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Development of Bio-Film on Metal and Polycarbonate as a Sulphate Reducing Bacteria

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In sulphur cycle Sulphate Reducing Bacteria (SRB) have an important role and therefore in wastewater treatment system, they are able to form biofilms on metallic surfaces, leading to fouling and corrosion problems. Though last two decades researchers have discovered that SRB can, infact, tolerate and even respire oxygen. In this study the SRB biofilms were discovered in order to evaluate surface effect on biofilms formation.

The present investigation results showed that the biofilms formed on stainless steel presented higher metabolic activity, confirmed by lactate and sulphate removals. Studies performed with suspended cultures of *Desulfovibrio desulfuricans* also showed that the presence of Ni in the media have the positive impact on bacterial activity. This will show satisfactory results in wastewater treatment plants of the industries.

Key words: Effluents, *Desulfovibrio desulfuricans*, Sulphate reducing bacteria.

SRB are important for their fundamental role in the sulphur cycle and also for their ability for growing in biofilms, on metallic surfaces or in aerobic wastewater treatment systems (Wani and Thorat, 2007). In recent years, the application of this and related technologies has been proposed (trials performed) for use in oilfield situations.

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 processed equipments. Hydrogen sulphide production from SRB respiration can pose a serious health risk. Despite numerous publications, much research is still needed, particularly on the interaction between SRB and surfaces underflow condition. In the present study, SRB biofilms formation was studied on metal. The goal of this paper is to summarize current knowledge regarding the role of SRB in microbial systems and review research that links activity to lithification of microbial mats.

Edyvean *et al.* (1996) showed that stainless steel 304 was colonized by a significantly higher number of bacteria (viable and total) than stainless steel 316, in a potable water system. Stainless steel 304 was characterized by a rougher

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surface and the presence of molybdenum in SS 316 could explain the lower bacterial adhesion. In the present study, SRB bio film formation was studied on metal (stainless steel 304) and polycarbonate coupons under turbulent conditions in a flow system. The present investigation proved that the physicochemical properties of surfaces influence bio film development for surface material like polycarbonate and stainless steel.

MATERIAL AND METHODS

SRB biofilm was grown under turbulent flow in a polycarbonate flow cell system within a recirculation loop. Desulfovibrio desulfuricans (DSM 642) was used to inoculate the reactor, which was first operated in batch mode for three days and then switched to a continuous flow mode at a dilution rate of 0.5 h as investigated by Bryant *et. al.,* 1993.The culture media were selected from Atlas (1993) and evaluated for cultivation of SRB cultures and trace elements like B, Co, Cu, Mn, Zn. The temperature in flow cell was approximately 27°C and pH was around 7. Total bacterial count in the biofilm was scraped in sterile buffer, depressed and treated for bacteria and SRB cultures for this DAPI technique and SRB were estimated by MPN. Lactate, acetate concentration both in influent an influent streams were determine by HPLC. This study shows that the bacteria is reducing pollution load from the wastewater treatment systems.

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

Fig. 1 to 3 present the development of biofilm on metal and on polycarbonate, as a total bacteria (fig. 2 and 3) and SRB (MPN counts fig. 1). Biofilm formation followed the same trend on stainless steel or on polycarbonates in all replicates at steady state they reached similar value for total bacteria per surface area.

Figs. 1 to 3 present the development of biofilm on metal (stainless steel 304) and on polycarbonate coupons, as total bacteria (fig. 2 and 3) and SRB (MPN counts; Fig. 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).Other studies have presented additional evidence of sulphate reduction on polycarbonate and stainless steel (*e.g.,* Canfield and Des Marais, 1993; Teske *et al.,* 1998; Wierenga *et al.,* 2000; Jonkers *et al*., 2003 and Buhring *et al*., 2005).

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

Table 1. Average rates of sulphate reduction and sulphide generation for SRB isolates

	Average sulphate reduction rate (mg SO_4^{2-}/L/day)					Average sulphide generation rate (mg HS^-/L/day)				
Isolate	A27	B2	C3	E28	F7	A27	B2	C3	E28	F7
Day 0 to 15 (total)	73	76	68	87	81	3.4	2.5	2.7	4.4	3.2
Day 11 to 15 (most rapid)	473	475	456	393	488	9.8	7.3	8.3	11.3	8.3

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.

SRB have a high requirement of Fe, which affect the production and activity of specific enzymes (Wani and Thorat, 2008). However, in the present study Fe was supplied in the liquid medium and therefore not considered to be limiting for the SRB development on polycarbonate. Besides Fe, other metal elements that we present in the studied alloy may have an effect on SRB activity.

Table 1 gives the average sulphate reduction and sulphide generation rates for all the culture isolates over the duration of the experiment (15 days) and compares these to the average rates over day 11 to day 15 (4 days), where the most rapid changes in sulphate reduction and sulphide generation were observed (see figures 2 and 3).

During days 11 to 15, the period of the most rapid activity, the sulphate reduction rate for isolate E28 was lower at 393 mg SO_4^{2-}/L/ day, compared to the highest reduction rate of 488 mg SO_4^{2-}/L/day for isolate F7. However isolate E28 showed the highest sulphide generation rate during the full period of 11.3 mg HS^-/L/day compared to 9.8 mg HS^-/L/day for isolate A27 with the next highest rate of sulphide generation.

Overall, isolate E28 showed the highest average sulphate reduction and sulphide generation rates of 87 mg SO_4^{2-}/L/day and 4.4 mg HS^-/L/day compared to 81 mg SO_4^{2-}/L/day and 3.2 mg HS^-/L/day for isolate F7, the next best isolate, and compared to 76 mg SO_4^{2-}/L/day and 2.5 mg HS^-/L/day for B2, which was the worst performing isolate.

