Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87* (3): 329-337, jul./sep. 1992

SULPHATE-REDUCING BACTERIA ASSOCIATED WITH BIOCORROSION – A REVIEW

TANIA C. DE ARAUJO-JORGE/+; CLÁUDIA M. L. MELO COUTINHO & LUIZ EDMUNDO VARGAS DE AGUIAR

Laboratório de Biologia Celular, Departamento Ultraestrutura e Biologia Celular Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Biocorrosion means any process of corrosion in which microorganisms are somehow involved. As far as the petroleum industry is concerned, the anaerobic type is the more important, with Sulphate-Reducing Bacteria (SRB) accounting for half of the described processes. SRB are obligate anaerobes that use sulphur, sulphate or other oxidized sulphur compounds as oxidizing agents when decomposing organic material. A typical product of SRB metabolism, hydrogen sulphide -H2S-, is extremely toxic. In the present work we review the literature on mechanisms underlying biocorrosive processes in which SRB are involved and summarize some of the ultrastructural and electrochemical work developed using SRB obtained from water injection flow in wells located on PETROBRAS offshore marine platforms, sampled directly in the field over metallic probes, or cultured under laboratory conditions.

Biofilms develop when SRB adhere to inert surfaces. A high diversity of morphological types is found inside these biofilms. Their extracellular matrix is highly hydrated and mainly anionic, as shown by its avid reaction with cationic compounds like ruthenium red. We have noted that variations in iron content lead to interesting changes in the ultrastructure of the bacterial cell coat and also in the rate of corrosion induced in metallic test coupons. Since routine methods to prevent and treat SRB contamination and biodeterioration involve the use of biocides that are toxic and always have some environmental impact, an accurate diagnosis of biocorrosion is always required prior to a treatment decision. We developed a method that detects and semi-quantifies the presence of living or dead SRB by using free silver potentials as an indicator of corrosive action by SRB-associated sulphides. We found a correlation between sulphide levels (determined either by spectrophotometry, or using a silver electrode – E(Ag) – that measured changes in free potentials induced by the presence of exogeneously added sulphide) and SRB concentration (enumerated by a culturing method). E(Ag) was characterized under a variety of conditions and was found to be relatively immune to possible interference resulting from aeration of media or from the presence of iron corrosion products. The method offers a simple, rapid, and effective means of diagnosing biocorrosive processes prior to their control.

Key words: sulphate-reducing bacteria – SRB - biocorrosion – ultrastructure

1. SULPHATE-REDUCING BACTERIA (SRB) AND THE PROBLEM OF BIOCORROSION

Corrosion means the deterioration of different materials, generally metals, under chemical or electrochemical action by surrounding media (Gentil, 1983; Pourbaix, 1987). The phenomenon can occur in air, water or land environments. The deterioration of non-metallic materials, such as polymers, paper, plastic, rubber, cement or wood is also considered as a kind of corrosion. Aeration and deaeration, electrolyte concentration, metal composition of alloys and thermodynamic conditions are some of the many variables that give rise to different types of corrosion, defining the nature and rate of chemical reactions.

Metallic corrosion may be described as the oxidation of metal, with consequent mass loss arising from the formation of metallic ions

This work was supported by PETROBRÁS (Petróleo Brasileiro S.A.) and FIOCRUZ (Fundação Oswaldo Cruz).

+Corresponding author.

Received 4 November 1992.
Accepted 29 June 1992.

and the concomitant loss of electrons to the oxidizing agent. The reaction involving loss of electrons – the oxidizing reaction – is known as anodic reaction, while the one characterized by a gain of electrons – the reducing reaction – is known as the cathodic reaction. In aerated aqueous environments oxygen and water are cathodic, gaining electrons from metal to form hydroxyl ions, while in deaerated aqueous environments hydrogen ions are cathodic, gaining electrons to form gaseous hydrogen (Fig. 1).

