Prevention and protection of the effects of biocorrosion and biofouling minimizing the environmental impact

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Abstract

Biocorrosion and biofouling processes are mediated by microorganisms adhered to the metal surfaces or embedded in a gelatinous matrix called biofilm. Biofilms affect the interaction between metals and the environment not only in deleterious processes like corrosion but also in several biological processes applied to materials recovery and handling. The growth of the microorganisms capable to induce biocorrosion is conditioned by favorable environmental conditions. However, 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. Four different approaches developed in our laboratory to prevent and control biocorrosion but minimizing the environmental impact, are successively presented in this paper: a) the use of ozone as an environmentally friend biocide for cooling water systems; b) the assay of the effectiveness of natural biocides on planktonic and sessile bacteria; c) the potential use of film forming corrosion inhibitors; d) the use of innovative preventing substances.

Keywords

Biocide. Biocorrosion. Biofilms. Ozone. Environment.

Prevención y protección de los efectos de la biocorrosión y el biofouling con mínimo impacto ambiental

Resumen

Los procesos de biocorrosión y *biofouling* están mediados por microorganismos que adhieren a las superficies metálicas embebidos en una matriz gelatinosa llamada biofilm. Los biofilms afectan a la interacción entre metales y el medio ambiente, no solo a través de procesos deletéreos tales como la corrosión sino, también, en el manipuleo de diversos materiales. El crecimiento de los microorganismos capaces de inducir biocorrosion esta condicionado por un medio ambiente favorable. Sin embargo, generalmente, los agentes químicos usados para prevenir o proteger las estructuras metálicas de la biocorrosion son altamente tóxicos y su uso puede tener un impacto negativo para el ambiente. En este trabajo se presentan cuatro vias diferentes, desarrolladas en nuestro laboratorio, para prevenir y controlar la biocorrosión minimizando el impacto ambiental: a) uso del ozono en sistemas de enfriamiento de agua; b) ensayos de efectividad de biocidas naturales sobre bacterias sésiles y planctónicas; c) uso de inhibidores de corrosión formadores de film; d) uso de nuevas sustancias preventivas.

Palabras clave

Biocida. Biocorrosión. Biopelículas. Ozono. Medio ambiente.

1. INTRODUCTION

Biocorrosion and biodeterioration are directly related to the presence of biofouling deposits on the surfaces. These deposits are mediated by microorganisms adhered to metal surfaces or embedded in a gelatinous organic matrix called biofilm. A biofilm can be considered as a gel containing c.a. 95 % or more water, made of a matrix of extracellular polymeric substances (EPS) in which bacteria, other cells and inorganic detritus are suspended[1]. Thus, prevention and treatment of material biodeterioration should be mainly based on avoiding or minimizing the development of biofilms.

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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 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) easy use and safe storage; v) appropriate biodegradability; vi) cost effectiveness[2].

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 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 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 with this goal is the use of naturally-produced compounds, such as plant extracts that will be biodegraded more easily and will be more environmentally acceptable. A number of plant oils and aqueous plant extracts have been shown to have inhibitory activity against yeast, filamentous fungi and bacteria[3-5].

2. OZONE AS A NON-POLLUTING BIOCIDE

Ozone has attracted special interest in recent years as an effective and non-polluting biocide for cooling water systems. Taking into account environmental concerns, the use of ozone offers several advantages over other biocides[6]: i) minimal on-site chemical inventory; ii) non-toxicant discharge; iii) potential for water conservation. The unique combination of high toxicity during treatment with non-toxicant discharge could make ozone the leading choice biocide in the near future, if an appropriate balance between positive effects, problems caused and cost, is reached.

Dissolved ozone is able to reduce planktonic Pseudomonas fluorescens (P. fluorescens) bacterial numbers below detectable limits in only 15 min at 0.28 ppm and in 30 min at 0.14 ppm[7] in agreement with previously reported results for Escherichia coli and Legionella pneumophilia[8 and 9].

