# LABORATORY STUDIES OF BIOCORROSION CONTROL USING TRADITIONAL AND ENVIRONMENTALLY FRIENDLY BIOCIDES: AN OVERVIEW.

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*Abstract*- **Metal surfaces immersed in natural or industrial waters undergo a sequence of biological and inorganic changes that may result in biocorrosion due to the formation of a biofilm. Microbial adherence to metallic surfaces affects different industrial systems, such us cooling water systems, off shore oil industry, thermal power stations, hydroelectric, chemical and process industry, etc. The chemical agents generally used to prevent or protect metallic structures from biocorrosion are highly toxic and, after use, can have a negative impact on the environment. Environmental concerns have led to legislation which encourages the replacement of toxic biocides, widely used in the past (e.g. chlorine) with more readily degradable antimicrobial chemicals that are compatible with system operation and less toxic to the environment. One innovative attempt to accomplish this goal is the use of naturally-produced compounds, such as plant extract that are environmentally acceptable. The aim of this paper is to give an overview of different laboratory studies which used both traditional and environmentally friendly biocides against planktonic bacteria and sessile bacteria on different metal surfaces.**

***Keywords.*** **Biocorrosion, Environment, Glutaraldehyde, Natural biocides, Traditional biocides, Ozone.**

## I. INTRODUCTION

Biocorrosion is directly related to the presence of microorganisms that by adhering to different industrial surfaces can damage the metal. Bacterial cells can encase themselves in a hydrated matrix of polysaccharides and protein, and form a slimy layer known as a biofilm. The later can be considered as a gel containing c.a. 95% or more water, microbial metabolic products including enzymes, extracellular polymeric substances (EPS), organic and inorganic acids, as well as volatile compounds such as ammonia or hydrogen sulphide and inorganic detritus (Geesey, 1982, Beech and Gaylarde, 1999). The capacity of EPS to bind metal ions is important to biocorrosion (Kinzler *et al.*, 2003; Rohwerder *et al.*, 2003; Sand, 2003) and depends both on bacterial species and on the type of metal ion (Beech and Coutinho, 2003). Biocorrosion occurs in aquatic and terrestrial habitats that differ in nutrient content, temperature, pressure and pH. It results from the presence and physiological activities of microbial consortia on the metallic surface. Such biofilms promote interfacial physiochemical reactions, not normally favoured under abiotic conditions (Beech and Sunner, 2004).

Therefore, prevention and treatment of biocorrosion should be mainly based on avoiding or minimizing the development of biofilms. Chemical treatments applied to control biofilms involve the use of biocides and other products such as penetrating or dispersive agents (which enhance the efficacy of the treatment). The mechanisms of resistance in biofilms are different from the now familiar plasmids, transposons, and mutations that confer innate resistance to individual bacterial cells. In biofilms, resistance seems to depend on multicellular strategies (Stewart and Costerton, 2001). The lack of efficacy of biocides against sessile organisms, well documented in the past few years, is probably due to the inability of the chemical to penetrate the biofilm, in addition to physiological differences between sessile and planktonic cells (Brown and Gillbert, 1993). A number of workers have shown that biocides sensitivity can be altered up to 1000-fold by changes in nutrients and growth rates (Gillbert and Brown, 1978).

The classical criteria governing the selection of an effective biocide have been generally summarised as follows: i) proven efficacy against a broad spectrum of microorganisms; ii) ability to penetrate and disperse microbial slime; iii) chemical and physical compatibility with other products (e.g. corrosion inhibitors) and the environment (e.g. pH effects); iv) safe storage and easy use and storage; v) appropriate biodegradability; vi) cost effectiveness (Gaylarde and Videla, 1992).

Unfortunately, biocides are inherently toxic and frequently are difficult to degrade being persistent in the natural environment or able to accumulate in a variety of matrixes and often causing contamination of areas distant from the site of treatment. Thus, biocides may have a very negative impact on the environment if they are applied without a proper environmental risk assessment. Environmental concern has led to legislation which encourages the replacement of toxic biocides, widely used in the past (e.g. chlorine) with more readily degradable antimicrobial chemicals that

are compatible with system operation and less toxic to the environment.

