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Effects of selection and fate of substrates supplied to anaerobic bacteria involved in the corrosion of pipe-line steel

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SUMMARY

The corrosion of AISI C1020 carbon steel in an anoxic, marine, sulphide-containing environment was examined as a function of bacterial physiology and consortial complexity. The carbon steel was exposed to three organism; *Eubacterium limosum, Desulfovibrio* sp. and *Desulfobacter* sp. which were provided with H_2/CO_2, butanol, glucose, and acetate as carbon and electron sources. A consortium of these bacteria utilizing hydrogen gave rise to relatively high corrosion rates (5.7×10^{-4} mhos cm^{-2}) with respect to corrosion resulting from bacteria supplied with organic electron sources ($0.6–1.6 \times 10^{-4}$ mhos cm^{-2}). Disproportionation of electrons between sulphate reduction and fermentation had a significant effect on the corrosion rate in the case of *Desulfovibrio*. Surface examination using scanning electron microscopy coupled with electrochemical impedance spectroscopy supported the hypothesis that the corrosion rate was controlled by the relative intactness of a ferrous sulphide film in which the bacteria were embedded.

INTRODUCTION

The performance of carbon steels in petroleum-rich, anoxic environments has been of concern for several years. These materials are generally useful at neutral pH and low oxygen tension. The introduction of anaerobic microorganisms, however, has had a deleterious effect on their utility. While numerous types of microbiologically influenced corrosion (MIC) are possible [1] this article is solely concerned with the conditions common to gas-production wells and transmission pipes where carbon steel is habitually employed in quantity.

Major geochemical changes in the marine environment performed by microorganisms are affecting the performance and service lifetime of structural materials [1,2]. Operators of undersea pipelines in the Gulf of Mexico are required by environmental protection regulations to dispose safely of brine, accumulated liquid hydrocarbons and drilling fluids produced at the wellhead. This commonly entails reinjection of these waste products back into the conduit pipes for transmission to shore after compression of the natural gas. Assessment of the effects of this procedure after transmission of the heterogeneous mixture to shore showed sharply decreased pH levels resulting from the accumulation of specific short-chain organic acids [3]. Coincidentally, sulphate concentrations dropped from open ocean values (about 2800 ppm) to 10–15 ppm. Sulphide concentrations were elevated, and the undersea pipeline developed leaks. Two kinds of microorganisms were implicated: fermentative bacteria producing organic acids and sulphate-reducing bacteria. A study of the same system with radiolabels [2] demonstrated acid production by several possible means including acetogenesis via oxidation of H_2 (CO_2). It is proposed that in reducing systems sulphide-producing bacteria may utilize the H_2 and promote corrosion while deriving carbon from short-chain acids produced by acetogenic bacteria.

This paper examines the effect of substrate selection by the microorganisms on the corrosion rate in a model system. In the pipeline previously described, sulphate reduction and acid production were evident, but from unknown substrate precursors. The degradation of hydrocarbons in anaerobic systems is controversial and if possible, is considered unlikely to proceed quickly enough to contribute to deterioration of the steel. It is more likely that bacteria follow two paths: (i) fermentation of oxygen-substituted compounds in the bulk hydrocarbons; and (ii) sulphate reduction at the expense of hydrogen generated either in fermentation reactions, or present as geological contaminants of the natural gas, or produced by the cathodic half-reaction. This reasoning was examined by providing a range of oxygen-containing substrates to fermentative

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and sulphate-reducing bacteria in contact with carbon steel. Substrates that were not degradable by all the bacteria in the model system were deliberately provided. Correlations were drawn between the physiological status of the bacteria and the corrosion rates associated with them.

EXPERIMENTAL METHOD

Cultures and growth conditions

Desulfovibrio Florida strain was obtained by chemostat enrichment from Appalachicola Bay on the northern Gulf coast of Florida. The cells were highly motile short vibroids. This strain could utilize hydrogen and lactate as electron sources and lactate, CO_2 and acetate for carbon. The main products were sulphide (HS^-), CO_2, and water. *Desulfovibrio* species also engage in fermentation reactions with certain organic substrates under low sulphate concentrations.

Desulfobacter 2ac9 and 3ac10 were obtained from the Deutsche Sammlung von Mikroorganismen (Braunschweig, Germany). These strains were extremely similar 'brackish' and marine bacteria which oxidized acetate and ethanol only at the expense of sulphate. Sulphide (HS^-), CO_2 and water were the main products. These bacteria were morphologically distinguished by their distinctive figure-eight shapes of twin cells, or short fat immotile oval cells.

Eubacterium limosum was obtained from the American Type Culture Collection No. 8486. This bacterium may grow on H_2/CO_2 to produce acetate or engage in fermentation of other organic substrates such as glucose, alcohols and formate to short-chain acids, carbon dioxide and water. These bacteria were morphologically distinguished from the other types by their long rod-shaped cells.

