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Underground Corrosion by Microorganisms Part II : Role of Anaerobic Sulphate Reducing Bacteria-*Desulfotomaculum SP*

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ABSTRACT

During the course of studies on the corrosion causing soil microflora from different geoclimatic regions of India, several strains of anaerobic sulphate reducing bacteria belonging to genus *Desulfotomaculum* were isolated and characterised. Their corrosive action on mild steel, galvanised iron and structural aluminium, the three main metals of construction of underground structures, have been studied under laboratory conditions.

1. INTRODUCTION

Many strains of the anaerobic sulphate reducer belonging to genus *Desulfovibrio* have been reported to play a very active role in the corrosion of piple lines, underground storage tanks and such other structures throughout the world[1-3]. Some strains of this organism are known to corrode stainless steel also[4]. The mechanism of subsoil anaerobic corrosion of metals is a combined action of several factors including a consortium of several types of microorganisms[5]. The extent of involvement of some of these collaborators in microbial corrosion has been investigated by several workers[6,7]. In India, only limited studies with some strains of *Desulfovibrio* in respect of microbial corrosion of oil storage tanks[8], aircraft fuel tanks and oil and water pipelines has been done. However, no attempt to detect and study the role of another anaerobic sulphate reducer belonging to genus *Desulfotomaculum,* a much more resistant type of organism was made. A study on the occurance of this organism in Indian soils and their corrosion causing potential was undertaken. Panels of the three main metals of construction, viz.

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mild steel, galvanised iron and aluminium alloy (54-S- half hard) were subjected to underground exposures at 7 locations for 18 months, after which the potential corrosion causing microbes were isolated from the excavated panels. Soils of seven more types from other locations were also analysed for these studies (unpublished work). The isolated strains of the genus *Desulfotomaculum* were studied for their comparative corrosivity to the experimental metals under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Isolation, Purification and Characterisation of the Strains.

Winogradeskey's[9] technique modified by Larsen[10] for obtaining enriched population of soil bacteria was adopted, besides using the enrichment and isolation techniques recommended by Postgate[11] for sulfate reducers. Strain purification was done on API[12] agar medium stubs by serial dilutions and respective promient black colonies alongwith one cubic centimeter of agar blocks were transferred to API broth and put under anaerobic conditions innediately. Incubation was done at 35° ± 1°C and 55° ± 1°C. Characterisation was done using freshly prepared (24 hours old) subcultures. The desulfovirdine[13] and hydrogenase[14] positive strains (*Desulfovibrio*) were separated. The remaining strains were studied for their growth temperature, gram reaction, shape, motility, spore formation and thermotolerance as per the scheme recommended for their characterisation by Campbell and Postgate[15].

2.2 Preparation of Test Panels and Laboratory Exposures

The experimental panels was prepared from mild steel, galvanised iron and structural aluminium alloy. The panels were of uniform size viz. 75 × 15 mm although slight variation in their thickness from metal to metal was there. After identification marking the panel finishing for experiments was done as per standard technique[16].

For every strain, four replicates of each metal, prepared, numbered and weighed earlier were placed in separate test tubes (150 x 25 mm) and plugged with tight cotton plugs after putting 20 ml of Baar's medium[17] so as to keep the panels completely immersed. Four replicates of each metal, prepared under identical conditions were kept to serve as control. After sterilisation and cooling the control and set markings (for different strains) were made. Excepting the control, all other sets of panels were inoculated with their respective strains and incubated anaerobically at their respective optimum temperature of growth for a period of four weeks. After incubation the panels were cleaned under running water with a brush, dried in an oven at 60°C for one hour and weighed. The difference in the initial and final weights of the panels indicated loss in weight due to corrosion. The average of the replicates was taken as loss for each metal by the particular strain.

2.3 Confirmation of Corrosive Role of Microorganisms

In order to verify the corrosive role of the microbes, metal panels were exposed in sterile soil and presterilised extract of soil in water. The panels were incubated under identical conditions and temperature as used in other experiments. There was

practically no difference in the loss in weight as compared to control panels. This gives a conclusive indication that the microbes accelerate the corrosive action through their metabolic activity.

3. RESULTS AND DISCUSSION

It is an interesting observation that all the three known species of genus *Desulfotomaculum* have been recorded in the Indian soils. It appears that their occurance is linked with the particular types of soils. *D. nigrificans* has been isolated from seven locations having saline coastal or desert soils (except strain No. 7 from Hyderabad). Five strains of *D. orientis* were picked up from undifferentiated alluvial soils. The two strains of *D. ruminis* have been found to be associated with gravelly soils (Table 1).

The structural aluminium alloy proved to be quite resistant to corrosion by the strains of *Desulfotomaculum*. Mild steel and galavanised iron are quite susceptible to the corrosive action of all the isolates in varying degrees.

