THE EFFECT OF DISSOLVED STAINLESS STEEL ALLOY ELEMENTS ON THE ACTIVITY AND GROWTH OF SRB

F.A. Lopes* [(1)], P. Morin [(1)], R.Oliveira [(1)] and L.F.Melo [(2)]

[(1)]Centro de Engenharia Biológica, Instituto de Biotecnologia e Química Fina (IBFQ), Universidade do Minho, 4700 Braga, Portugal
[(2)] Faculdade de Engenharia do Porto, Departamento de Engenharia Química, Universidade do Porto, Porto, Portugal

ABSTRACT

Sulphate reducing bacteria have an important role in the sulphur cycle, and therefore in wastewater treatment systems. They are able to form biofilms on metallic surfaces, leading to fouling and corrosion problems. These bacteria are among the micro-organisms most frequently implicated in microbial corrosion of iron and ferrous alloys.
Alloying elements added to steels for the improvement of their corrosion resistance such as molybdenum and nickel can be dissolved in bulk liquid during the corrosion processes and therefore available to the micro-organisms. That may affect bacterial metabolism and adhesion.
In this study, suspended cultures of sulphate reducing bacteria (SRB) were subjected to several nickel concentrations in order to evaluate the effect of the dissolved metal on bacterial metabolism. Simultaneously, SRB biofilms were developed on stainless steel 304 and on poly-methylmethacrylate (PMMA) in order to study surface effect on biofilm formation. Results showed that nickel (Ni) in all tested concentrations between 0.006 and 5 mg/L had a positive effect on the growth of *Desulfovibrio desulfuricans*. Additionally, biofilms formed on stainless steel presented higher metabolic activity, confirmed by sulphate removal and acetate concentration in the effluent stream. Metal elements present in stainless steel may affect SRB activity. This can be the case of nickel that represents around 8% of stainless steel 304 and that had a positive impact on suspended SRB cultures, under the tested concentrations.

KEYWORDS

Biofilm, *Desulfovibrio desulfuricans*, nickel, sulphate reducing bacteria

INTRODUCTION

The use of stainless steel pipes for the distribution of water is interesting due to its resistance to corrosion. It has been demonstrated that stainless steel 316 (SS316) can be degraded only very slowly or not at all; this is also true when biofilms are attached to the material surface, even in the presence of sulphate reducing bacteria (SRB). Sulphate reducing bacteria are very important microbes from an environmental and industrial point of view. They belong to the group of micro-organisms most frequently associated with corrosion failures. Biofilms containing SRB are responsible for metal and concrete corrosion, as well as H_2S production in oil industries and wastewater treatment systems.
Although several mechanisms have been proposed to elucidate the role played by sulphate reducing bacteria in the corrosion of steels, little evidence is available concerning the effect of

the alloying elements in the prevention of the Microbiologically Influenced Corrosion (MIC). It is also important to study the effect of the metallic ions generated from the alloy degradation on bacterial behaviour as they may influence bacterial growth, and the attachment of bacterial cells to steel surfaces. Some alloying elements like chromium (Cr), nickel (Ni), molybdenum (Mo) are added to steel for the improvement of their properties but these elements may also be dissolved in the water phase or in the biofilm during corrosion processes making them available to the micro-organisms in the biofilm. The resistance of stainless steel 316 to corrosion is due to some of its alloy elements such as molybdenum that is considered toxic to microbial growth (Hanjansgsit *et al.*, 1994) In other stainless steel alloys such as SS 304, pit corrosion has been observed under certain conditions. Edyvean *et al.* (1996) showed that stainless steel 304 was colonised by a significantly higher number of bacteria (viable and total) than stainless steel 316, in a potable water system. Stainless steel 304 was characterised by a rougher surface and the presence of molybdenum in SS 316 could explain the lower bacterial adhesion.

The study of the role of these metallic elements on bacterial growth and adhesion is of importance in increasing the understanding of SRB metabolism and MIC mechanisms. Studies conducted on the effects of dissolved metals on SRB such as *Desulfovibrio spp.* showed that they generally have a detrimental impact on bacterial growth and that they are also able to inhibit hydrogenase activity (Cheung *et al.*, 1994, Feron, 1994).

The objective of this work was to study the effect of dissolved alloy elements on the growth and activity of *Desulfovibrio desulfuricans* in the bulk liquid and in the biofilm. For this purpose, SRB biofilms were developed on stainless steel and poly-methylmethacrylate (PMMA) coupons under turbulent flow conditions in a flow cell reactor and also several nickel concentrations were added to suspended cultures of SRB.

