Indian Journal of Experimental Biology
Vol. 41, September 2003, pp. 1023-1029

Control of metallic corrosion through microbiological route

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Involvement of biofilm or microorganisms in corrosion processes is widely acknowledged. Although majority of the studies on microbiologically induced corrosion (MIC) have concentrated on aerobic/anaerobic bacteria. There are numerous aerobic bacteria, which could hinder the corrosion process. The microbiologically produced exopolymers provide the structural frame work for the biofilm. These polymers combine with dissolved metal ions and form organometallic complexes. Generally heterotrophic bacteria contribute to three major processes: (i) synthesis of polymers (ii) accumulation of reserve materials like poly-β-hydroxy butrate (iii) production of high molecular weight extracellular polysaccharides. Poly-β-hydroxy butyrate is a polymer of D(-)β-hydroxy butrate and has a molecular weight between 60,000 and 2,50,000. Some extracellular polymers also have higher molecular weights. It seems that higher molecular weight polymer acts as biocoating. In the present review, role of biochemistry on corrosion inhibition and possibilities of corrosion inhibition by various microbes are discussed. The role of bacteria on current demand during cathodic protection is also debated. In addition, some of the significant contributions made by CECRI in this promising area are highlighted.

Keywords: Biofilm, Corrosion control, Heterotrophic bacteria, Metallic corrosion

Solid surfaces immersed in aqueous environments absorb organic matter from the surrounding environment. Bacteria through their extra cellular metabolites cause the formation of an extremely complex micro layer called slime or biofilm. The involvement of biofilm or microorganisms in corrosion processes is well known. The microorganisms can promote corrosion in different ways by differential aeration cell[1], extra cellular polymeric substances[2,3], or by their binding capability with metal ions[4-6]. According to the more recent studies by researchers, certain specific microorganisms which favour the formation of passive film on the metal surface could actually hinder the corrosion process. But no detailed study is available on microbiologically induced corrosion control (MICC) systems. This provides a vast scope for developing corrosion control techniques through microbiological route.

Process of corrosion control by bacteria

A biofilm can contain different types of bacteria, viz. (a) heterotrophic bacteria (b) sulphate reducing bacteria, (c) thiosulphate oxidizing bacteria and (d) manganese oxidizing bacteria. The active contributor in a biofilm is heterotrophic bacteria, which utilize energy from carbon source. These heterotrophic bacteria contribute to three major processes.

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1. Synthesis of polymers (anabolism)
2. Reserve materials (storage)
3. Catabolism

More than 95% of the cellular material of *E.coli* and other microorganisms consists of macromolecules. A typical analysis of microbial cells g/100g of dried cells[7] is as follows : protein 52.4 , polysaccharides 16.6, lipid 9.4, RNA 15.7, DNA 3.2. These constituents have their own distinct role in controlling metallic corrosion. Bacteria do not accumulate lipids as reserve material but they contain considerable amounts of lipids especially phospholipids and glycolipids as constituents of their membrane systems. These phospholipids are negatively charged. While glucose serves as growth substrate for *E.coli*, a number of hexose and pentose phosphates are intermediates in the breakdown of this substrate. Phosphates are also negatively charged. Hence, it becomes easier for the positively charged metal ions to combine with phosphates and form protective organometallic complex.

Synthesis of polymers

It has been mentioned that about 97% of the cellular material are macromolecules. Three groups can be distinguished, viz. (a) lipids, (b) periodic macromolecules such as peptidoglycan, polysaccharides and (c) macromolecules such as nucleic acids and

proteins. Many microorganisms excrete polysaccharides which may be retained as part of the cell structure. The macromolecules may become dispersed in the liquid phase or in the presence of a suitable solid substrate, may adhere to immersed surfaces to form an organic film.

