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The Influence of Sulphate-Reducing Bacteria on Hydrogen Absorption by Steel During Microbial Corrosion

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ABSTRACT

During the anaerobic corrosion of steel, hydrogen sulphide (H_2S) evolution by the Sulphate-Reducing Bacteria (SRB) promotes hydrogen absorption by enhancing cathodic hydrogen evolution and inhibiting the combination of hydrogen atoms. While the effects of hydrogen damage are well known, it is unclear whether surface SRB films influence hydrogen absorption any differently to the separate effects of H_2S. Also, for particularly hydrogen sensitive metals in soured environments, there is some doubt regarding what constitutes a safe but effective level of cathodic protection.

Using the electrochemical technique of Devanathan and Stachurski, special apparatus were used to monitor hydrogen permeation through steel foils exposed simultaneously to cells and cell-free dialysates in batch cultures of either Desulfovibrio vulgaris (Woolwich) or Desulfovibrio desulfuricans (Norway 4). Further experiments were performed using applied potentials of -850mV (SCE) and -1000mV (SCE), and polarisation scans were performed on replicate steel specimens under the same conditions. Changes in pH, Eh, cell numbers and H_2S levels were monitored during SRB growth.

Following a period of SRB growth and H_2S evolution, hydrogen permeation currents rose rapidly and then peaked. Subsequent hydrogen permeation declined markedly, and the potential of freely corroding specimens became less active. This was interpreted as evidence for anodic polarisation, which was consistent with the types of adherent sulphide films present. Polarisation data confirmed that partially protective films became established within the first 24 hours of SRB growth, and that corrosion was under anodic control.

Most importance has been placed on the observation that a surface hydrogen concentration (0.066ppm) generated during cathodic protection at -850mV (SCE) was up to 10 times Co values obtained by freely corroding specimens. A further lowering in potential to -1000mV (SCE) resulted in a doubling or tripling in hydrogen permeation rate.

The presence of SRB cells at the steel surface appeared to influence neither the peak hydrogen permeation rate, nor the protectiveness afforded by corrosion product films.

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NOTATION

A	amperes
AnalR	analytical reagent
ASTM	American Society for the Testing of Materials
BA	British Association
C	celsius (unless indicated differently in text)
C	coulomb (unless indicated differently in text)
cm	centimetre
Co	surface hydrogen concentration
conc	concentration
D	apparent diffusion coefficient for hydrogen
Da	daltons
DC	direct current
F	faraday (96480 coulombs/mol)
g	grammes (except in Figure references, or as "x g")
hrs	hours
i.d.	internal diameter
IPA	isopropyl alcohol
J	equilibrium hydrogen flux
l	litres
L	length
mA	milliamperes
mm	millimetres
mM	millimols or millimolar
M	mass
mdd	milligrammes per square decimetre per day

mg	milligrammes
mins	minutes
ml	millilitres
mV	millivolts
nA	nanoamperes
OFN	oxygen free nitrogen
o.d.	outside diameter
ppm	parts per million
PMB	Postgate's Medium 'B'
PMC	Postgate's Medium 'C'
PME	Postgate's Medium 'E'
%	percent
PTFE	polytetrafluoroethylene
SCE	saturated (KCl) calomel electrode
secs	seconds
SHE	standard hydrogen electrode
SRB	Sulphate-Reducing Bacteria
TEMED	tetramethylethylenediamine
V	volts
wt	weight
x g	force of gravity
µA	microamperes
µg	microgrammes
µM	micromols or micromolar

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1. INTRODUCTION

Most people have at some time experienced directly the consequences of bacterial sulphate reduction, whether from the familiar 'rotten egg' smell of hydrogen sulphide given off by poorly aerated soil or water, or perhaps by unwittingly revealing a body of blackened sulphide-containing soil or mud beneath a pond or sandy beach. In fact for the vast majority of man's existence, these types of experience probably accounted for the only direct interaction between human activity and the agents responsible for the generation of biogenic sulphide, namely the Sulphate-Reducing Bacteria (SRB). The limited perceived role of SRB in our daily lives is mostly attributable to their obligately anaerobic habit, which for respiration and growth to proceed, requires not only the complete absence of gaseous or dissolved oxygen, but also a chemically-reduced environment. In addition, there is a relatively limited range of simple carbon sources available to the SRB, and so to a large extent their growth in environments containing more complex nutrients, such as decaying plant or animal matter, depends upon the presence and activity of heterotrophic organisms.

In view of these latter factors, in a domestic situation the SRB might appear to be quite an innocuous group of bacteria, considering that they have no known pathogenic

effect on man, and during the normal preparation and storage of food, SRB growth is rarely a cause of concern (Postgate, 1984). Moreover, from an industrial viewpoint, we may owe much to past SRB activity, as mass isotopic fractionation studies have showed that many commercially useful deposits of elemental and reduced sulphur, and also metal sulphide ores owe their existence to the role of SRB in the overall sulphur cycle (Nriagu and Hem, 1980). However, compared to the number of any such benefits, in industrial situations which either involve the creation of anaerobic environments conducive to SRB growth or which intrude into existing SRB habitats, there are a great many instances where SRB growth and activity can have a very damaging effect. Some of the problems caused by SRB growth, including the separate and combined influences of hydrogen sulphide and extracellular biopolymer materials are listed below.

Corrosion of buried steel pipelines in anaerobic soils, and steel piers, sheet piling and ship hulls lying in estuarine muds (Miller and King, 1975; Miller, 1971).

Corrosion of interiors of oil platform legs and petroleum product storage tanks (Moosavi and Hamilton, 1987; Wilkinson, 1984; Postgate, 1984), and the corrosion of reinforced concretes used in offshore oil storage (Morgan and Steele, 1986).

The graphitisation of cast iron piping (Miller and King, 1975) and crevice corrosion of steels (Cragnolino and Tuovinen, 1984).

Pitting of copper based alloys used for heat exchanger tubes and piping (LaQue, 1975).

Corrosion of offshore structures exposed to contaminated sea-bed drilling cuttings (Edyvean, 1987) and corrosion of valves/pipes and distribution systems for natural gas production (Herbert, 1986).

Oil injection well plugging (Edyvean, 1987).

Stress corrosion cracking of steels (Cragnolino and Tuovinen, 1984) and the hydrogen embrittlement and corrosion fatigue of steel (Edyvean, Thomas, Brook and Austen, 1986; Thomas, Edyvean and Brook, 1988).

Corrosion of domestic hot water or central heating systems (Postgate, 1984).

Toxic effects of hydrogen sulphide on human activity in offshore operations, and on freshwater fauna (including the death of fish populations), and flora (including paddie field rice crop failure (Postgate, 1984).

Air pollution nuisance (smell) emanating from ponds, lakes, bogs, estuaries, harbours, beaches and from dumped refuse (Postgate, 1984).

Degradation of cutting emulsions (Postgate, 1984).

Discoloration of paints and coatings, and the blackening of pulp in the paper making industry (Postgate, 1984).

In economic terms, probably the worst affected activities listed are those relating to the use of metals in the extraction, storage and processing of petroleum products, and and also those involving either the laying of buried steel pipelines, or the operation of pipelines which may support SRB growth due to the nutritional status of the material being transported. Despite the inconvenience, danger and extra cost involved in handling materials and equipment which have become contaminated by SRB, probably the greatest economic loss attributable to such bacteria is incurred due to their corrosiveness towards steel.

Although the recent literature does not suggest a cost relating specifically to the effects of SRB on corrosion, Tiller (1982) estimated that the effects of biofouling in general were costing UK industry some £300-400M per year. Considering the continual introduction of new plant, machinery and pipelines into environments which are conducive to biofouling or SRB activity, and while allowing for inflation, one would expect this figure to have at least doubled in the past 8 years.

It is very important at this stage to appreciate that the biodeterioration of metals, and especially steel (which is by far the most widely used metal and the most important economically), may be caused by the activities of a great many different types of organism. These include a wide

range of species of bacteria, microalgae, protozoa and fungi, as well as larger forms such as macroalgae (seaweeds), molluscs and marine benthic organisms. Furthermore in reality, mechanisms of biological corrosion often involve consortia of different microbial species. These can be supported by the cycling of various chemical factors, such as iron, oxygen, hydrogen, sulphur and carbon, which participate simultaneously in corrosion processes and metabolic reactions alike. The broad range of species involved in the corrosion of a range of different metals has been reviewed by a number of workers (Costello, 1969; Miller, 1971; Miller, 1981; Tiller, 1982), but this work will be concerned mainly with the microbial corrosion of steel, the most important microbial agents of which are widely recognised as being the SRB (Hamilton, 1985). Reference will, however be given to the influences of other member species of microbial consortia where relevant to the discussion of corrosion mechanisms.

As in all base metals, the corrosion of steel in aqueous environments proceeds by the electrochemical oxidation and dissolution of metal atoms into ions, and the simultaneous consumption of charge due to reduction reactions occurring at the metal surface (section 2.1). According to the ease with which these two processes take place at different points on the metal, the resulting metal loss may occur either uniformly, or as is more often the case, in a more

localised fashion which results in pitting. Thus the corrosion associated with reduced performance of a structure such as a buried pipeline may appear obvious to the observer, especially if it were to perforate.

Unlike such clear manifestations of metal loss though, in acidic or anaerobic conditions, the less apparent absorption and accumulation within steel of hydrogen generated during corrosion causes an unseen reduction in ductility of the metal and an increased susceptibility to cracking. The absorption of hydrogen can have a devastating effect on a steel structure, especially when under a load or an applied stress. Since the possibility of such failures occurring is directly related to the corrosion reactions, it would appear appropriate to prevent corrosion. However, the electrochemical means of protection commonly employed also generate hydrogen, and thus can also encourage embrittlement.

The complexity of the relationships between corrosion processes, corrosion protection and hydrogen absorption may be increased in the presence of SRB activity, since hydrogen can play a central role in SRB growth and respiration. In order to deduce the relative importance of microbial influences, and to establish effective means of corrosion prevention and control it is therefore necessary to adopt an integrated approach, which addresses the

nature of anaerobic corrosion and hydrogen absorption in terms of SRB activity. For a complete understanding of the situation observed in the field, such an approach should also address the influence of the biological community as a whole. In practice though, the ecological complexity of real situations can make this very difficult to achieve, and more can be learnt by an initial study of simple monoculture systems. Moreover, the subject of SRB induced hydrogen absorption by steel is still at a relatively early stage of development, and it is therefore appropriate that simple systems are employed in its investigation. Such is the nature of the following study, which attempts to shed some light upon the role of SRB and biogenic hydrogen sulphide in the tendency for steel to absorb hydrogen during anaerobic corrosion.

ANAEROBIC CORROSION AND HYDROGEN ABSORPTION

2.1 ANAEROBIC CORROSION

In moist or wet environments, elemental iron tends to oxidise and dissolve into its ions (Fe^{2+}).

$$Fe = Fe^{2+} + 2e^{-}$$

The sites at which the oxidation takes place act as anodes, and thus tend to become negatively charged. However, such anodic dissolution can only proceed if this charge can be consumed by a simultaneous reduction process, which occurs at cathodic sites in electrical contact with the anodes. In situations at approximately neutral pH, where oxygen is dissolved in the aqueous solution surrounding the metal, the predominant cathodic reaction is:

$$O_2 + 2H_2O + 4e^{-} = 4OH^{-}$$

But where oxygen has become depleted, for instance due to the above oxygen reduction reaction, or perhaps following a period of aerobic microbial activity, the above reaction is replaced by the cathodic reduction of hydrogen ions (from the dissociation of water) and the evolution of gaseous hydrogen:

$$2H^{+} + 2e^{-} = 2H = H_2$$

An important consequence of the above reaction is that a surface film of cathodic hydrogen becomes established upon the steel surface, which acts as a physical and electrical barrier between metal and cathodic reactant, thus hindering any further discharge of hydrogen ions (cathodic polarisation). Because the anodic and cathodic reactions can only proceed in balance with each other, the reduced cathodic activity results in a smaller current flowing between anode and cathode, thus lowering the corrosion rate (cathodic polarisation). At a neutral pH, and in the absence of oxygen or any externally applied current, the only means by which steel may corrode faster is by a continual removal of the surface film of cathodic hydrogen, either mechanically, or by some chemical means. In a similar way, the anodic reaction may become polarised, such that the dissolution of iron atoms is impeded in some way. This can be effected by the establishment of protective films, which may be formed for instance by a reaction between the metal surface and reactive species dissolved in an aqueous medium.

The surface sites at which anodic dissolution and cathodic reduction reactions occur may change constantly, owing to temporary, randomly distributed irregularities in the metal surface. The continual removal of these is effected by dissolution of the metal, resulting in a generalised pattern of corrosion. Less superficial irregularities may

however provide more permanent sites for the anodic and cathodic reactions. For instance an irregular distribution of corrosion product may lead to the formation of concentration cells, where areas of metal which become screened from cathodic reactant (for example O_2) tend to become anodic with respect to adjacent cathodic sites located on the bare metal surface. Alternatively, films of corrosion product may confer enhanced cathodic properties when in contact with the metal. When covering most but not all of the metal surface, such cathodic films may lead to very localised corrosion, where the relatively small remaining areas of bare metal act as anodes and thus tend to corrode preferentially, owing to the galvanic effects of the film material.

In either case, it follows that if either the anodic or cathodic reactions become polarised before permanent anodic and cathodic sites become established, then a limited extent of generalised corrosion will result. However, a fixed differentiation of anodic and cathodic sites may lead to localised metal loss in the form of pits, which despite often being quite small in area, may have very much more influence upon the performance of a metal structure than an equivalent loss of metal resulting from generalised corrosion (section 2.2).

In addition to the effects of concentration cells on metal loss, any physical or chemical agent which causes the removal of polarising hydrogen from the steel surface will enhance the overall rate of corrosion. In anaerobic environments at neutral pH, such cathodic depolarisation of steel is most clearly illustrated by the influences of SRB and biogenic hydrogen sulphide on steel (section 3). These latter factors, when combined with the possible role of bacteria in the generation of concentration cells and localised corrosion, may give rise to much more serious rates of metal loss than those which would be applicable if the polarisation of the cathodic reaction persisted.

2.2 CORROSION PROTECTION

While seeking to prevent such corrosion from occurring, a number of different approaches can be taken. Clearly any adverse bacterial influence on corrosion might be mitigated by the use of biocides or metabolic inhibitors, but these are often expensive, and can have serious environmental effects on surrounding communities (Brunt, 1987). Moreover bulk environments such as a body of soil, freshwater or sea water are in a state of continual circulation and change, and it is only with great difficulty that a lasting means of controlling biological growth can be established which will remain effective throughout the normal design life of a steel structure.

It is for this reason that when buried in soils, steel installations such as pipelines are usually pre-treated with combinations of a large number of different types of coating, including those consisting of plastics, glass fibre, and materials based on coal tar and bitumen, as well as an armoury of specially formulated paints which can afford an additional degree of protection from corrosion.

When combined with some of the measures described above, perhaps the most efficient (and cost effective) means of protecting steel structures immersed in natural corrosive environments is by the application of cathodic protection. The basic principle of cathodic protection involves the application of sufficient electrical current from an external source (which forms an anode), to ensure that only cathodic processes (such as the reduction of hydrogen ions) can occur at the steel surface, to the exclusion of the anodic process of metal dissolution. The principle can be illustrated most clearly by means of an Evans diagram showing the polarisation curves for both the anodic dissolution reaction, and the cathodic hydrogen evolution reaction, which occur together in the model anaerobic situation illustrated in figure 2.1.