METHODS

The SRB biofilm was grown under turbulent flow (Reynolds number = 7000) in a poly-methylmethacrylate (PMMA) flow cell system within a recirculation loop. Eleven independently removable coupons located in the flow cell allowed for biofilm observation. The coupons used in these assays were either stainless steel 304 or poly-methylmethacrylate (PMMA). *Desulfovibrio desulfuricans* DSM 642 was used to inoculate the reactor, which was first operated in batch mode for 3 days and then switched to a continuous flow mode at a dilution rate of 0.5 h^{-1}. The culture medium contained mineral salts with 2.5 g/L sodium lactate (50%), 1.5 g/L K_2SO_4, 7 mg/L $FeSO_4.7H_20$, 0.25 g/L yeast extract, 0.022 g/L Na_2 EDTA.$2H_2O$ and trace elements (B, Co, Cu, Mn, Zn). The temperature in the flow cell was approximately 27°C and the pH was around 7. Periodically, coupons coated with biofilm were removed from the reactor. The biofilm was scraped in sterile buffer, dispersed and treated for total bacteria and SRB. Total bacteria counts in the biofilm were determined using the DAPI technique and SRB were estimated by the Most Probable Number (MPN) technique.

Suspended cultures of *Desulfovibrio desulfuricans* were grown in the referred culture medium with 12.6 g/L sodium lactate (50%), 7.8 g/L K_2SO_4, 0.48g/L $Na_2S.9H_2O$ and 1 mg/L rezasurin. The influence of dissolved nickel was tested by adding nickel at various concentrations to the standard culture medium during its preparation.

Three to four days old cultures were used as a source of bacterial inoculum. After inoculation (10% or 20% v/v inoculum), the cultures were incubated at 27°C. Growth was followed by optical density at 620 nm. In the assays with suspended cultures and biofilm, lactate and acetate concentrations were determined by HPLC. Sulphate concentrations were measured by capillary electrophoresis.

RESULTS AND DISCUSSION

Figures 1 and 2 present the SRB suspended growth under the effect of several nickel concentrations. Growth observed during incubation of *Desulfovibrio desulfuricans* in the standard medium (reference - without Ni) is given as a reference.

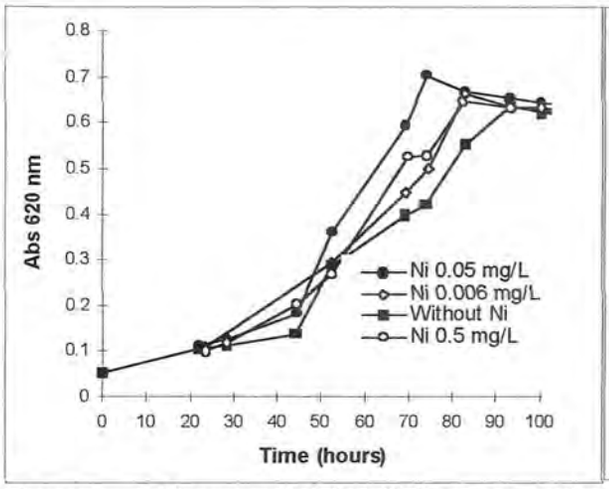

Figure 1 - Influence of nickel (Ni) concentration (0.006, 0.05 and 0.5 mg/L Ni) on suspended culture growth (assay 1)

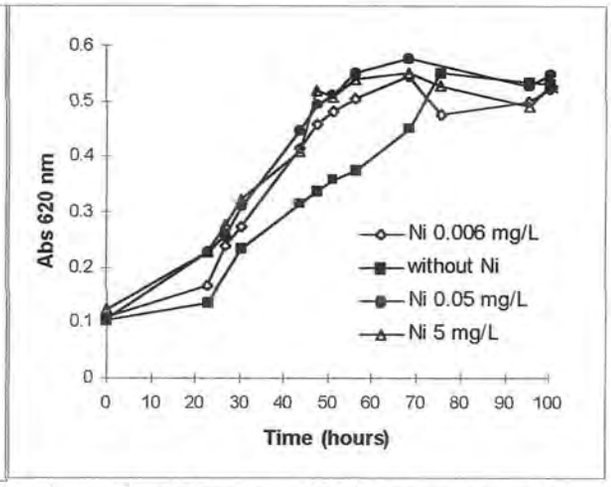

Figure 2 - Influence of nickel concentration (0.006, 0.05 and 5 mg/L Ni) on suspended culture growth (assay 2)

Results showed that nickel (Ni) in all tested concentrations had a positive effect on the growth of SRB as compared to the culture medium without nickel. Results from another assay with two concentrations of nickel (Figure 3), showed the same profile of growth but also indicated that identical lactate removal (Figure 4) is obtained at stationary phase for the two concentrations of nickel as well as for the control. Acetate concentration and sulphate removal also presented similar values for all the tested concentrations of nickel at the stationary phase (data not shown). This suggest that the overall values of product (acetate) yield and bacterial yield (identical values of optical density are observed in stationary phase) are similar with and without nickel. Nickel seems to affect mainly the growth rate of SRB.