Lipids are not true macromolecules, as the monomers are not linked to one another by covalent bonds. However, in aqueous environment phospholipid molecules such as phosphatidyl choline associate in such a way that a double layered structure is formed . Membrane contains up to 60% of protein; some are embedded in the lipid layers and have specific functions in the various transport processes. Peptidoglycan is the cell wall which is synthesized from two building blocks: UDP–N–acetyl muramic acid, pentapeptide and UDP–N–acetyl glucosamine. The formation of these compounds in the cytoplasm is transferred to a membrane lipid carrier–undecaprenyl phosphate. It is then linked to N–acetyl glucosamine. This disaccharide pentapeptide is transferred by the lipid carrier to the extracellular site of the cell membrane and used on a building block in peptidoglycan chain elongation. Besides these, molecules of fatty acids are linked to glucosamine disaccharide of lipid through ester and amide linkages.

Bacteria differ in the reserve material they accumulate under certain conditions. Polyphosphate accumulation is widespread among bacteria. It essentially functions as a phosphorus storage material and is utilized for nucleic acid and phospholipids synthesis under conditions of phosphate starvation. Besides, glycogen and poly-β-hydroxy butyrate serve as energy storage compounds. Poly-β-hydroxy butyrate (PHB) is a typical prokaryotic storage material. It is widespread in bacilli, in chemolithotrophic and phototropic bacteria, and in *Pseudomonas* sp. PHB is a polymer of D(-)β-hydroxy butyrate and has a molecular weight between 60,000 and 2,50,000. It is accumulated in the cells of granules surrounded by membranes. Under appropriate conditions, bacterial cells may accumulate so much Poly-β-hydroxy butyric acid (PHB) that accounts for approximately 60% of their dry weight [8]. *Alcaligenes* sp accumulates up to 80% of its dry weight as polymer. In eubacteria the synthesis of large amounts of PHB is triggered by different environmental stimuli including limitation of oxygen, nitrogen, phosphorus and potassium individually or collectively depending on the organism[9,10]. Propionic and pentanic acids are required as substrates in the production of poly-β-hydroxy butyric co-β-hydroxy valoric acid. Since it has high molecular weight, it is possible that the compound may act as a very good bio-coating. Page[11] has described the isolation of a mutant strain of *Azotobacter vinelandii*, which rapidly produces large amounts of PHB.

Production of siderophores

Escherichia coli develops four different Fe^{3+} chelator transport systems which are termed siderophores. It occurs in all aerobic and facultative anaerobic microorganisms. In gram negative microorganisms, specific receptor proteins are present for the siderophores in the outer membrane. It may act as inhibitor particularly in acid medium.

Enzymes

Nitrate reducer is a molybdenum containing membrane bound enzyme which reduces nitrate to nitrite. Molybdenum is present in this enzyme in the form of the so called molybdenum cofactors (Mo Co) in which molybdenum is bound to a protein moiety; the so called molybdopterin Mo Co is non covalently bound to the protein and its function is that of a prosthetic group. It is interesting that this cofactor occurs in all molybdoprotein except the nitrogenase in which one type of subunit is a molybdenum iron-sulphur protein[8]. Besides nitrate reducers molybdenum cofactor has been detected in formate dehydrogenase, sulfite oxidase, Xanthine dehydrogenase, trimethylamine–n-oxide reductase and co-oxides. It is possible to inhibit the production of hydrogenase enzyme by molybdate. It is also well known that sulphate reducing bacteria can accelerate corrosion by conversion of sulphate to sulphide in presence of hydrogenase enzyme. By using appropriate nutrient, it is possible to control the production of hydrogenase enzyme thereby nullifying the effect of sulphide conversion. On the other hand sulphate reducing bacteria will be made to form protective film which will be tenacious and adherent.