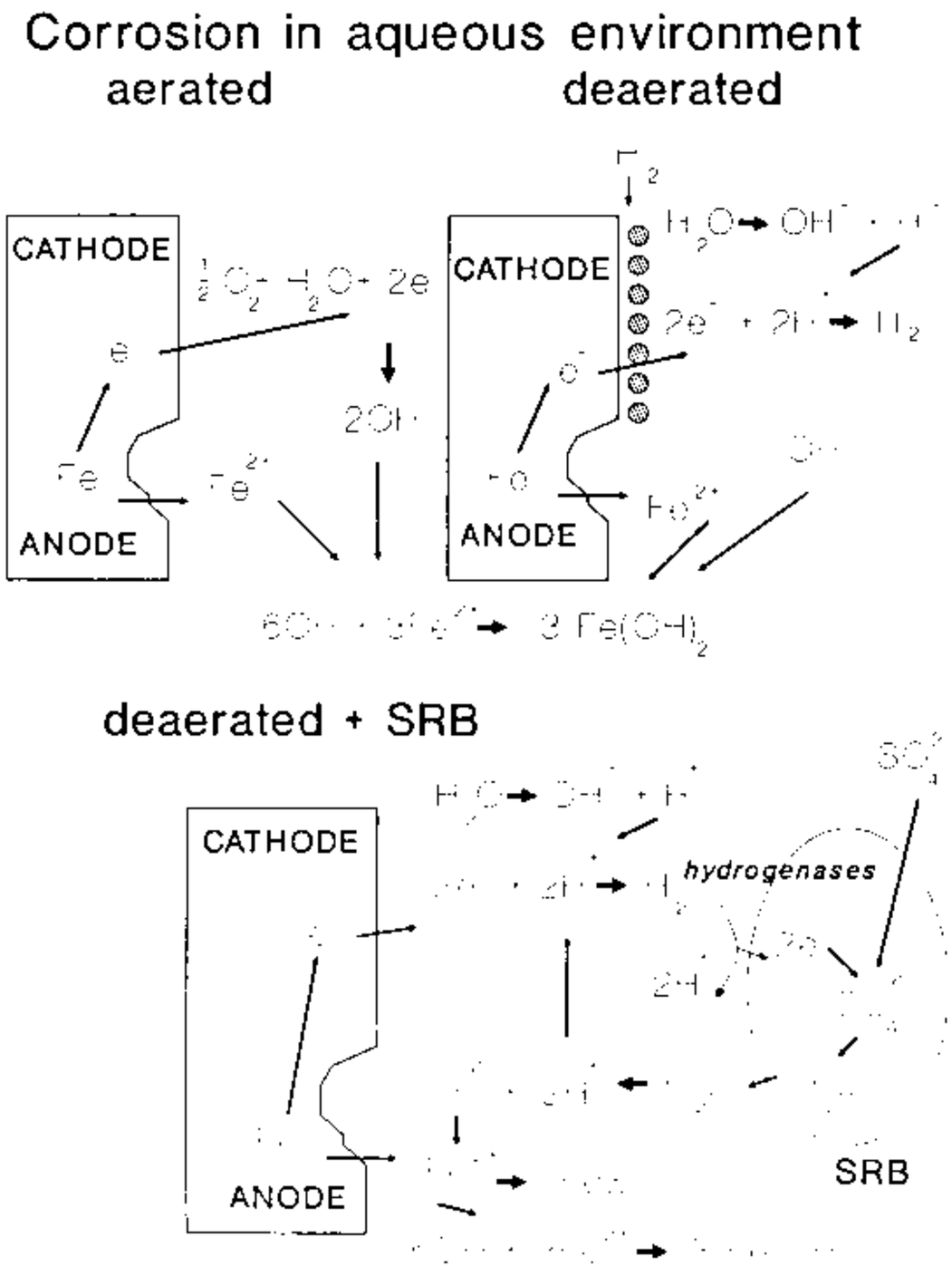

Fig. 1: proposed mechanisms for corrosion in aqueous environments, in aerated and deaerated media, in the absence (top) and presence (bottom) of Sulphate-Reducing Bacteria.

Biocorrosion, or microbiologically induced corrosion (MIC), signifies any process of corrosion in which microorganisms are somehow involved, either in aerated or deaerated media (Tiller, 1983). As far as the petroleum industry is concerned, the anaerobic type is the more important, with Sulphate-Reducing Bacteria (SRB) accounting for half of the described processes (Hamilton, 1983, 1985). The costs to industry take two forms: repair of damaged equipment and oil pipelines, and preventive maintainance. The United Kingdom spends 300 to 500 million pounds/year in this area (Tiller, 1983); the United States something in the region of 16 to 18 billion dollars/year (Skerebowski, 1978); PETROBRÁS estimates that 6.5 million dollars/year is spent on infusion of biocides on offshore platforms in the Campos basin (state of Rio de Janeiro). These calculations, moreover, do not include the cost of environment pollution. How to cut the growing costs arising from prevention of corrosion and from replacement of corroded materials on offshore marine oil platforms is then a question of strategic interest, especially for countries dealing with petrol extraction off its marine coast, such as Brazil.

Sulphate-Reducing Bacteria form a heterogeneous group of taxonomically and morphologically diverse genera and species, that are related in terms of ecology and physiology (Postgate, 1984; Gibson, 1990). They are obligate anaerobes that use sulphur, sulphate or other oxidized sulphur compounds as oxidizing agents when decomposing organic material, just as aerobes use oxygen in conventional respiration. They grow in environments that are rich in sulphate ($SO4^{-2}$) and have the metabolic machinery to reduce it gradually to sulphide (S^{-2}), passing through intermediate steps of incomplete sulphur oxidation in which sulphite, thiosulphate and other non-stoichiometric sulphides are formed (Gibson, 1990). Since SRB assimilate only relatively small amounts of reduced sulphur, releasing most of it into the external media, they are called dissimilatory sulphate reducing microorganims (Postgate, 1984). In the external aqueous environment, the released sulphide ions are generally hydrolyzed to free gaseous or dissolved hydrogen sulphide (H_2S). This compound, a typical product of SRB metabolism, is extremely toxic (more dangerous than hydrogen cyanide), and easily identifiable by its characteristic and unpleasant odour. In partially closed systems, such as storage tanks and concrete pillars on offshore oil platforms, gaseous H_2S is sometimes produced in considerable quantities, posing a serious hazard for oil industry workers (Hamilton, 1983).

