A possible role for phototrophic sulphur bacteria in the promotion of anaerobic metal corrosion

Bacteria associated with the biological sulphur cycle play a particularly dominant role in the process of metallic corrosion. This is largely due to the ubiquitous presence of these bacteria in natural environments[1,2]. Unsurprisingly, sulphate-reducing bacteria have been the main highlight in numerous bio-corrosion investigations. A vast number of mechanisms[3–8] have been proposed in the literature to explain the rather multifarious process of anaerobic corrosion. The deterioration of iron and concrete by aerobic sulphur bacteria (thiobacilli) is more straight-forward. These bacteria oxidize sulphur and/or sulphide to sulphuric acid[9].

The propensity of other sulphur bacteria to influence corrosion reactions has not been amply understood. Monero and Laborda[10] isolated sulphite-reducing bacteria (in addition to sulphate reducers) from corroded materials found in power stations, and speculated a possible symbiotic relationship between the two groups. Reviewing the biology of sulphur bacteria in relation to corrosion mechanisms, Bos and Kuenen[11] hypothesized an indirect role for the phototrophic bacteria. The authors suggested that these bacteria might, by virtue of their ability to oxidize sulphide, contribute to an increased risk of the corrosion caused by the sulphate reducers.

Here, we present preliminary evidence of a possible role for phototrophic sulphur bacteria in the anaerobic corrosion process. The present work is basically a sequel to our recently published paper[12] in which we showed that the decay of macro-organisms in sea water, when exposed to light, could produce and sustain copious enrichments of phototrophic sulphur bacteria. In order to examine what might be the effect of these bacteria on corrosion rate and mechanism, we performed three series of experiments.

The first category of tests was an inspection of the rates of corrosion of mild steel in decomposing cultures of an oyster, *Crassostrea cucullata* and a green alga, *Caulerpa peltata*. These tests were performed in 3 l culture flasks with 5% w/v ratios of the biomass with freshly sampled coastal sea water from Tuticorin harbour (8°47′N; 78°9′E). The basic idea here was to compare the rates of corrosion with or without the development of phototrophic sulphur bacteria. The two types of lighting, namely a fully darkened condition that inhibited the development of the phototrophs, and a natural 1 : 1 diurnal cycle that supported copious growths, were accomplished essentially as described previously[12]. Coupons of dimension 50 mm × 15 mm × 1.1 mm were cut from a sheet of commercial-grade mild steel (C = 0.1; Mn = 0.46; Si = 0.07; S = 0.03; P = 0.07; Fe = remainder). They were pickled in Clarke's solution, polished to a mirror-finish, degreased in acetone, and weighed to an accuracy of 0.01 mg. Three coupons were immersed in each flask; they were positioned in PVC holders and placed at the bottom of the flask. At the end of the test (30 days), the average corrosion was calculated from weight loss and surface area data for six replicate coupons.

Weight-loss tests were also performed in Pfennig's medium[13] with or without a purified culture of *Chlorobium* sp.[12] (green sulphur bacterium), employing 250 ml stoppered conical flasks. Two polished and pre-weighed mild-steel coupons (25 mm × 5 mm) were suspended in each flask from glass-hook supports. The prepared deoxygenated broth was added, leaving a small air space at the top for pressure compensation. Flasks were autoclaved at 15 psi, 120°C for 15 min and cooled. The autoclaving caused a black oxide film on the coupons indicating a minor amount of corrosion prior to inoculation, as in studies by Booth and Tiller[3]. One batch of flasks served as the control while the other received 5 ml portions of a 7–10-day-old *Chlorobium* culture. In these tests, the level of sulphide in the media was varied, but a constant light level (250 lux; Kyoritsu, Model 5200) was employed. The sulphide levels used were 6, 10 and 15 ml l^{-1} of the 5% Na_2S stock solution relative to Pfennig's medium. At the end of the test period (10 days), corrosion rates were averaged from four replicate coupons. In parallel with these tests, one batch of the flasks with no coupons provided for the estimation of the produced sulphur[14].

The third series of tests dealt with polarization characteristics of steel in the *Chlorobium* culture. These tests were done at ambient laboratory light (natural, diffuse daylight; 400 to 500 lux). Two 250 ml corrosion cells were constructed with provisions for four working electrodes, a platinum counter electrode, luggin capillary to a saturated calomel electrode (SCE), and a port for inoculation. The working electrodes were flag-shaped mild-steel coupons having an exposed area of 1 cm^2 and the stem masked with insulating lacquer. The entire assembly was autoclaved after addition of the medium, and allowed to cool. One cell served as the control, while the other received 5 ml of the *Chlorobium* culture. Corrosion potentials were measured using a high-impedance voltmeter vs SCE. Potentiodynamic scans (1 mV/s) were made at 1, 5 and 12 days from an EG & G Model 173 potentiostat and Model 175 Universal Programmer (Princeton, NJ). The cathodic and anodic scans were plotted on a Rikandenki *X-Y* Recorder.