The biocidal action of ozone on bacterial biofilms formed on stainless steel coupons after 7 h of incubation in a P. fluorescens culture was studied within the 0.2 to 0.5 ppm concentration range for several contact times[7]. 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 viable microorganisms was approximately 30 times lower than that found for 0.28 ppm ozone concentration. Viable micoorganisms 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 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[7]. Many sessile cells of P. fluorescens remained viable on biofilm formed on stainless steel and carbon steel surfaces after similar treatment conditions. Neither the type of steel, nor the presence of corrosion products altered the biocidal effectiveness differing from results previously obtained with glutaradheyde[10].

Biocidal action of ozone on SRB anaerobic biofilms was assayed using carbon and stainless steel coupons previously incubated in Desulfovibrio vulgaris and Desulfovibrio desulfuricans mixed cultures. After a 10 min contact time the number of bacteria decreased in two orders of magnitude[7].

The limited reduction in sessile cells numbers found in our work agrees with experimental results reported by Characklis[11] for the disinfectant action of chlorine on biofouling deposits.

The lesser effectiveness of ozone against sessile than planktonic bacteria can be attributed to several causes: a) the high oxidizing 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[12] in biofilms. This result shows that chlorine concentration within the biofilm was only 20 % or less of the concentration in the bulk liquid; b) present models for biofilm consider it as formed by cell clusters surrounded by channels were the liquid movements would be controlled by convective flow[13 and 14]. In this model a blockage

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of biofilm channels by the oxidation product of ozone may impede the further access of ozone to the inner layers of biofilm structure. Supporting this assumption, it was recently reported[15] that the channels of *P. fluorescens* biofilms were disrupted by the anti-microbial agent fleroxacin. Moreover, cells near to the biofilm-liquid interface suffered more morphology changes 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 have altered physiological status that improve their resistance against biocidal action.

3. CORROSION INHIBITORS TO PREVENT BIO-FILM FORMATION AND BIOCORROSION

Two innovative attempts to replace toxic biocides through the use organic film-forming corrosion inhibitors have been reported[16 and 17]. Some of the advantages of this approach, as claimed by the authors, are: a) lower operational costs due to the lower concentration of the chemicals used; b) less frequent dosages and environmental control measures; c) simultaneous action on corrosion inhibition and bacterial adhesion.

There have been reports that some quaternary amines form a film on a substratum and inhibit the bacterial attachment onto it[18], thus inhibiting the formation of biofilm and subsequently retarding the corrosion rate of that metal[16]. Today, in addition to quaternary amines, a wide variety of corrosion inhibitor formulations are available. The inhibitors are complex mixtures or reaction products designed to meet the demands of an industry. Thus, depending on the application demand, the inhibitor mixture contains films formers, surfactants, oxygen scavengers, and demulsifiers. It was reported[17] that among the four different groups of corrosion inhibitors (quaternary salts, imidazolines, amides and amide/imidazolines) tested, some were able to control or even prevent biocorrosion by inhibiting biofilm formation. Whereas the toxicity of corrosion inhibitors (expressed as LC 50 range) were in all cases similar or lower than commercial biocides, microbial attachment was better inhibited by imidazoline/amide mixtures than by quaternary amines. Organic film-forming inhibitors used in the oil an gas industry are generally of the cationic type. Their mechanism of action is to form a persistent monolayer film adsorbed at the metal/solution interface. Thus, any alteration of the molecule of the inhibitor caused by microbial degradation during their use can affect their specific performance on corrosion inhibition.

Corrosion inhibitors could act as a nutrient source and enhance populations of bacteria. In oil fields and water floods that are treated with corrosion inhibitors, these effects are obviously important because bacteria are frequently present and can influence corrosion[19].

The performance of an organic film forming inhibitor blend to inhibit carbon steel corrosion was assessed in the presence of two microbial contaminants: *P. fluorescens* and a mixture of SRB[20].