Petroleum extraction is performed in two ways on marine platforms. In primary recovery, the pressure in the oil reservoir is sufficient to induce the flow of petroleum through the drill. In secondary recovery, this natural pressure is too low, and therefore a water injection system has to be installed providing the additional force needed to push out the oil.

Marine water is taken from sites close to the platform, deaerated to prevent oxygen corrosion, treated, filtered and injected to maintain the reservoir pressure. The resulting environment favours bacterial growth, in particular SRB growth, in that it offers conditions of anaerobiosis with sulphate and nutrients, as well as a metallic substrate for bacterial adhesion (the pipeline alloy). This is the most significant biocorrosive problem in the oil industry, but it is not the only one. In the course of production and storage of petroleum, there are many points at which biocorrosion is a problem, ranging from the well drill, through the processes of primary and secondary production, to the separation of oil fractions, transport and storage.

2. METABOLISM OF SRB AND MECHANISMS OF ANAEROBIC BIOCORROSION

The biology of SRB, which were first described in 1895 by Beijerinck, was reviewed in 1984 by Postgate and in 1990 by Gibson. The main genera are *Desulfovibrio, Desulfobacter, Desulfomonas, Desulfotomaculum* and *Desulfobacterium,* and these display a variety of forms: rod, vibrio, filamentous, rounded and coccoid. They grow using sulphate, or sometimes undergo fermentative growth in its absence, but are unable to use oxygen as an electron acceptor. Although they are tolerant to oxygen (Wall et al., 1990), their growth is inhibited in aerobic conditions and is activated whenever the local environment becames anaerobic (Postgate, 1984). Their nutritional range is restricted: they can not use carbohydrates or hydrocarbonides, but instead use CO_2, or a series of compounds like benzoates or fatty acids (from acetate to stearate).

Two types of enzymes are very important in the biology and ecology of SRB: surface *hydrogenases,* which allow the use of hydrogen as an electron donor in the chain of reactions leading to sulphate reduction, and *APS reductase,* the first enzyme in the cascade of sulphate reduction, which catalyses the reduction of adenosine-5'-phosphosulphate (the phosphorylated form of sulphate which is formed when ATP reacts with sulphate) to sulphite. SRB hydrogenases seem to be involved in the mechanism of biocorrosion (Pankhania, 1988; Bryant & Laishley, 1990), while APS reductases are used as specific markers for the detection of SRB (Odom et al., 1987).

The mechanisms underlying biocorrosive processes in which SRB are involved are not fully understood (reviewed in King & Miller, 1971; Hamilton, 1985; Pankhania, 1988; Videla, 1989). Corrosion of metallic alloys in an aqueous anaerobic environment (Fig. 1, Table) is an electrochemical phenomenon, in which H^+ ions generated through the dissociation of water (reaction 1) and electrons generated during the anodic reaction with metal (reaction 2) combine (cathodically) to form hydrogen atoms (reaction 3) which then form molecular H_2 (reaction 4). The process is susceptible to a mechanism of self-control ("passivation", in which the metal becomes "immune" to further corrosion) that take effect when a layer of H_2 forms over the metal surface, thus reducing the electron flow from the anodic to the cathodic area. The main corrosion products are metal hydroxides (reaction 5) or other metal compounds, depending on what additional dissolved cations are present. SRB seem to act by inhibiting the mechanism of self-control, in at least three ways (Fig. 1): (a) their

TABLE

Reactions occurring during anaerobic corrosion in aqueous environment

(1)	water dissociation	$8\ H_2O \rightarrow 8\ OH^- + 8\ H^+$
(2)	anodic reaction	$4\ Fe \rightarrow 4\ Fe^{2+} + 8\ e^-$
(3)	cathodic reaction (formation of atomic hydrogen)	$8\ H^+ + 8\ e^- \rightarrow 8\ H$
(4)	formation of molecular hydrogen	$8\ H \rightarrow 4\ H_2$
(5)	anodic depolarization (formation of corrosion products –iron hydroxides)	$6\ OH^- + 3\ Fe^{2+} \rightarrow 3\ Fe(OH)_2$
(6)	cathodic depolarization (SRB sulphate reduction and formation of sulphides)	$SO_4^{2-} + 8\ H \rightarrow S^{2-} + 4\ H_2O$
(7)	dissociation of hydrogen sulphide	$H_2S \rightarrow S^{2-} + 2\ H^+$
(8)	anodic depolarization (formation of corrosion products –iron sulphides)	$S^{2-} + Fe^{2+} \rightarrow FeS$

hydrogenases consume the H_2 formed in reaction 4, thus destroying and/or impeding the formation of a passivation film of hydrogen (in a process known as cathodic depolarization caused by SRB); (b) they generate H_2S as a metabolic product (reaction 6), that by dissociation (reaction 7) increases the concentration of H^+ ions in the cathodic area, changing the kinetics of reaction 3; and (c) they indirectly interfere with anodic depolarization, since sulphide anions (released in reaction 7) will combine with Fe^{+2} (released in the anodic area in reaction 2) to form iron sulphides as new corrosion products (reaction 8). The dissociation of H_2S as HS^- and S^{-2} depends on the environmental pH (Pourbaix, 1987). SRB can thus displace the chemical reactions that normally occur in an aqueous anaerobic environment, thereby accelerating both the anodic and cathodic depolarizations. These may not be the only ways in which SRB influence the corrosive process, and phenomena like direct metal binding by bacterial surface components (Bradley et al., 1984; Ferris et al., 1989), differential aeration by heterogeneous growth over the metallic surface (Costerton et al., 1987) or the direct action of extracellular bacterial polymers on the metal (Geesey et al., 1987) may also be implicated.