Table 1. Characters of the strains of *Desulfotomaculum* isolates from different regions of India

Location of the predominate strain	Strain number	Shape	Gram reaction	Spore formation	Motility	Heat stability at 100°C 10 mts.	Optimum growth temp. (°C)	Organism
Kanpur		Thin long curved rods.		+	+		35	D. *orients*
Jodhpur	2	Straight long thin rods		+			55	D. *nigrificans*
		Thin long curved rods		+		+	35	D. *orients*
		Straight long thin rods		+			55	D. *nigrificans*
Mysore	5	Thick short straight rods					35	D. *ruminish*
	6	Thin long curved rods		+	+		35	D. *orientis*
Hyderabad	7	Straight long thin rods		+	+		55	D. *nigrificans*
Puri	8	Straight long thin rods		+	+	+	55	D. *nigrificans*
Jammu	9	Short thick straight rods					35	D. *ruminis*
	10	Thin long curved rods		+			35	D. *orientis*
Jorhat	11	Thin long curved rods		+	+		35	D. *orientis*
Digha	12	Straight long thin rods		+			55	D. *nigrificans*
Madras	13	Straight long thin rods		+		+	55	D. *nigrificans*
Kandla	14	Straight long rods		+			55	D. *nigrificans*

Legend : Positive +
Negative

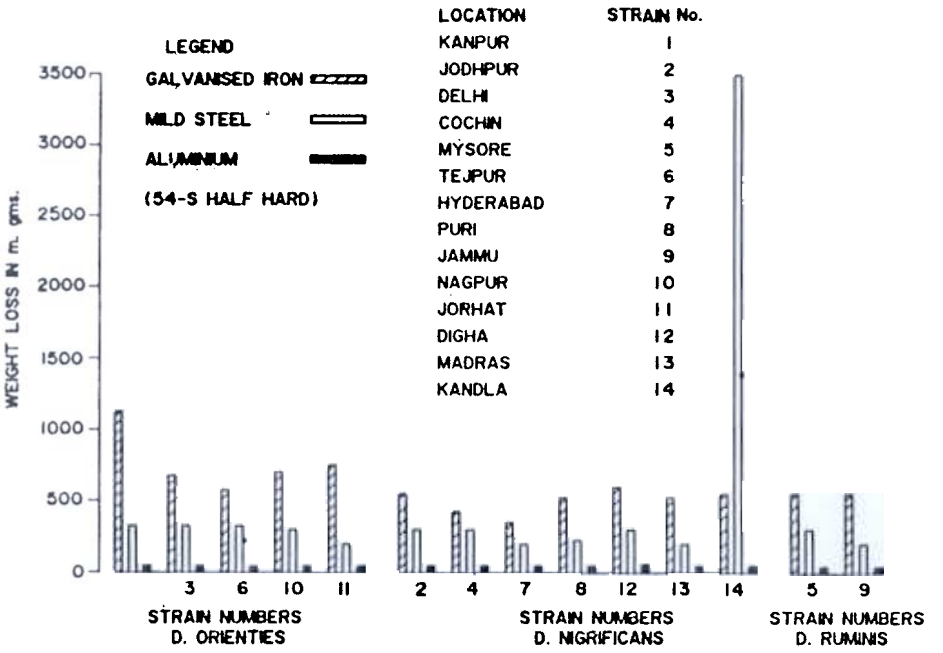

Figure 1 Variation in corrosivity of the different strains of the panels of galvanised iron mild steel and aluminium alloy.

The three species varied in their optimum growth temperatures. In the first 36 hours after inoculation their degree of growth at 35°C and 55°C varied considerably. However, incubation for a prolonged period upto 96 hours resulted in growth at 55°C for all strains. Strain variations within the three species are clearly marked. Isolates of the same species from different locations vary in corrosivity to the same metal (Fig. 1).

The highest amount of corrosion to mild steel has been caused by strain No. 14 from Kandla, (*D. nigrificans*), other strains of the same species viz. strain Nos. 7, 8 and 13 from Hyderabad, Puri and Madras respectively caused very little corrosion. Similar is the case with strain No. 1 from Kanpur (*D. orientis*) causing heavy corrosion to galavanised iron as compared to strain No. 3, 6, 10 and 11 from Delhi, Tezpur, Nagpur and Zorhat respectively. The corrosive action of the two strains of *D. ruminis* on mild steel shows a similar tendency though their corrosivity towards galvanised iron is fairly comparable.

A significant observation has been about the initial inhibitory action of galvanised iron against all the isolates of *Desulfotomaculum.* Within 18 hours of inoculation the inoculum turns colourless indicating cessation of its metabolic activity. However, addition of a heavier dose of inoculum revitalises the growth and the culture becomes almost normal in 72 hours of incubation.

The corrosivity of the culture is appreciably increased when the aerobes are also introduced in a mixed form. This observation is corroborative to the findings of Hamilton[5] that the process of corrosion is due to combined effort of a group of microbes.

4. CONCLUSION

All the three species of the genus *Desulfotomaculum* have been recorded in the various geoclimatic regions of India and have been found to play a definite role in corroding the underground structures. Their corrosivity varies from metal to metal and strain to strain. The strains of this anaerobic sulphate reducer had almost no effect on the aluminium alloy. This is quite in contrast to the corrosivity of this metal caused by the strains of genus *Desulfovibrio* which are well known corroders of aluminium alloys.

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