The selected growth medium consisted of basal salts containing (g/l): Na_2SO_4 3, KH_2PO_4 0.2, NH_4Cl 0.25, NaCl 20, $MgCl_2 \cdot 6H_2O$ 4, KCl 0.5, $CaCl_2 \cdot 2H_2O$ 0.15. Resazurin was added as redox indicator as well as complex vitamins, sodium sulphide [4] and trace elements [5]. The final pH was between 7.0–7.5. Operating temperatures were at ambient (approx. 23 °C). The gas mix was 5% H_2 with the balance CO_2 or N_2 (90%)/CO_2 (10%). Some carbon was supplied in small amount of yeast extract (0.001%) which was required by the *E. limosum* for growth. In one experiment 3-*N*-morpholinopropanesulfonic acid (MOPS, 0.5 g/l) was added to control the pH.

Fermentation balance study

In order to take inventory of substrates likely to affect the corrosion mechanisms, a fermentation balance was conducted. This involved the batch monoculture of all bacteria with known substrate concentrations prior to inoculation. All balance studies were conducted in triplicate in 30 ml of liquid in closed bottles. Thus, *E. limosum* was separately provided with H_2/CO_2, butanol, and glucose. The *Desulfovibrio* strain was provided with H_2/CO_2, and butanol, while *Desulfobacter* was provided with acetate alone. A coculture of all three organisms was also tested in the same system.

Materials selection

A carbon steel AISI (American Iron and Steel Institute) C1020 was selected. This concentration (%) C 0.2, Mn 0.47, P 0.012, S 0.013 and remainder Fe. Carbon Steel C1020 was selected since much prior data is available and its performance is well-known.

Description of test system and electrochemical methods

Corrosion exposure tests were carried out in 2-l glass reaction kettles using a classical three-electrode system: the working (corroding) electrode, a titanium counter electrode and saturated calomel reference electrode. Gas (H_2/CO_2 or N_2/CO_2) was introduced via heated copper columns and sterilized 0.2-μm pore-size filters into gas dispersion tubes. Mild steel coupons were electrically connected and embedded in Epoxy resin (Buehler, IL). The edges were subsequently sealed with an acetone-soluble enamel. The exposed area was 1.3 cm^2. The bulk phase was sampled by drawing liquid into a hypodermic syringe inserted via thick-walled neoprene tubing connected to the reaction vessel. Low redox potentials were confirmed by inclusion of resazurin indicator in the electrolyte. Due to the evolution of H_2S gas, the study was carried out in a chemical fume cabinet.

Open circuit potentials were taken with a Princeton Applied Research EG&G model 273 potentiostat. Electrochemical impedance spectroscopy (EIS) was used to analyse the corrosion rate due to necessity of minimizing the biofilm disturbance while deriving as much information as possible [6]. The same potentiostat was used with a Solartron-Schlumberger 1255 frequency response analyzer. Impedance analysis in this case required a 5 mV rms signal applied to the working electrode while monitoring the resultant current between the frequencies 10 KHz and 5 mHz. Phase angle and impedance were calculated. No faradaic rectification was observed for small amplitude signal (5 mV). Application of a 10 mV signal gave an apparently identical result. Stability was confirmed by noting two important points: (i) current demand for application of the open-circuit potential was a few nanoamperes at both the beginning and the end of the measurement interval; and (ii) immediate repetition of the measurement produced an almost identical impedance spectrum. The instruments were controlled and the data manipulated using software by Scribner Associates (Charlottesville, VA).

Mathematical argument for impedance data

The basis for comparing corroding systems in this article is given by impedance (Z), a complex quantity with real and imaginary components. For a review of the application of electrochemical impedance spectroscopy see Refs. 7 and 8.

In electrical terms the impedance is derived from the relationship of the overpotential to current for a range of frequencies:

$$Z(s) = V(s)/I(s) \quad (1)$$

A simplified expression for such an electrochemical system where the exchange current density is dominated by the charge transfer resistance (R_{ct}) associated with the faradaic process (metal dissolution) double layer capacitance (C_{dl}, the charged layer surrounding the corroding metal) may be expressed as:

$$Z = R_u + R_{ct}/1 + (j\omega \cdot C_{dl} \cdot R_{ct})^{\alpha} \quad (2)$$

omitting any diffusional constraints where ω is the frequency in radians, R_u is the solution (uncompensated) resistance, $j^2 = -1$, and α is a character reflecting the dispersion of frequency across the surface with the values between 0–1. As the frequency decreases, approaching a DC limit, the contribution from capacitance is likewise reduced until the impedance resolves as the sum of R_u and R_{ct}. For the purposes of this article, we have assumed that subtraction of the uncompensated resistance at some sufficiently low frequency will provide the charge transfer resistance. Thus, EIS permits a more reasonable estimation of the corrosion rate that is unavailable without exhaustive direct current methods. In this system, the corrosion product film was composed of iron sulphides in various crystalline states. Impedance data provided single relaxations in all cases with the exception of the incorporation of MOPS ($pK_a = 7.2$). We used this model to estimate values for the corrosion rate in terms of the admittance, Y (in mhos cm^{-2}).

$$Y = 1/Z \quad (3)$$

and at sufficiently low frequency

$$Y_{ct} = 1/R_{ct} \quad (4)$$

where Y_{ct} is the charge transfer admittance, offering the convenience of a direct relationship between corrosion and the measured quantity.