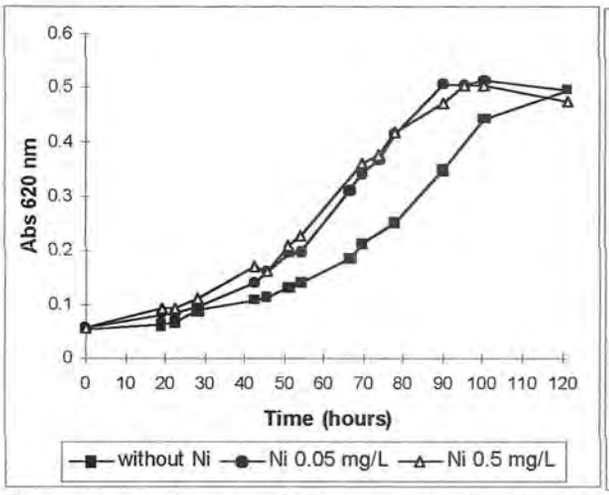

Figure 3 - Influence of Ni concentration (0.05 and 0.5 mg/L Ni) on bacterial growth (assay 3)

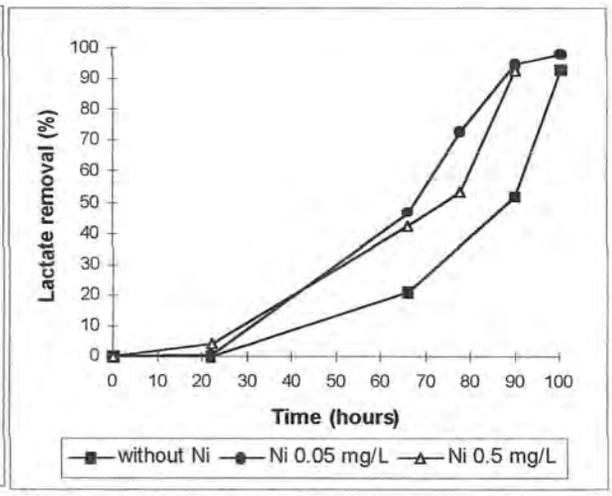

Figure 4 - Influence of nickel concentration on lactate removal. (assay 3)

These results are different from those of other groups, Cheung *et al.* (1994) studied metal effect on two marine strains of sulphate reducing bacteria and they observed that the growth rates and biomass production of planktonic SRB population were adversely affected by Cr, Ni and Mo ions present in the culture medium.

Simultaneously with the suspended cultures experiments, SRB biofilm was developed on stainless steel 304 and on PMMA coupons under turbulent conditions in a flow cell reactor. Figures 5 and 6 present the development of biofilm on metal (stainless steel 304) and on PMMA coupons, as total bacteria (Figure 5) and SRB (MPN counts; Figure 6).