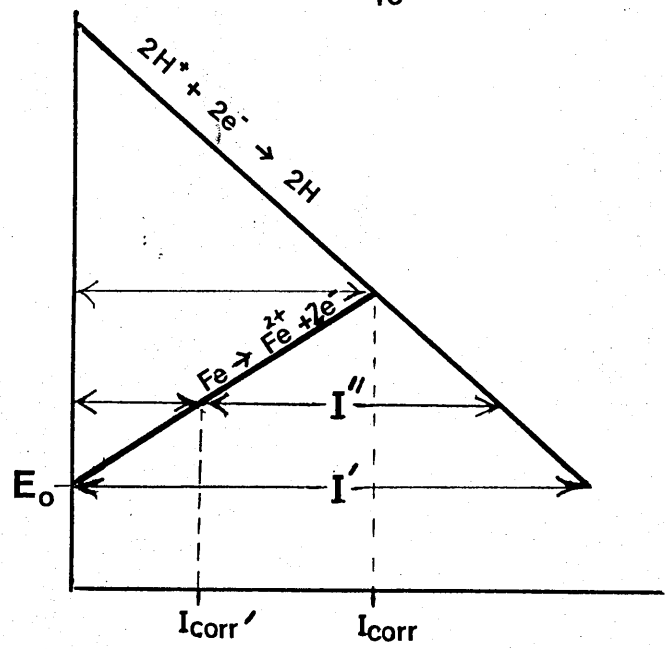


Figure 2.1 Evans diagram indicating the polarisation of anodic and cathodic reactions, with the reversible (equilibrium) potential (Eo) for the metal dissolution reaction and the current required for full (I') and partial (I'') cathodic protection.

For freely corroding steel, the rates of anodic and cathodic reactions are in balance with each other, and the corrosion current, Icorr flowing between them is determined by the intersection of anodic and cathodic polarisation curves. The steel is afforded with complete protection from corrosion when the metal is cathodically polarised to such an extent that net anodic dissolution cannot occur. This condition is met by supplying current I', such that the metal assumes a potential equivalent to the reversible anodic potential (E_o), where the anodic reaction ceases to be polarised, with iron atoms in equilibrium with ferrous ions in solution.

The success of the technique in preventing corrosion is dependent upon two important criteria, which come to notice by a further consideration of figure 2.1. In order to achieve full cathodic protection, the reversible anodic potential (E_o) of the metal in the chosen environment must be known. Having established this, it is then equally important to know that when applying the protection, an adequate level of current is actually being supplied to all parts of the steel structure. If the true value of E_o were not recognised, and instead the current I'' was supplied to the metal, then an incomplete level of protection will be given, resulting in the level of corrosion indicated by Icorr'. Clearly the incomplete level of protection would be of some benefit in reducing the rate of metal dissolution, but the design of many types of structure or installation for prolonged service in corrosive environments may not tolerate the occurrence of such 'reduced' levels of corrosion.

In practice, to achieve the two criteria mentioned is not straight forward. Sacrificial anodes composed of alloys of more electronegative metals than iron, such as magnesium and aluminium (for example alloyed with zinc or manganese) may become damaged or even detached, for instance due to fouling, thus leaving areas of an installation incompletely protected. Large structures such as offshore oil rigs require complex systems for

delivering and monitoring the required level of current to all parts of an installation. The influence of the corrosiveness of the environment on the efficacy of cathodic protection is evident when considering the effects of changing soil types, moisture contents, pH and oxygen concentration on very long installations such as buried pipelines, which may extend across a range of different environments, each making different demands on cathodic protection. In addition, the influence of the time factor becomes important when considering changes in the reversible anodic potential, which during cathodic protection may become more active, due to a fall in the concentration of ferrous ions immediately surrounding the metal, owing to the lack of anodic dissolution. In accordance with the Nernst equation for iron (an approximation of which at 25 C is given below),

$$E = -440mV + 30 \log [Fe^{2+}]$$

where -440mV refers to the standard potential of iron versus the normal hydrogen electrode (NHE) at a unit activity of ferrous ions; the potential (E) of the metal will become more active as the concentration of ferrous ions falls, and thus the current demand for full protection tends to increase.

A similar effect might occur in the presence of species such as hydrogen sulphide, which may remove ferrous ions from solution by the formation of insoluble precipitates, such as those of iron sulphide. Increased current demands for full protection have also been attributed to the metabolic activity of bacteria (Booth and Tiller, 1960), due to the uptake of cathodic hydrogen generated during cathodic protection (section 3). In practice, the level of protection applied to steel takes account of the low concentration of Fe^{2+} present, which in sea water occurs at approximately 10^{-6}M. According to the Nernst equation above, the departure of sea water Fe^{2+} concentrations from unit activity results in a lowering in the equilibrium potential of iron by approximately 180mV, and thus a minimum 'safe' protection potential in sea water might be attained at -620mV, NHE (equivalent to -861mV, versus the saturated calomel electrode, SCE). Lower potentials may be selected in the presence of SRB and H_2S.

It may therefore seem appropriate to consider a rationale whereby cathodic currents were applied to the metal which were in excess of the minimum levels thought necessary to prevent metal loss. However, three problems can be associated with such 'over-protection'; whether using a system based on sacrificial anodes, or using an impressed current system, where anodic reactions take place upon an inert electrode, located separately from (but in

electrical contact with) the protected structure. Firstly, it is clearly more expensive to generate and deliver current in excess of that which is necessary, and the increased cost rises with the size of the protected structure. Also, the constant reduction of either oxygen or hydrogen ions leads to an increase in pH of the medium surrounding the structure. In some situations, the increase in alkalinity near to the steel surface serves to enhance the cathodic protection by causing the localised precipitation of calcium hydroxide and magnesium carbonate in the form of 'calcareous scales'. These serve to protect the steel from corrosion, and thus reduce the current needed for protection. Moreover, at higher pH values and in the presence of oxygen, further protection from corrosion is afforded to the metal by the formation of passive iron oxide films. However, the types of paints and coatings often used in combination with cathodic protection, tend to suffer damage in highly alkaline conditions, and the failure of such physical measures would only lead to a greater current requirement.

Perhaps most importantly for the safety of the protected structure though, is a consideration of the effects of lower potentials and cathodic over-protection on the rate of the hydrogen reduction reaction, and the increased tendency for hydrogen to diffuse into the metal. This latter effect will now be examined further.

2.3 HYDROGEN ABSORPTION BY STEEL

2.3.1 The Absorption of Cathodic Hydrogen

The mechanism of hydrogen absorption by steel and the possible influence of SRB activity are best understood by considering further the anaerobic cathodic hydrogen evolution reaction given in section 2.1. In what may appear to be a relatively simple process, McCright (1977) identified up to seven separate stages, from the diffusion of hydrated protons in the bulk medium, to the evolution of molecular hydrogen at the steel surface (figure 2.2).

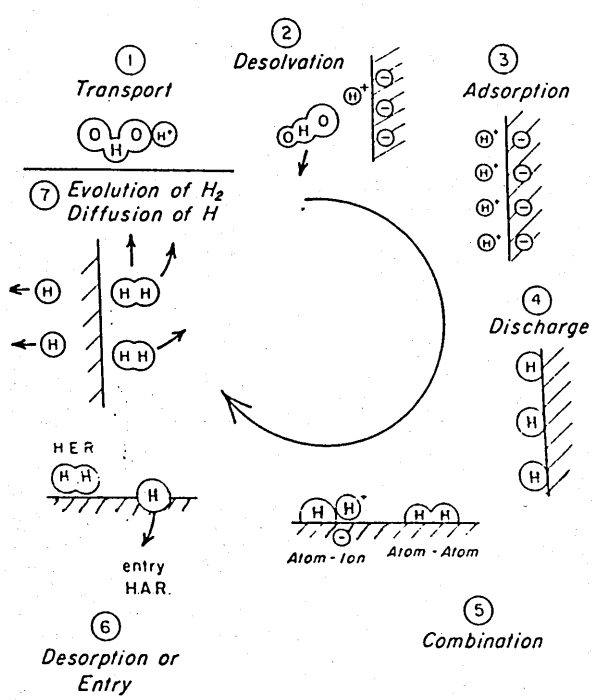


Figure 2.2 Stages of cathodic hydrogen evolution, and hydrogen absorption by steel (from McCright, 1977).

Each of steps 1,2,3 and 7 in the scheme shown in figure 2.2 generally do not limit the overall rate of hydrogen evolution. In addition, the combination and desorption steps (steps 5 and 6) can be considered to occur together, owing to the weak tendency for molecular hydrogen to remain adsorbed on the metal surface (McCright, 1977). Thus for clarity the overall reaction may be presented in terms of two processes; the discharge of hydrated protons (sometimes referred to as the Volmer discharge step):

$$H_3O^+ + e^- = H_{ads} + H_2O \qquad (1)$$

followed by the combination of adjacent adsorbed hydrogen atoms to molecules (The Tafel recombination step):

$$H_{ads} + H_{ads} = H_{2ads} \qquad (2)$$

An alternative to reaction 2 (above) involves the combination of adsorbed atomic hydrogen with a hydrated proton, which then becomes reduced to hydrogen molecules (the Heyrovsky electrochemical desorption step):

$$H_{ads} + H_3O^+ + e^- = 2H + H_2O \qquad (3)$$

At ambient temperatures, hydrogen in atomic form is the only element small enough to enter and diffuse into the crystal lattice of iron atoms within a body of steel. An important feature of the hydrogen discharge step (reaction 1, overleaf) is that for a short time, nascent hydrogen atoms remain adsorbed at the steel surface, and have a tendency to become absorbed by the metal (reaction 4).

$$H_{ads} = H_{abs} \qquad (4)$$

Hydrogen absorption can thus be thought of as proceeding in competition with the hydrogen combination reaction (reaction 2, overleaf), and the overall evolution of cathodic hydrogen. For iron, the rate of proton discharge (reaction 1 overleaf) normally limits the overall rate of hydrogen evolution, owing to the limited tendency for hydrated protons to adsorb onto the metal surface (Scully, 1975). However, there are a number of elements which inhibit the hydrogen combination step (reaction 2 overleaf), the most notable of which are sulphur, phosphorus, arsenic, selenium, antimony, bismuth and tellurium. Such substances are often termed 'cathodic poisons', owing to their influence of reducing the overall rate of hydrogen evolution; and the poisoning effect is found to be most marked when these elements are in the form of hydrides (such as H_2S), or when ionised (for example to HS^- or S^{2-}, McCright, 1977).

In practical terms, the consequences of increased hydrogen absorption due to cathodic poisoning can be serious, and are most important in relation to hydrogen embrittlement of steel and an increased susceptibility to hydrogen induced cracking and stress corrosion cracking (section 2.2.4), which may be evident in situations where H_2S is present as a result of SRB activity. It should also be mentioned here that despite its 'cathodic poisoning' property, biogenic H_2S enhances the corrosion of steel, and stimulates the cathodic hydrogen evolution reaction. Thus in addition to increasing the proportion of hydrogen entering the metal, H_2S also effects an absolute increase in hydrogen absorption by increasing the overall rate of hydrogen discharge (the influences of hydrogen sulphide and SRB are reviewed in Chapter 3).

2.3.2 The Measurement of Hydrogen Absorption

Three general approaches may be taken for measuring hydrogen absorption (McCright, 1977), from which the tendency for different types (composition and microstructure) of steel to exhibit hydrogen embrittlement can be deduced. Firstly, the effects of exposure to a particular environment can be measured in terms of changes in the physical or mechanical properties of the metal, such as a reduction in ductility or tensile strength. Alternatively, an accurate measurement of the bulk

hydrogen content may be obtained by the extraction of hydrogen while heating the metal under a vacuum. A far more convenient method though, is one in which the rate of hydrogen absorption and the hydrogen concentration can be measured while corrosion is in progress. Although this can be achieved by measuring changes in gas pressure during vacuum extraction, a more convenient approach became available following the work in the 1960's by Devanathan and Stachurski (1962 and 1964), who developed an accurate and reliable, yet relatively simple and inexpensive electrochemical technique for measuring hydrogen permeation through metal membranes. In this technique, one side of a piece of steel becomes charged with hydrogen (generated either from corrosion, or due to an applied cathodic potential), which diffuses through the metal under a concentration gradient (figure 2.3).

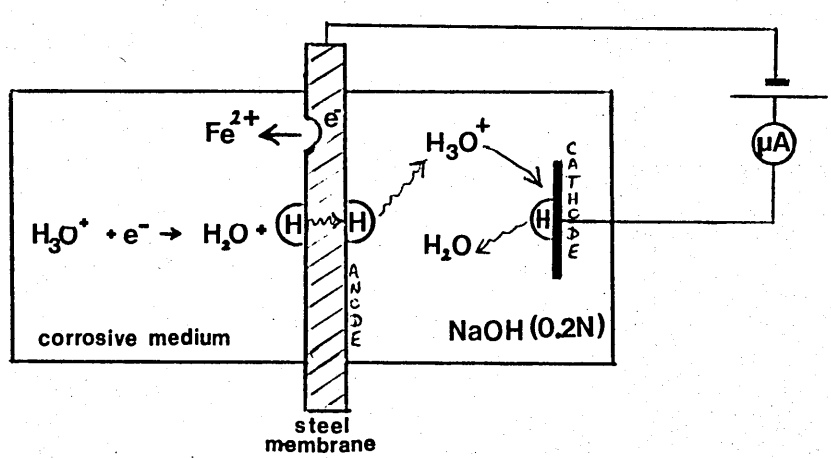


Figure 2.3 Principle of the electrochemical measurement of hydrogen permeation through a steel membrane.

Using a potentiostat, the hydrogen exit surface is polarised anodically with respect to an inert cathode, both of which are bathed in a common electrolyte solution so that emergent hydrogen oxidises and diffuses away from the steel, to become reduced at the cathode. The measurement of hydrogen permeation current thus generated then provides a direct measure of the rate of hydrogen absorption at the corroding metal surface. To ensure that the oxidised hydrogen measured originates exclusively from the corroding surface, an alkaline electrolyte solution is used, thus maintaining the metal in a passive condition. In addition, the exit surface is sometimes coated with an electro-deposited layer of palladium or platinum, which may act as a further corrosion prevention measure (Bockris, 1977), or which may also serve to catalyse the oxidation of diffused, emergent hydrogen (Mergey, 1984).

Prior to the exposure of steel to a corrosive environment, the total quantity of diffusible hydrogen present is distributed uniformly within the steel membrane (shown by by the area beneath the horizontal line in figure 2.4a). The application of an anodic potential at the exit surface results immediately in a zero hydrogen concentration (figure 2.4b). The continual depletion of lattice hydrogen results in the establishment of a uniform concentration gradient (2.4c), which becomes less steep as hydrogen escapes from the metal.

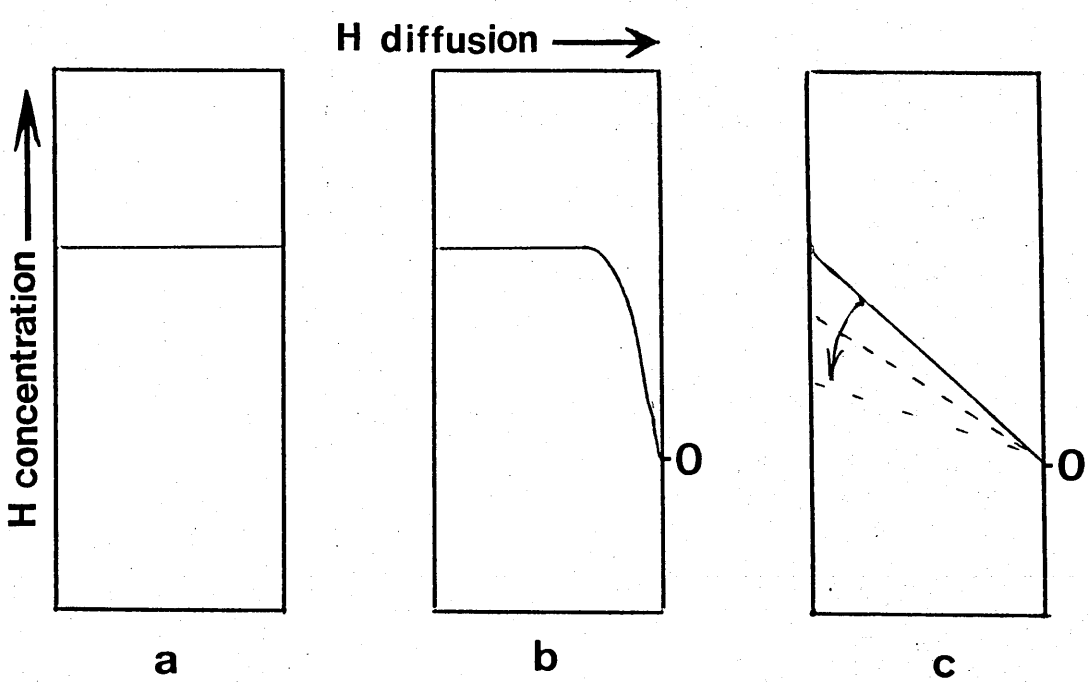


Figure 2.4 Sequence of changes in hydrogen concentration gradient within a steel membrane before (a), and during (b), electrolytic depletion of hydrogen; and after a uniform concentration gradient has become established (c).