Biocorrosive processes involving SRB can be studied in the laboratory by exposing metal coupons to the corrosive environment containing SRB previously grown in specially designed culture media (Postgate, 1984). The bacteria can growth both in iron-rich and in iron-poor media, and we have noted that variations in iron content lead to interesting changes in the ultrastructure of the bacterial cell coat (Coutinho, 1991) and also in the rate of corrosion induced in metallic test coupons. Figure 2 shows the black precipitate of iron sulphides formed when similar inocula of SRB are applied to vessels filled with iron-poor culture medium in the absence (Fig. 2a) or presence (Fig. 2b) of a metallic coupon. Without the coupon, the low availability of Fe^{+2} (Fig. 2a) in the medium leads to the formation of only a thin black film after seven days in culture, whereas, in the presence of a coupon made with an iron-based alloy, SRB induces formation of a much greater quantity of corrosion products (Fig. 2b). The rate of corrosion, which is higher in the presence of SRB (King et al., 1973) or even of non-living SRB extracts (Bryant & Laishley, 1990), seems to be dependent on the sulphides formed during the corrosive process (Hamilton et al., 1989). Biogenic and inorganic sulphides have the same corrosiveness (Rickard 1969; Miller & King 1975; Videla 1986) and most of them do not confer protection by passivation, except for short periods (Pourbaix, 1987; Hamilton et al., 1989).