Chemical assays

Sulphate analysis was carried out using the method of Tabatabai [9]. Volatile fatty acids and butanol were examined by gas chromatography using a Schimadzu Model GC8A with flame ionization detection. The packing utilized was SP-1200 (Supelco, Bellefont, PA) at 120 °C column temperature and injector/detector at 170 °C. Carrier gas flow rate was 30 ml/min. The gases H_2 and CO_2 were assayed using a Schimadzu 8A-TCD (thermal conductivity detector) at 80 °C with a carrier gas rate of 25 ml/min.

Microscopic analyses

Cells in the bulk phase were enumerated using a Helber (Weber Scientific, Teddington, UK) counting chamber under phase-contrast light microscopy at 1000 × magnification.

Scanning electron microscopy (SEM)

The post-exposure coupons were placed in a large anaerobic plastic sack constantly gassed with N_2 immediately after recovery from the medium to preserve cell structure which is subject to lysis in sudden transition from reduced to aerobic systems. The biofilm/corrosion product matrix was fixed in anaerobic 2.5% glutaraldehyde/5% cacodylate at pH 7.0 and subsequent desiccation series were set up in 50%, 75% and 100% acetone prior to critical point drying. The coupons were subsequently gold-sputtered.

RESULTS

Fermentations

In order to obtain an appreciation of the nutritional capabilities of the different microorganisms, a fermentation balance was carried out with each organism and a range of substrates. *E. limosum* fermented glucose with the production of hydrogen and acetate. Butanol also gave rise to hydrogen gas. *Desulfovibrio* sp., however, was able not only to utilize hydrogen with concomitant reduction of sulphate (21.0 to 0.5 mM), but also incompletely oxidized butanol to succinate (5.5 mM) during the reduction of sulphate (21.0 to 15.9 mM). *Desulfobacter* sp. provided with 10 mM acetate as both electron and carbon source caused a decrease in the sulphate concentration (21.0 to 13.0 mM). The sulphate-reducing bacteria in all cases caused an increase in sulphide (HS^-) concentration. Chemical analysis of the triculture supernatant and gas headspace initially provided with 10 mM butanol, 5 mM glucose, 10 mM acetate and 2.64 μmol hydrogen demonstrated a low use of the hydrogen (excess hydrogen was present after 18 days). In the same culture 14.0 mM sulphate, 2 mM sulphide, 14 mM CO_2 and 2 mM succinate were detected after 18 days. These data show that the *E. limosum* was principally engaged in glucose fermentation, the *Desulfovibrio* incompletely oxidized butanol to succinate with perhaps some sulphate reduction, while the

Desulfobacter sp. could only undergo sulphate reduction at the expense of acetate. No acetate was detected at the termination of the culture period. Notably, the amount of sulphate reduced by the triculture was approximately the same as that performed by the monocultures of *Desulfobacter*. This supports the hypothesis that while the *E. limosum* and *Desulfovibrio* were engaged in principally fermentative functions, the *Desulfobacter* performed all or most of the sulphate reduction in the triculture.

General observation on corrosion

All experiments were carried out with a basic system requiring two corrosion electrodes per exposure vessel and two vessels per condition. Thus, a sterile control had four electrodes distributed between two vessels. The results of all four electrodes were required to obtain a mean value for that condition.

Impedance data demonstrated a single relaxation which is due to the double-layer capacitance. This is consistent with results obtained by others [10]. The electrochemical data imply that the corrosion mechanism is relatively simple. At approx. −0.7 V/SCE (saturated calomel electrode) and bulk pH 6.8–7.2, the main cathodic reactant is the reduction of water to hydrogen and hydroxyl ions. Bacteria interfere in this event by controlling the pH at the surface and, as detailed above, oxidizing hydrogen. The anodic half reaction was assumed to be simply the loss of two electrons from iron to give ferrous ions. The operating redox conditions precluded oxidation to ferric ions. Rapid formation of an impermeable sulphide film slowed this process by blocking the anodic sites. McNeil and Little [11] examined mineral films on corroding ferrous alloys and concluded that Mackinawite and possibly Green Rust II (GRII) may be associated exclusively with the impact of sulphate-reducing bacteria in natural environments.