Metal ion and exopolymer interaction

Microorganisms growing on water-immersed metal surfaces form biofilms that are held together by extra cellular polymeric substances (EPS) or biopolymers. Widespread evidence indicates that many extra cellular polymers produced by bacteria are acidic and contain functional groups that easily bind

al ions in natural environment. The naturally med biofilms can be strengthened by addition of propriate nutrients so as to form a tenacious and erent protective film on the metallic substrate. A direct beneficial effect of this approach will be a considerable reduction in the current requirement of cathodically protected structures, since the tenacious film being less porous will reduce the cathodic area and lead to lesser current demand for cathodic polarization. Thus this approach may prove to be an energy saving system in the case of cathodic protection.

International status

Only few references are available with regard to corrosion control by microorganisms[12-15]. The protective action of *Serratia marcescens* on aluminium has been reported. These polymers combine with corrodible metal ions and form organometallic complexes. These metal/exopolymer complexes in biofilm provide sites for differential aeration cells[16] and anaerobic zones for the growth of SRB[14]. Many workers have examined the role of bacterial exopolymers in metal binding[5] by many ways. Differential binding abilities help to establish ion concentration cells[3-5,17] Bacterial film can prevent diffusion of corrosion species such as oxygen to the metal surface thereby reducing the corrosion rate. The similar reduction in corrosion of aluminium and copper obtained by the two different biofilms (*Bacillus brevis* and *Pseudomonas fragi*) suggests that the protection of these metal surfaces is a general phenomenon which occur due to oxygen removal. Pederson and Hermansson[18] found that protection was caused by cellular metabolic activity. The gram positive bacteria excretes hydrophobic mycolic acids to its exterior, whereas the outer membrane of the gram-negative consists mainly of hydrophilic lipopolysaccharides, probably covered with protein[8] . Besides, these same groups produce "Vivianite" formation which inhibits corrosion[19]. Syrett *et al.*[20] reported that the genetically engineered bacteria can release corrosion inhibiting compounds or antimicrobial compounds. Ornek *et al.*[21] observed that the *Bacillus subtilis* biofilm reduces the corrosion rate of the passive aluminium alloys at *p*H 6.5. Vorster and Wanders[22] emphasized the need for non toxic environmentally benign compounds such as derivatives of naturally occurring polysaccharides, acetyl amines that can protect metal against corrosion. Corrosion inhibition by neutralization of corrosive substances is related to the corrosion inhibition of mild steel by aerobic bacterial biofilms under flow conditions[23].

CECRI's contribution on corrosion inhibition

Maruthamuthu *et al.*[24,25] suggested that improved passivity of metals could be attributed to negatively charged cell walls of bacteria. It was shown that reduction in anodic current (i_p) is accomplished by passivators present within the biofilm. The actual concentration of potential passivators such as phosphates and nitrate have been measured in the biofilm. Besides, Mohanan *et al.*[26] observed that biofilm reduces the growth of inorganic phase but promotes the formation of organometallic complex thus improving the passivity of metal. Recently Ponmariappan *et al.*[27] noticed that *Pseudomonas maltophilia* and *Serratia* sp. inhibited (communicated) mild steel corrosion by about 5 times. It can be seen from Table 1 that CECRI has identified 10 bacterial species which can confer effective corrosion protection and raise the durability of steel substrate by different factors ranging from *1.17* to *5.33*[28]. They have also established for the first time that gram negative strains have more inhibitive capability than gram positive strains (Table 1). All the bacteria studied have corrosion inhibiting property but to varying degree. The reason for the more inhibitive capacity by negative strains is the presence of phospholipids in their cell wall when compared to positive strains. Hence, it can be concluded that it is possible that improvement of cell wall may inhibit the corrosion. The corrosion rate is activation controlled and not diffusion controlled. Hence it can be concluded that the inhibitive bacteria act as "anodic inhibitor". Since the literature available on the role of enzymes in biologically inhibiting corrosion can be said to be nearly scarce, investigation has been done

Table 1 — Corrosion Inhibitive property of various strains in 1000 ppm chloride environment[28]

