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THE INFLUENCE OF SURFACE MATERIAL ON THE DEVELOPMENT OF *DESULFOVIBRIO DESULFURICANS* BIOFILMS

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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. Additionally, hydrogen sulphide that is a product of their metabolism can cause serious health risks. In this study, sulphate reducing bacteria (SRB) biofilms were developed on stainless steel 304 and on polycarbonate in order to evaluate surface effect on biofilm formation.
Results showed that the biofilm formed on stainless steel presented higher metabolic activity, confirmed by lactate and sulfate removals. 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. Studies performed with suspended cultures of *Desulfovibrio desulfuricans* also showed that the presence of nickel in the media had a positive impact on bacterial activity.

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

INTRODUCTION
Dissimilatory sulphate-reducing bacteria (SRB) are important for their fundamental role in the sulphur cycle and also for their ability for growing in biofilms, on metalic surfaces or in aerobic wastewater treatment systems (sulfate reduction accounts for up to 50% of the mineralization of the organic matter), leading to enormous fouling problems. The number of studies about this group of bacteria recently increased, especially stimulated by the recognition of their importance in the oil industry, where they are considered responsible for corrosion of process equipment. Also, hydrogen sulphide production from SRB respiration can pose a serious health risk. Despite numerous publications, much research is still needed, particularly on the interactions between SRB and surfaces under flow conditions. It has been proved that the physico-chemical properties of surfaces influence biofilm development. 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. In the present study, SRB biofilm formation was studied on metal (stainless steel 304) and polycarbonate coupons under turbulent conditions in a flow system.

METHODS
The SRB biofilm was grown under turbulent flow (Reynolds number = 7000) in a polycarbonate 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 polycarbonate. *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 27ºC 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). Lactate and acetate concentrations both in the influent and in the effluent streams were determined by HPLC. Sulfate concentrations in the two streams were measured by capillary electrophoresis.

RESULTS AND DISCUSSION

Figures 1 to 3 present the development of biofilm on metal (stainless steel 304) and on polycarbonate coupons, as total bacteria (figures 2 and 3) and SRB (MPN counts; Figure 1). Biofilm formation followed the same trend on stainless steel or on polycarbonate in all replicates. At steady state they reached similar values for total bacteria per surface area (above $1x10^8$ cells/cm^2; Figs. 2 and 3).

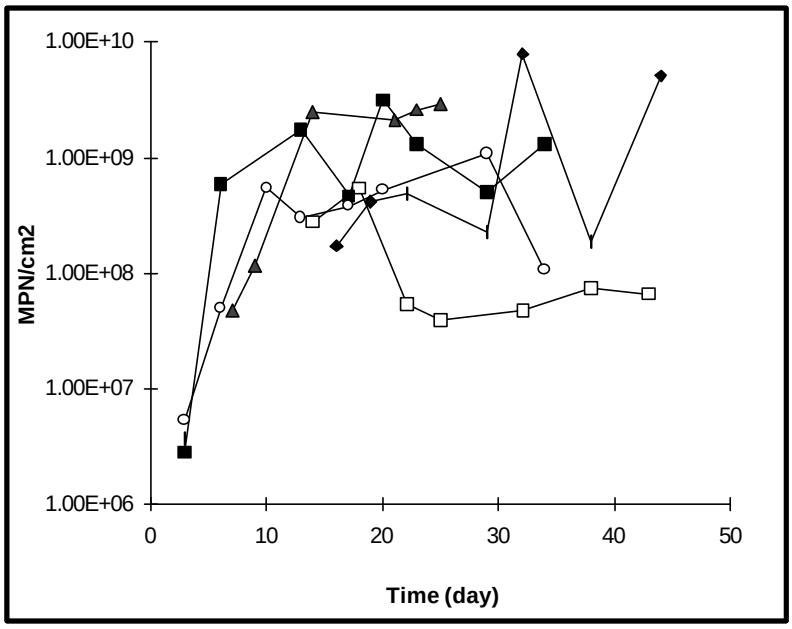

Figure 1- Number of SRB versus time on stainless steel and on polycarbonate surfaces (Σ - stainless steel assay 1,◆ - stainless steel assay 2; ■ - stainless steel assay 3; - polycarbonate assay 1; O - polycarbonate assay 2).

