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INHIBITION OF ANAEROBIC BACTERIAL ADHERENCE TO METAL SURFACES

P.S.Guiamet[1] and S.G.Gómez de Saravia[2].
[1]INIFTA. CONICET. UNLP. C.C. 16, Suc.4, 1900 La Plata. ARGENTINA.
E-mail: pguiamet@inifta.unlp.edu.ar
[2]INIFTA.CICPBA.UNLP. C.C. 16, Suc.4, 1900 La Plata. ARGENTINA.
E-mail: sgomez@inifta.unlp.edu.ar

Abstract
The aim of this paper is to study the capacity of immunoglobulin combination (IgA, IgG and IgM) to prevent the adhesion of sulphate reducing bacteria (SRB) (*Desulfovibrio vulgaris and Desulfovibrio desulfuricans*) on carbon steel and stainless steel surfaces. An inhibition of bacterial adhesion was found when immunoglobulins solution was use to cover metal surfaces under different experimental conditions. Scanning electron microscopy (SEM) was used to evaluate the inhibition of adhesion. Corrosion potential evolution vs time and potentiodynamic runs were used to study the electrochemical behavior of the carbon and stainless steel in the presence and the absence of the immunoglobulins coverage.

Keywords: biofilms, inhibition adhesion, immunoglobulins, stainless steel, carbon steel, sulphate-reducing bacteria.

Introduction
Metal surfaces immersed in industrial or natural aqueous medium undergo biological and inorganic changes that lead to biofilm formation and corrosion respectively (Videla & Characklis, 1992).
The sequence of biological changes (Characklis, 1981) is due to the irreversible adhesion types of microorganisms: aerobic and anaerobic bacteria, microalgae, fungi and protozoans on the metal substrata through extracellular polymeric substances (EPS) production.
Among the methods commonly employed to prevent microbial adhesion to metals: mechanical cleaning, chemical cleaning, biocides, coatings, corrosion inhibitors, cathodic protection and biological substances can be mentioned. It has been reported some quaternary amine form a film on a substratum and inhibit the bacterial attachment onto it (Pope & Skultety, 1995), inhibiting the formation of biofilm and subsequently retarding the corrosion rate of that metal (Ensien et al., 1996). Besides some inhibitors are capable of inhibiting the bacterial attachment on the coupon surfaces (Prasad, 1998).
It has been recently published that secretory immunoglobulin A (IgA) inhibits *Pseudomonas aeruginosa* bonding to cornea through in vitro experiment and scanning electron microscopy (SEM) observation to quantitate bacterial adherence (Masinick et al., 1997). The bacterial adhesion inhibitory action of IgA was found to be concentration dependence. IgA is one of the five major classes of immunoglobulin in the human body. It is present selectively in sero-muccus secretions as a dimer stabilized against proteolysis by combination with an other protein (the secretory component) that has a single peptide chain of molecular weight 60.000 (Roitt, 1988).
The aim of this paper is to study the capacity of immunoglobulins combination (IgA, IgG and IgM) to prevent the adhesion of sulphate reducing bacteria (SRB) *Desulfovibrio vulgaris and Desulfovibrio desulfuricans* on carbon steel and stainless steel surfaces.

Experimental
A mixed culture of SRB containing *Desulfovibrio vulgaris* (*D. vulgaris*) and *Desulfovibrio desulfuricans* (*D. desulfuricans*) was used. SRB were maintained en Postgate B medium (Postgate, 1984) at 28 °C in anaerobic condition. The inoculum was prepared with 9 ml of SRB cultures (7 days old) in liquid Postgate C medium e incubated in an anaerobic jar. A commercial immunoglobulins solution (IgAbulin, Austria) was used and each ml of the solution contained: c.a. 60 mg of IgA, 30 mg of IgG and 2 mg of IgM, total protein (100 mg) and glucose (100 mg) in a saline solution. Occasionally, 1:100 and 1:1000 dilutions of immunoglobulins solution were also used to check if the inhibition capacity was diminished by dilution.
Metal specimens used to assess microbial biofilms and electrochemical measurements were SAE type 1020 carbon steel, AISI type 316 and AISI type 304 stainless steel. Metal samples were immersed in BRS cultures. They were tested either without immunoglobulins coverage or after forming a film of immunoglobulins (exposing the surfaces to immunoglobulins solution for an hour) prior to the immersion in the bacterial culture. The coverage was corresponding to 15 µl. After 15 day of incubation, bacterial biofilms were removed from metal coupons by scraping and later poured into 10 ml of NaCl isotonic solution. SRB cells were enumerated by the dilution to extinction method in liquid Postgate B medium. The experiments were made in triplicate. Samples were examined by scanning electron microscopy (SEM), using a JEOL JSMT 100 microscope. The presence of immunoglobulins film adsorbed to the metal surfaces was also verified by using an electron microprobe (CAMECA SX 50).
To assess any effect of immunoglobulins on the electrochemical behavior of the metals tested, potentiodynamic