One innovative attempt is the use of naturally–produced compounds, such as plant extracts that will be more easily biodegraded and will be environmentally acceptable. A number of plant oils and aqueous plant extracts have been shown to have inhibitory activity against yeast, filamentous fungi and bacteria (Heisey and Gorman, 1992; Masood *et al.*, 1994; Baranoski *et al.*, 1980). Many plants of the family Cruciferae have been found to have antimicrobial properties against clinically important organisms. Their action may be due to isothiocyanates, of which allyl isothiocyanate (AITC) was the first identified from plant tissues (Gilliver and Osborn, 1994). Indian species such as clove, cinnamon, horse raddish, cumin, tamarind, garlic, onion, are use as preservaties, disinfectants and antiseptics (Krishna and Banerjee, 1999).

The aim of this work is to give an overview of different laboratory studies aimed to investigate the activity of various traditional and environmentally friendly biocides against planktonic bacteria and sessile bacteria on different metal surfaces.

## II. BIOCIDAL ACTION ON SESSILE AND PLANKTONIC BACTERIA

### A. Traditional biocides

*Glutaraldehyde (GA), formaldehyde and quaternary ammonium compound (quats)*

Bacteria used in the experiments were either of the genus *Pseudomonas* (aerobic specie isolated from hydrocarbons) or a strain of *Pseudomonas fluorescens* (*P. fluorescens*) isolated from cutting oil emulsions. Biofilmed metal samples (with c.a. $10^8$ CFU/cm$^2$) were transferred into flasks containing synthetic cooling water (Videla *et al.*, 1996) with different concentrations of the biocides tested. Contact times assayed were 10, 15, 30 or 60 min. and 3, 6 or 24 h. Thereafter, bacterial cell enumeration was performed using the standard plating method for viable counts. Biofilms were removed from metal coupons by scraping and later poured into 10 ml of sodium chloride isotonic solution. The number of colony forming units (CFU) were determinated after 48 h. of incubation at 28 °C, using the standard plating method for viable counts (Videla *et al*., 1996).

Of the two aldehyde–based biocides assayed (glutaraldehyde (GA) and formaldehyde) using *Pseudomonas* sp., only GA was able to kill planktonic bacteria within three hours at the lower concentration used (10 ppm). Both biocides were less effective against sessile bacteria. A total concentration per treatment time of 30 ppm x h for GA was increased to 6.000 ppm x h. in the case of the sessile cells, whilst the equivalent figures for the formaldehyde were 150-240 ppm x h and 4.800 ppm x h, indicating that the later is slightly more effective in biofilms than the former. On the other side, results with *P. fluorescens* revealed a good biocidal activity on sessile cells when a concentration of 500 ppm x h. of formaldehyde was used. The quat (ammonium didecyldimethyl chloride) used in these studies was effective against planktonic *Pseudomonas* sp. with a concentration x contact time of 24-30 ppm. x h., but did not achieve the required viable cell reduction at 4800 ppm x h. against sessile organisms. Quats compounds are surfactant agents, and might be expected to promote the removal of deposits from a surface, although some previous results from our laboratory have shown that even sessile cells apparently killed by the biocide were not always removed from the biofilm (Videla *et al.*, 1996). The mixture of quats and GA, a combination frequently tested in the field, may be more effective for attaining a better detachment of the biological deposit (Eagar *et al*., 1986). The action of different biocides (glutaraldehyde, sodium hypochloride, formaldehyde and isothiazolinones mixtures) on *Pseudomonas fluorescens* strain showed similar trends, *i.e.* a high effectiveness on planktonic cells and a less effective performance (restricted to reduction of 3 to 7 orders cell numbers) for sessile population (Videla *et al.*, 1996).

Contact time and biocide concentrations are not the only variables to control when biocide efficacy is considered. A great amount of EPS, arranged as a dense and uniform film was produced by *Pseudomonas fluorescens* on AISI 304L stainless steel. Smaller amount of EPS and a patchy biofilm distribution was observed on SAE 1020 mild steel. EPS may thus act as a diffusional barrier for biocide penetration, and may influence biocidal action of GA (Videla *et al*., 1991).