The introduction to hydrogen-depleted steel (figure 2.5a), of a corrosive medium results in the establishment of a higher surface hydrogen concentration, Co (figure 2.5b). A uniform hydrogen concentration gradient subsequently becomes established with an increased rate of hydrogen diffusion, as indicated by the area beneath the line Co-0 (figure 2.5c).

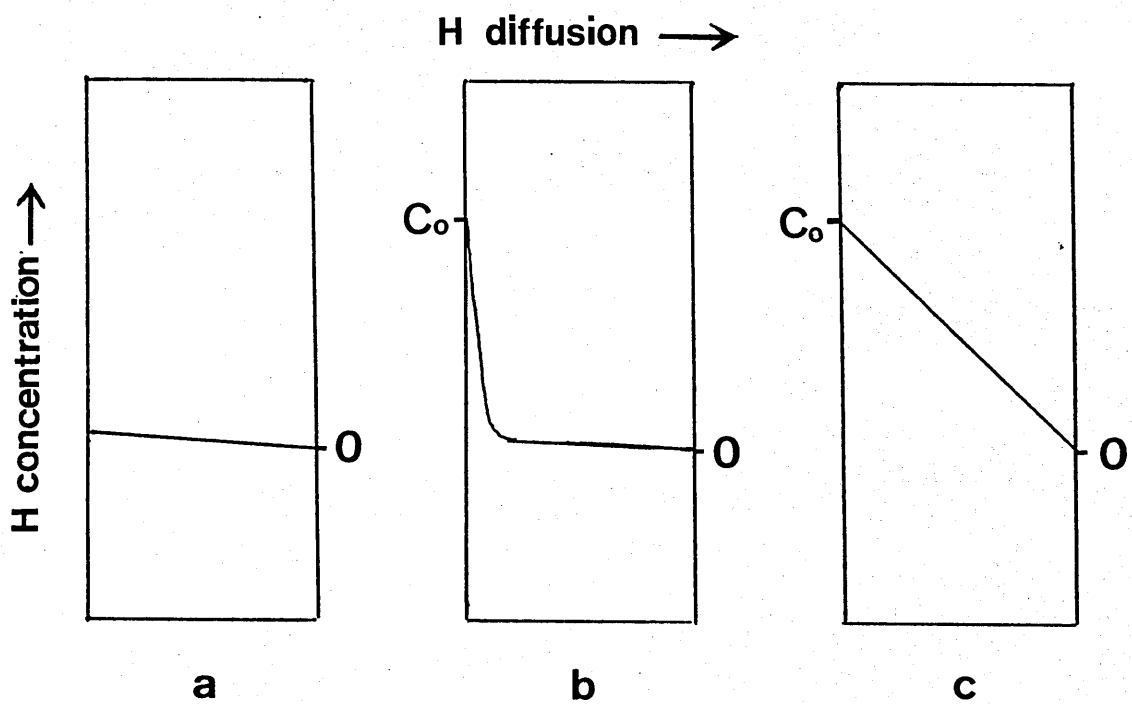


Figure 2.5 Effect of exposing hydrogen depleted steel to a corrosive environment on the surface hydrogen concentration (Co) and the hydrogen concentration gradient before exposure (a), just after initial exposure (b), and after a uniform hydrogen gradient is established (c).

The hydrogen permeation currents generated using this technique are plotted as transients, a typical example of which is shown in figure 2.6.

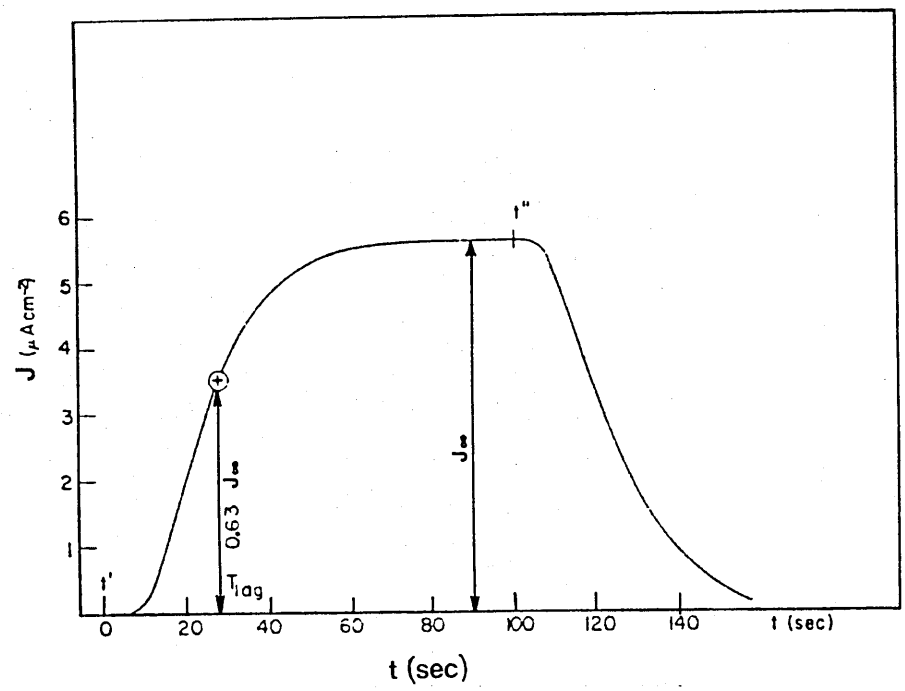


Figure 2.6 A typical permeation transient (Adapted from Devanathan and Stachurski, 1964).

At time 0 in the above transient, a non-corroding metal membrane which has been electrolytically depleted of hydrogen in the manner described previously, and which corresponds to the situation shown in figure 2.5a, is exposed to a corrosive medium (for instance sea water containing H_2S). This results in a rapid increase in the rate of hydrogen absorption, as depicted in the situation shown in figure 2.5b. The hydrogen permeation current subsequently flattens out, such that a uniform

hydrogen concentration gradient is established within the metal, giving a 'steady state' hydrogen permeation current (situation 2.5c). Under such conditions, the maximum surface hydrogen concentration, Co (attained at the hydrogen entry side of the metal) is related to the steady state hydrogen permeation current, J_∞ as follows:

$$J_\infty = F\ D\ Co\ /\ L$$

where F is Faraday's Constant (96480 coulombs/mol hydrogen reduced) and D is the apparent hydrogen diffusion coefficient for the material in use (cm^2/sec). The diffusion coefficient, D is a physical characteristic for the particular metal in use, and can be deduced from permeation transients in a number of ways (Devanathan and Stachurski, 1962). Two simple methods of determining D from transients similar to that in figure 2.6 can be identified. The time taken for nascent atomic hydrogen to diffuse completely through the steel, the 'breakthrough time', t_b, is related to D and the metal thickness, L by:

$$t_b = L^2\ /\ 15.3\ D$$

Alternatively, D can be determined by the 'time lag', T_{lag} method, in which:

$$T_{lag} = L^2\ /\ 6\ D$$

Where T_{lag} corresponds to the time at which the hydrogen permeation current is 0.63 times the steady state value.

A useful facility of the hydrogen permeation technique is that values of D and Co may also be obtained during a decline in permeation current (while hydrogen is being depleted from the metal) after a source of hydrogen, such as a cathodic charging potential, has been removed. This results in a decay transient, as represented by the decline in current following t" in figure 2.6. The value in measuring decay transients is greatest though, when seeking to determine hydrogen concentrations in metals which for a certain duration, have already been exposed to a source of hydrogen (for example that generated during electroplating, or from in-service corrosion or cathodic protection). Moreover, a device based upon the technique described, which is known as the 'Barnacle Electrode', has been developed specifically for this task (Berman et al, 1979). In addition to providing a Co value, by applying Faraday's Law (section 5.4) the device can deduce the total mass of hydrogen extracted from the metal by integrating the decay transient electronically, rather than by the more laborious manual method.

2.4 THE EFFECTS OF HYDROGEN ABSORPTION ON STEEL

2.4.1 Hydrogen Damage

A variety of cracking mechanisms are associated with the absorption and accumulation of hydrogen within steel. When present as an interstitial atom, diffusible hydrogen can lead to what is generally referred to as 'hydrogen embrittlement', which during corrosion and in the presence of stress can promote 'stress corrosion cracking'. In the absence of stress though, various forms of 'hydrogen induced cracking' may occur, owing to the accumulation of 'trapped' (molecular) hydrogen within the metal. As these terms imply, the presence of hydrogen generally tends to make the metal more prone to cracking. The factors which influence the likelihood of hydrogen damage occurring are, however extremely varied, and depend on a host of inter-related physical and chemical factors (material and environmental effects), including the corrosiveness of the environment (including the pH, temperature and the concentration of H_2S and Cl^-) (Treseder, 1977); the level of cathodic protection and the degree of stress applied to the metal, and whether such stress is constant or fluctuating, as in fatigue (Brown, 1971); the nature and distribution of impurities within the metal, and the microstructure, hardness and degree of cold working the metal has received during manufacture (Gooch, 1982).

Although the deleterious effects of hydrogen in metal have been well known in the oil industry for some three decades (Treseder, 1977), and despite the fact that the potential implications of a major structural failure continue to be of great concern in many industrial situations (and the conditions of service make ever increasing demands on materials performance), the exact mechanisms of hydrogen damage are still not fully understood (Oriani, 1987). These factors, combined with the multidisciplinary nature of study into the effects of hydrogen in metals, account for the great breadth, scale, detail and complexity of the subject (for example, Staehle et al, 1977). For convenience, the effects of hydrogen in metals can be considered in terms of microstructure and composition, the nature of the environment, and the possible influences of static or cyclic stress. The following sections shall consider the effects of hydrogen on steel in anaerobic, aqueous (marine) situations containing SRB, or where past SRB activity has resulted in the generation of H_2S (ie 'sour' conditions have become established). Under such conditions, steel can be particularly susceptible to hydrogen damage, owing to both the poisoning effect of sulphide on the hydrogen combination reaction, and the increased generation of hydrogen during either corrosion or cathodic protection.

2.4.2 Hydrogen Induced Cracking

A major determinant of the susceptibility of steel to hydrogen damage is the nature and abundance of impurities within the metal. In terms of hydrogen damage, probably the most important contaminating element in steel is sulphur, which is often combined with manganese (a common alloying element in steels) in the form of discrete inclusions of manganese sulphide (MnS). During rolling of the metal, such MnS inclusions become flattened and elongated, with a consequent increase in surface area.

The generation of cathodic hydrogen at a steel surface (for example due to anaerobic corrosion) results in the diffusion of atomic hydrogen into the metal under a concentration gradient (section 2.3.1). When meeting a discontinuity such as an MnS inclusion though, atomic hydrogen ceases to be 'dissolved' within the metal, and tends to combine to form molecular hydrogen, which cannot diffuse interstitially. Inclusions may thus act as hydrogen 'traps' (grain boundaries and dislocations within the metal may also act in this way), which serve to impede the overall diffusion of hydrogen through the metal (Pumphrey, 1980). The redistribution and accumulation of hydrogen within inclusions may eventually exert an outward pressure sufficient to distort the surrounding metal. In superficial inclusions, the effect of an internal hydrogen

pressure is manifested in 'blistering' at the steel surface. But when located deeper within the metal, the stress exerted by molecular hydrogen becomes concentrated at the sharper extremities of inclusions, the relief of which results in cracking. In the absence of an externally applied stress, the path taken by further such cracking is largely determined by the presence of weaknesses (including other inclusions) within the metal. In this way, the joining of two or more adjacent inclusions, located at slightly different levels, can give an overall effect known as 'step-wise cracking'.

2.4.3 The Control of HIC

Obvious approaches to preventing HIC involve a control of the hydrogen generating process. During corrosion, the rate of cathodic hydrogen discharge can be reduced by controlling the aggressiveness of the external environment; by removing corrosive species, or perhaps by dehydration. In a marine situation though (such as on an offshore oil platform), such an approach is clearly unlikely to be practical. Furthermore, a corrosive agent such as H_2S may be especially difficult to remove once high levels have become established, for instance within a poured oil-bearing reservoir. Alternatively, hydrogen absorption due to corrosion may be prevented by physical or chemical means of separating steel from the

environment, using corrosion inhibitors, or a combination of paints, coatings and wrappings (section 2.2). The complete effectiveness of such measures though, depends on the full coverage of the structure, which may not be maintained, for example due to mechanical damage received during installation, or while in service. Alternatively, the possibility of corrosion occurring may be excluded by the application of cathodic protection, but this has the obvious effect of increasing the rate of hydrogen absorption, especially in the presence of H_2S in its capacity as a cathodic poison.

Because of the difficulties involved in controlling the external environment of a steel structure, it is often more practical (and economic) to prevent HIC by selecting steel materials whose physical properties render it less susceptible to hydrogen damage. To this end, the diffusion rate of hydrogen within steel and its tendency to become absorbed can be reduced by certain additives to the metal, such as copper. Alternatively, the abundance of MnS inclusions which serve as hydrogen traps can be reduced by using higher purity steel. In addition to these measures, it has been found that by adding calcium to the metal, the MnS inclusions produced during manufacture are less pointed, and thus concentrate to a lesser extent, the stresses exerted by trapped hydrogen.

2.4.4 The Effects of Hydrogen Under an Applied Stress

Many of the factors which affect the incidence of HIC are also relevant to the cracking of steel under stress. Furthermore, the effects of stress may be manifested in similar ways, for example where the direction of stress coincides with the orientation of weaknesses such as a line of adjacent MnS inclusions. However, in situations where an element of both stress and corrosion exist, cracking can occur at right angles to the direction of the stress. The initiation of such cracks may be promoted by hydrogen embrittlement or by pitting at the steel surface, and their propagation involves the anodic dissolution of metal at the crack tip, by what is referred to as an 'active path' mechanism (Uhlig and Revie, 1985). The rate of crack growth under a given stress intensity is also influenced by the presence of interstitial hydrogen near to the crack tip, and debate surrounding the relative importance of the active path and hydrogen embrittlement processes accounts for much of the past and present interest in mechanisms of stress corrosion cracking.

The tolerance of steel to the above mentioned material and environmental influences on hydrogen embrittlement can be deduced by measuring the effect of different levels of stress (the stress intensity, K) on the rate of crack growth. Over a range of stress intensities, and for a

particular environment, a threshold value (K_{th}) can be found, below which no cracking is seen to occur. Similarly, using a range of environments exhibiting varying degrees of aggressiveness towards steel, a threshold surface hydrogen content in steel (C_{th}) can be identified (using the methods described in section 2.3.2), below which no cracking is seen to occur (Van Gelder et al, 1986). The two parameters together, C_{th} and K_{th} can give an indication of the susceptibility of steel to cracking for a given application and environment.

In aqueous anaerobic situations at approximately neutral pH (for example offshore, or in a waterlogged soil), perhaps the most important single environmental influence on stress corrosion cracking is the presence and level of H_2S. At gas phase H_2S concentrations as low as 0.01 atm, steel can be be susceptible to cracking (Treseder, 1977), and a similar concentration of H_2S (0.01bar) has been shown to correspond to a C_{th} value of 0.2ppm hydrogen for a pipeline steel (Van Gelder et al, 1986).

The active path element of stress corrosion cracking might be prevented by cathodic protection, but this in itself generates hydrogen and encourages embrittlement. Gooch (1984) reported that there was little evidence of failures resulting from cathodic protection as normally applied (-900mV, SCE), although it was uncertain what effects over-protection, or cathodic protection in the

presence of biogenic H_2S might have upon the level of hydrogen in steel. Lucas and Robinson (1986) demonstrated that in sea water, a cathodic protection potential of -1150mV (SCE) gave a similar surface hydrogen concentration (0.253ppm in steel) to the C_{th} value cited above, using a similar method of determination.