The profiles of lactate and sulfate (substrates) removal are presented in Figures 4 and 5, respectively. There was much higher lactate and sulfate consumption in the assays with stainless steel as biofilm substratum than with polycarbonate. Acetate (product) concentration in the effluent stream was also higher in the assays with stainless steel (data not shown). That shows that the metabolic activity of the biofilm was markedly higher on stainless steel than on polycarbonate. However, MPN counts presented on figure 1 did not appear much higher on stainless steel , this also indicates that SRB cells on stainless steel may have higher specific activity than the ones on polycarbonate.

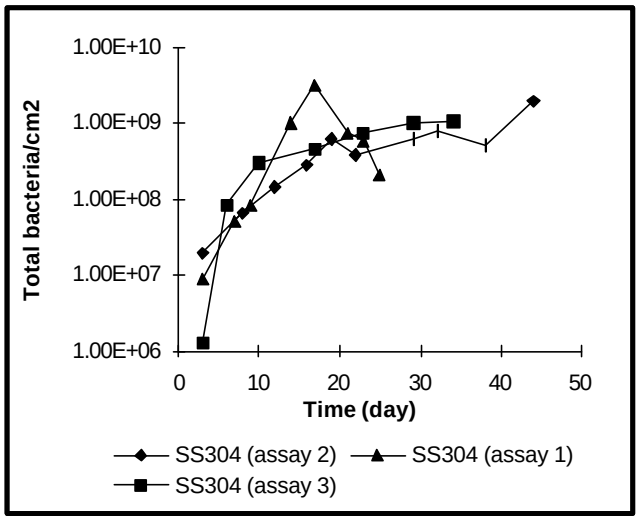

Figure 2- Total bacteria versus time in stainless steel assays

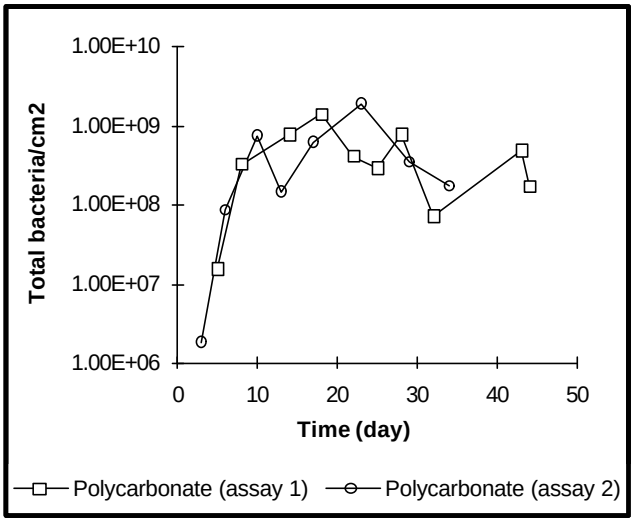

Figure 3 -Total bacteria versus time in polycarbonate assays

(Σ - stainless steel assay 1,◆ - stainless steel assay 2; ■ - stainless steel assay 3; - polycarbonate assay 1; O - polycarbonate assay 2).

Figure 6 presents the growth curves obtained with biofilms scraped into a batch medium from the two different surfaces. It is possible to see that the biofilms developed on stainless steel had a higher growth rate. 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.

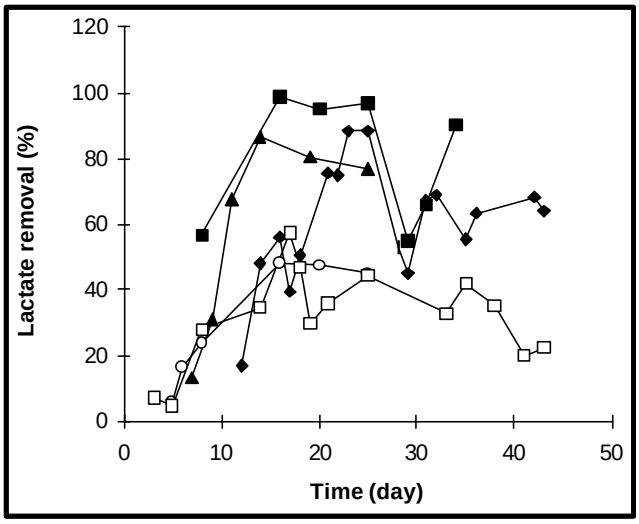

Figure 4 - Lactate removal versus time in stainless steel and polycarbonate assays (Σ - stainless steel assay 1,◆ - stainless steel assay 2; ■ - 1; O - polycarbonate assay 2).