Accelerated corrosion with H_2/CO_2

The corrosive effect of *E. limosum* was compared to both sterile conditions and a coculture of *E. limosum* with *Desulfovibrio* sp. provided with H_2/CO_2 as electron and carbon sources. Impedance data in the Bode format (Fig. 1) may be interpreted simply by extrapolating the low frequency impedance data to the *y*-axis and determining the total impedance. This value corresponds to the sum of the uncompensated resistance (a negligable value under these conditions) and the charge transfer resistance (R_{ct}) as described in Eqn. 2. Fig. 1 presents a low total impedance when the sulphate-reducing bacteria were present. Since impedance and the corrosion rate have an inverse relationship, the corrosion rate associated with the coculture was demonstrably faster than either the sterile condition or the exposure to *E. limosum*.

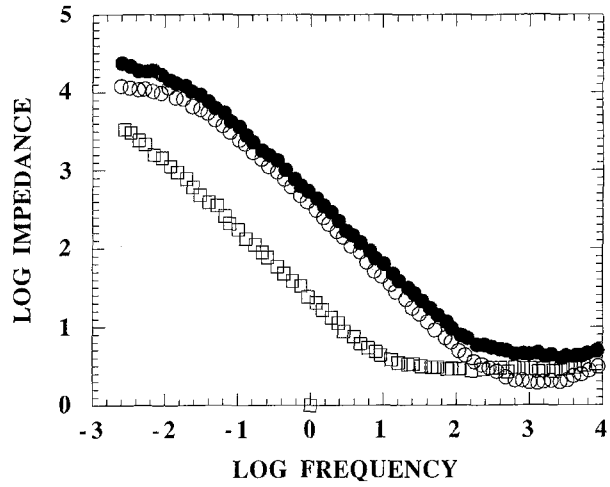

Fig. 1. Bode plot impedance diagrams displaying impedance as a function of frequency for carbon steel variously in contact with sterile medium (●), *E. limosum* alone (○) and a coculture of *E. limosum* with *Desulfovibrio* (□). The medium contained excess H_2/CO_2. The low frequency impedance data for each diagram (approx. 10–2 Hz) indicate that the coculture has the lowest impedance, which corresponds to the highest corrosion rate.

Examination of the phase angle plot (a method of observing the phase shift between the overpotential and resulting current) for the same data (Fig. 2) showed a significant shift in this relaxation (f_{max} being the peak phase angle) to lower frequencies (10 Hz to 0.2 Hz). It is deduced from this that the double-layer capacitance of the electrode surfaces was affected, i.e., the bacteria changed the electric field surrounding the corroding electrode. The corrosion rate (measured by the admittance) in sterile conditions decreased rapidly as an inert sulphide film formed and reduced further corrosion to about 0.2×10^{-4} mhos cm^{-2}. *E. limosum* growing on H_2/CO_2 did not significantly change this rate, however, the *Desulfovibrio* evidently prevented the formation of a uniform sulphide film and the corrosion rate stabilized at about 1.3×10^{-4} mhos cm^{-2} after 11 days.

Corrosion rates associated with cocultures of *E. limosum* with *Desulfobacter* sp. and *Desulfovibrio* sp. were also compared with a triculture of all three organisms. *E. limosum* and *Desulfobacter* sp. did not prevent the formation of the sulphide film, and consequently the corrosion rate dropped from approx. 2.2×10^{-5} to less than 1.0×10^{-4} mhos cm^{-2}. Exposure to a coculture of *E. limosum* and *Desulfovibrio* increased corrosion slightly from 2.0×10^{-4} to 2.8×10^{-4} mhos cm^{-2} (Fig. 3). In contrast, exposure to a triculture of all three organisms dramatically increased the corrosion rate between days 5 and 9 to a maximum of 5.7×10^{-4} mhos cm^{-2}. Subsequently

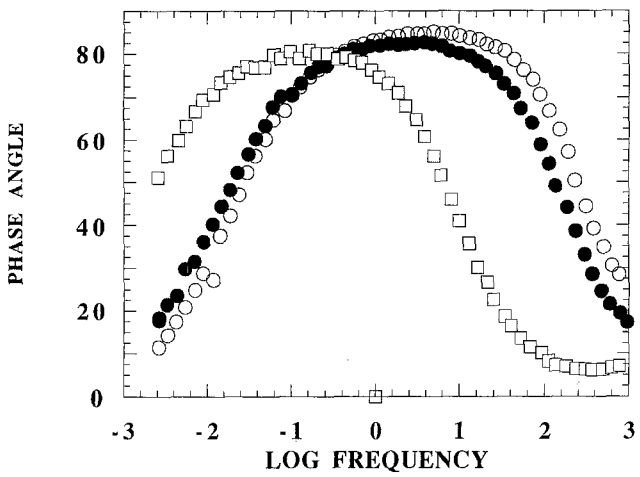

Fig. 2. Phase angle plot as a function of frequency which displays the phase relation between the current and voltage for a series of frequencies, for sterile conditions (●) *E. limosum* (○) and a coculture of *E. limosum* and *Desulfovibrio* (□). These data allow an estimation of the potential for charge storage by the metal/biofilm/medium interface. The coculture of *E. limosum*/*Desulfovibrio* (Dv) shows a shift of the maximum phase angle (f_{max}) to lower frequencies indicative of active and perhaps localized corrosion which is often implicated. The bacteria were provided with H_2/CO_2 as sole carbon and electron sources.