Of equal importance in determining the susceptibility of steel to cracking, is the metal microstructure and hardness. During the manufacture of a particular component or installation, it is possible to control these factors to give a material exhibiting C_{th} and K_{th} values which are compatible with the intended environment. However, an important exception to this can arise from the effects of welding (for instance when joining sections of a pipeline) during which localised heating causes an alteration in hardness and microstructure in an area adjacent to the weld, known as the 'heat affected zone' (HAZ), which by the nature of its microstructure becomes more susceptible to hydrogen embrittlement and cracking (Coe, 1973). Less hydrogen-sensitive microstructures can be established by heating the weld and then allowing it to cool slowly. Even after such 'post weld heat treatment' though, a zone of greater hardness than the surrounding metal may remain, and thus it is important to ensure that the HAZ can tolerate the most aggressive environmental conditions likely to be encountered in service (Gooch, 1984).

2.4.5 Corrosion Fatigue

Hydrogen effects in steel are also implicated in corrosion fatigue, where crack initiation, growth and failure results from the combined effects of metal dissolution, hydrogen embrittlement and a cyclic stress, the latter of which may for instance be applied by the regular wave action on an offshore structure. Similar types of material and environmental factors influence corrosion fatigue as for stress corrosion cracking, but with the important additional effects of a constantly changing stress intensity, and also a varying stress intensity range. For instance, on an offshore structure, the loading on a component may increase as a result of fouling, which has the combined effects of increasing the area of the structure exposed to wave action, while also increasing the drag factor (Edyvean et al, 1986).

The measurement of corrosion fatigue for steel in offshore environments is performed using media which attempt to reproduce a 'worst possible' environment (Edyvean et al, 1986), consisting of H_2S saturated (3140ppm) artificial sea water, and using 'notched' specimens which mimic an initiated crack (crack initiation may effectively be provided by superficial damage to a structure prior to, or during installation). Thomas et al (1988) reviewed the levels of H_2S reported to be present in a variety of

offshore situations exhibiting SRB activity, which in closed systems reached up to 150ppm. In their own investigations, H_2S levels of between 600ppm and 900ppm were obtained in the optimum growth conditions of a laboratory batch culture of SRB, and it was concluded that levels approaching saturation as defined in corrosion fatigue test media were non-representative of conditions observed in the field. Moreover, it was suggested by the same workers that the use of saturated H_2S levels in test media could mask any possible effects of other biological processes, or it might perhaps prevent them occurring at all. For example, a fundamental aspect of microbial corrosion is the inter-species cycling of substrates for growth, energy metabolism and reducing equivalents, without which sustained SRB growth and H_2S evolution would not be possible within a closed or partially closed system such as the interior of a platform leg, or perhaps within a complex macrofouling layer. In relation to the possible merits in using saturated H_2S test media, an interesting feature of the work of Edyvean et al (1986) is that biogenic H_2S levels above 600ppm appeared not to have any additional effect on crack growth rate, further suggesting that test conditions of lesser severity may provide a more representative and useful indication of the 'worst possible' environment.

3 THE SULPHATE-REDUCING BACTERIA AND MICROBIAL CORROSION

3.1 THE SULPHATE-REDUCING BACTERIA

3.1.1 Species Diversity

The term 'SRB' is used to group together bacterial species which utilise sulphate as respiratory terminal electron acceptor, and liberate hydrogen sulphide as the final product of the dissimilatory process (Postgate, 1984). All species are obligately anaerobic, although there is a widespread ability amongst the group to tolerate aeration (Cypionka et al, 1985), and super-oxide dismutase and catalase activity have both been demonstrated (Hardy and Hamilton, 1981). Further means of distinguishing SRB from other anaerobic bacteria are less straight forward however, owing to the great variety of morphology, motility, and metabolism exhibited within the group (Pfennig et al, 1981), as well as the variation in genetic and staining characteristics (Postgate, 1984).

The variety of types of SRB species is reflected by early attempts of classification, according to different types of morphology and motility, using genus names such as Spirillum, Vibrio and Clostridium. The development of selective enrichment culture media in the 1930's facilitated a wider appreciation for the group, and a more rationalised scheme of classification became accepted according to the common ability for dissimilatory respiratory sulphate reduction (which it could be argued

is the most important common activity). This comprised two genera, in which the type species were Desulfovibrio desulfuricans and Clostridium nigrificans (Starkey, 1958), the former of which represented non-sporulating species, and the latter included spore-formers (later to be renamed as the genus Desulfotomaculum). Largely due to the use of novel substrates for growth and respiration, the last few decades has seen a steady growth in the number of SRB species, from which at least nine separate genera have variously been recognised as follows:

Desulfovibrio	(listed by Iverson and Olson, 1982)
Desulfotomaculum	"
Desulfobacter	"
Desulfobulbus	"
Desulfococcus	"
Desulfonema	"
Desulfosarcina	"
Desulfomonas	"
Desulfobacterium	(Brysch et al, 1987).

While the above definition of SRB may seem unambiguous, sulphate is only one of many inorganic and organic electron acceptors available to SRB species. Moreover, a separate genus, the Desulfuromonas, reduces sulphur but not sulphate to sulphide (Pfennig and Biebl, 1977), though in their important capacity to liberate H_2S, they can be included in discussion of SRB activities (Hamilton, 1985).

3.1.2 Dissimilatory Sulphate Reduction

The reduction of sulphur in its most oxidised form (SO_4^{2-}) to its most reduced state (S^{2-}) occurs in a wide variety of bacteria, fungi and many green plants, as a means of assimilating sulphur for the synthesis of amino acids and proteins (Peck, 1974). The dissimilatory process though, is a characteristic shared by the relatively small number of genera listed above. Both processes begin with the activation of sulphate by reaction with ATP in the presence of the enzyme ATP sulfurylase, to form adenosine phosphosulphate (APS) and pyrophosphate (PPi):

$$\mathbf{ATP} + \mathbf{SO_4^{2-}} \longrightarrow \mathbf{APS} + \mathbf{PP_i}$$

The biochemical basis for distinguishing between the dissimilatory and assimilatory processes is that in the latter case, reduction can only proceed after a further phosphorylation of APS, to give phospho-adenosine phosphosulphate (PAPS). The second stage in the dissimilatory process involves the reduction of APS to adenosine monophosphate and sulphite, which is catalysed by the enzyme APS reductase:

$$\mathbf{APS} \longrightarrow \mathbf{AMP} + \mathbf{SO_3^{2-}}$$

Further reduction of the sulphur atom occurs to give sulphide, either as a single step, or with one or more intermediates. Despite work performed by many different

workers over the past two decades, the exact sequence of reactions and intermediate sulphur ions involved in the reduction of sulphite is not yet certain, but Postgate (1984) postulated that intermediates may comprise in order metabisulphite ($S_2O_5^{2-}$), dithionite ($S_2O_4^{2-}$), trithionate ($S_3O_6^{2-}$) and thiosulphate ($S_2O_3^{2-}$). SRB may also utilise alternative forms of sulphur or other ions to sulphate as terminal respiratory electron acceptor, namely thiosulphate, dithionite, trithionate and tetrathionate ($S_4O_6^{2-}$) (Postgate, 1951), and also nitrate (Postgate, 1984). In addition, organic substrates may be used, such as pyruvate and fumarate (Miller and Wakerley, 1966). Bak and Cypionka (1987) isolated a species (which they later named Desulfovibrio sulfodismutans), capable of oxidising and reducing simultaneously thiosulphate and sulphite.

3.1.3 Carbon Metabolism

The most commonly used carbon source in the cultivation of SRB is lactic acid, which is oxidised with the reduction of sulphate to yield sulphide and acetate:

$$2CH_3.CHOH.COO^- + SO_4^{2-} \longrightarrow 2CH_3.COO^- + 2CO_2 + 2H_2O + S^{2-}$$

Cultures are also grown routinely in media containing pyruvate or fumarate, which may serve as combined carbon and energy source as with lactate, but in addition may act

as an alternative terminal electron acceptor to sulphate (fumarate), or may be involved in energy metabolism by substrate level phosphorylation (pyruvate). A great many other carbon sources have been identified though, including methanol and homologues thereof, as well as the equivalent series of carboxylic acids. Using hydrogen as sole energy source, Badziong and Thauer (1978) demonstrated the combined use by Desulfovibrio vulgaris (Marburg) of CO_2 and acetate in what has been termed 'mixotrophic growth' (Postgate, 1984), and the same strain has since been widely used in the demonstration of hydrogen utilisation by SRB, particularly in relation to the study of cathodic depolarisation mechanisms in anaerobic corrosion (section 3.2).

It is adequate in this very brief review to merely give an indication that the recent major growth in number of separate SRB species (mostly attributable to the work of Widdel and Pfennig) is largely attributable to the use of novel carbon sources. An exception to this though, is afforded by the recent isolation of a truly autotrophic SRB, Desulfovibrio autotrophicum, which utilises CO_2, H_2 an SO_4^{2-} (Brysch et al, 1987).

3.1.4 Hydrogen Metabolism

The activation and utilisation of hydrogen by SRB during the reduction of sulphate was first described by Stephenson and Stickland (1931), who assigned the name 'hydrogenase' to the class of enzyme involved. The enzyme is reversible, and can mediate the production or evolution of hydrogen in a very broad range of bacterial species and algae (Mortenson and Chen, 1974). In environments where SRB occur naturally, hydrogen generated during heterotrophic activity may only be available as an energy source in low concentrations, owing to the competition for hydrogen between different species, including methanogenic bacteria (Abram and Nedwell, 1978). However, even in anaerobic microbial consortia (for example containing methanogenic as well as sulphate-reducing activity), the energetics of sulphate reduction with hydrogen are favourable towards the uptake of hydrogen as an energy source (Thauer et al, 1977; Badziong et al, 1978). Most SRB species exhibit hydrogenase activity, which results from the action of up to three differently located enzymes within the same cell (Reider et al, 1984), comprising periplasmic, cytoplasmic, and membrane-bound forms, each of which may be involved in either hydrogen utilisation or evolution (Pankhania, 1988).

Odum and Peck (1981) proposed a hydrogen cycling mechanism for energy coupling, which could account for the ability of SRB cells to consume or evolve hydrogen, and which was consistent with the observations of hydrogen production by Hatchikian et al (1975), and also the observation by Tsuji and Yagi (1981) that early growth in lactate based medium resulted in a short 'hydrogen burst', possibly serving to catalyse and initiate sulphate reduction and substrate level phosphorylation during the oxidation of lactate. Lupton et al (1984) provided evidence that SRB hydrogen metabolism in lactate-sulphate media was necessary for regulating the oxidation state of electron carrier proteins involved in electron transport phosphorylation, according to the model shown in figure 3.0b.

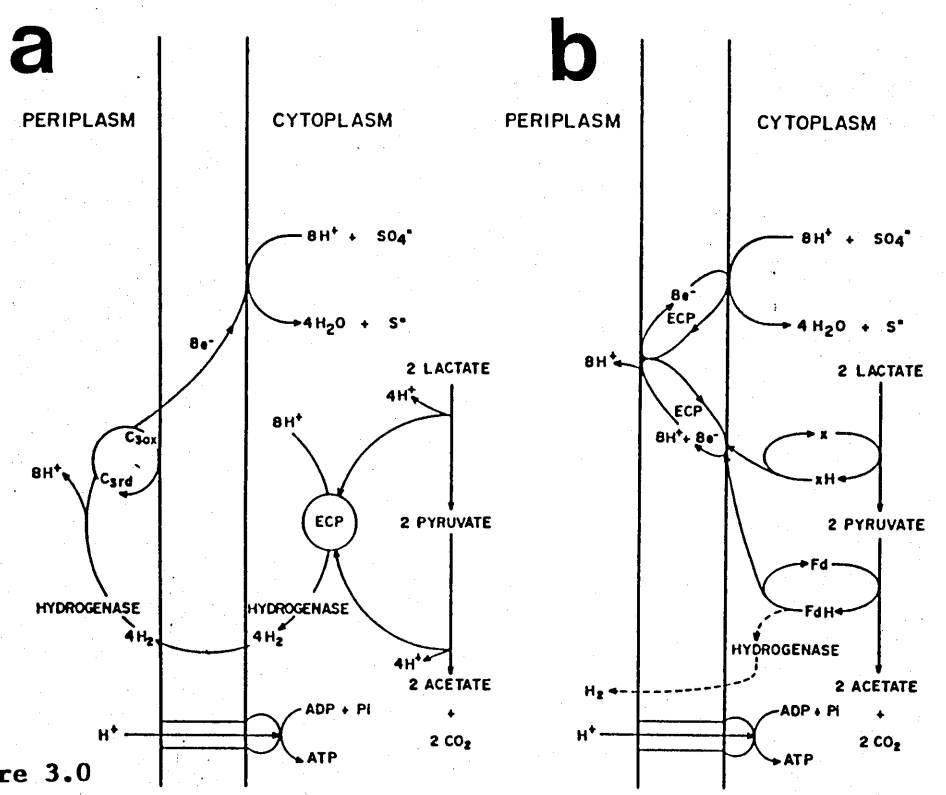


Figure 3.0

. Physiological function of hydrogen metabolism during growth of sulfidogens on lactate and sulfate. (A) Obligate H_2 cycle. Hydrogen is an obligate intermediate in energy metabolism as hydrogen production via the cytoplasmic hydrogenase is coupled to hydrogen consumption by a periplasmic hydrogenase, which generates a proton motive force. (B) Trace H_2 transformation model. A cytoplasmic hydrogenase acts to control the redox level of ferredoxin to regulate vectorial electron and proton transfer which generates a membrane-bound proton motive force. The flow of electrons from ferredoxin to H_2 may be mediated by other electron carriers (e.g., cytochrome c_3) and enzymes (i.e., oxidoreductases) in addition to hydrogenase. ECP, Electron carrier protein; Fd, ferredoxin.

'From Lupton et al, 1984)

The same workers argued that hydrogen metabolism did not occur exclusively according to the hydrogen cycling hypothesis proposed by Odum and Peck (1981) (scheme shown in figure 3.0a), and their results suggested that periplasmic hydrogenase activity played a minor role in the oxidation or reduction of hydrogen during growth on lactate or pyruvate with sulphate as electron acceptor.

3.2 THE ROLE OF SRB IN THE ANAEROBIC CORROSION OF STEEL

3.2.1 The Classical Theory of Cathodic Depolarisation

In the previous chapter it was explained that at a neutral pH and in the absence of oxygen, iron base alloys normally corrode rather slowly, due to the formation of a protective film of cathodic hydrogen. At around the turn of the century though, after only a few years service, many iron pipes which had been buried in waterlogged, neutral soils were found to have suffered appreciable corrosion, which at the time was inexplicable. The possibility that sulphur metabolising bacteria were somehow involved in the corrosion of buried ferrous metals was first suggested by Gaines (1910). The later development of selective and enrichment SRB culture media (Baars, 1930) facilitated the work of Stephenson and Stickland (1931), who first demonstrated and described the hydrogenase-mediated uptake of gaseous hydrogen during sulphate reduction. In light of these developments and the well-known association between SRB and the blackening of anaerobic polder soils, von Wolzogen Kuhr and van der Vlugt (1934) were able to demonstrate the coincidental occurrence of active SRB populations and severe corrosion of cast iron water mains, which despite having being buried only 2-3 years previously, were in need of replacement owing to extensive graphitisation corrosion. The same workers'

theory proposed that during sulphate reduction (reaction 4 below), and by means of the recently discovered hydrogenase enzyme, SRB could accelerate corrosion by removing polarising hydrogen from the corroding iron surface, yielding ferrous hydroxide and iron sulphide as corrosion products. The overall reaction sequence postulated was as follows:

$$4Fe = 4Fe^{2+} + 8e^{-} \quad (1)$$

$$8H_2O = 8H^{+} + 8OH^{-} \quad (2)$$

$$8e^{-} + 8H^{+} = 8H \quad (3)$$

$$SO_4^{2-} + 8H = S^{2-} + 4H_2O \quad (4)$$

$$Fe^{2+} + S^{2-} = FeS \quad (5)$$

$$3Fe^{2+} + 6OH^{-} = 3Fe(OH^{-})_2 \quad (6)$$

$$4Fe + SO_4^{2-} + 4H_2O = 3Fe(OH)_2 + FeS + 2OH^{-} \quad (7)$$

Because of the major economic consequences of SRB activity in terms of corrosion (Chapter 1), and the possibility that the corrosion rate may be controlled by a purely metabolic process (and might therefore be prevented by inhibiting respiration or growth of the culprit organism) there has been a considerable effort by many workers over the past 56 years to test this so-called 'Classical Theory' of cathodic depolarisation. Despite this, there is still no overall consensus on the importance of hydrogen uptake for increased corrosion in the presence of SRB.