This product is a mixture of aliphatic and aromatic hydrocarbons, dimethylamine, imidazoline, morpholine, cicloexylamine and quaternary ammonium compounds.

A moderate microbial growth, using the inhibitor as the sole carbon source was observed (Table I). The biodegradation of the corrosion inhibitor in the case of SRB started later than in the case of *P. fluorescens*[20]. Gomez de Saravia *et al.*[21] observed that the inhibitor capacity of the inhibitor blend used in oil product systems was preserved and that it was not affected by the partial microbial degradation of the product.

4. NATURAL BIOCIDES

Many plants of the family Cruciferae such as *Brassica nigra* (mustard seed) have been found to have

Table I. Statistical evaluation of results for P. fluorescens growth in SMM with and without corrosion inhibitor (CFU/ml)[20].

Tabla I. Evaluación estadística de los resultados del crecimiento de P. fluorescens en Medio Mineral Simplificado (MMS) con y sin inhibidor de corrosión en Unidades Formadoras de Colonias (UFC/ml)[20].

Time of incubation (days)	Without corrosion inhibitor	With 400 ppm of corrosion inhibitor
3	X: 2.2×10^9 SD: 1.2×10^9	X: 1.1×10^8 SD: 1.0×10^8
7	X: 5.1×10^7 SD: 4.8×10^7	X: 1.1×10^9 SD: 1.15×10^9
15	X: 8.5×10^6 SD: 7.6×10^6	X: 8.4×10^8 SD: 1.50×10^9
30	X: 7.3×10^6 SD: 1.0×10^6	X: 3.8×10^{10} SD: 4.7×10^{10}
60	X: 8.2×10^6 DS: 1.5×10^6	X: 6.8×10^9 SD: 5.6×10^9

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antimicrobial properties against clinically important organisms. Their action may be due to isothiocyanates, of which allyl isothiocyanate (AITC) was identified from plant tissues[22].

The activity of an aqueous extract of *Brassica nigra* on planktonic and sessile *Pseudomonas sp.*, the fungus *Aspergillus fumigatus* (*A. fumigatus*) and a mixture of SRB revealed a promissory biocidal action against microorganisms frequently found in industrial biofilms. Its main active ingredient is the allyl isothiocyanate (AITC) which react with amines and other active groups in proteins. This action results in the inactivation of enzymes.

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 tratment. SRB approx. 58 % reduction after 24 h with 500 ppm were the most resistant sessile organisms[23].

Pseudomonas sp. was the most sensitive and *A. fumigatus* the most resistant microorganisms in the planktonic phase[23].

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

The ability of AITC to affect cell binding suggests that it reacts with surface groups important for cell adhesion. The ability of the seed extract to deplete the numbers of microorganisms in biofilms, together with its lack of corrosive activity[24], is an excellent sign of its potential for use in the control of microbial contamination, biocorrosion and biofouling.

5. INNOVATIVE SUBSTANCES TO PREVENT BACTERIAL ADHESION AND BIOFILM FORMATION

Microbial adhesion is widely accepted as the main stage prior to the induction or initiation of biocorrosion[25].

An innovative method for preventing biocorrosion through microbial adhesion inhibition has been proposed recently by forming an immunoglobulin film on metal surfaces. This procedure has been effective to prevent the formation of *P. fluorescens* biofilms on two different types of stainless steel[26].

The bacterial adhesion inhibitory action of IgA was found to be concentration dependent[27]. IgA is one of the five major classes of immunoglobulins in the human body. It is present selectively in sero-mucous secretions as a dimer stabilised against proteolysis by combination with an other protein (the secretory component) that has a single peptide chain of molecular weight 60.000[28].

The coverage of different steels with immunoglobulin solution has been effective to prevent the adherence of *P. fluorescens* cells to the metal surface under different experimental conditions (i.e. several immersion times, different metallic substrata, various immunoglobulin dilutions).