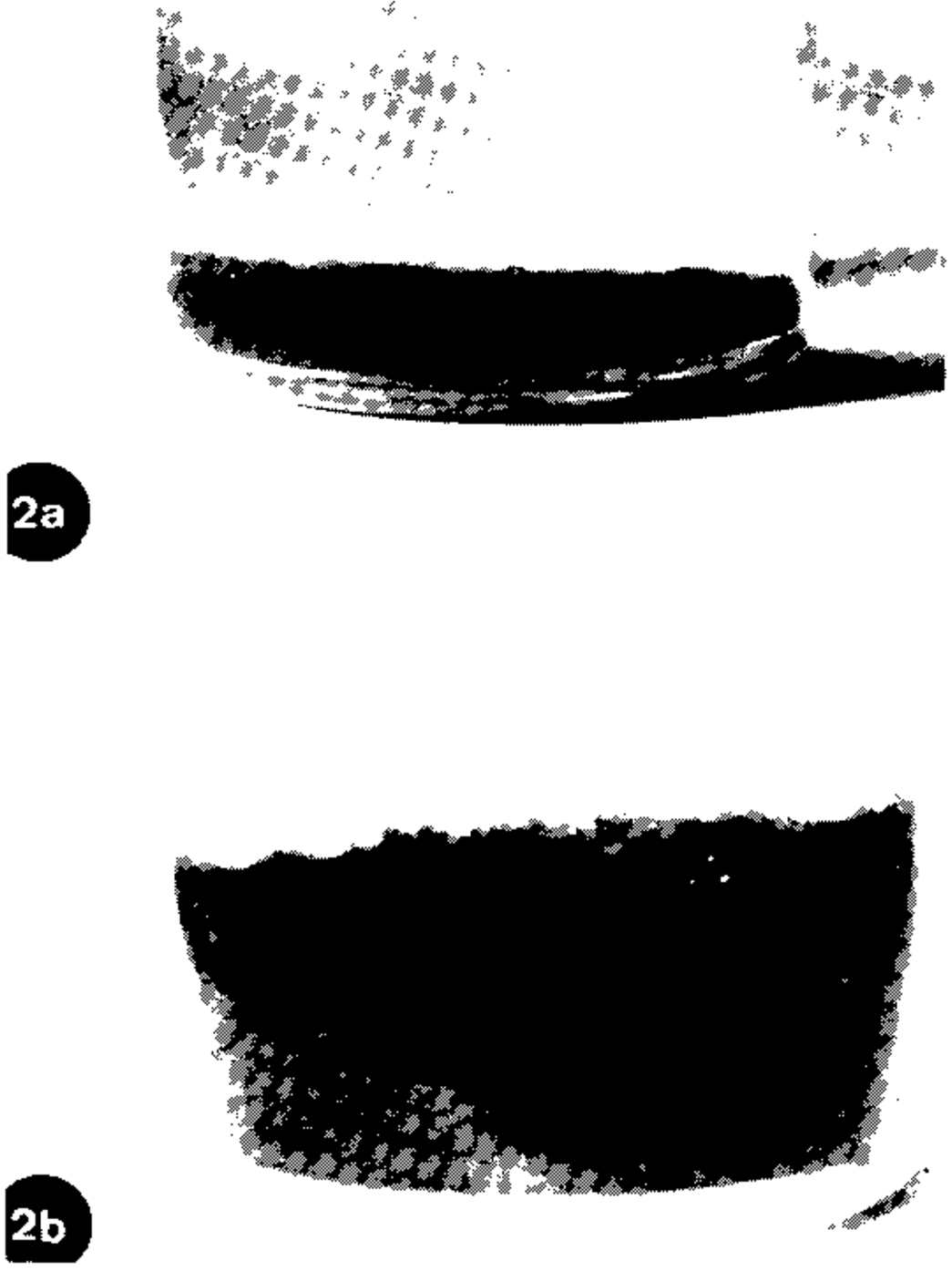

Fig. 2: iron sulphide precipitates in SRB samples grown for seven days in Postgate Medium C (iron-poor medium) in the absence (a) and presence (b) of an inserted metallic test coupon. Note that similar inocula of SRB were able to use either the low level of iron available in the media (Fig. 2a) or the iron released by the metallic coupon (Fig. 2b), with the latter situation showing a far greater release of corrosion products.

3. BIOFILMS AND BIOCORROSION

Following the universal strategy of microorganisms to attach firmly to sbstrates and to grow in consortia with a variety of species, SRB adhere to inert surfaces and develop biofilms (Characklis & Cooksey 1983; Costerton et al., 1987). Biofilms mediate the interaction between metal surfaces and the environment in biodeterioration processes like corrosion, but also in several biotechnological processes applied to materials recovery and handling (Videla, 1989). Sulphate reducers in

nature are adapted to ambient sulphate concentrations and, in a thick biofilm, they show varying affinities for sulphate, depending on their location within the biofilm (Nielsen, 1987). Biofilms containing mixed aerobic and anaerobic populations display stratified structures with specific anaerobic niches in which SRB growth is comfortably supported. Extracellular polymeric exopolysaccharidic material, which is produced in larger amounts by wild bacterial strains than by test tube bacteria (Costerton 1985), mediates cell-substrate and cell-cell attachment, forming an extensive network of extracellular matrix in which bacteria are embedded. We studied the ultrastructure of SRB (Coutinho, 1991; Coutinho et al., 1992) sampled directly over metallic probes exposed to water injection flow in wells located on PETROBRÁS offshore marine platforms (Fig. 3a), or cultured as mixed SRB populations under laboratory conditions in SRB- designed media (Fig. 3b). Figure 3 shows the ultrastructure of such biofilms as seen by transmission and scanning electron microscopy, and illustrates the high diversity of morphological types found inside them (Fig. 3b). The biofilm surface is highly adsorptive and can trap sediments, nutrients and ions in significant quantities. The extracellular matrix of SRB biofilms is highly hydrated and mainly anionic, as shown by its avid reaction with cationic compounds like ruthenium red (Fig. 3a). It concentrates and conserves extracellular enzymes necessary to the processing of high molecular weight substrates for subsequent nutrition of bacteria. Biofilms are thus complex structures consisting of microcolonies of proliferating cells, dead cells, extracellular material (metabolic products and secreted polymers) and, at the metallic surface, corrosion products. Since bacteria constitute a minor mass inside an enormous hydrated bioresin made with the extracellular polysaccharidic matrix, the interfacial system (metal/water) will be governed by the different transport processes taking place through the biofilm (Videla, 1989). Reactions between metabolites derived from bacteria and metal take place within the biofilm.

The organization of SRB in biofilms changes the classical electrochemical concept of electrical interface employed in corrosion studies and confers new properties which may also be implicated in additional corrosive mechanisms, such as the creation of areas of differential aeration over the metallic surface (Hamilton 1985) or the direct action of extracellular polymers (Geesey et al., 1987). Corrosion may occurs even in the absence of living bacteria, when two polymers of different metal binding affinities are adsorbed over adjacent areas on the same metallic surface (Geesey et al., 1987). In general, the biocorrosive processes involving SRB offer fascinating models in the area of applied cell biology and constitute an open field of study with regard to the functions of extracellular polymers, their synthesis under different conditions by a variety of single-species or mixed bacterial populations, and their consequent influence on rates and mechanisms of corrosion.

4. DETECTING AND PREVENTING SRB-ASSOCIATED BIOCORROSION

Suspicion of SRB-associated biocorrosive processes arises when pit-type corrosion is found under anaerobic conditions, and, as mentioned above, when an odour of H_2S is detected in conjunction with a black film of FeS precipitate. Since routine methods to prevent and treat microbial contamination and biodeterioration involve the use of biocides (Gaylarde & Johnston, 1984), that are toxic and always have some environmental impact, an accurate diagnosis of biocorrosion is always required prior to a treatment decision. It is therefore necessary at least (1) to confirm the presence of biofouling, i. e., living and/or dead microorganisms associated with corrosive processes, (2) to identify the type of microorganisms involved, be they fungi, nitrate-reducing bacteria, iron and sulphur-oxidizing bacteria, sulphate-reducing bacteria, *Pseudomonas* spp, etc., and if possible (3) to identify the types of corrosion product present in the biofilm material, since they may hinder the action of biocides and thus call for the additional use of a dispersant. Furthermore, biocides can be problematical both in terms of the high resistance of bacterial biofilms (Watkins & Costerton, 1984; Gaylarde & Johnston, 1984) and in terms of the direct corrosive action of certain biocides upon metal (Videla et al., 1991). Besides the use of bacteriostatic or bacteriocide substances to eliminate biocorrosion in closed systems, another method which is also frequently used is to change environmental characteristics (such as pH variation or aeration) in order to avoid growth of microorganism (Videla, 1986).