S.No.	Bacterial species	Corrosion rate (mpy)	Durability factor
1.	*Actionobacillus lignieresii*	0.1597	3.44
2.	*Actinobacter calcoaceticus*	0.1303	3.83
3.	*Acinetobacter radioresistens*	0.1030	5.30
4.	*Bacillus amyloliquefaciens*	0.3299	1.87
5.	*Bacillus brevis*	0.5283	1.17
6.	*Escherichia coli*	0.1172	4.60
7.	*Kluyvera cryocrescens*	0.1383	4.76
8.	*Pseudomonas aureofaciens*	0.2022	3.10
9.	*Salmonella typhimurium*	0.1152	4.78
10	*Xanthomonas campestris*	0.1031	5.33

by using α-amylase enzyme in presence of *Pseudomonas putida* and *Bacillus amyloliquefaciens*. Polarization study indicated that both *Pseudomonas putida* and *Bacillus amyloliquefaciens* act as cathodic inhibitors. The impedance behaviour of mild steel with and without various bacterial species is shown in Figs 1-6.

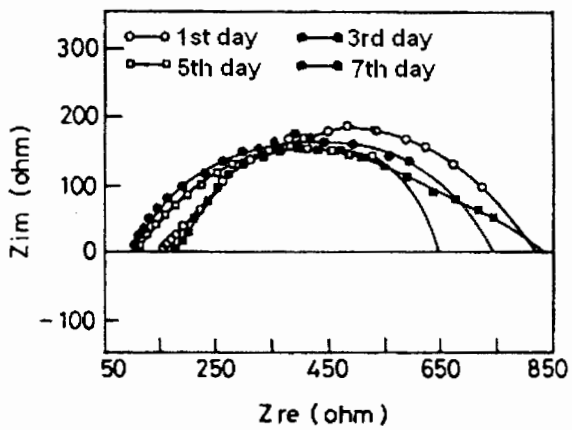

Fig. 1 — Nyquist plot showing relationship of resistance (Zre) with reactance (Zim) of without bacteria (control)

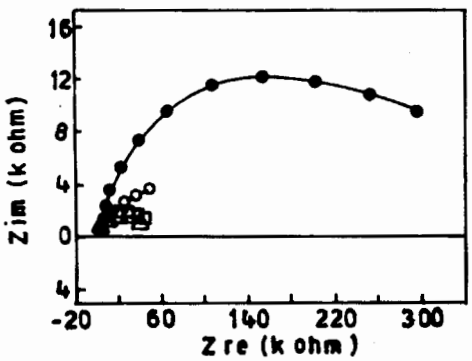

Fig. 2 — Nyquist plot showing relationship of resistance (Zre) with reactance (Zim) of *Bacillus brevis*

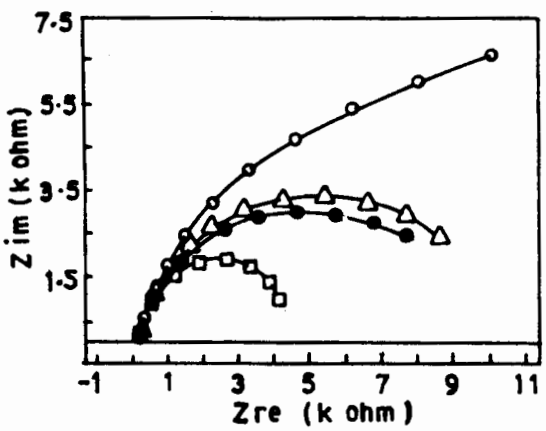

Fig. 3 — Nyquist plot showing relationship of resistance (Zre) with reactance (Zim) of *Xanthomonas campestris*

The figures indicate that the electrochemical kinetics changes greatly with time. Surface charges are attributed to the formation of protective film and the continued improvement in the protective ability of the film. It needs further research to explain cell wall physiology on corrosion inhibition. Corrosion