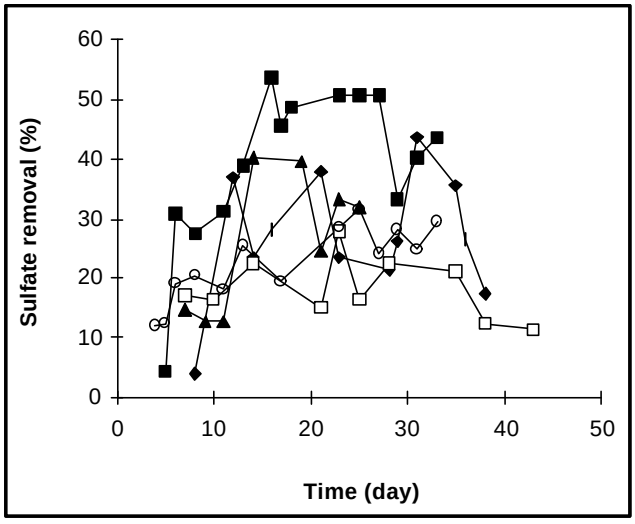

Figure 5 - Sulfate removal versus time in stainless steel and polycarbonate assays . stainless steel assay 3; - polycarbonate assay

Sulphate reducing bacteria have a high requirement for iron, which affects the production and the activity of specific enzymes such as the periplasmic hydrogenase (Bryant *et al.*, 1993). However, in the present study iron was supplied in the liquid medium and therefore not considered to be limiting for SRB development on polycarbonate. Beside iron, other metal elements that were present in the studied alloy may have an effect on SRB activity. This is the case of nickel (Ni) that represents around 8% of stainless steel 304. Its presence may explain that SRB attached to stainless steel 304 coupons showed higher activity than when they attach to polycarbonate. The effect of Ni on growth and activity of SRB is currently under investigation in our laboratory. Preliminary results showed that the presence of nickel in the growth medium of SRB has a positive impact on their activity. When nickel at several concentrations is added to suspended SRB culture, the bacteria present higher growth rate compared to the control (medium without added nickel).

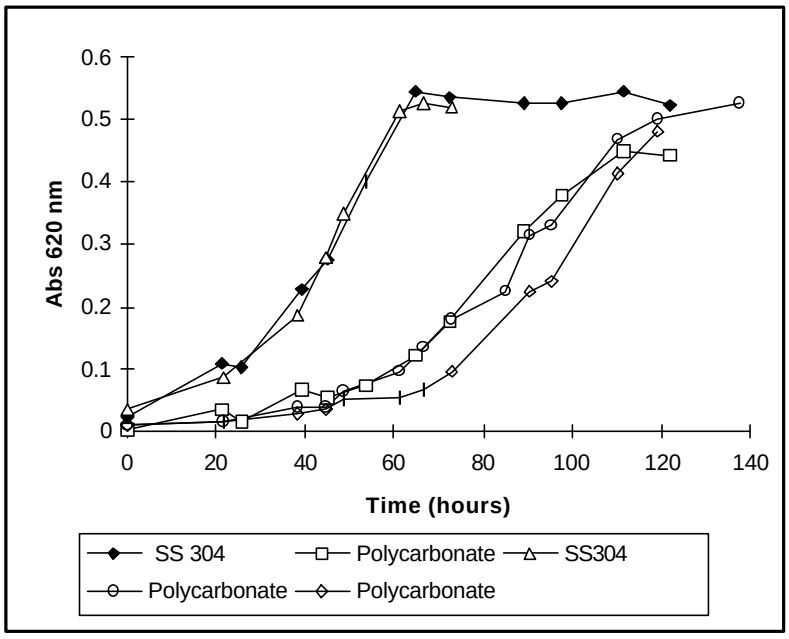

Figure 6 - Growth curves of bacteria from biofilm developed in stainless steel and polycarbonate (stainless steel assay 3 and polycarbonate assay 2)

CONCLUSIONS

The conclusions of the study are as follow:

- The sulphate reducing bacteria showed a higher activity as biofilm on stainless steel surface than on polycarbonate.
- The apparent positive influence of the metal substratum on the SRB activity may be related to its composition. Preliminary results show that nickel may be involved in the higher activity of the SRB growing on stainless steel

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