Figure 1 presents corrosion rate versus time data for steel in decomposing cultures of *Cr. cucullata* and *Ca. peltata* with or without lighting (illuminated and dark, respectively). Figure 1 also illustrates the evolution in colour of the sea water solutions, associated with the enrichments of phototrophic bacteria in the decomposition vessels that received illumination. The decomposing cultures in dark did not develop pigmentation and the data are coloured grey for clarity. Regardless of lighting, the average rates of corrosion in the early periods were low, between 10.1 and 14.2 mg dm^{-2} day^{-1} (mdd). While no further change in the steel behaviour was apparent in the dark, a marked increase in corrosion occurred under the illuminated condition, concurrent with the development of phototrophic bacteria. Here, the average rates of corrosion measured after 20 and 30 days of decomposition were much higher, between 41.8 and 47.5 mdd.

Table 1 summarizes weight loss data for mild-steel coupons immersed in *Chlorobium* cultures and corresponding controls. The average rates of corrosion in the sterile controls were extremely low, between 3.9 and 4.6 mdd. No sulphur was detected in the sterile flasks, and the rates of corrosion were independent of the solution sulphide levels. On the other hand, rates of corrosion were much higher (up to 22.1 mdd) in *Chlorobium* cultures, wherein noticeable concentrations

of elemental sulphur (4.5 to 7.0 mg l^{-1}) were measured.

Corrosion potential trends in Figure 2 show a minor negative shift during the early periods in the sterile control cell as well as in the *Chlorobium* culture. Subsequently, the steel was rapidly ennobled by about 100 mV in the sterile control, whereas in the *Chlorobium* culture, there was no further change in potential.

Results of polarization experiments are illustrated in Figure 3. Extrapolations from the Tafel slopes yielded corrosion currents of about 5.0 μA cm^{-2} for 1 day and 5 day data corresponding to the electrode in the sterile cell. At 12 days, the corrosion current dropped down to about 1.7 μA cm^{-2}. The anodic curve appears almost vertical at this point in time, indicating passivation of the electrode. The electrode in the *Chlorobium* culture showed sharp increase in corrosion current from 4.1 μA cm^{-2} at day 1 to about 29 and 40 μA cm^{-2} at 5 day and 12 day respectively. The shapes of the curves suggested that both anodic and cathodic current increased, which is consistent with the small variation in corrosion potential (Figure 2).

The results seem to suggest that in the present work, enhancement of corrosion by the phototrophs was linked to their ability to produce elemental sulphur according to the following equation:

$$HCO_3^- + H_2S + HS^- \rightarrow$$
$$(CH_2O) + S^0 + 2OH^-,$$
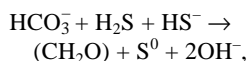
a pathway that we proposed in our previous work[12]. As reviewed by Schaschl[15] and Schmitt[16], elemental sulphur is highly corrosive to steel. The lack of replicate data in this work does not allow straightforward correlations between the amounts of sulphur and corrosion. Nevertheless, the fact that an acceleration of corrosion occurred at once with the growths of phototrophic bacteria, both in decomposing enrichments and in pure cultures, provides reasonable basis for the suggested mechanism.

The low rates of corrosion measured in sterile media (Table 1) are consistent with several early works[3,5,17–19] with regard to the passivating effect of sulphide under conditions of anaerobiosis. Typically, the corrosion potential became more positive (Figure 2), as often observed in sulphide solutions[3,19]. The lower amounts of corrosion in *Chlorobium* cultures (Table 1) compared to the rates in decomposing cultures (Figure 1) appear to be because of the batch culture problem that appar-

ently limited the cycling of sulphide. The tests with decaying biomass were also deficient in fluid replenishment. Nevertheless, the decomposition system satisfied many conditions that are important in biocorrosion experiments. Notably, the cultures (i) produced rates of corrosion relevant to practical field conditions, (ii) most likely permitted growths of synergistic bacterial communities that conduct combined

processes (e.g. cycling of sulphide and sulphur) that individual species cannot, and (iii) were biologically active for time periods appropriate for biocorrosion testing.