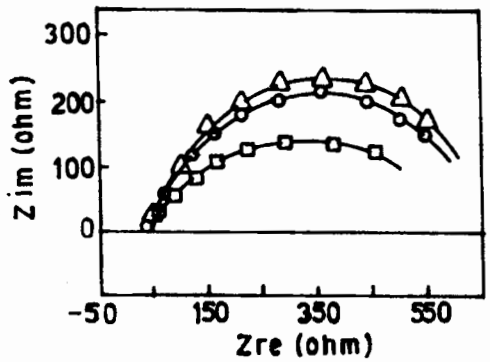

Fig. 4 — Nyquist plot showing relationship of resistance (Zre) with reactance (Zim) of 5000 ppm chloride

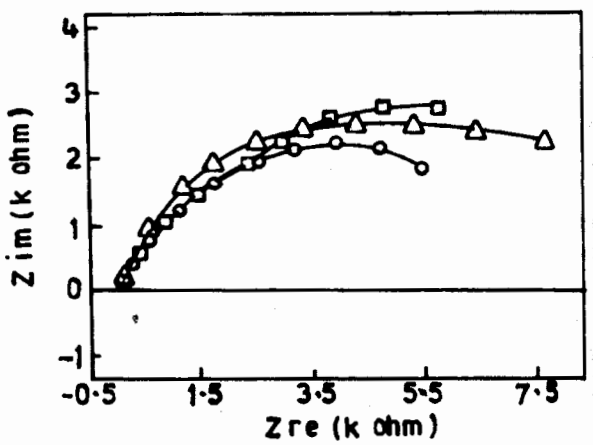

Fig. 5 — Nyquist plot showing relationship of resistance (Zre) with reactance (Zim) of *Bacillus amyloliquefaciens*

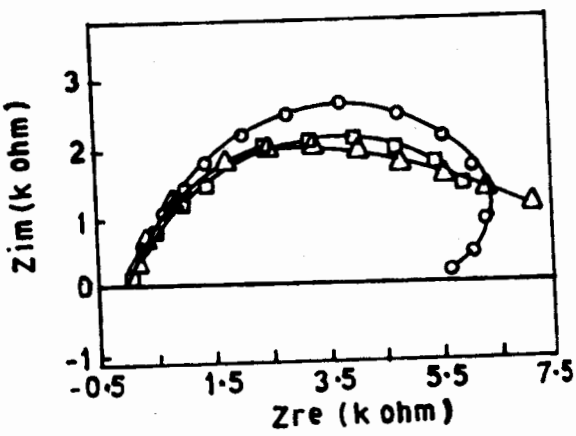

Fig. 6 — Nyquist plot showing relationship of resistance (Zre) with reactance (Zim) of *Pseudomonas putida*

inhibition has also been observed in presence of *Vibrio* sp. which leads to solubilization of inorganic phosphate by the production of organic acid and conversion of the same as free phosphate ions which may get adsorbed on the material and inhibit the corrosion[29]. The immobilized strains of *Bacillus brevis, Xanthomonas campestris* have also been identified as good corrosion inhibitive species.

Influence of fresh water heterotrophic bacteria on reinforced concrete has also been studied[30]. whereas the presence of HB in the surrounding medium adversely affects the compressive strength of concrete due to the destructive action of glucose derived from bacterial action, polarization study (Figs 7 & 8) revealed that microbes improves the passivity of

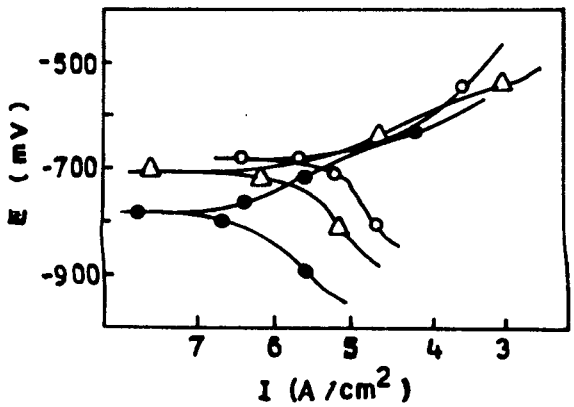

Fig. 7—Tafel's plot showing relation of current(I) with potential (E)