While the differences in the average sulphate reduction and sulphide generation rates between the isolates are not very large, overall, isolate E28 showed superior growth and performance characteristics compared to the other four culture isolates with 55 % sulphate reduction. Therefore, isolate E28 was selected as the culture isolate of choice for all subsequent experiments.

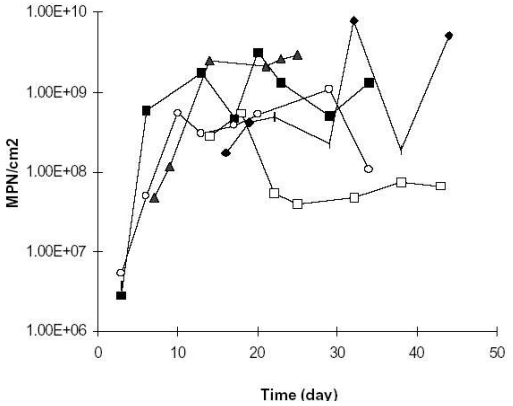

Fig. 1. Number of SRB versus time on stainless steel and on polycarbonate surfaces (Σ-stainless steel assay 1,u - stainless steel assay 2; η - stainless steel assay 3; ■ - polycarbonate assay 1; O - polycarbonate assay 2)

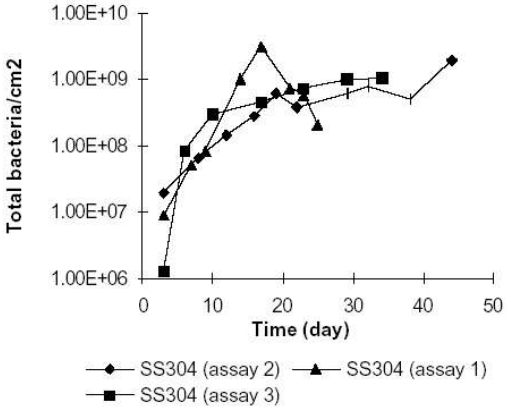

Fig. 2. Total bacteria Vs time in stainless steel assays

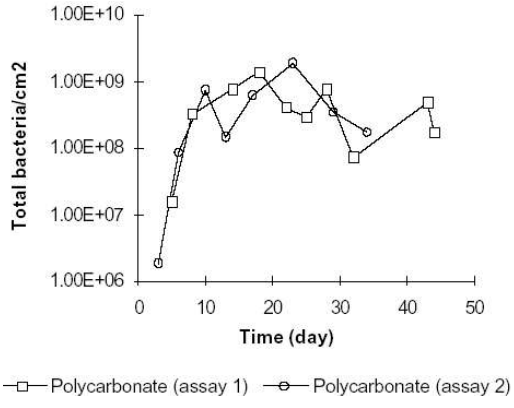

Fig. 3. Total bacteria Vs time in polycarbonateassays

REFERENCES

1. Atlas, R.M, *Handbook of microbiological media.* Parks, L.C. (Ed.). CRC Inc. Florida. 1993; **71**: 566-567, 856-858.
2. Bryant R.D., Kloeke F.O. and Laishley E. J., Regulation of the periplasmic Fe hydrogenase by ferrous iron in *Desulfovibrio vulgaris* (Hilenborough). *Appl. Environ. Microbiol*. 1993; **59**: 491-495
3. Bu¨ hring, S.I., Elvert, M., Witte, U., The microbial community structure of different permeable sandy sediments characterized by the investigation of bacterial fatty acids and fluorescence in situ hybridization. *Environ. Microbiol*. 2005; **7**: 281– 293.
4. Canfield, D.E., Des Marais, D.J., Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. *Geochim. Cosmochim. Acta* 1993; **57**: 3971– 3984.
5. Edyvean R. G. J., Percival S., Knapp J. S., Wales D. S., Beech I. B. and Videla H., 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* 1996.
6. Jonkers, H.M., Ludwig, R., De Wit, R., Pringault, O., Muyzer, G., Niemann, H., Finke, N., De Beer, D., Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: dLa Salad de ChipranaQ (NE Spain). *FEMS Microbiol. Ecol.* 2003; **44**: 175-189
7. Wani P.P. and Thorat S. R., "Isolating metal tolerance bacteria capable to remove chromium from tannery waste". *J. Biosciences, Biotechnology Research Asia,* 2007; **4** (2): 733-736.
8. Wan P.P. and Thorat. S.R., "Heavy metal adsorption by clinoptilolite from aqueous solutions," *J. of Current World Environment* 2008; **3**(1), 135-141.
9. Teske, A., Ramsing, N.B., Habicht, K.S., Fukui, M., Ku¨ver, J., Jørgensen, B.B., Cohen, Y., Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). *Appl. Environ. Microbiol*. 1998; **64**: 2943- 2951.
10. Wierenga, E.B.A, Overmann, J., Cypionka, H., Detection of abundant sulphate-reducing bacteria in marine oxic sediment layers by a combined cultivation and molecular approach. *Environ. Microbiol*. 2000; **2**: 417-427.
11. Baumgartner, L.K., Reid, R.P., Dupraz C. Decho, A.W, Buckley, D.H., Spear, J.R, Przekop, K. M., Visscher, P.T., Sulfate reducing bacteria in microbial mats: Changing paradigms, *new discoveries Sedimentary Geology* 2006; **185**: 131-145