3.2.2 Bacterial Depolarisation of the Cathodic Reaction

Early attempts to test the Classical theory were commonly based upon the demonstration of positive shifts in cathodic polarisation curves, using mild steel electrodes exposed to pure batch cultures or cell suspensions of hydrogenase positive SRB. Booth and Tiller (1960) were amongst the first workers to demonstrate depolarisation in this way, using the freshwater species Desulfovibrio desulfuricans (Hildenborough) (figure 3.1a).

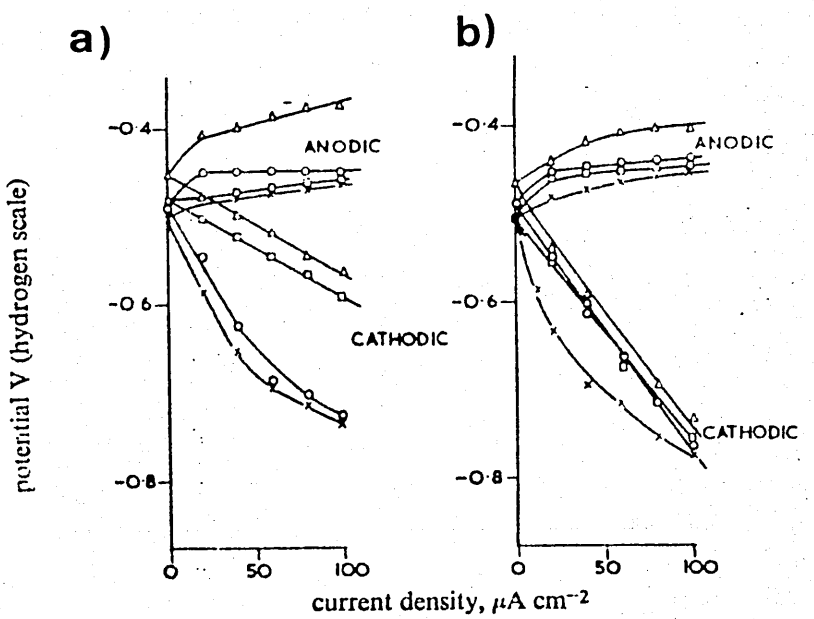
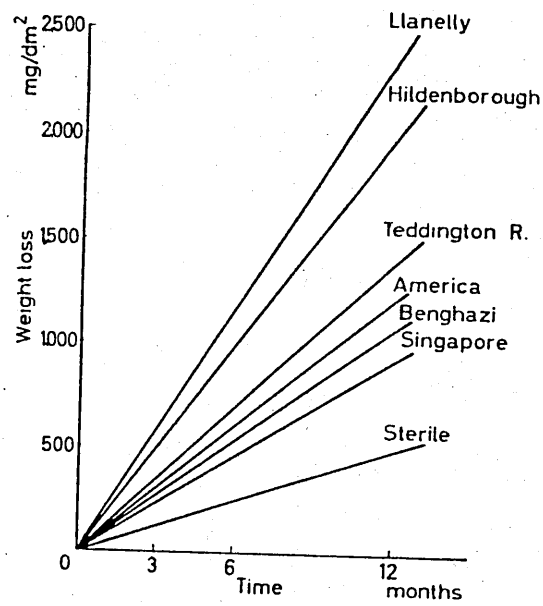


Figure 3.1 a) —Anodic and cathodic polarization curves for mild steel in a culture of *D. desulphuricans* (Hildenborough) at 30°C.
O, initially; ×, after 1 day incubation; □, after 3 days incubation; △, after 6 days incubation.

b) —Anodic and cathodic polarization curves for mild steel in a culture of *D. orientis* (Singapore)at 30°C.
O, initially; ×, after 1 day incubation; □, after 3 days incubation; △, after 6 days incubation.

(From Booth and Tiller, 1960)

Similar changes were not observed using the hydrogenase negative species Desulfovibrio orientis (Singapore) (figure 3.1b), suggesting that cathodic depolarisation was dependent upon the presence of hydrogenase. Seeking to validate these observations using batch cultures of a variety of SRB species (including those mentioned above), Booth and Wormwell (1961) performed a combined study of metal weight loss and hydrogenase activity, and noticed a positive correlation between these factors (figure 3.2).



Hydrogen absorption coefficients of sulphate-reducing bacteria

Organism	$-Q_{H_2}^{SO_4}$
D. desulphuricans	
Llanelly	340
Hildenborough	240
Teddington R	140
America	30
Benghazi	12·5
D. orientis	
Singapore	0
D. desulphuricans var. *aestuarii*	
California 43:63	2·5
El Agheila C	36
El Agheila Z	8·5

Figure 3.2 The relationship between corrosion rate and hydrogenase activity of various strains of SRB (from Booth and Wormwell, 1961).

Using batch cultures of the halophilic (marine) organism Desulfovibrio salexigens (California 43:63), Booth and Tiller (1962) demonstrated a marked depolarisation of the cathodic reaction (figure 3.3), despite recording a

relatively low hydrogenase activity during sulphate reduction. These results were consistent with the observation by Booth and Wormwell (1961), who showed high weight losses using the same organism, but in the presence of minimal hydrogenase activity. In previous work though, a much higher hydrogenase activity had been obtained using benzyl viologen as an alternative electron acceptor to sulphate, and this was interpreted as confirmation of the importance of cathodic hydrogen uptake, and seemed to vindicate the Classical theory.

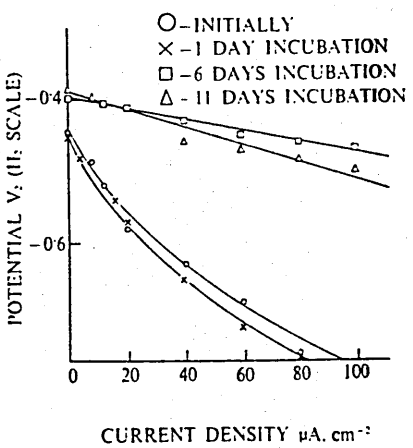


Figure 3.3 Cathodic Polarisation Curves for Mild Steel at 30 C in cultures of Desulfovibrio salexigens (California 43:63), from Booth and Tiller (1962).

In recognition of the possible influences of nutrient limitation on SRB activity, and the possible effects of sulphide on the cathodic reaction (Booth et al, 1968), Booth and Tiller (1968) adopted a different approach for

testing the Classical theory. Using mild steel specimens exposed to resting cell suspensions of both hydrogenase positive and negative SRB, and with benzyl viologen as final electron acceptor, a positive logarithmic relationship was observed between the extra current required to maintain a fixed cathodic potential of steel (compared to the current required in sterile medium) and the hydrogenase activity of the cell suspension.

Earlier work by Iverson (1966), who also employed benzyl viologen as terminal electron acceptor, had also demonstrated increased cathodic activity in the presence of washed SRB cell suspensions. In these experiments, an electrochemical cell was set up between a pair of steel electrodes immersed in two electrically connected agar gels, each impregnated with the redox dye, but only one of which contained SRB cells. After 17 hours exposure in anaerobic conditions, the electrode exposed to SRB cells was found to have become a cathode, as evident from the reduction of the dye, and metal loss was observed only at the sterile electrode (the anode). At the time, this was interpreted as evidence for the Classical mechanism of cathodic depolarisation. However, further consideration of results obtained using redox dyes (Costello, 1974) gave an indication that hydrogen uptake was not necessarily a direct function of SRB activity at the steel surface.

3.2.3 The Limitations of Evidence for the Classical Theory

Possible difficulties in interpreting the polarisation behaviour of steel using redox dyes (Booth and Tiller, 1968) were identified in the work of Costello (1974), who demonstrated that cathodic depolarisation was dependent upon the concentration of simple additions of benzyl viologen in solution (figure 3.4). Moreover, the effect was shown to be significant using a range of molarities (from 0.001mM to 0.5mM) which included the molarity used (0.073mM) in the work of Booth and Tiller (1968).

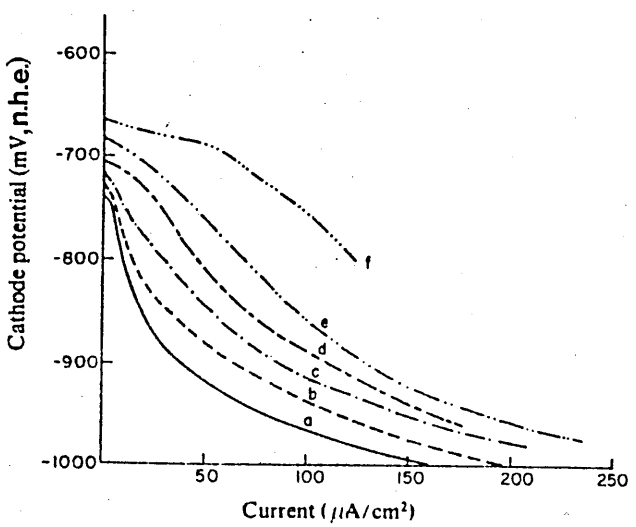


Figure 3.4 Cathodic polarization curves of mild steel in tris buffer at *p*H 7.0, containing benzyl viologen at molarities (a) 0, (b) 10^{-6}, (c) 10^{-5}, (d) 10^{-4}, (e) 2×10^{-4}, and (f) 5×10^{-4}.

(From Costello, 1974):

In addition, Costello suggested that because hydrogenase is a reversible enzyme, the dye could also become oxidised in the presence of SRB cells, and remarked that

oxidation of the reduced dye was the principle of a standard hydrogenase assay technique. Thus it was proposed that benzyl viologen could act as a mediator for transporting hydrogen from the steel surface into the bulk solution by becoming reduced at the steel surface, and then oxidised (liberating hydrogen, and thus being recycled) by SRB cells present within the bulk medium.

In the redox dye impregnated agar experiment mentioned previously, Iverson (1966) noted that the small currents generated by the electrochemical cell were not consistent with the very high equivalent corrosion rates observed in the field. Booth and Wormwell (1961) also noted this anomaly, and their results (from which the plots shown in figure 3.2 were derived), indicated rates of metal weight loss from only 2.1 to 6.6mdd using hydrogenase positive SRB. For comparison, values of metal weight loss observed for steel in industrial service were provided by Costello (1969), who listed examples ranging from 235mdd for a buried steel water main and 443mdd for an oil well steel casing, to up to 855mdd due to the corrosion of ship plates exposed to SRB contaminated bilge water.

A major anomaly in the experiments of both Booth and Wormwell (1961) and Booth and Tiller (1962) was that the most marked cathodic depolarisation, and the highest rates of metal loss were exhibited by the hydrogenase 'negative' SRB strain Desulfovibrio salexigens (California 43:63),

whereas using freshwater organisms, the same workers had claimed that the corrosion rate rose in proportion to hydrogenase activity in sulphate media.

Booth and Tiller (1960 and 1962) made the further important observation that although exposure of steel to SRB culture resulted in cathodic depolarisation, the effect was transitory, and longer term cathodic currents returned towards preinoculation values (figure 3.5b below). It was suggested that this transitory depolarisation was due to nutrient limitation and a consequent decrease in SRB activity (Booth and Wormwell, 1961).

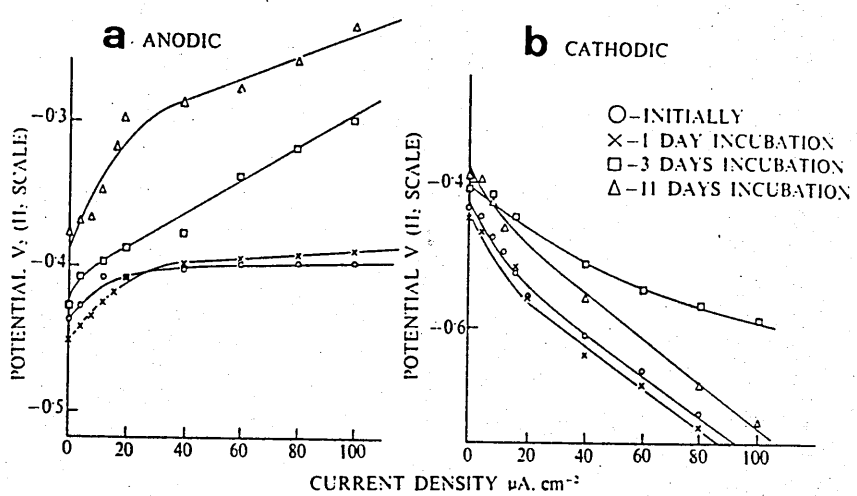


Figure 3.5 *Polarization curves for mild steel at 30°C in cultures of* Desulfovibrio desulfuricans *(strain El Agheila Z_1, a hydrogenase-positive strain), showing the formation of a protective sulphide film, and cathodic depolarization during active growth.*

(from Booth and Tiller, 1962).

In addition to these possible latter effects, Booth and Tiller (1962) noticed that following SRB growth, the anodic reaction often became polarised (figure 3.5a, above).

Such anodic polarisation had previously been described by Wanklyn and Spruit (1952), and was associated with the formation of protective sulphide films on the steel surface. An interesting aspect of the experiments of Booth and Tiller (1962), was that the high corrosion rates obtained using the hydrogenase negative organism (mentioned previously) coincided with the development of an especially non-protective sulphide film compared to the adherent films laid down in the presence of organisms exhibiting high hydrogenase activities. At the time, no connection was made between protective film formation and the low corrosion rates obtained, and rather more emphasis was placed upon the possible effects of reduced SRB activity on cathodic polarisation. In order to help establish the importance of sustained SRB activity, further investigations were conducted using continuous and semi-continuous cultures.

3.2.4 An Alternative Cathodic Depolarisation Mechanism

In the work of Booth et al (1965), mild steel specimens were exposed for up to 60 weeks to cultures of a variety of SRB species, using similar lactate-sulphate media as before (Booth and Tiller, 1960; Booth and Wormwell, 1961), but where each day following initial inoculation, one fifth of the culture volume was replaced with fresh medium. In addition, following each medium replenishment,

any H_2S present was sparged out with nitrogen gas. Using this semi-continuous culture technique, the corrosion rate (metal weight loss) versus time curves obtained were either linear, or consisted of an initial linear phase, followed by a phase of accelerated corrosion. It was noticed that the specimens exhibiting linear corrosion time curves developed thin, adherent sulphide films, but that accelerated corrosion corresponded to film breakdown over much of the steel surface. In cases where film breakdown had occurred, protective films never became re-established, and overall corrosion rates were much higher than those previously obtained in batch SRB cultures by Booth and Wormwell (1961). Compared to the batch culture work though, there was no clear relationship between hydrogenase activity and corrosion rate. Although one freshwater species exhibiting no hydrogenase activity gave the lowest corrosion rate, the second highest metal loss was obtained using the hydrogenase negative marine strain Desulfovibrio salexigens (California 43:63). The latter strain also gave rise to a non-protective sulphide film (thus repeating the anomalous result obtained by Booth and Tiller, 1962; using the same organism). Moreover, the highest corrosion rates were obtained when preventing protective film formation by adding an excess of ferrous ions over dissolved H_2S. Having recognised the varying degrees of protectiveness afforded by sulphide films using different SRB strains, it was suggested that the types of

films established depended upon aspects of the metabolism of the strains of organisms involved.

In order to test any possible metabolic influences of SRB on corrosion, similar semi-continuous culture experiments were conducted using a ferrous ion concentration (12.7mM) sufficient to precipitate as iron sulphide all H_2S evolved during SRB growth (Booth et al, 1966), thus avoiding the formation of protective sulphide films. The corrosion rate versus time curves produced under these conditions were all linear due to the development of loose unprotective films, and the corrosion rates obtained were higher than those obtained in batch culture. However, at this higher Fe^{2+} concentration, metal weight losses were almost constant, irrespective of SRB strain used and the presence of hydrogenase activity. In addition, while the corrosion rates obtained were higher than in the minimal iron batch culture experiments (excess H_2S present), they were well below those obtained in similar minimal iron media using semi-continuous cultures. It was suggested that the reduced corrosion rate was either a consequence of the lowered tendency for iron to dissolve at the higher Fe^{2+} concentration (according to the Nernst equation, section 2.2), or was due to the lack of anodic stimulation by H_2S (shown by Wanklyn and Spruit, 1952). At this stage though, there was no discussion of the possible effects of iron sulphide on the cathodic reaction.