Schaschl[15] raised the question whether there is a biological mechanism for the formation of elemental sulphur in deaerated waters. The answer is readily provided in the present work; yes, sulphur can indeed result from the activities of

Table 1. Summary of corrosion rates and sulphur levels in Pfennig's medium with and without *Chlorobium* sp. (10 d study)

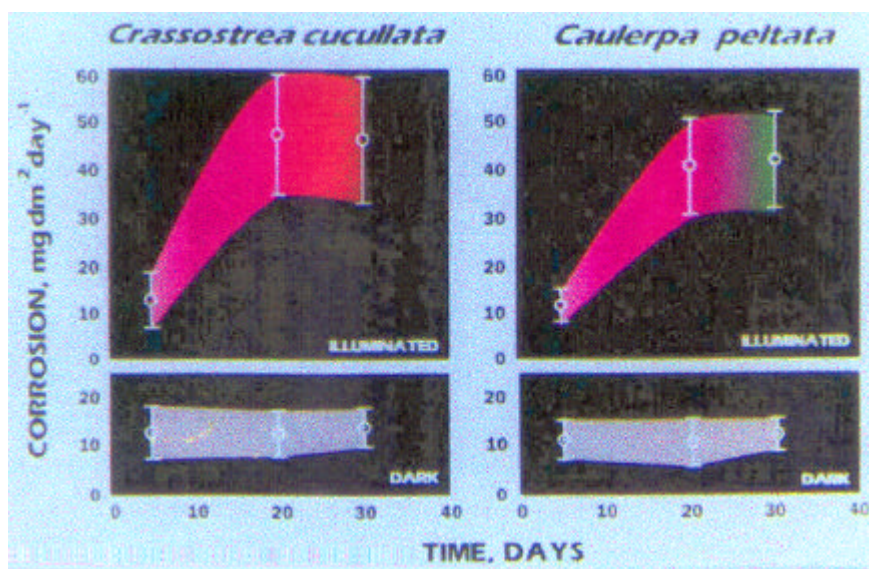| Sulphide level (mg l^{-1})	Sterile control		*Chlorobium* culture	
	Corrosion (mdd)	Sulphur (mg l^{-1})	Corrosion (mdd)	Sulphur (mg l^{-1})
6	4.2 ± 1.9	BD	12.2 ± 4.0	4.5
10	4.6 ± 1.6	BD	17.8 ± 4.3	6.8
15	3.9 ± 1.4	BD	22.1 ± 5.5	7.0

BD, Below detection.

Figure 1. Illustration showing corrosion rate versus time for mild steel in decomposing cultures of *Cr. cucullata* and *Ca. peltata* in sea water with or without lighting (illuminated and dark respectively). Evolution of the colour of sea water from the enrichments of phototrophic bacteria is also shown. Data corresponding to the exposure in dark are coloured grey for clarity.
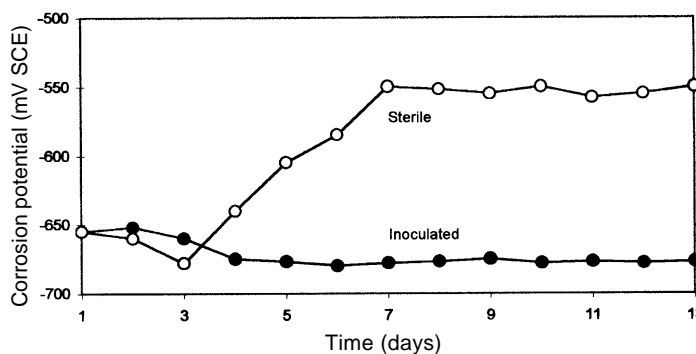

Figure 2. Corrosion potential variations for mild steel exposed to Pfennig's medium with (inoculated) and without (sterile) *Chlorobium* sp.
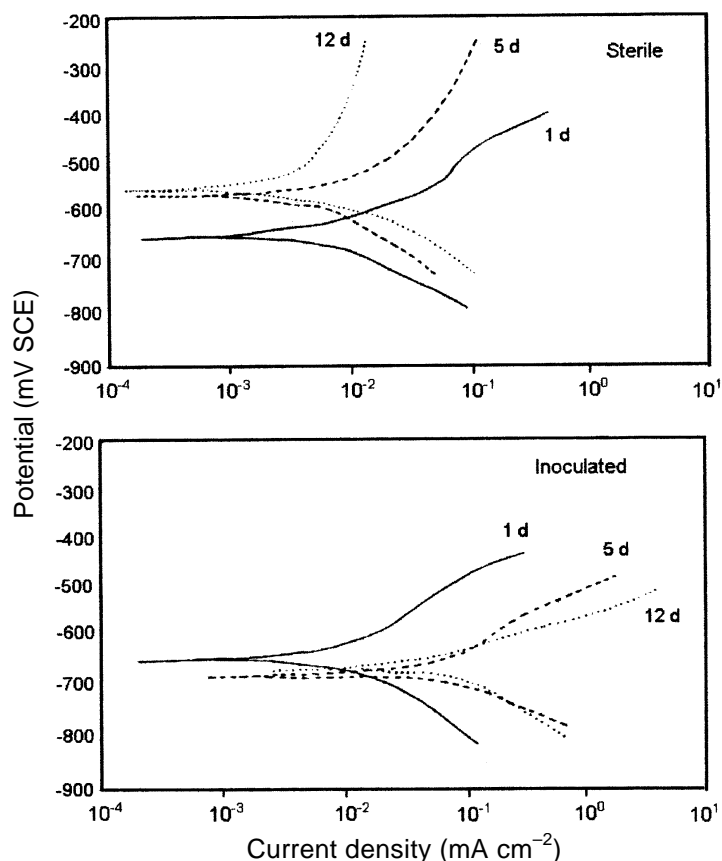
Figure 3. Polarization characteristics of mild steel in Pfennig's medium with (inoculated) and without (sterile) *Chlorobium* sp.