runs at 0.003 V/s, corrosion potential evolution vs. time (for a period of 1 hs.) and linear polarization resistance experiments (at +/- 0.010 V of the corrosion potential) were made with the two metals tested in this paper using Postgate C medium + 3 % NaCl + abiotic or biogenic 10^{-4} M Na_2S, as electrolyte.

Results and discussion

Figure 1 show a biofilm on carbon steel of *D. vulgaris* and *D. desulfuricans* after 15 days of exposure to the bacterial culture. The number of sessile population was in the order of 10^5 cells/cm^2 for stainless steel and carbon steel. Metal coupons, previously conditioned with the immunoglobulin solution, revealed an important decrease in the number of attached bacteria (Figure 2) according to extinction method up to 10^2 cells/cm^2 for both steels. These results are in agreement with a previous investigation made with *Pseudomonas fluorescens* (Guiamet et al., 1999). In this case, the effect of immunoglobulins solution on *Pseudomonas fluorescens* biofilms formation revealed a decrease in the cells numbers (Guiamet et. al., 1999).

To determinate if the immunoglobulins were able to affect the electrochemical behavior of the steels used in this paper, several electrochemical experiments were made in the laboratory. For both steels, the pre-conditioned samples showed a passive behavior region slightly more extended than non-treated coupons (Fig. 3). Similar results were found in the case of *Pseudomonas fluorescens* (Videla et. al., 1998).

The evolution of open circuit potential of the different steels in Postgate C medium + 3% NaCl + inorganic or biogenic sulphate showed values more positive for samples with a coverage of immunoglobulins.

Conclusions

- The immunoglobulin solution coverage of stainless steel and carbon steel has been effective to prevent the adherence of BRS cells to the metal surface under different experimental conditions (several immersion times, different metallic substrata, various immunoglobulin dilutions).
-These results were in agreement to recent findings published in the literature that reporting the inhibition of *Pseudomonas fluorescens* adherence to the metal surfaces.

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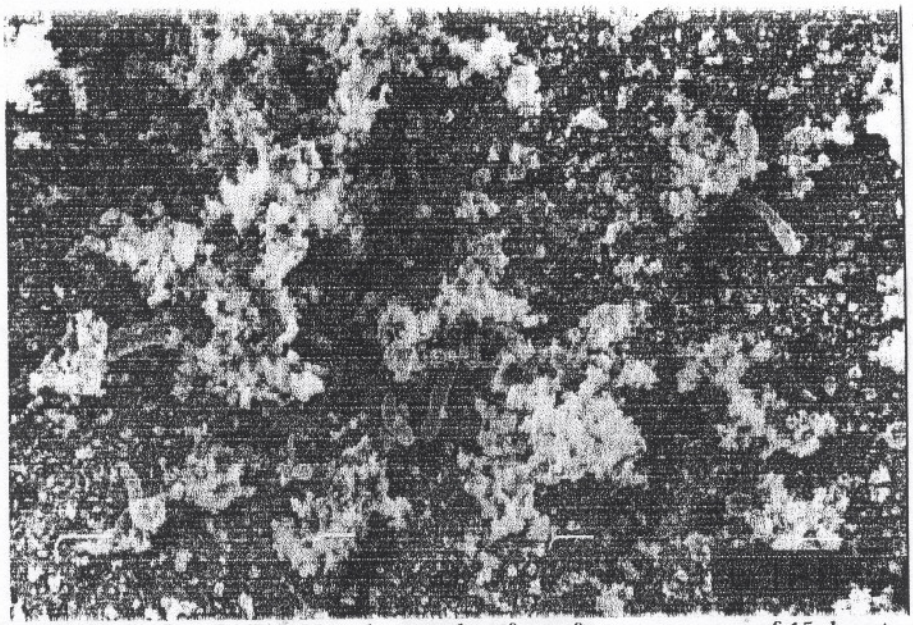

Figure1: SEM micrograph of an SAE 1020 carbon steel surface after an exposure of 15 days to a BRS culture (magnification X