Fig 3: sulphate-reducing bacteria biofilms sampled directly under field conditions (with six months exposure of metallic probes to water injection systems on offshore oil platforms (3a) or grown for four days over metallic coupons in Postgate C Medium inoculated with mixed SRB populations (3b). The transmission electron micrograph shows extensive extracellular material (stars) in which bacteria (b) are embedded, and which is strongly reactive with the cationic electrodense compound ruthenium red (Fig. 3a). The scanning electron micrograph (Fig. 3b) shows a high concentration of morphologically diverse bacteria (rod -r-, spirilar -s-, filamentous -f-, vibrio -v- and coccoid -c- forms) associated with elements of extracellular matrix (arrow) that are condensed (arrow heads) during the dehydration process required for electron microscopy.

Current methods used in the identification and enumeration of SRB are mainly of 2 types (Tatnall et al., 1988): (a) specific culturing methods and (b) biochemical and immunoenzymatic methods involving direct detection of SRB. Both types of technique suffer from limitations. Culturing anaerobic SRB in the laboratory is difficult, since the bacteria take a long time to grow on specialised media necessary for their particular requirements (Postgate, 1984); indeed, often they do not grow at all in pure culture if environmental reduction is insufficient. In addition to aseptic and anaerobic conditions, and special media, the standard culturing methods require long periods of follow-up. Meanwhile, biochemical methods – such as gas-liquid chromatographic identification of specific fatty acids in phospholipids of SRB, or the radiorespirometric method that measures radiolabelled sulphide produced by SRB samples when incubated with radiolabelled sulphate – all require sophisticated laboratory conditions. Their application in field conditions is therefore difficult and involves great expense in the collection, preparation and analysis of samples. Other biological tests – such as the ATP assay, epifluorescence with acridine orange and tests based on aerobic respiratory activity – are not specific to anaerobic SRB. Increasingly, therefore, efforts are being focused on the development of rapid serological methods to identify SRB (Odom et al., 1987; Tatnall et al., 1988; Gaylarde & Cook, 1990) and on research into rapid electrochemical techniques to detect SRB-generated sulphide (Al-Hitti et al., 1983). In a recent report on a long-term field study of SRB-associated corrosion in the presence of prevental electrochemical equipment (such as sacrifice anodes), Hamilton's group, which developed the radiorespirometric method for identifying and monitoring SRB-associated biocorrosion, concluded that sulphide levels may offer the most reliable parameter for the detection of long-term ongoing corrosive processes in which SRB are implicated, prior to effective control (Hamilton et al., 1989).

Our laboratory has been developing a method that detects and semi-quantifies the presence of living or dead SRB by using free silver potentials as an indicator of corrosive action by SRB-associated sulphides (Aguiar, 1991). The method offers a simple, rapid, and effective means of diagnosing biocorrosive processes prior to their control. We found a correlation between sulphide levels (determined either by spectrophotometry, or using a silver electrode -E(Ag)- that measured changes in free potentials induced by the presence of exogeneously added sulphide) and SRB concentration (enumerated by a culturing method) (Fig. 4). E(Ag) was characterized under a variety of conditions and was found to be relatively immune to possible interference resulting from aeration of media or from the presence of iron corrosion products.