GA and formaldehyde, which are highly reactive compounds, produce cytoplasmic coagulation in vegetative bacteria, act at the core in bacterial spores and the amino groups at fungal cell wall and in the case of formaldehyde, act on the cell wall of vegetative bacteria. Cationic biocides, such as quats, coagulate all types of intracellular constituents in vegetative bacteria causing leakage of intracellular components. They also inhibit spore outgrowth, but not germination, and act on the cytoplasmic membrane in fungi (Russell *et al*., 1999).

*Ozone as a non- polluting biocide*

Ozone generation under laboratory conditions was achieved using U.V radiation on pure oxygen, which was suitable to produce ozone solutions in the 0.2 to 0.5 ppm concentration range. The feed gas was pre-treated to provide a gas free from impurities. The ozone levels were measured for each experiment using the standard N-N-diethyl-p-phenylendiamine (DPD) method (Oldfield, 1981). Strains of sulphate-reducing bacteria (SRB) *Desulfovibrio desulfuricans* (*D. desulfuricans*) and *Desulfovibrio vulgaris* (*D. vulgaris*) and a strain of *Pseudomonas fluorescens* were used in these experiments. Biofilm were generated by incubating metals coupons (SAE 1020 carbon steel and AISI 304L stainless steel) in *P. fluorescens* cultures containing c.a

$10^5$ cells/ml. Incubation time were 7 and 24 h for *Pseudomonas* and 14 days at 28 °C for SRB. After incubation, coupons were transferred into flasks containing synthetic cooling water (Viera *et al*., 1993) with the following ozone concentrations: 0.20; 0.28 and 0.5 ppm. Contact times assayed were 5, 10, 15 and 30 min. Enumeration of bacterial cells was performed after exposing the biofilms to different ozone concentrations. Biofilms were removed from steel coupons by scrapping with a sterile scalpel and poured into 10 ml of sodium chloride physiological solution or 10 ml liquid Postgate's medium C for *P. fluorescens* and SRB respectively (Viera *et al.*, 1993).

Dissolved ozone was able to reduce planktonic *Pseudomonas fluorescens* bacterial numbers below detectable limit in only 15 min. at 0,28 ppm and in 30 min. at 0,14 ppm (Viera *et al*., 1993), in agreement with previously reported results for *Escherichia coli* and *Legionella pneumophilia* (Domingue *et al.*, 1988; Pope *et al.*, 1984).

The reduced effectiveness of ozone against sessile compared with planktonic bacteria can be due to several factors: a) the high oxidising power of ozone could alter the EPS outer layer of the biofilm matrix creating a barrier to further penetration of the dissolved ozone within the biofilm. This assumption is supported by micro-electrode measurements made for chlorine in the biofilm (De Beer *et al.*, 1994). These results showed that chlorine concentration within the biofilm was only 20% or less than the concentration in the bulk liquid; b) present model considers biofilm as a structure formed by cell clusters surrounded by channels where the liquid movement would be controlled by convective flow (Stoodley *et al.*, 1994; Lewandowski, 1998). In this model a blockage of biofilm channels by the oxidation product of ozone may impede the further access of ozone to the inner layers of biofilm structure. Indeed, it has been reported (Korber *et al.*, 1994) that the channels of *Pseudomonas fluorescens* biofilms were disrupted by the anti-microbial agent fleroxacin. Moreover, cells near to the biofilms –liquid interface suffered more morphology change than those located in the deeper part of the biofilm; c) the existence of compact microbial aggregates in the deepest region of the biofilm, which may alter physiological status of cells so that the resistance against biocidal action is enhanced.