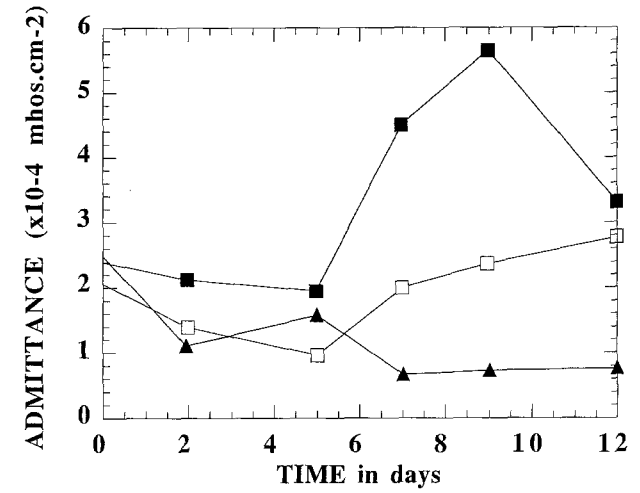

Fig. 3. Corrosion rate displayed as admittance (mhos cm^{-2}) comparing the corrosion rates of a coculture of *E. limosum*/*Desulfovibrio* (▲), a coculture of *E. limosum*/*Desulfovibrio* (□) and the triculture of all three organisms growing on H_2/CO_2 (■).

the corrosion rate dropped sharply, perhaps due to exhausted substrate. The phase angle data showed similar trends of the f_{max} to low frequencies implying that the bacteria substantially modified the electrical double layer. Previous data from sterile systems provided f_{max} values of about 10 Hz, while a coculture of *E. limosum* and *Desulfobacter* sp. shifted the f_{max} to 1.0 Hz. Interestingly, the maximum phase angle frequency for the triculture and the coculture of *E. limosum* with *Desulfovibrio* sp. were at about 0.2 Hz two decades lower than the sterile condition.

Observations of corrosion with organic electron and carbon sources

The use of butanol as carbon and electron source instead of H_2/CO_2 favored a fermentation process to shorter carbon chains and possibly provided acetate for *Desulfobacter* which cannot grow on butanol. The sparging gas during this part of the study was changed to N_2/CO_2 (90:10). The experiment compared the effect of *E. limosum* alone with cocultures of *E. limosum* with *Desulfobacter* sp., *Desulfovibrio* sp., and both sulphate-reducing bacteria. Bacteria grew in all vessels, and during a 12-day exposure corrosion rates in all cases were quite low ($< 2 \times 10^{-4}$ mhos cm^{-2}) with no large differences between the experimental conditions. Microscopic examination of the bulk phase liquid during the study revealed massive growth by the *Desulfovibrio* in all cases, and only moderate growth by the *E. limosum* and *Desulfobacter* sp. Morphological distinctions in most cases were sufficient to give a qualitative analysis of the relative numbers of types of bacteria.

Combinations of bacteria were also tested as described above using glucose as substrate. Glucose is used only by *E. limosum* among the three bacteria selected. Growth of either *Desulfovibrio* sp. or *Desulfobacter* sp. would indicate fermentation products were made available to them via fermentation from the *Eubacterium*. Microscopic examination of the bulk phase liquid revealed mostly *Eubacterium* with some *Desulfovibrio* and *Desulfobacter* cells. Corrosion rates were also quite low (generally less than 2.0×10^{-4} mhos cm^{-2}) for the duration of the experiment. Despite the similarity in the corrosion rates all the conditions containing *Desulfovibrio* had the highest average corrosion rates midway through the exposure.

Finally, all substrates were combined: butanol, glucose, acetate and hydrogen to provide optimal conditions for growth of all the bacteria selected in the combinations described. Since the evolution of volatile acids was expected to be vigorous, MOPS buffer was added to stabilize the pH. As expected *E. limosum* performed fermentative reactions with acetic and some propionic acid waste products. Coculture with the *Desulfobacter* sp. had the principal results of removing both acetate and sulphate (Fig. 4).