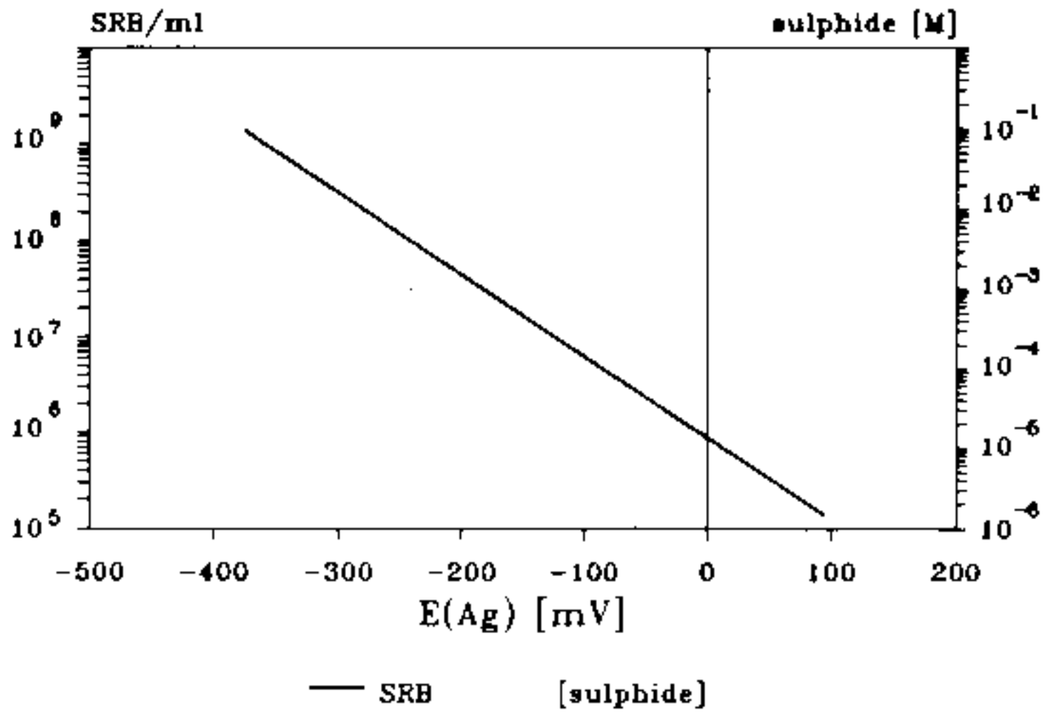

Fig. 4: detection and semi-quantification of sulphate-reducing bacteria and of sulphide levels by measuring free silver potentials -E(Ag). Note the high negative potential recorded by silver electrodes immersed in deaerated medium containing high concentrations of SRB and/or exogeneously added sodium sulphide.

5. PERSPECTIVES

Microbiologically-induced corrosion pose a set of industrial and technological problems that require basic multidisciplinary research in order to explain phenomena that are not exclusively chemical, electrochemical or biological. Furthermore, problems for basic research in this area are among the most fascinating and challenging questions concerning cell biology and biophysics. Of special interest are questions relating to the mechanisms of metabolite transport inside anaerobic biofilms, the nature of the extracellular matrix in SRB biofilms, and its possible interference with ongoing mechanisms of corrosion.

A recent report by Prof. Hector Videla, from the University of La Plata, describes the state-of-the-art of biological corrosion research in Latin America today (Videla, 1990) and notes that present and future research in this area in Brazil seems to be confined mainly to one laboratory in the University of Rio Grande do Sul and to a research center in a private insti-

tution (Aquatec). The author also correctly calls attention to the fact that research on anaerobic corrosion by SRB and on biofilm effects in water injection systems will be increasingly important for Brazil's expanding offshore oil industry. Our work intends to increase Brazilian contributions to this field. It bridges the gap between metallurgy and cell biology, and shows that an area of national strategic importance, such as petroleum production, can pose problems which are also biological in nature and which merit the attention of more research groups.

ACKNOWLEDGEMENTS

To Nilda B. L. da Mota and Amalia Furtado, from the Center for Research and Development of PETROBRÁS (CENPES) for help with experiments involving SRB, and to Fatima C. M. Magalhães (CENPES), Antoine Pourbaix (CEBELCOR) and Vicente Gentil (UFRJ) for helpful discussions over the course of this project. To the staff of CENPES and FIOCRUZ for photographic services.

REFERENCES

AGUIAR, L. E. V., 1991. *Estudos eletroquímicos para desenvolvimento de um método de identificação e semi-quantificação de bactérias redutoras de sulfato envolvidas em processos de biocorrosão, com base na detecção de sulfetos.* MSc Thesis, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil, 71 p.

AL-HITTI, I. K.; MOODY, G. J. & THOMAS, J. D. R., 1983. Sulphide ion-selective electrode studies concerning *Desulfovibrio* species of sulphate-reducing bacteria. *Analyst, 108:* 1209-1220.

BRADLEY, G.; GAYLARDE, C. C. & JOHNSTON, J. M., 1984. A Selective interaction between ferrous ions and lipopolysacchridae in *Desulfovibrio vulgaris. J. Gen. Microbiol., 130:* 441-444.

BRYANT, R. D. & LATHLEY, E. J., 1990. The role of hydrogenase in anaerobic biocorrosion. *Can. J. Microbiol., 36:* 259-264.

CHARACKLIS, J. W. & COOKSEY, K. E., 1983. Biofilms and microbial fouling. *Adv. Appl. Microbiol., 29:* 93-138.

COSTERTON, J. W., 1985. The role of bacterial exopolysaccharide in nature and disease. *Devel Indust. Microbiol., 26:* 249-261.

COSTERTON, J. W.; CHENG, K-J.; GEESEY, G. G.; LADD, T. I.; NICKEL, J. C.; DASGUPTA, M. & MARRIE, T., 1987. Bacterial biofilms in nature and disease. *Ann. Rev. Microbiol., 41:* 435-464.

COUTINHO, C. M. L. M., 1991. *Estudo ultraestrutural de biofilmes bacterianos envolvidos no processo da corrosão microbiológica em plataformas marítmas da PETROBRÁS.* MSc Thesis, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil, 168p.

COUTINHO, C. M. L. M.; MAGALHÃES, F. C. M. & ARAUJO-JORGE, T. C., 1992. Scanning electron microscopic study of biofilm formation at different flow rates over metal surfaces using sulphate-reducing bacteria. *Biofouling,* in press.

FERRIS, F. G.; SCHULTZE, S.; WITTEN, T. C.; FYFE, W. S. & BEVERIDGE, T. J., 1989. Metal interactions with microbial biofilms in acidic and neutral pH environments. *Appl. Envir. Microbiol., 55:* 1249-1257.