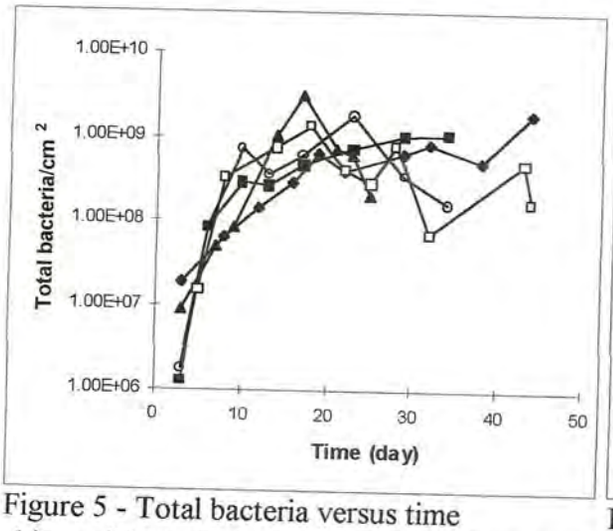

Figure 5 - Total bacteria versus time

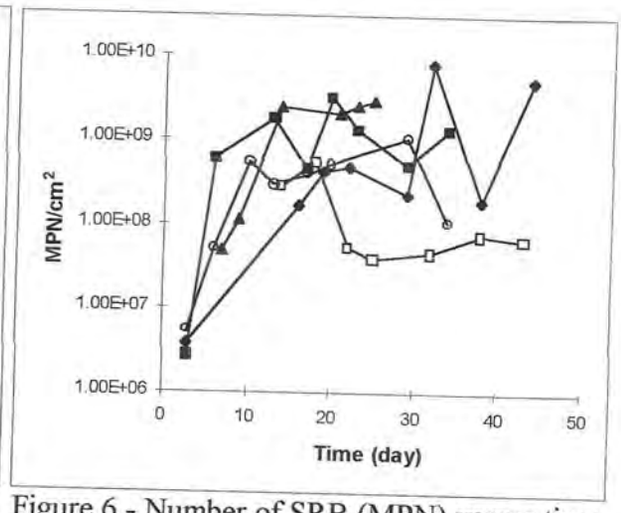

Figure 6 - Number of SRB (MPN) versus time

(▲- stainless steel assay 1,◆ - stainless steel assay 2; ■ - stainless steel assay 3; □- PMMA assay 1; O - PMMA assay 2).

Biofilm formation followed the same trend on stainless steel or on PMMA in all replicates. At steady state they reached similar values for total bacteria per surface area (above $1x10^8$ cells/cm^2; Fig 5).

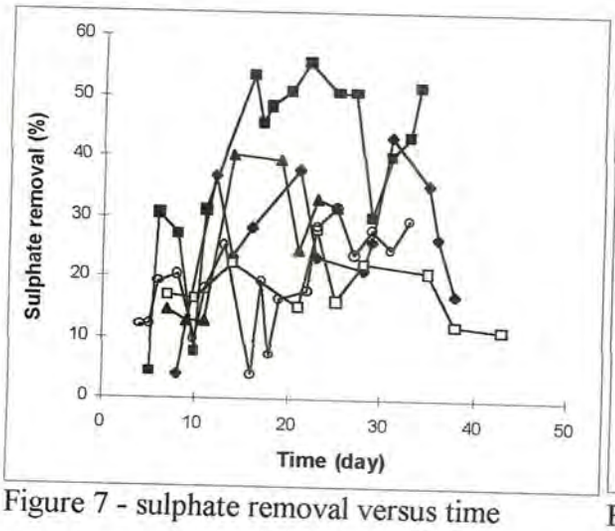

Figure 7 - sulphate removal versus time

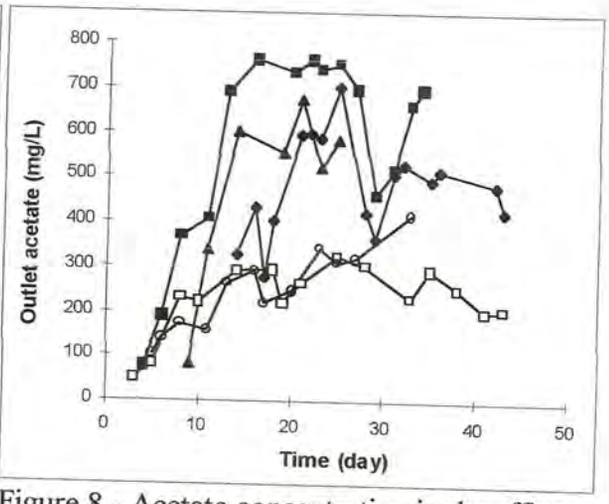

Figure 8 - Acetate concentration in the effluent stream versus time.

(▲ - stainless steel assay 1,◆ - stainless steel assay 2; ■ - stainless steel assay 3; □ - PMMA assay 1; O - PMMA assay 2).

Figures 7 and 8 present sulphate removal and the acetate concentration in the effluent stream, respectively. Results showed that there was a much higher sulphate consumption and acetate concentration in the assays with stainless steel as the biofilm substratum than with PMMA, although the total bacteria number (Figure 5) were quite similar in both substrates. Additionally, the MPN counts (Figure 6) did not appear much higher on stainless steel surface. All these statements may prove that the specific activity of SRB was higher on the metal surface.

Biofilm from the stainless steel and PMMA coupons were scraped into a batch medium and the suspended growth of the biofilm bacteria were followed the same way as for the batch previously referred. (Figure 9).