Further work with continuous cultures enabled Booth et al (1967) to observe the effects of sustained SRB growth and constant removal of dissolved H_2S (by media replacement or by reaction with Fe^{2+} at 7.3mM concentration). After twelve weeks' exposure, loose (non-protective) corrosion products were observed on all specimens, and the corrosion rates were the highest recorded so far (up to 221mdd, compared to a maximum of 50.9mdd in the semi-continuous cultures of Booth et al, 1965). The rates of metal loss observed in the field (section 3.2.3) had finally been reproduced experimentally, and it was thought that these corrosion rates depended upon sustained SRB activity.

Using media based upon the fumarate medium developed by Miller and Wakerley (1966), Booth et al (1968) were able to demonstrate cathodic depolarisation during SRB growth in the complete absence of sulphide (figure 3.6a). However, cathodic depolarisation was greater in cultures containing H_2S (using sulphate media), and was most marked when adding sterile sulphide to equivalent levels obtained in SRB cultures (figure 3.6b,overleaf). In addition, the capacity for iron sulphide to enhance corrosion was greatest when able to accumulate on steel surfaces, compared to the lesser effect upon vertically mounted specimens. Thus it became clear that iron sulphide was at least as important as any possible effects of hydrogenase in the cathodic depolarisation mechanism.

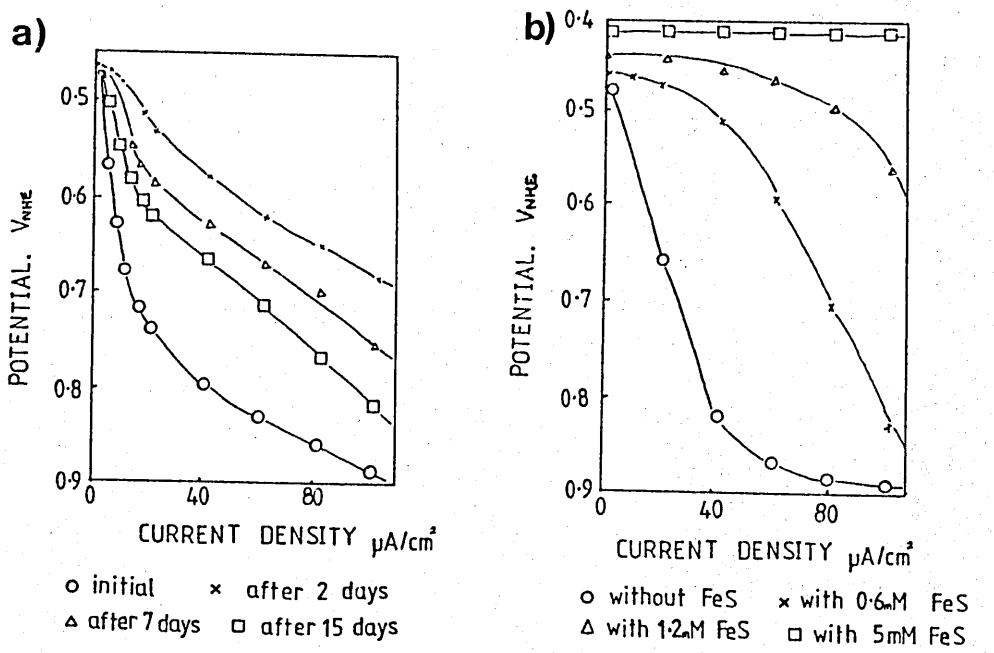


Figure 3.6 Cathodic Polarisation Curves for mild steel at 25 C in a) Semi continuous cultures of Desulfovibrio desulfuricans (Teddington R) in Lactate-Fumarate-Yeast Extract based medium & b) Sterile suspension of FeS in 1% NaCl solution **(From Booth et al, 1968)**

3.2.5 Cathodic Depolarisation by Iron Sulphide Films

King and Miller (1971) were amongst the first to suggest that iron sulphide corrosion products (loose or adherent) could serve as cathodic surfaces for hydrogen evolution at which SRB-mediated hydrogen uptake could take place. The same workers had noticed that the cathodic activity of sulphide films was only temporary (possibly because of the accumulation of adsorbed hydrogen within the material), but that this cathodic activity was restored in the presence of SRB cells.

The work of King and Miller (1971) was followed by a succession of later investigations using combinations of different Fe^{2+} concentrations and additions of sterile sulphide to corroding steel, in order to deduce the relative roles of SRB and sulphide in cathodic depolarisation (King and Wakerley, 1973). Using a sterile system, the latter workers demonstrated a dependence of high sustained corrosion rates on the continuous replenishment of iron sulphide, and also upon the quantity of sulphide present, thus supporting the previous theory that iron sulphide becomes polarised with hydrogen unless somehow regenerated; perhaps by SRB hydrogen uptake.

In further corrosion experiments in SRB culture, King et al (1973a) demonstrated a major increase in metal weight loss following the addition of sulphate (and a consequent evolution of H_2S) to cultures growing in fumarate dismutation medium (figure 3.7a). In addition, it was shown that during SRB growth in sulphate media, a lowering of the soluble Fe^{2+} concentration resulted in much reduced corrosion rates (figure 3.7b), and that increases in corrosion were dependent upon the quantity of iron sulphide produced. No relationship was found between hydrogenase activity and corrosion rate, and thus it was concluded that iron sulphide had a greater influence on corrosion than any direct effects of the SRB themselves.

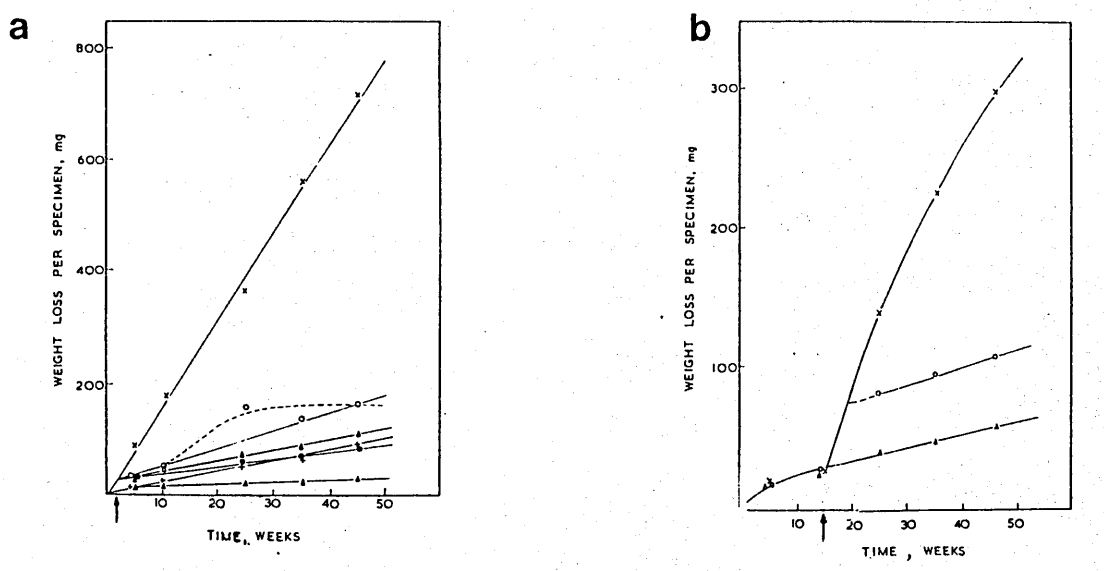


Figure 3.7 **(From King et al, 1973a)**

a) *Corrosion of mild steel in semi-continuous cultures of sulphate-reducing bacteria and the effect of changing from an iron-rich to a minimal-iron medium*

× Strain Teddington R in iron-rich medium
○ Strain Teddington R in iron-rich then minimal iron medium (---alternative plot, if weight loss at 25 weeks is not aberrant)
△ Strain Hildenborough in iron-rich then minimal-iron medium
● Strain Singapore in iron-rich then minimal-iron medium
+ Sterile media: iron-rich then minimal-iron medium
▲ Sterile media with added Na_2S: iron-rich then minimal-iron medium
↑ Point at which the medium was changed (2 weeks after beginning of experiment)

b) *Corrosion of mild steel in cultures of sulphate-reducing bacteria: effect of changing from a fumarate (sulphate-free) medium to a sulphate medium with high or low ferrous ion content*

× Strain Teddington R in LFY medium then high-iron sulphate medium
○ Teddington R in LFY medium then minimal-iron sulphate medium
△ Sterile LFY medium
↑ Point at which the medium was changed (15 weeks after beginning of experiment)

A common characteristic of corrosion experiments performed either in batch culture or using minimal iron media was that on initial exposure to biogenic H_2S, primary films of the corrosion product mackinawite, $FeS_{(1-x)}$ formed on the steel surface. Polarisation and weight loss studies had demonstrated previously the protectiveness of such films (Booth and Tiller, 1960; Booth and Wormwell, 1961), but it was not fully appreciated how, in the presence of high Fe^{2+} concentrations, such primary films broke down and transformed into a more corrosive product. In the polarisation experiments of Mara and Williams (1972a), it

was shown that out of a wide range of different iron sulphides, the most corrosive was the product pyrrhotite, $Fe_{(1-x)}S$ (figure 3.8).

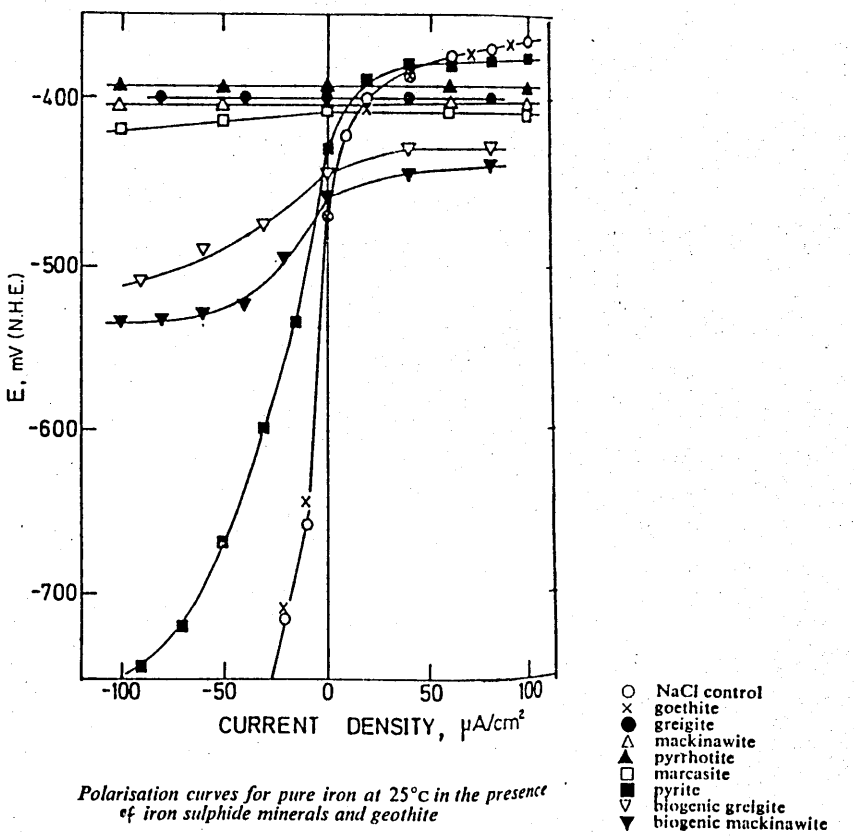


Figure 3.8 *Polarisation curves for pure iron at 25°C in the presence of iron sulphide minerals and geothite*

(From Mara and Williams, 1972a)

It was then suggested that loose, corrosive films of pyrrhotite (and the intermediate products greigite and smythite, (both Fe_3S_4) resulted from the progressive sulphidation of initially protective films of mackinawite and siderite ($FeCO_3$), according to the scheme presented by King and Wakerley (1973) shown in figure 3.9 (overleaf).

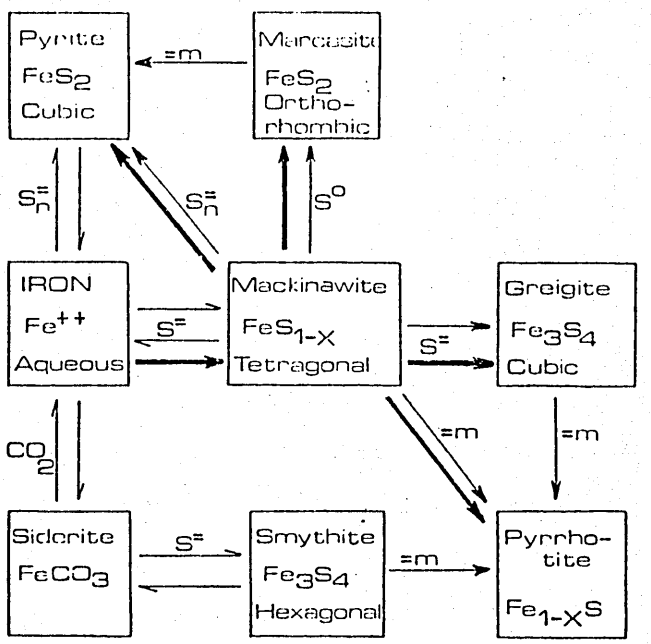


Figure 3.9 *Summary of the major iron sulphide inter-relationships in aqueous solutions and their biogenic counterparts*

Reactions involving ferric ions are not included
(S⁻) Sulphide (Sn⁻) Polysulphide
(S°) Sulphur (-m) Equilibration
→ abiogenic formation
⟶ biogenic formation

(From King and Wakerley, 1973).

From a consideration of crystalline structure in a range of sulphide compounds, Mara and Williams (1972b) postulated that the corrosiveness of iron sulphides rose in proportion to the number of cationic defects present. However, in a rigorous study of the effects of Fe^{2+} concentration on the corrosiveness of iron sulphides in SRB culture, King et al (1976) found that corrosion rates were higher than the latter theory predicted. Moreover, X-ray diffraction analysis of biogenic sulphides showed that primary film breakdown accompanied the

formation of corrosive smythite rather than greigite (as suggested by Mara and Williams, 1972a), and that greigite was in fact a constituent of primary sulphide films (which characteristically formed in minimal iron media, 0.025mM), rather than being present exclusively in the loose, non-protective films which formed at higher concentrations of Fe^{2+} (0.125mM to 1.025mM).

From the various corrosion experiments conducted with iron sulphides (especially those of King and co-workers), it can be concluded that in the short term at least, sulphide effects a more significant cathodic depolarisation than the separate influence of SRB cells. This effect becomes more marked at increased Fe^{2+} concentrations present during SRB growth, due to both the physical breakdown of primary protective sulphide films, and also due to the chemical transformation of films to more corrosive iron sulphide products. However, the capacity for sulphides to act in this way is limited, and the 'Classical' theory may be valid to the extent that sustained cathodic depolarisation appears to be dependent upon the presence of SRB cells.

In this context, several separate or combined roles of SRB cells can be identified. Firstly, sustained cathodic depolarisation may be effected by SRB-mediated hydrogen uptake at the surface of an iron sulphide cathode, rather

than from the bare steel surface, as suggested by King and Miller (1971). Alternatively, the rate of corrosion may be related simply to the rate of iron sulphide production (which in turn is determined by the rate of SRB sulphate reduction and the Fe^{2+} concentration). In addition to these factors though, it has been shown that the physical mixing of sulphide corrosion product brings 'fresh' (non-polarised) sulphide to the cathodic surface of the steel (King and Wakerley, 1973), and it has been proposed that the motile cells themselves may serve to physically turn-over surface iron sulphide material; either acting 'en masse', or perhaps by transporting sulphide particles which become attached to the surface of bacterial cells (Miller and King, 1975).