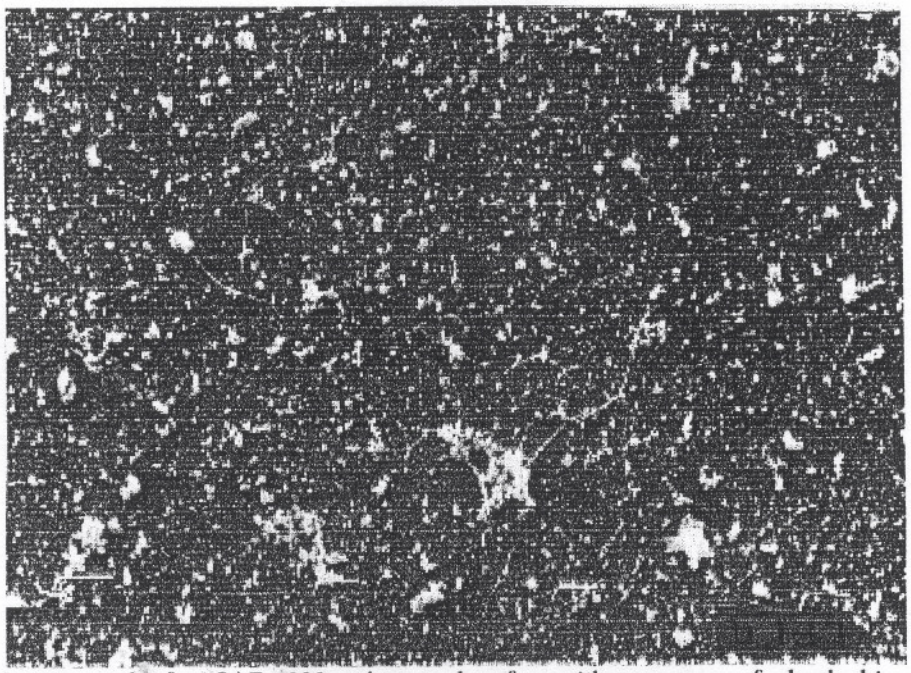

Figure 2: SEM micrograph of an SAE 1020 carbon steel surface with a coverage of adsorbed immunoglobulins after an exposure of 15 days to a BRS culture (magnification X 3500).

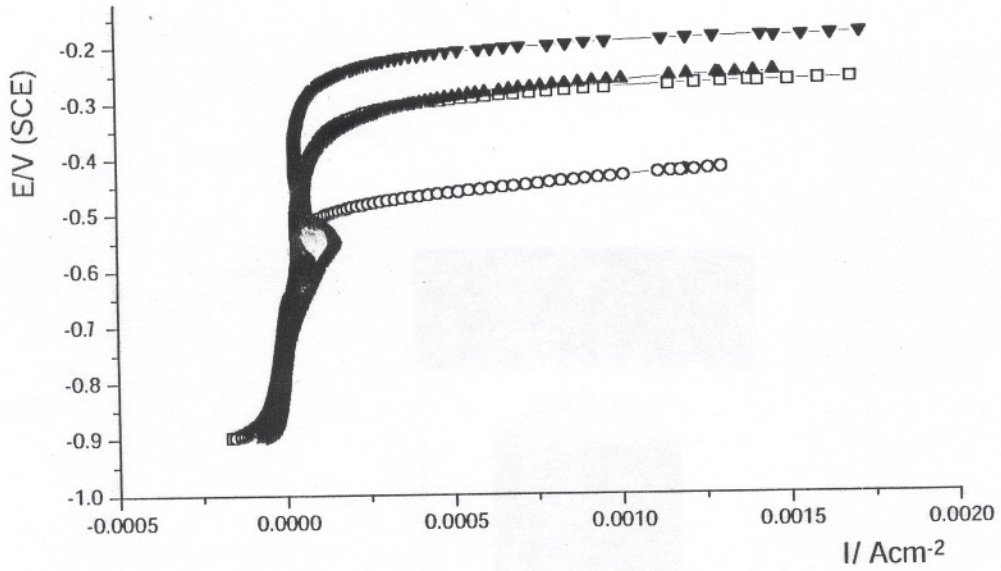

Figure 3: Potentiodynamic polarization curves of SAE 1020 carbon steel at 0.003 V/s in Postgate medium C + 3% NaCl + abiotic 10^{-4} M SNa_2 without adsorbed immunoglobulins (o); with adsorbed immunoglobulins ( ) and Postgate medium C + 3% NaCl + biogenic 10^{-4} M SNa_2 without adsorbed immunoglobulins (▲); with adsorbed immunoglobulins (▼).