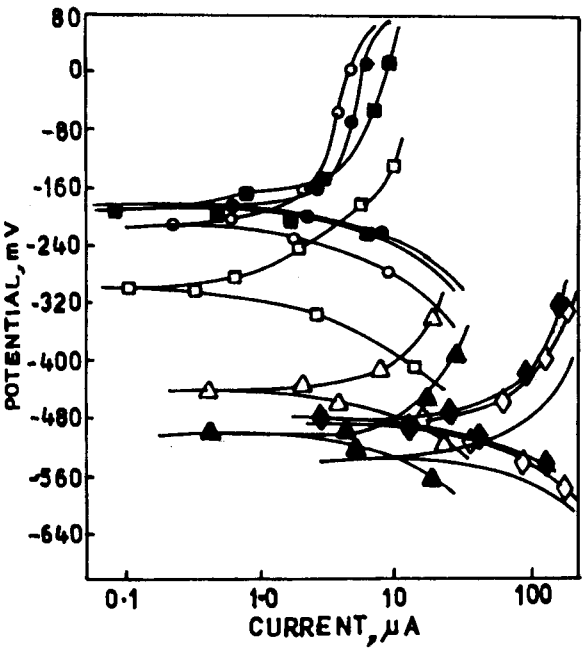

Fig. 8—Potentio dynamic polarization for reinforced steel in presence and absence of Heterotrophic bacteria

reinforcing steel by adsorption of organic species on steel which has been confirmed by XRD observation. The XRD pattern of mild steel in presence of bacterial species is shown in Fig. 9.

Underground pipelines normally traverse through different soil strata which might be aerobic or anaerobic in character. Cathodic protection is one of the well known techniques of protecting pipelines against soil corrosion. Various standards (NACE standard, DNV standard) suggest that maintaining the potential of pipeline at higher negative value (-950 mV vs $Cu.CuSO_4$) is needed in anaerobic environment. Recently CECRI has established that *Pseudomonas* sp. and *Vibrio* sp. dramatically reduces the current demand at various potentials during cathodic protection. These bacteria are organic nutrients utilizing bacteria, while attaching on structure, the first attached cells may became less active cells because oxygen reduction may be higher on cathodic surface. At the same time, these groups consume hydrogen formed at cathodic surface (Table 2). The possible routes of corrosion inhibition through the microbiologically secreted products is graphically represented in chart form.

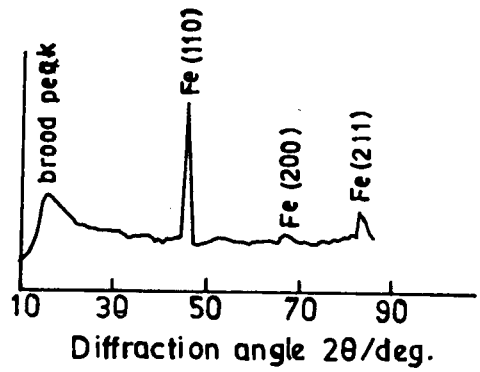

Fig. 9—XRD patterns of thin films on reinforced steel specimen

Table 2—Current demand during cathodic protection in presence of bacterial species (Ponmariappan *et al.*)[31]

Control			Bacterial species	
Potential (mV vs SCE)	1000 ppm (chloride alone) ($\mu A.cm^{-2}$)	3% nutrients 1000 ppm chloride ($\mu A.cm^{-2}$)	Pseudomonas ($\mu A.cm^{-2}$)	*Vibrio* sp. ($\mu A.cm^{-2}$)
-700	15 ± 2	16 ± 2	2 ± 0.1	2.0 ± 0.2
-800	28 ± 3	26 ± 2	3 ± 0.1	4.0 ± 0.5
-900	32 ± 3	30 ± 3	5 ± 0.1	5.5 ± 0.5
-1000	28 ± 2	30 ± 3	7 ± 0.2	6.0 ± 0.5
-1100	68 ± 4	69 ± 5	25 ± 2.0	16 ± 2.0