The biocidal action of ozone on bacterial biofilms formed on stainless steel coupons after 7 hours of incubation in a *Pseudomonas fluorescens* culture was studied within the 0.2 to 0.5 ppm concentration range for several contact times. No biocidal action was found for 0.28-ppm ozone concentration at the lowest contact time assayed (5 min.). Conversely, for 10 min contact time and 0.5 ppm ozone concentration, the number of variable microorganisms was approximately 30 times lower than found for 0.28 ppm ozone concentration. Viable microorganisms were approximately $10^5$ CFU/cm$^2$ for both 15 and 30 min. contact times at 0.5 ppm of ozone concentration. Thus biocidal action was not significantly increased when the contact time was extended. It was also observed that ozone action on stainless steel biofilms was not only able to kill sessile bacteria, but to detach them as well (Viera *et al.*, 1993). Many sessile cells of *Pseudomonas fluorescens* remained viable within biofilms formed on stainless steel and carbon steel metals after similar treatment condition. Neither the type of steel, nor the presence of corrosion product altered the biocidal effectiveness, contrary to the results obtained previously with GA (Videla *et al.*, 1991). However, in spite of the decrease in viable cell numbers, the amount of cells attached to carbon steel observed with scanning electron microscopy (SEM) did not decrease. Thus, it can be assumed that dead cells remained attached to the metal surface (Viera *et al.*, 1993).

It has been demonstrated that 10 min. ozone treatment of SRB biofilms formed on stainless steel and carbon steel reduced the number of bacteria by two orders of magnitude (Viera *et al.*, 1993).

**B. Natural biocides**

*Aqueous extracts of Brassica nigra and Allium cepa*

The activity of natural biocide consisting of aqueous extract of *Brassica nigra* on planktonic and sessile *Pseudomonas sp.*, the fungus *Aspergillus fumigatus (A. fumigatus*) and mixed cultures of SRB species (all strains isolated from contaminated diesel oil) revealed a promising biocidal action against microorganisms in industrial biofilms. A main active ingredient of *Brassica nigra* is the allyl isothiocyanate (AITC) which reacts with amines and other activated groups in proteins. This action results in the inactivation of enzymes, although the penetration of isothiocyanates into cells is poor. However, the ability of this extract to deplete a planktonic cells and *Pseudomonas sp.* and *Aspergillus fumigatus* in biofilms reveals a good potential for it practical use.

The organisms were more resistant in the sessile state, but significant reductions in the numbers of adhered cells of *Pseudomonas* were found at all times and concentrations assayed. A reduction of approximately 89 % from initial levels was achieved after 24 h contact with 500 ppm of active ingredient. The extract was also very effective against sessile *A. fumigatus* attaining a reduction of about 87 % with the same treatment. SRB which showed approx. 58 % reduction after 24 h with 500 ppm was the most resistant of the sessile organisms. *Pseudomonas sp.* was most sensitive and *A. fumigatus* most resistant of the microorganisms in the planktonic phase (Gómez de Saravia and Gaylarde, 1998).

A comparison with the sensitivity of planktonic cells shows that not extrapolation can be made about the relative sensitivity of suspended and biofilm organisms, since the most resistant planktonic type was the fungus.

It has been reported that aqueous extract of garlic (*Allium sativum*) and onion (*Allium cepa*), were able to

inhibit growth of Gram positive and Gram negative bacteria and fungi, although the activity of garlic extract was greater as compared to the onion extract (Elmina *et al.,* 1983). The biocidal activity of onion extract against several species of the genus *Aspergillus* (Yin and Tsao, 1999), decreased with increasing incubation and heating temperature.

In a similar study, aqueous extracts of *Allium cepa* (onion bulb) were tested in our laboratory against planktonic and sessile aerobic bacteria (Videla *et al.,* 2004). Onion extracts (100, 75 and 50 %) were tested against *Pseudomonas fluorescens* cultures with different concentrations of cells ($10^5$ to $10^9$ microorganisms/ml) at 8 different contact times ranging between 5 min. and 24 h. In addition, experiments using *Pseudomonas fluorescens* biofilms (1.25 x $10^5$ cfu/cm$^2$) on AISI 304 type stainless steel were conducted to study the biocidal efficacy of *Allium* extract on bacterial adhesion. No significant biocidal action of onion extract on sessile and planktonic cells was observed (Videla *et al.,* 2004).