3.2.6 Recent Evidence for Bacterial Mechanisms of Cathodic Depolarisation.

The 1980's saw something of a resurgence of interest in the Classical theory of cathodic depolarisation, which was largely facilitated by the demonstration and characterisation of SRB growth on CO_2 and acetate, using sulphate and hydrogen as sole energy sources (Badziong *et al*, 1978) as follows:

$$4H_2 + SO_4^{2-} \longrightarrow 2H_2O + 2OH^- + H_2S$$

Using the strain *Desulfovibrio vulgaris* (Marburg), Badziong and Thauer (1978) demonstrated logarithmic SRB growth by maintaining a high sparging rate of a gas mixture containing 80% H_2 and 20% CO_2, and using defined media containing either sulphate or thiosulphate as electron acceptor. By 'training' species to grow with hydrogen (Badziong and Thauer, 1981; Hardy, 1983), similar culture systems have been used to grow a wide variety of SRB species (Badziong and Thauer, 1981; Pankhania, 1988), and this has provided a convenient means of testing directly the depolarisation theory according to the production of H_2S and SRB growth.

Hardy (1983) was the first to report the respiration of SRB using cathodically generated hydrogen as sole energy source, by demonstrating in the presence of corroding steel, the reduction of radio-labelled sulphate ($Na_2{}^{35}SO_4$) to $^{35}S^{2-}$. Polarisation scans taken at the same time indicated that hydrogen utilisation accompanied a small depolarisation of the cathodic reaction, but that the metal gradually re-polarised following H_2S production (as observed in the early work of Booth and Tiller, 1960). Hardy concluded that in view of the small amounts of hydrogen likely to be utilised during growth, the depolarising effects of sulphides were likely to be more significant.

Pankhania et al (1986) attempted to demonstrate cathodic depolarisation during SRB growth using cathodic hydrogen as energy source. The degree of cathodic depolarisation of a single steel specimen exposed to SRB cells appeared to be greater than that for a further specimen exposed to sterile sulphide alone. The value of the sterile sulphide control was however doubtful, in view of the very similar cathodic activity exhibited by a similar control electrode in deaerated media but in the absence of sulphide or SRB cells. It was notable though, that in the same workers' results, sulphate depletion due to respiration and growth did not commence until the steel electrode was polarised continuously to -1400mV (SCE).

Owing to the implications for hydrogen embrittlement (chapter 2), a potential of -1400mV (SCE) could be highly undesirable for metals in engineering use, and in practice such a potential is unlikely ever to be encountered in the field, and especially in a natural aqueous (soil or marine) environment in which high corrosion rates are common. Despite these possible limitations, the demonstration of cathodic hydrogen utilisation during SRB growth has been considered to be of major significance in the elucidation of anaerobic microbial corrosion mechanisms (Pankhania, 1988).

In experiments conducted at the free corrosion potential, Cord-Ruwisch and Widdel (1986) demonstrated that in lactate-sulphate based medium and in the presence of steel wool, growth and respiration of the Marburg strain of Desulfovibrio vulgaris resulted in the evolution of more sulphide than was expected from lactate oxidation alone (equation below).

$$2CH_3\text{-}CHOH\text{-}COO^- + SO_4^{2-} \longrightarrow 2CH_3\text{-}COO^- + H_2S + 2HCO_3^-$$

In addition, the overall increase in sulphate reduction appeared to depend upon the surface area of steel wool added. The same workers also found that cathodic hydrogen utilisation occurred only during lactate oxidation, and that corrosion rates declined as lactate became depleted.

It was not clear however, whether the reduction in corrosion rates (which were low to begin with, and were comparable to those obtained by Booth and Wormwell, 1961 - section 3.2.2), was caused by lactate depletion (and a lower quantity of H_2S evolved), or was due to the formation of a protective sulphide film. Pankhania (1988) considered that this apparent inability to utilise hydrogen after lactate depletion was due to the omission by the former workers to 'train' their culture to take up hydrogen as a source of energy. Reference was made to a previous demonstration (Pankhania et al, 1986) in which following lactate depletion, further growth of Desulfovibrio vulgaris (Hildenborough) was possible by switching to acetate and CO_2 as carbon source, and hydrogen and sulphate as sole energy source, so that hydrogen served to increase total growth yields (no such comparison was made using the same organism as that used by Cord-Ruwisch and Widdel, 1986). However, in contrast to the findings of Cord-Ruwisch and Widdel (1986), Pankhania (1988) found no increase in the growth rate, nor in the rate of respiration; and the failure of hydrogen to act as a supplementary energy source during growth on lactate was found to be consistent with the earlier observations of Khosrovi et al (1971), who grew the same strain of organism in lactate-sulphate media (but lacking CO_2 in the sparge gas), and demonstrated that lactate suppressed the oxidation and utilisation of hydrogen as an energy source.

3.2.7 Other Mechanisms of Cathodic Depolarisation

Costello (1974) performed a series of polarisation experiments using mild steel electrodes exposed to washed cells and cell-free centrifugates from 8 day old batch cultures of a Desulfovibrio species. Cells present in cultures exhibiting cathodic depolarisation also possessed a high hydrogenase activity, but after washing and re-introduction to the steel, the cathodic activity was lost. It was thus concluded that the mechanism proposed in the Classical theory did not operate. Depolarisation was observed though when the experiment was repeated using cell-free centrifugates, but this effect too was lost, after purging with nitrogen. From this, it was suggested that the depolarising species was a gas, and referring to the work of Bolmer (1965), who studied the polarisation characteristics of steel in sulphide buffer solutions, Costello accounted for all of his observations (and cathodic depolarisation by SRB) by the reaction:

$$H_2S + e^- \longrightarrow HS^- + \tfrac{1}{2}H_2$$

In relation to this mechanism, Hamilton (1985) suggested that bacterial hydrogenase activity might stimulate corrosion indirectly, by promoting sulphate respiration and the production of H_2S. However, in comparison with the polarisation data presented by other workers (for

instance, Booth and Tiller, 1962; figure 3.3 in this work) the range of cathodic potentials used to demonstrate cathodic depolarisation by Costello (1974) was probably too low to be of much practical significance, as the effect of cathodic depolarisation was only evident below a potential of -760mV, NHE (-1001mV, SCE).

3.2.8 The Influence of Corrosive Metabolites

In addition to his various demonstrations of cathodic depolarisation using redox dyes (section 3.2.2), Iverson (1968, 1984), and also Iverson and Olson (1984) have reported evidence for the existence of an extracellular corrosion product based on phosphorus. The phosphorus compound is thought to be excreted as a normal byproduct of SRB metabolism, and in the presence of H_2S, becomes rapidly transformed into a more volatile phosphorus-containing substance which may react with a bare steel surface to give a highly corrosive film of iron phosphide. The mechanism largely depends upon the direct exposure of steel to the corrosive compound, and therefore it would be inactive in cases where a protective film of corrosion product (for example, iron sulphide) had already become established. However, since first reporting their so-called 'Corrosive Metabolite' theory (Iverson, 1968), neither of the intermediate phosphorus compounds have been

positively identified. Gas chromatographic analysis has successfully identified phosphine as a product of decomposition of the volatile species, and iron phosphide as the final corrosion product, but the techniques employed do not appear to have facilitated analysis of the other phosphorous species (Iverson and Olson, 1984). Clearly, since the formation of the volatile phosphorus compound depends upon a reaction with dissolved H_2S, the validity of the theory could be tested in the absence of sulphide, using an alternative electron acceptor, such as in the fumarate dismutation medium of Miller and Wakerley (1966).

3.2.9 Anodic Effects of Hydrogen Sulphide

In the polarisation experiments of Booth and Tiller (1960 and 1962; figures 3.1 and 3.5 respectively), it was shown that cathodic depolarisation corresponded to the formation of protective sulphide films, the composition of which was later identified as mackinawite. It is a common feature of such polarisation experiments though, that immediately following initial H_2S production and prior to film formation, the anodic reaction becomes more active, due to a breakdown of oxide films which normally afford the metal with a degree of protection. The effect has been reported in terms of changes in corrosion potential towards more negative (active) values (Wanklyn and Spruit, 1952; Togano

et al, 1975), and also in terms of shifts in the anodic curve which cause an increase in current density at a given potential (Booth and Tiller, 1960 and figure 3.1; Hoar and Farrer, 1961). The electrochemical mechanism of passive film breakdown during the initial exposure of steel to SRB culture was discussed by Salvarezza and Videla (1984). In the absence of protective films though, and in anaerobic conditions, corrosion is likely to be under cathodic control, and it is accepted that the enhancement of corrosion due to SRB activity is mostly a result of cathodic depolarisation.

3.2.10 Effects of Cell Adhesion and Biofilms.

In considering mechanisms of anaerobic corrosion, it is becoming increasingly evident that the influences of SRB activity are inextricably linked to the activities of other groups of organisms which co-exist within microbial consortia, based for instance upon the cycling of carbon and energy sources (for instance sulphur and hydrogen); or which may be structured according to local variations in redox potential (Hamilton, 1985). Such consortia represent a mature stage in the formation and development of biofilms. The processes of cell adhesion and biofilm development are not peculiar to the SRB, and a great variety of bacteria, algae and larger organisms routinely

colonise metal surfaces in aqueous environments. Indeed, the microbial corrosion of steel invariably occurs beneath some kind of biofilm, and therefore the free floating (planktonic) cell suspensions used in experiments described in the previous sections are in many ways non-representative of biological situations which give rise to corrosion (Costerton and Lashen, 1984).

The process of biofilm development on a wetted surface broadly takes place in a number of stages (Characklis and Cooksey, 1983), beginning with the transport of suspended organic particles and microbial cells to the surface, followed by a 'conditioning' of the wetted surface by adsorption of organic particles, the adhesion of microbial cells, and then the commencement of microbial metabolic activity within the nutrient-containing organic film. Cell adhesion is commonly accompanied by the production of an extracellular polymeric substance (EPS), the possible functions of which appear to be varied, but which include the binding together of individual cells into a matrix, which provides the cells with a collective facility for the absorption of nutrients and a physical means of resistance to external physical and chemical stresses (Characklis and Cooksey, 1983). Subsequently, the metabolically active film may be subjected to a range of physical and chemical factors, including changes in temperature, oxygen tension, nutrient availability, and

also mechanical stresses (which may for instance be exerted by the flow of liquid near to the film surface), all of which may cause the periodic detachment (sloughing) of biofilm, resulting for instance, in the exposure of a region of bare metal.

In view of the latter background, a number of significant influences on anaerobic corrosion become apparent, which might not be appreciated if considering experimental systems employing SRB cell suspensions. Although most field situations in which steel is used are not anaerobic, SRB growth and respiration may be possible due to the depletion of oxygen during aerobic microbial metabolism within a biofilm. Infact low redox potentials which may facilitate SRB respiration can become established within a bacterial film thickness of only 12 microns (Costerton and Geesey, 1979). Furthermore, heterotrophic aerobic growth provides the SRB with a ready supply of the cocktail of simple carbon and energy sources on which they thrive, such as acetate and CO_2, hydrogen, lactate, pyruvate and propionate. In view of these factors, the development of visibly thick biofilms is clearly not a prerequisite for SRB growth to become established.

The combination of this apparent facility for SRB activity within biofilms, and the patchiness of surface growth provides a regime well-suited for a mechanism of localised anaerobic corrosion, due to the formation of galvanic couples between anodic areas of bare metal and adjacent areas on which sulphide films develop and act as cathodes. Moreover, White et al (1986) and also Gilbert et al (1987) each reported an enhancement of corrosion in the presence of fouling organisms due to an irregular distribution of biofilm and EPS, rather than due to the specific nature of the organisms themselves. In addition to the possible role of EPS in creating differential concentration cells, it has been suggested that due to its electrical conductivity, the EPS may serve as a sink for free electrons generated during corrosion (Characklis and Cooksey, 1983).

While the relationship between cell adhesion, EPS and microbial corrosion is currently of much interest, there appear to have been few attempts to demonstrate clearly how the influence of dissolved H_2S on steel might differ from identical bulk concentrations and sources of H_2S, but in the complete absence of cells. The simple application of dialysis membranes would appear to satisfy these conditions, acting as an inert, yet permeable screen from molecules above a certain fixed size (for instance, with a mass 'cut-off' at 20 000 daltons). Gaylarde and Johnston

(1980 and 1982) sought to achieve this using mild steel coupons sequestered within visking tubing membranes, which when immersed in active cultures of Desulfovibrio vulgaris suffered a small but significant reduction in weight loss compared to specimens exposed to SRB cells. Using mixed cultures containing Desulfovibrio species and Vibrio alginolyticus or Vibrio anguillarum, metal weight losses were found to be significantly greater than in pure SRB culture (Gaylarde and Johnston, 1982; Gaylarde and Videla, 1987). Moreover, the latter workers demonstrated the marked pitting of steel beneath discrete colonies of the Vibrio organism, which became enhanced in the presence of SRB. However, no such pits formed in the pure SRB cultures, and instead a protective sulphide films became established over the steel surface, suggesting that initially at least, pure SRB cultures had a protective effect. While performing corrosion tests on steel, in view of the possible synergistic influence of the two types of organism, there may be much to be gained by adopting routinely a test regime using mixed, as well as pure cultures.

4. EXPERIMENTAL APPARATUS

4.1 INTRODUCTION

For the study of hydrogen absorption by steel in SRB culture, the need to achieve both sterility and anaerobiosis serves as a major practical constraint on the types of simple and cost efficient apparatus designs available. Important considerations include the additional facilities required for the measurement and control of culture redox potential and pH, as well as the specimen corrosion potential. Also required are facilities for intermittent culture sampling, and a means of delivering culture media and inocula aseptically. However, as a minimum specification for hydrogen permeation measurement, the containment vessel need only provide for the entry of media and inoculum, and accommodate a steel membrane attached to a hydrogen detector. In practice the most convenient, adaptable and cost effective material is glass, which can fulfil the criteria of air tightness, tolerance to high temperatures during sterilisation and resistance to the effects of hydrogen sulphide. Using an open-ended apparatus, a steel membrane can be fastened or clamped to provide a flat surface for the development and interaction of SRB and corrosion product films (fig 4.1).

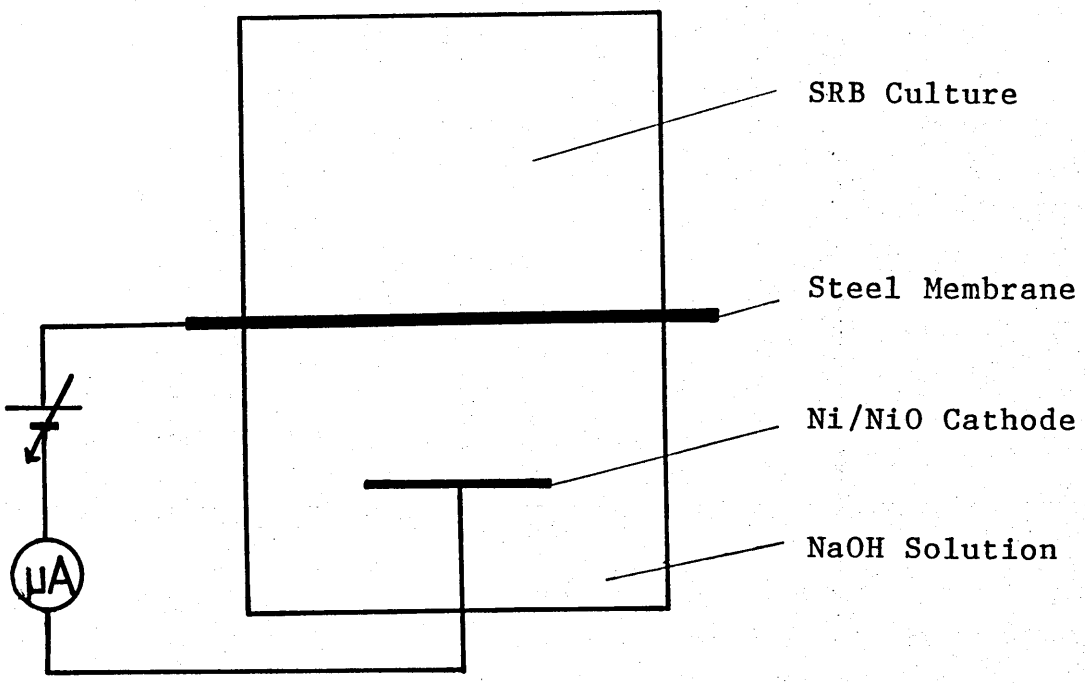


Figure 4.1 Scheme for the measurement of hydrogen permeation through steel exposed to SRB culture

The following sections describe in detail the principal apparatus used (hereafter referred to as 'bioreactors') for the containment of SRB cultures during the analysis of microbially corroding steel membranes. Descriptions also include the various electrodes and monitoring devices attached to bioreactors. Any apparatus associated with separate techniques, and which does not form an integral part of bioreactor design, are explained in the separate 'Experimental Methods' section (chapter 5). The suppliers and manufacturers of materials and equipment used are listed in Appendix 1.

