous extracts of plants with antimicrobial effects (Heisey and Gorman 1992; Masood *et al.,* 1994; Baranowski *et al.,* 1980; Gilliver and Osborn, 1994; De and Banerjee, 1999; Mercedes *et al.,* 2011; Lavania *et al*., 2011; Prabhakar *et al.,* 2010).

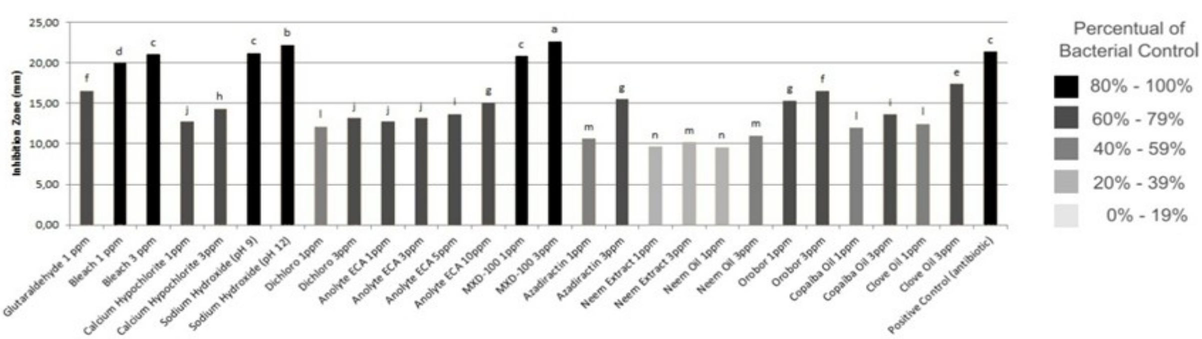


**Figure 1 – Sensitivity of bacteria to microbial biocontrol agents on solid media – Disc diffusion test.**

* Significant at 1% probability ($p < 0.01$)

** Results presented with same capital letter not represent significant differences between of samples

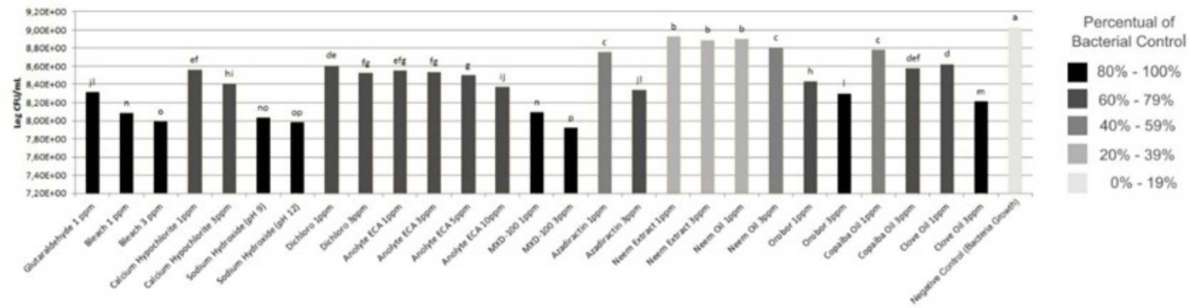


**Figure 2 - Sensitivity of bacteria to microbial biocontrol agents in liquid medium - Turbidimetry**

* Significant at 1% probability ($p < 0.01$)

** Results presented with same capital letter not represent significant differences between of samples

These compounds were as effective as bleach to 3 ppm (Figure 1 and Figure 2), which is used as surface sanitizer. It is usually applied in power plant products with the same principle of action of bleach, such as calcium hypochlorite, sodium hypochlorite, dichloro, gaseous chlorine, sodium dichloroisocyanurate, and other chemical compounds such as sodium hydroxide, ozone, copper sulfate and antifouling paints (Giordani *et al.,* 2005), The chlorinebased compounds follow environmental laws, establishing a limit of 0.01 mg / L for total residual chlorine (free + combined) downstream the power plants, when the receiving water body is classified as Class I freshwater, that meets the standard of human consumable supplies with primary treatment and protection of aquatic communities. The same law provides a limit of 250 mg / L Chloride for Class II rivers that meet the former requirements and are also destined to human and animal consumption after primary treatment, balneability, and use in agricultural activities (Conselho Nacional do Meio Ambiente 2005).