## III. BIOCIDAL EFFECT ON THE ELECTROCHEMICAL BEHAVIOR OF STEELS

Data from different electrochemical tests performed with carbon and stainless steel in the presence of various biocide solutions tested, revealed that none of the biocides assayed, were able to alter the passive behaviour of the steels in the synthetic cooling water solution (Table I). Corrosion current extrapolated from linear Tafel polarization was non-significant in all cases. Corrosion potential vs. time experiments using stainless steel specimens were made separately in ozonized and non-ozonized solutions. Corrosion potential remained almost stable at noble values (c.a 0.20 V) for colonized solutions, whereas it became stable at more negative potentials (c.a –0.05 V) for non-ozonized solutions. Conversely, corrosion potential vs. time evolution for carbon steel specimens shifted in the active direction in ozonized solutions (Viera *et al.*, 1993).

Potentiodynamic polarization experiments carried out with carbon steel specimens, showed a similar anodic behaviour of the metal substratum whether in the presence (0.28 ppm) or in the absence of ozone. Dissolved ozone enhanced the passive behaviour of stainless steel and glutaraldehyde displaced the pitting potential of carbon steel in the noble direction in sulphide containing media (Videla *et al*., 1991; Viera *et al*., 1993). The first effect may be due to stabilisation of the passive oxide layer as a result of a high oxidising power of glutaraldehyde and sulphide on the steel surface, which would hinder the action of the aggressive sulphides on the iron oxide passive layers.

Table 1. Open circuit potential (OCP), corrosion current (Icorr) and break down potential (EV) of carbon steel and AISI 304 stainless steel in different aqueous solutions. (From reference, Videla *et. al.,* 1996).

| Metal | Synthetic cooling water | Isothiozolinones | Sodium hypochloride | Formaldehyde |
|---|---|---|---|---|
| Carbon steel | OCP= - 0.624 V<br>Icorr= 5.634 μA/cm$^2$<br>EV= -0.450 V | OCP= -0.654 V<br>Icorr= 8.394 μA/cm$^2$<br>EV= -0.420 V | OCP= -0.631 V<br>Icorr= 15 μA/cm$^2$<br>EV= -0.470 V(*)<br>EV= -0.300 V (**) | OCP= -0.629 V<br>Icorr= 6.733 μ/cm$^2$<br>EV= -0.350 V |
| Stainlesstee l | OCP= -0.199 V<br>Icorr= 93.66ηA/cm$^2$ | OCP= - 0.149 V<br>Icorr= 20.24 ηA/cm$^2$ | OCP= -0.133 V<br>Icorr= 5.649 ηA/cm$^2$ | OCP= -0.038 V<br>Icorr= 8.737ηA/cm$^2$ |

(*) 1 ppm; (**) 10 ppm; OCP values were measured after 24 h.

Electrochemical tests of different concentrations of aqueous extract of mustard seed of the geneus *Brassica nigra* in a buffer solution (Gaylarde and Gómez de Saravia, 1999) has shown that it was able to alter the passive behaviour of the metal. The open circuit potentials in the presence of 0.50 % of the final concentration of aqueous extract were more cathodic for carbon steel than for carbon steel (-0.7 V) than for stainless steel (- 0.4 V). Nevertheless, for both metals these values increased within 10 h, indicating passivation. Potentiodynamic polarization curves for carbon steel and stainless steel in the presence of 0.50 % of the final concentration of aqueous extract also suggested a passivating effect, although the difference between these curves and those for the control (buffer solution plus NaCl only) was small. In spite of this, the fact that the passivating effect is seen for both carbon steel and stainless steel specimens suggests that it would be advisable to test higher concentrations of the extract to confirm this activity (Gaylarde and Gómez de Saravia, 1999).

## IV. CONCLUSIONS

- Results shown in this overview, demonstrate that biocides, such as glutaraldehyde, formaldehyde, sodium hypochloride, isothiozolinones mixture and quats differ significantly in their ability to control biocorrosion hazard depending on the conditions present at the metal surfaces at the time of their application.
- The ozonation appears to be a promising bio-control treatment for circulating cooling water, although it is

not a total replacement for all treatment chemicals. Effective and reliable monitoring instruments and analytical techniques should be employed to identify the best conditions for success.

- The ability of the seed extract of *Brassica nigra* to deplete the numbers of microorganisms in biofilms, together with its lack of corrosive activity, is an excellent sign for its potential use in the control of microbial contamination, biocorrosion and biofouling. Natural biocides are more easily biodegraded and, consequently, more environ-mentally acceptable.

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