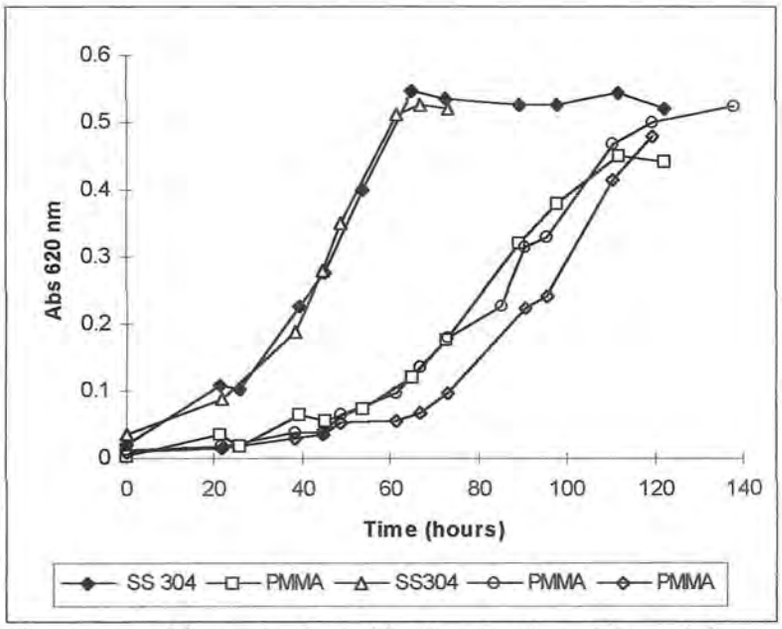

Figure 9 - Growth curves of bacteria from biofilm developed in stainless steel and PMMA (stainless steel assay 3 and PMMA assay 2)

The biofilms developed on stainless steel presented higher growth rate than the ones previously developed on PMMA. This may signify that stainless steel has an effect on biofilm development. Probably, metal elements present in stainless steel may be the reason for these results.

Literature present few studies that investigated the influence of metal ions present as alloying elements of steel (such as Cr, Ni and Mo) on the growth of planktonic population of SRB and on the activity of the enzyme hydrogenase. Probably, iron is one of the most studied elements and its influence in hydrogenase enzymes is well known. These enzymes found in the cytoplasm, periplasmic space or attached to the membrane of some SRB *spp.* play an important role in the generation of energy in bacterial cells. Studies showed that hydrogenase activity can be affected by the presence of some surfaces. (Cheung *et al.*, 1994). Cheung *et al.* (1994) studied metal effect on two marine strains of sulphate reducing bacteria and they noticed that Cr, Ni and Mo ions present in the culture medium had a negative impact on the growth rates and biomass production of planktonic SRB population.This study also demonstrated that those ions were able to inhibit the hydrogenase activity of a SRB *sp.*.

In the present study, it has been shown that Ni has a positive impact on SRB suspended growth. Nickel (Ni) represents around 8% of stainless steel 304 so its presence may explain

that SRB attached to stainless steel 304 coupons showed higher activity than when they attach to PMMA.

CONCLUSIONS

The conclusions of the study are as follow:

- All the tested nickel (Ni) concentrations studied had a positive impact on the growth of suspended cultures of *Desulfovibrio desulfuricans*. Nickel may contribute to an increase in the growth rate of the planktonic cells.

- The sulphate reducing bacteria showed a higher activity as biofilm on stainless steel surface than on PMMA.

- The apparent positive influence of the metal substratum on the SRB activity may be related to its composition.

ACKNOWLEDGEMENT

The authors acknowledge the financial support of Programme Praxis XXI (Grant Praxis XXI/ BD /13462 /97.

REFERENCES

- Cheung C.W.S. and Beech I.B. (1994). The influence of metal ions on the activity of hydrogenase in sulphate reducing bacteria. In: Proc of the 3rd International EFC Workshop. Portugal.
- Edyvean R. G. J., Percival S., Knapp J. S., Wales D. S., Beech I. B. and Videla H. (1996). Physical and chemical differences between grades AISI 304 and 316 stainless steels and their effect on bacterial fouling and corrosion. In: Proc: 2nd NACE Latin American Region Corrosion Congress.
- Feron D. (1994). Attachment of *Desulfovibrio vulgaris* to steels: influence of alloying elements. In: Proc of the 3rd International EFC Workshop. Portugal
- Hanjangsit L., Beech I.B., Edyvean R.G.J. and Hammond C. (1994). Biofilm development on stainless steels in a potable water system. In: Proc of the 3rd International EFC Workshop. Portugal