In closed systems, glutaraldehyde can be used. However, this compound exhibits high toxicity. Thus, in the present work, it was used only for comparison purposes, since it is normally used at 50 ppm in cooling tower systems, where there is no open circulation (Sondossi 2004). This compound showed 76.76% efficiency at a concentration of 1ppm (Figure 1) and 80.40% in a liquid medium (Figure 2). Nevertheless, the use of the anolyte of the electrified water system "Radical Water" to 10ppm (Figure 1 and Figure 2) proved to be a substitute for Calcium Hypochlorite in controlling biofilm formation in hydroelectric power plants. Its active principle is based on the action of hypochlorous acid, which is less harmful than hypochlorite, and does not require storage of any chemicals such as sodium hydroxide. Its production is based on the electrolysis of water and requires only electricity and conductive solution of 2.5% NaCl iodine free (Thantsha and Cloete 2006). As hydroelectric power plants have periods of low electricity production due to reduced demand during certain periods, for example, at night, the turbines operate in "standby" mode and therefore, the energy required for water electrolysis could be enhanced by using this energy. The control efficiency of the tested bacteria in the liquid medium and disc diffusion test were respectively 72.09% (Figure 1) and 77.62% (Figure 2), results that are close to those obtained with Calcium Hypochlorite, which is currently used by hydroelectric power plants to control fouling.

The addition of sodium hydroxide solution 50% to change the pH of the solution to pH 9 resulted in reducing the growth of bacteria in disc diffusion test 85.81% (Figure 1), and in liquid media test it was 89.75% (Figure 2).

Comparing to other products, MXD-100 showed the best results in controlling the growth of bacteria in disc diffusion test and turbidimetry in liquid medium. It was observed a reduction of 87.57% (Figure 1) in the growth in solid medium using a concentration of 3 ppm, and a reduction of 92.06% (Figure 2) in a liquid medium using the same concentration.

Other compounds based on natural sources have shown effectiveness in reducing the growth of bacteria in disc diffusion and turbidimetry tests: azadiractin 3ppm (respectively 73.52% and 79.37%), orobor 3ppm (respectively 76.49% and 81.29%) and clove Oil 3 ppm (respectively 78.81% and 84.49%). However, further studies are necessary to allow its use in industrial systems, in order to correct problems such as cost and / or solubility in water (Figure 1 and Figure 2).

Most hydroelectric power plants use Calcium Hypochlorite as control fouling agent and sanitizer of cooling systems (Giordani *et al.,* 2005; Mäder Netto 2011). This compound is currently suffering some restrictions due to the ability to form trihalomethanes and haloacetic acids in the presence of residual chlorine with organic matter (Hong *et al*., 2007; Xue *et al.,* 2009; Hong *et al*., 2013; Palacios *et al.,* 2000; Gagnon *et al.,* 2005), being potential carcinogens (Liu *et al*., 2011; Takanashi *et al*., 2001).

The mechanism of action of chlorine when added to water follows the following reaction: $Cl_2 + H_2O$ à $HOCl + HCl$, forming hypochlorous acid, HOCl, and hydrochloric acid. HOCl dissociates into $H^+$ ions and $ClO^-$ ion, hypochlorite, which is the active oxidizing agent. The action of the hypochlorite oxidant is most effective at pH values between 6.5 and 7.5, becoming ineffective above pH 9. This compound reacts rapidly with inorganic reducing agents, such as sulfides, sulfites and nitrites, and organic matter (Gentil 2011; López-Galindo *et al*., 2010). Some studies show the formation of these compounds in natural waters, both marine environment (López-Galindo *et al.,* 2010) and freshwater (Hong *et al*., 2013), but particularly in water treatment systems for both human consumption and industrial cooling systems (López-Galindo *et al*., 2010; Murthy *et al*., 2005; Gagnon *et al*., 2005; Eguía *et al.,* 2008).

The results observed in this study suggest potential alternatives to the use of Calcium Hypochlorite in the primary treatment and improvement of natural waters used in cooling system in Hydroelectric Power Plant located in northern Brazil. The replacement of this compound for products such as MXD-100, anolyte ECA and NaOH which have better efficiency in the control of biofilms, can help to reduce costs with maintenance and repairs on equipment immersed or in contact with natural waters, in addition to improving the quality of water released downstream from the power plant and that serves the local population.

## CONCLUSION

Among evaluated microbiological control agents, three options may be considered for treating natural waters in heat exchanger systems in hydroelectric power plants, due to greater efficiency in microbial control: MXD-100, anolyte ECA and NaOH. Removing biofilms in natural water treatment systems in thermal exchange systems of power plants is important due to the clogging and biocorrosion processes, which interfere the production of electricity in hydroelectric plants. Brazil has most of its energy matrix based on hydropower, being approximately 67% of generated energy. Thus, the results obtained are relevant in order to minimize problems and economic losses in the energy sector, arising from the exposure of equipment to the influence of natural waters.

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