GAYLARDE, C. & COOK, P., 1990. New rapid methods for the identification of sulphate-reducing bacteria. *Internat. Biodeterior., 26:* 337-345.

GAYLARDE, C. C. & JOHNSTON, J. M., 1984. Some recommendations for sulphate-reducing bacteria biocide tests. *J. Oil & Colours Chemists' Assoc., 67:* 305-309.

GEESEY, G. G.; IWAOKA, T. & GRIFFITHS, P. R., 1987. Characterization of interfacial phenomena occuring during exposure of a thin copper film to an aqueous suspension of an acidic polysaccharide. *J. Colloid Interface Sci., 120:* 370-376.

GENTIL, V., 1983. *Corrosão.* 2a. ed. Ed. Guanabara Dois SA, Rio de Janeiro. 453 p.

GIBSON, G. R., 1990. Physiology and ecology of sulphate-reducing bacteria: a review. *J. Appl. Bacteriol., 69:* 769-797.

HAMILTON, W. A., 1983. Sulphate-reducing bacteria and the offshore oil industry. *Trends Biotechnol., 1:* 36-40.

HAMILTON, W. A., 1985. Sulphate-reducing bacteria and anaerobic corrosion. *Ann. Rev. Microbiol., 39:* 195-217.

HAMILTON, W. A.; MOOSAVI, A. N. & PIRRIE, R. N., 1989. Mechanism of anaerobic microbial corrosion in the marine enviroment, p. 13-19. In C. A. C. Sequeira & A. K. Tillers (eds), *Microbial Corrosion, Proc. First EFC Workshop,* Elsevier Applied Science, London & New York.

KING, R. A. & MILLER, J. D. A., 1971. Corrosion by the sulphate-reducing bacteria. *Nature, 233:* 491-492.

KING, R. A.; MILLER, J. D. A. & WAKERLEY, D. S., 1973. Corrosion of mild steel in cultures of sulphate-reducing bacteria: effect of changing the soluble iron concentration during growth. *Br. Corr. J., 8:* 89-93.

MILLER, J. D. A. & KING, R. A., 1975. Biodeterioration of metals, p. 91-103. In R. J. Gilbert & D. W. Lovelock (eds), *Microbial aspects of the deterioration of materials,* Academic Press, Soc. Appl. Bact. Tech., Ser. 9.

NIELSEN, P. H., 1987. Biofilm dynamics and kinectics during high- rate sulphate reduction under anaerobic conditions. *Appl. Environ. Microbiol., 53:* 27-32.

ODOM, J. M.; GAWEL, L. J. & NG, T. K., 1987. Patent application ICR 7780-USSN 946, 547: sulphate reducing bacteria detection, Du Pont Co.

PANKHANIA, I. P., 1988. Hydrogen metabolism in sulphate-reducing bacteria and its role in anaerobi-corrosion. *Biofouling 1:* 27-47.

POSTGATE, J. R., 1984. *The sulphate-reducing bacteria.* Cambridge University Press, Cambridge.

POURBAIX, M. 1987. *Lições de corrosão eletroquímica.* Third Ed. CEBELCOR, Brussels.

RICKARD, D. T., 1969. The microbiological formation

of iron sulphides. *Stockholm Contr. to Geology, 20:* 67.

SKREBOWSKI, C., 1978. Is britain's oil industry on shaky legs? *New Scientist, 47:* 714-716.

TATNALL, R. E.; STANTON, K. M. & EBERSOLE, R. C., 1988. Testing for the presence of sulfate-reducing bacteria. *Mat. Performance, 27:* 71-80.

TILLER, A. K., 1983. Aspects of microbial corrosion, p. 115-159. In R. N. Parkins, *J. Corrosion Processes,* Appl. Science Pub.

VIDELA, H. A., 1986. *Corrosão microbiológica. Série Biotecnologia,* vol. 4, Ed. E. Blucher Ltda, São Paulo.

VIDELA, H. A., 1989. Metal dissolution/redox in biofilms, p. 301-320. In W. G. Characklis & P. A. Wilderer (eds), *Structure and Function of Biofilms,* John Wiley & Sons Ltd.

VIDELA, H. A., 1990. Biological corrosion research in Latin-America – past, present and future. *Biodeterior. Abstr., 4:* 1-11.

VIDELA, H. A.; SAUTU, A. E.; SARAVIA, S. G. G.; GUIAMET, P. S.; MELE, M. F. L.; GAYLARDE, C. C. & BEECH, I. B., 1991. Impact of glutaraldehyde on biofouling and MIC of different steels: a laboratory assessment. Corrosion' 91, NACE, paper 105, Cincinnati, Ohio, mach 11-15.

WALL, J. D.; RAPP-GILES, B.; BROWN, M. F. & WHITE, J. A., 1990. Response of *Desulfovibrio desulfuricans* colonies to oxygen stress. *Can. J. Microbiol., 36:* 400-408.

WATKINS, L. & COSTERTON, J. W., 1984. Growth and biocide resistance of bacterial biofilms in industrial systems. *Chem. Times & Trends, 23:* 35-40.