Coculture of the *E. limosum* with *Desulfovibrio* sp. produced a sharp increase in the succinate concentration (0–18 mM) with concomitant decrease in butanol (20–0 mM). Acetate concentrations appeared to rise slightly (14–

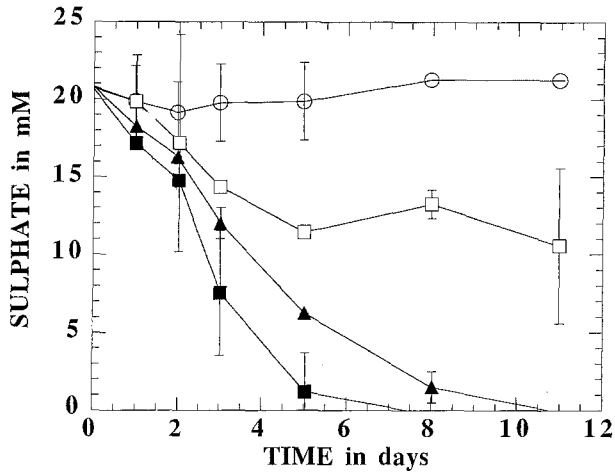

Fig. 4. Evolution of sulphate as a function of time during a corrosion study with the three anaerobic bacteria. *Eubacterium limosum* alone (○), *E. limosum* with *Desulfovibrio* sp. (□) and *E. limosum* with *Desulfobacter* sp. (▲) vs. a triculture of all three organisms (■). The medium was supplemented with glucose (5 mM), H_2/CO_2 (excess), acetate (10 mM), butanol (10 mM) and initially 21 mM sulphate.

18 mM) and propionate was also formed (maximum concentration = 1.5 mM). The concentration of sulphate decreased to only 12 mM (Fig. 4) after 11 days.

A triculture of the fermenter and both sulphate-reducing bacteria produced a rapid decrease in butanol (20–2.0 mM) and rapid removal of acetate which was reduced to 2 mM within 24 h. After 24 h, however, acetate increased to a maximum of 9 mM after 5 days by fermentation. Sulphate concentrations for the triculture decreased rapidly such that effectively it was exhausted after day 8 (Fig. 4). Despite these considerable quantities of volatile fatty acids, the corrosion rates remained exceptionally low in all cases (the admittance was below 1.0×10^{-4} mhos cm^{-2}) with no apparent difference between conditions.

Variation in corrosion

Chemical corrosion habitually demonstrates large variation in surface condition. The additional variable of biologically dependent systems undoubtedly has a deleterious effect on an already uncertain situation. In Fig. 5, a bar graph is used to compare the variation associated with data sets derived from four replicate electrodes, each at particular time points when it was important to distinguish whether treatment differences were 'real'. The data for several of the studies is provided in terms of the impedance. When H_2/CO_2 was provided, a comparison of sterile conditions, the presence of *E. limosum* and a coculture of *E. limosum* and *Desulfovibrio* showed significant differences in the corrosion rate. Means and standard deviations indicated large differences between the sterile cases (large R_{ct}) and the fermenter/sulphate-reducing bacteria cocultures. The trend to low R_{ct} values during exposures to sulphate-reducing bacteria, in particular *Desulfovibrio*, was continued. In experiments where organic carbon was provided, the means and standard deviations showed no reliable trends other than generally higher R_{ct} values (lower corrosion rates) than associated with the bacteria grown on H_2/CO_2.

Scanning electron microscopy (SEM)

The distribution of microorganisms on corroding surfaces is exceptionally important with respect to the corre-

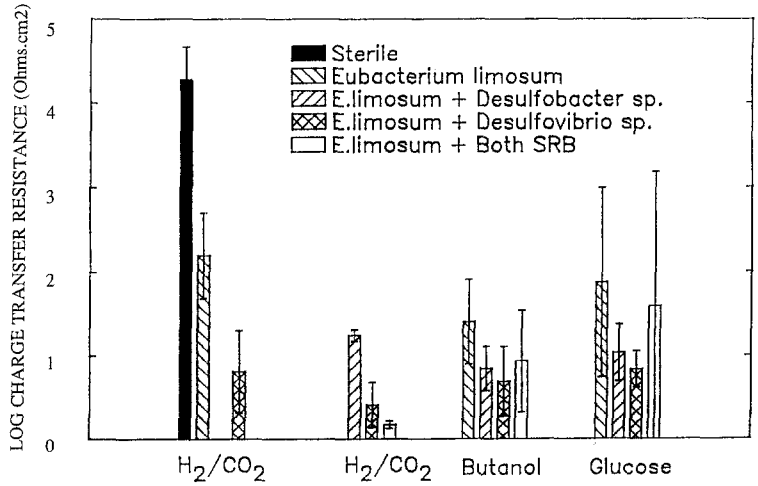

Fig. 5. Histogram of the distribution of corrosion rates with each of the experiments depending upon the substrates provided. Bars indicate standard deviations from four electrode sets. Note: charge transfer resistance is inversely proportional to the corrosion rate.