phototrophic bacteria that have hitherto remained unfamiliar to the corrosion engineer. Interestingly, Edyvean (pers. commun.) had sometimes noticed bright colouration of sea water in decomposition vessels constructed with perspex, but no opaque materials. At no time, however, did he investigate its cause and effect.

A major effect of the phototrophs on corrosion is possible in sea-bed sediment and within natural biofilms where their anaerobic counterparts, the sulphate-reducing bacteria, are known to flourish[14,20]. Although light can be a limiting factor, extreme low-light adaptation in certain phototrophs[21] and their ability to oxidize ferrous iron[22] might be of importance. It would be interesting to investigate as to what extent these bacteria contribute to the corrosion of metals under practical conditions. Further studies are required to establish more methodically the mechanism proposed here, besides elucidating the other possible pathways by which the phototrophs could contribute to the overall process of anaerobic corrosion.

1. Kelly, D. P., *Nature*, 1987, **326**, 830.
2. Postgate, J. R., *New Sci.*, 1988, **1621**, 5862.
3. Booth, G. H. and Tiller, A. K., *Trans. Faraday Soc.*, 1960, **56**, 1689.
4. Salvarezza, R. C. and Videla, H. A., *Corrosion*, 1980, **36**, 550.
5. Iverson, W. P., In *Underground Corrosion* (ed. Escalante, E.), American Society for Testing and Materials, Philadelphia, 1981, p. 33.
6. Pankhania, I. P., *Biofouling*, 1988, **1**, 27.
7. Deshmukh, M. B., In Proceedings of the International Conference on Frontiers of Electrochemistry, SAEST, Karaikudi, 1995, IL-7.
8. Beech, I. B., Zinchevich, V., Tapper R., Gubner R. and Avci, R., *J. Microbiol. Methods*, 1999, **36**, 3.
9. Sand, W. and Bock, E., In *Biodeterioration 7* (eds Houghton, D. R., Smith, R. N. and Eggins, H. O. W.), Elsevier, Barking, 1988, p. 113.
10. Monero, D. A. and Laborda, F., In *Microbial Corrosion* (eds Sequeira C. A. C. and Tiller, A. K.), Elsevier, London, 1988, p. 84.
11. Bos, P. and Kuenen, J. G., In *Microbial Corrosion*, The Metals Society, London, 1983, p. 18.
12. Eashwar, M., Maruthamuthu, S. and Venkatakrishna Iyer, S., *Curr. Sci.*, 2002, **82**, 329.
13. Pfennig, N. and Truper, H. G., In *The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria* (eds Starr, M. P. *et al.*), Springer Verlag, New York, 1981, p. 1635.
14. Stal, L. J., van Gemerden, H. and Krumbein, W. E., *FEMS Microbiol. Ecol.*, 1985, **31**, 111.
15. Schaschl, E., *Mater. Perform.*, 1980, **19**, 9.
16. Schmitt, G., *Corrosion*, 1991, **47**, 285.
17. Hardy, J. A. and Bown, J. L., *Corrosion*, 1984, **40**, 650.
18. Starkey, R. L., In *Biologically Induced Corrosion* (ed. Dexter, S. C.), National Association of Corrosion Engineers, Houston, 1986, p. 8.
19. Eashwar, M., Chandrasekharan, P., Subramanian, G. and Balakrishnan, K., *Corrosion*, 1993, **49**, 108.
20. Little, B. J., Ray, R., Wagner, P. A., Lewandowski, Z., Lee, W. C. and Characklis, W. G., *Biofouling*, 1990, **3**, 45.
21. Overmann, J., Cypionka H. and Pfennig, N., *Limnol. Oceanogr.*, 1992, **37**, 267.
22. Heising, S., Richter, L., Ludwig, W. and Schink, B., *Arch. Microbiol.*, 1999, **172**, 116.

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