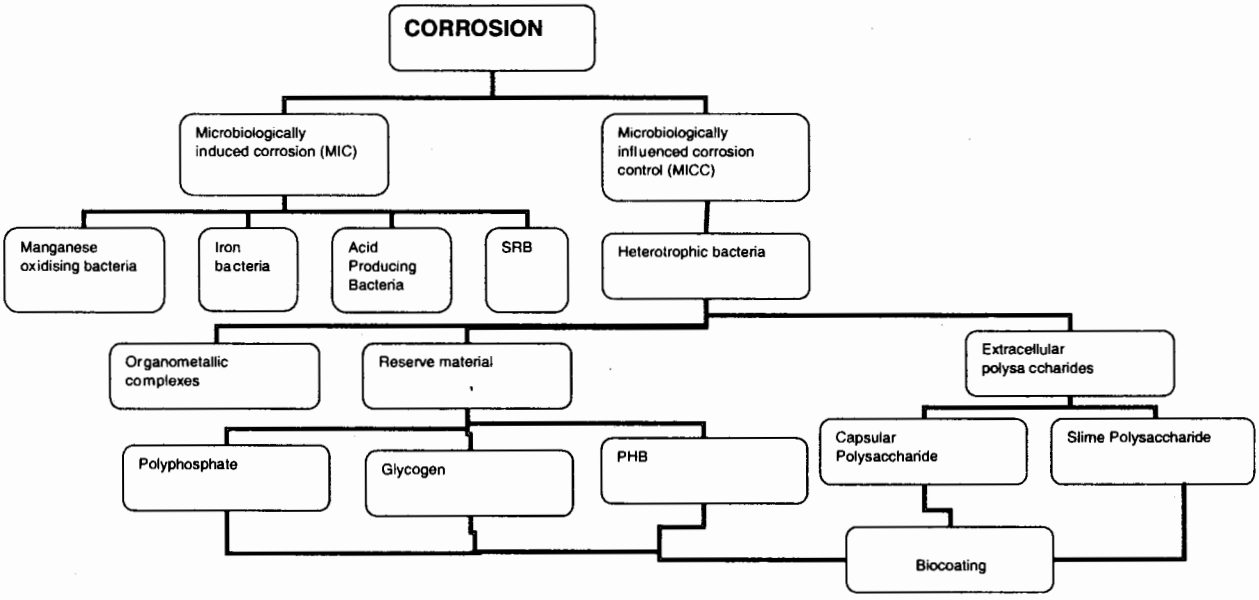

Chart I — Microbiological route for corrosion control

Conclusion

The present review clearly shows that there is vast scope for developing newer technologies of controlling metallic corrosion through microbiological route. Heterotrophic bacteria as a class are capable of developing a tenacious and adherent biocoating on the metal surface which can confer efficient protection. In this regard, production of high molecular weight polysaccharide becomes very important. The fact that molecular weight can be as high as 2,50,000 makes this approach quite promising.

Another aspect is the influence of gram negative strains on the durability factor. CECRI has identified some species which can give a durability of five and more in 1000ppm chloride. This observation could be quite useful in extending the life of the water pipelines. The future R&D efforts should concentrate on ensuring sustainability.

CECRI'S observation that certain bacteria can actually reduce the current demand for catholic protection is bound to have a beneficial impact on cost of cathodic protection. Large scale field data are to be generated for assessing the actual economic impact.

Acknowledgement

This research was supported by the Department of Science and Technology, New Delhi. The authors are thankful to the Director, Central Electrochemical Research Institute, Karaikudi for giving permission to communicate the review paper. One of the authors (S.Ponmarippan) expresses his sincere thanks t CSIR, New Delhi for the award of SRF.

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