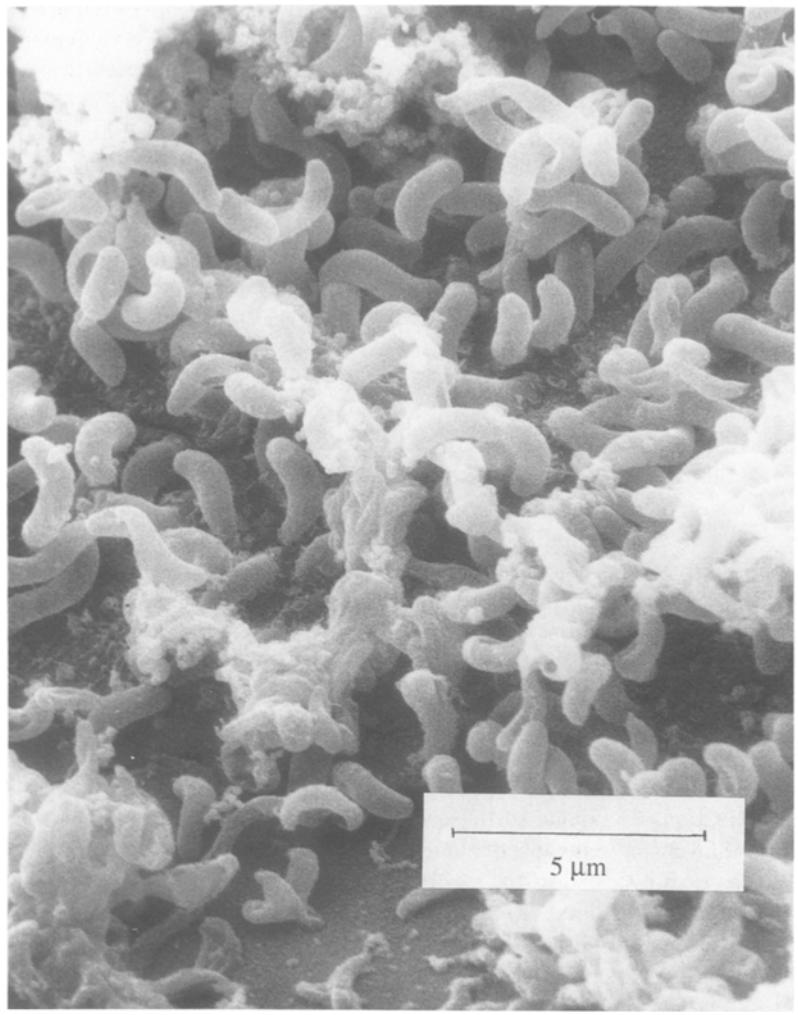

Fig. 6. Scanning electron micrograph of *E. limosum* with *Desulfovibrio* and corrosion debris on the surface of a C1020 carbon steel coupon after 11 days exposure under reduced conditions.

lation of anodic (dissolutive) and cathodic (reductive) sites. SEM was carried out in an attempt to determine qualitatively the ratio of one morphological type from another and determine the degree of 'coverage', by the biofilm/corrosion product, of the surface. Fig. 6 is a micrograph of a *E. limosum* and *Desulfovibrio* coculture attached to the carbon steel surface. The comma-shaped *Desulfovibrio* cells predominate. Clearly in this case the 'coverage' is extensive with, at these outer layers, a high biomass/corrosion product ratio. Studies of bacteria utilizing H_2/CO_2 showed a considerably reduced biomass associated with a higher corrosion rate.

DISCUSSION

The relationship between sulphate-reducing bacteria and corroding surfaces has been described [12–15]. The actual mechanism of microbiological corrosion is most often described in terms of the cathodic depolarization theory [16] where hydrogenophilic bacteria remove cathodically produced hydrogen to some low level accelerating the cathodic process and, by definition, the anodic (dissolution) reactions. In short, a higher net corrosion rate.

The case which we wish to study is the impact on carbon steel of sulphate-reducing bacteria in coculture with other strictly anaerobic bacteria. In the undersea pipeline, excess geologically produced hydrogen was available over that produced chemically at the metal surface or by fermentative bacteria. Phelps et al. [2] showed high activities of hydrogen-mediated acetogenesis in pipe systems involving active corrosion. We, therefore, propose a model for fermentative acetogenesis and sulphate-reduction in this system following the thermodynamics [17,18] of three physiological types:

(i) Fermentation of acetate
$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$
$[G^{o'} = -22.7\ kJ/mol\ H_2]$

(ii) Respiration of sulphate with acetate
$CH_3COO^- + 3H^+ + SO_4^{-2} \rightarrow 2CO_2 + H_2S + 2H_2O$
$[G^{o'} = -63\ kJ/mol\ SO_4^{-2}]$

(iii) Respiration of sulphate with hydrogen
$4H_2 + SO_4^{-2} + H^+ \rightarrow HS^- + 4H_2O$
$[G^{o'} = -152\ kJ/mol\ SO_4^{-2}]$

The values for free energy exchange given above show that respiration of sulphate at the expense of hydrogen is more energy efficient than at the expense of acetate per mole of sulphate. Likewise the respiration of sulphate at the expense of hydrogen is more efficient than acetogenesis from H_2/CO_2 per mole of H_2. Assuming that the cathodic depolarization hypothesis is correct, and all other parameters remain constant, it follows that organisms performing hydrogen oxidation would impact corrosion more or less depending upon the efficiency of removal. Thus, if corrosion corresponds to the affinity for 'hydrogenscavenging', then respiration at the expense of sulphate would most affect the corrosion rate. Serious problems arise, however, when the production of HS^- is taken into account, since a uniform distribution of sulphide at the surface would tend to form a relatively impermeable film and reduce the corrosion rate (as demonstrated in the sterile condition, Fig. 5). Thus, a competitive situation emerges between cathodic depolarization, which tends to increase corrosion, and sulphide production, which tends to reduce it.