After 24 h of exposure to a bacterial culture of *P. fluorescens*, the coupons of different stainless steels showed copious biofilms of P. fluorescens by SEM[26]. The average sessile population was in the order of 10^9 CFU/cm^2 (Table II). Metal coupons, previously conditioned with the immunoglobulin solution, revealed a dramatic decrease in the number of attached bacteria (that according to the plate count), was in the order of 10^4 to 10^5 CFU/cm^2.

Table II. Statistical evaluation of results of *P. fluorescens* growth on stainless steel surfaces with and without adsorbed immunoglubulins solution[29].

Tabla II. Evaluación estadística de los resultados del crecimiento de P. fluorescens sobre superficies de acero inoxidable con y sin la solución de inmonoglobulina adsorbida[29].

METAL	BIOFILM CFU/cm²	IMMUNOGLUBULIN SOLUTIONS CFU/cm²		
		Pure solution	(1:100 dil)	(1:1000 dil)
AISI 316	X: $4.2 \cdot 10^9$ SD: $3.2 \cdot 10^9$	X: $1.1 \cdot 10^5$ SD: $1.1 \cdot 10^5$	X: $1.0 \cdot 10^6$ SD: $3.6 \cdot 10^5$	X: $7.5 \cdot 10^5$ SD: $1.0 \cdot 10^6$
AISI 304	X: $4.0 \cdot 10^8$ SD: $7.7 \cdot 10^7$	X: $5 \cdot 10^4$ SD: $1.3 \cdot 10^4$	X: $4.6 \cdot 10^4$ SD: $5.4 \cdot 10^3$	X: $8.4 \cdot 10^5$ SD: $8.7 \cdot 10^4$

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Different electrochemical techniques were applied in the presence and in the absence of the immunoglobulins to asses any effect of these on the electrochemical behavior of the different stainless steel[30].

The evolution of open circuit potential with time of the different stainless steel samples in Postgate C medium + 3 % NaCl showed that the potential variation was similar for samples with and without a coverage of immunoglobulins. Potentiodynamic runs for stainless steel samples in the same medium, showed no significant differences in the breakdown potential (Eb) of the stainless steel, independently of the presence or absence of immunoglobulin coverage of the samples. Pre-conditioned samples showed a passive behavior region slightly more extended than non-treated coupons.

The ability of avoiding bacterial adherence to metal surfaces is specifically related to immunoglobulin fraction and, in particular, to IgA immunoglobulin. This finding is supported by several recent publications in the area of medical implants[27 and 31] and by a well documented knowledge on the specific effect of IgA against bacterial adherence to surfaces[28].

6. CONCLUSIONS

Dissolved ozone could be an excellent biocide for treating cooling water systems without negative effects on the environment. Although its effectiveness against planktonic bacteria is high, it decreases when applied to sessile bacteria in biofilms. For this case its use complemented with surfactant agents is recommended.

A natural biocide (as the aqueous extract of *Brassica nigra*) has potential for use against planktonic microorganisms in cooling water and in industrial biofilms, although the biocidal efficiency on the latter is slightly lesser. Natural biocides are more easily biodegraded and consequently more environmentally acceptable.

Film-forming corrosion inhibitors (such as mixtures of different amines, imidazoline, aliphatic and aromatic hydrocarbons and quaternary ammonium compounds) form a persistent monolayer adsorbed on the metal surface. Thus they could be simultaneously used to avoid biofilm formation and to inhibit corrosion. These compounds are potentially interesting because of their lower operational costs and lesser environmental risk. However, for their practical use they should have a proper resistance to microbial biodegradation.

Although momentarily restricted to the use in the medical field, immunoglobulin solutions containing a higher percentage of IgA immunoglobulin showed high effectiveness to inhibit microbial adhesion on metal surfaces.

Acknowledgements

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