While hydrogen could be utilized by either *E. limosum* or *Desulfovibrio* sp., the latter could grow efficiently on butanol producing the short-term intermediate succinate. *E. limosum* grew on butanol and hydrogen at a considerably reduced rate but was the only organism tested that could utilize glucose. *Desulfobacter* sp., on the other hand, could use neither hydrogen, butanol nor glucose and grew solely on acetate as electron and carbon source.

The introduction of *E. limosum* growing on hydrogen increased the corrosion rate over sterile conditions (Fig. 5), however, the coculture of *E. limosum* and *Desulfovibrio* sp. demonstrated an even higher dissolution rate. Following the hypothesis of Von Wolzogen Kuhr and Van der Vlugt, the introduction of the *Desulfovibrio* should have exerted a greater effect on the corrosion rate than the *E. limosum* due to the higher affinity of the sulphate-reducing bacterium for hydrogen (mean $K_m = 1.4$; [19]) over the *E. limosum* ($K_m = 0.34$; [20]). Although an acceleration of the corrosion rate with the presence of the *Desulfovibrio* was observed, it was not possible to correlate the cause directly with the hydrogenase activity.

Anomalous behaviour was, however, observed in a triculture study when an H_2/CO_2 mix was provided (Fig. 3). In this case a rise in the corrosion rate after day 5 with the triculture indicated that some other, as yet undescribed, process was operating. While the *E. limosum* and *Desulfovibrio* sp. are both hydrogenophilic, the non-hydrogen-utilizing *Desulfobacter* sp. was provided with no carbon or electron source other than that derived from the other bacteria. If the increase in corrosion rate with the triculture was an event with a real physiological basis, then it must have been due to a closer interaction of the *Desulfobacter* sp. with one or both of the other bacteria. This is evident by comparison with the exposure to the *E. limosum*/*Desulfobacter* coculture which demonstrated a decreasing corrosion rate. After day 9 (Fig. 3), however, the corrosion rate decreased with no indication of cause.

When all substrates (H_2, glucose, butanol and acetate) were provided to stimulate all strains without interdependency, the MOPS buffer depressed the corrosion rate such that deductions from the impedance data became unreasonable. Changes in the volatile fatty acid (VFA) concentrations, however, showed the preference of the individual organisms for specific substrates and interactions. While *E. limosum* apparently grew at the expense of glucose, the production of succinate in coculture with *Desulfobacter* indicated some unknown facility. Acetate consumption by the *Desulfovibrio* was evident with concomitant rapid total loss of sulphate by day 11 (Fig. 4). Coculture of *E. limosum* and *Desulfovibrio* did not remove all the sulphate, even though excess hydrogen was available (12 mM SO_4^{-2} remained after 11 days). Instead, the butanol was rapidly removed with the production of succinate, indicating that disproportionation had occurred. More energy is available from the reduction of fumarate to succinate [21] with hydrogen (-86.2 kJ/mol H_2) than the reduction of sulphate with hydrogen (-38.0 kJ/mol H_2). No intermediates such as fumarate were detected in this system. It is assumed, however, that the *Desulfovibrio* incompletely oxidized the butanol and shunted electrons to the four-carbon skeleton to form succinate, possibly via the fumarate reductase system [22]. In the case of the triculture, acetate was probably oxidized by the *Desulfobacter* sp. alone, since it was apparent from the fermentation tests and the *E. limosum*/*Desulfovibrio* coculture that *Desulfovibrio* incompletely oxidized butanol. Scanning electron microscopy (Fig. 6) of the metal surfaces and suspensions showed that the number of cells associated with the corrosion debris were more numerous when sulphate-reducing bacteria were present. *Desulfovibrio* sp., an organism previously implicated in many corrosion cases [1], appeared to be an important component of a corrosion-influencing

consortium. This contrasted with hydrogenase-negative sulphate-reducing bacteria such as the *Desulfobacter* sp. in the study.

These results show that hydrogenophilic bacteria have an important role in the corrosion of carbon steel under reduced conditions. An interesting synergistic interaction with respect to the corrosion rate was observed between two sulphate-reducing bacteria, one of which possessed no hydrogenase enzyme. The influence of substrate was also demonstrated for sulphate-reducing consortia when the provision of H_2/CO_2 increased the corrosion rate for a limited duration over that produced by organic substrates such as butanol and glucose. This suggests that removal of hydrogen from undersea pipelines would provide economic benefit with respect to this problem in addition to addressing the more usual concerns of hydrogen embrittlement.

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