4.2 BIOREACTOR DESIGN

4.2.1 Single Chamber Bioreactor Apparatus

In microbial corrosion studies utilising freshwater SRB, cultures were contained within improvised commercially available laboratory glassware adaptor components ('Quick Fit QVF Systems' [hereafter referred to as 'QuickFit'], and 'Corning Process Systems', Corning Ltd). The first of two experiments using stagnant SRB cultures employed the simple apparatus shown in figure 4.2 below.

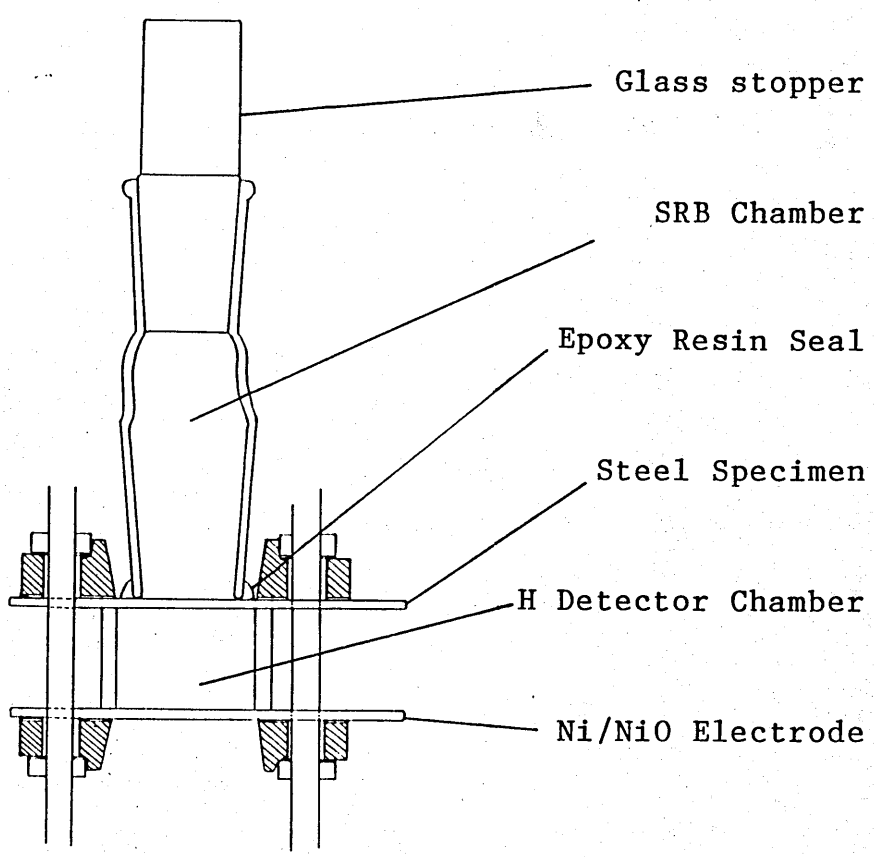


Figure 4.2 Simple hydrogen permeation apparatus for use with unwashed SRB cultures.

A hydrogen detector assembly comprising steel and nickel electrodes and a polypropylene cylinder (25mm bore) was clamped together with an improvised pipeline coupling (Corning Process Systems) insulated with butyl rubber, and fastened with steel studding. The steel sheet and SRB chamber were fastened together with epoxy resin (Araldite 'Rapid', Ciba Geigy), and then sodium hydroxide solution (0.2N) was delivered by syringe through a 1mm hole in the plastic cylinder. Once full, the hole was heat-sealed.

Further studies with freshwater SRB employed a second, larger apparatus design (figure 4.3, and plate 4.1), comprising improvised pipeline adaptor and spacer units (Corning Process Systems), each with ground glass end faces, and providing a total culture volume of 75ml. Sealing rings between glass and metal were cut from either butyl rubber (2mm thick, Corning Ltd) or PTFE sheet (0.4mm thick, Norton Performance Plastics), with a void internal area of approximately $15cm^2$. Sodium hydroxide was delivered by syringe through a 1mm diameter hole in the nickel plate, which when full was given an epoxy resin seal (Araldite 'Rapid' adhesive). Inoculated culture media were delivered under nitrogen (OFN), using a double-vented conical flask with one long glass delivery tube. Autoclavable plastic air vents (Gelman Ltd) were used throughout. All gases and media were delivered through 6.5mm bore, 1.5mm thick butyl rubber ('neoprene') tubing.

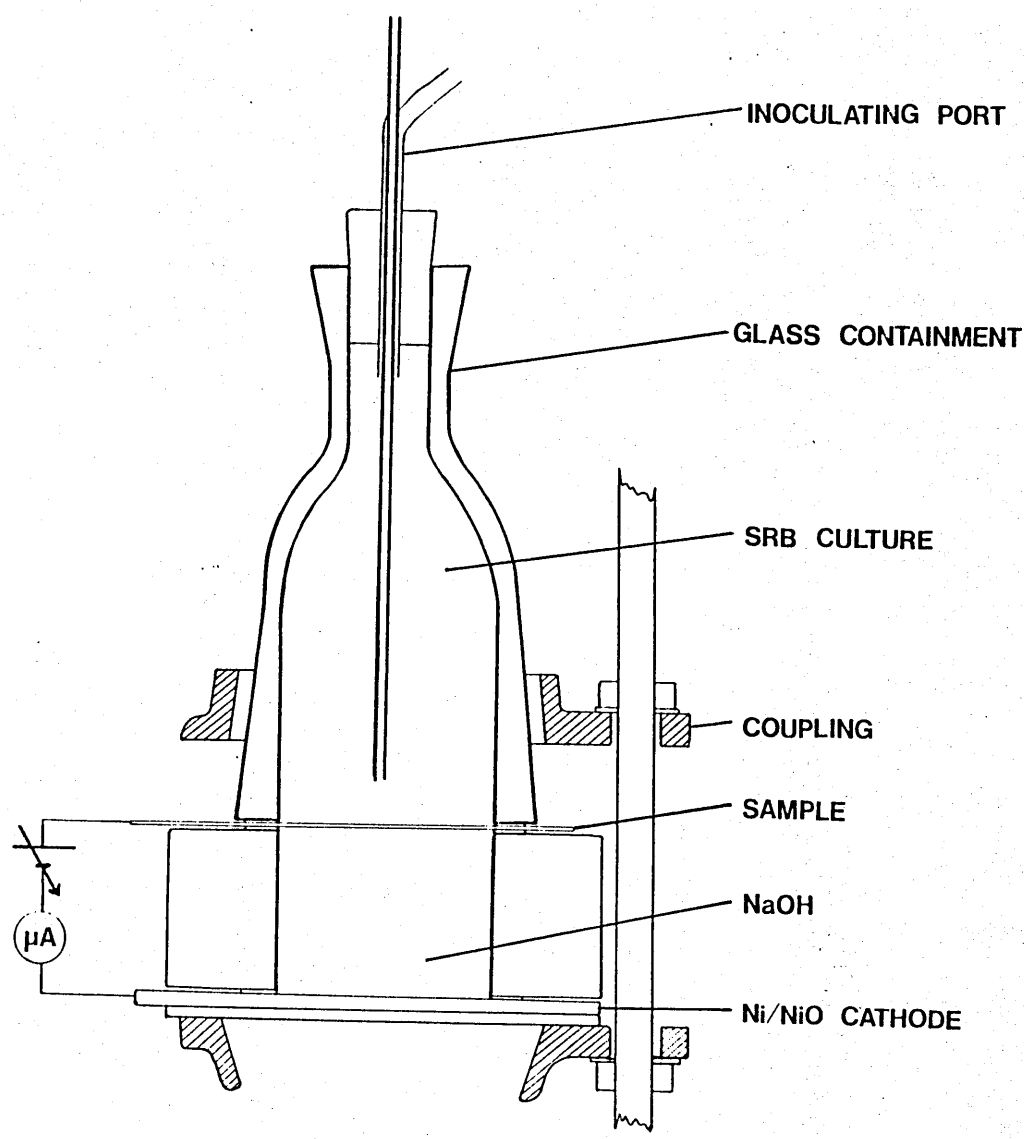
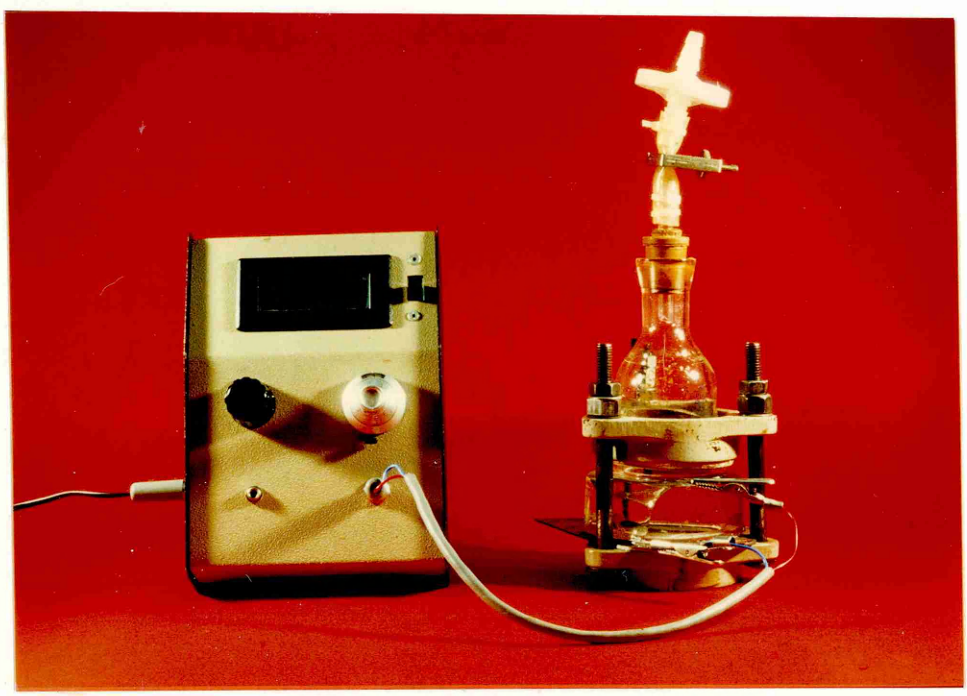


Figure 4.3 Revised single chamber bioreactor design used for hydrogen permeation studies with freshwater SRB (also refer to plate 4.1)

Plate 4.1 Revised single chamber bioreactor apparatus connected to hydrogen permeation detector unit



4.2.2 Double Chamber Bioreactor Apparatus

Corrosion studies involving cultures of the marine SRB Desulfovibrio desulfuricans (Norway-4) were conducted using a double chamber bioreactor, designed specially by the author, and constructed by Wesley Coe [Wingents], Cambridge (figure 4.4 and plate 4.2). This incorporated a variety of commercially available glass laboratory 'Quickfit' apparatus, welded onto sections of worked glass tubing. The apparatus was constructed in four sections, comprising two culture chambers and two hydrogen detectors, each in annealed borosilicate glass. The main culture chambers were shaped and adapted to include a gas head space chamber of approximately 50ml capacity and a recessed flat bed for magnetic stirring. Adjacent sections were coupled together by adjustable wire clips and ground glass flange joints lined with neoprene rubber washers (0.5mm thick x 95.25mm o.d. x 82.5mm i.d., S.J. Gaskets Ltd, Alcester), glued on with silicone sealant (Dow Corning Ltd), such that they became exactly opposed when coupled together. Conical ground glass and screw-threaded joints provided a similar range of facilities in each pair of chambers. Ports for the delivery of hydrogen detector electrolyte, culture media and inocula, consisted of dreschel bottle head units. Electrode or salt bridge ports were provided by cone jointed screw-cap units, with internal bores of

Figure 4.4 Section, side elevation and plan of double chamber bioreactor apparatus drawn to 1/3 scale, showing the arrangement of flange joints, the recessed housings for magnetic stirring, separate gas head space chambers, centrally opposed SRB culture/test medium chambers, end-to-end hydrogen detector chambers, and the screw-cap and ground glass cone type joints.

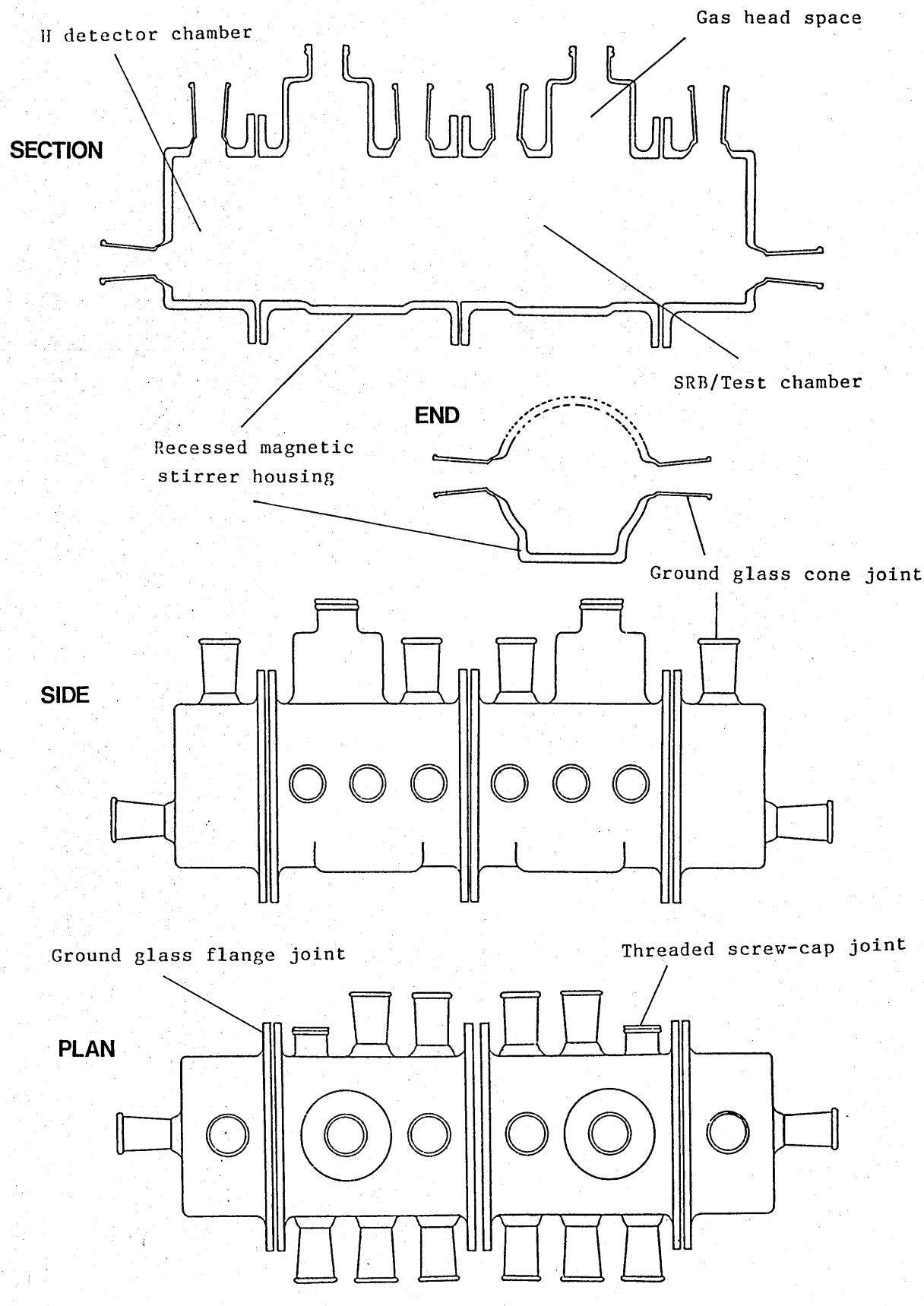
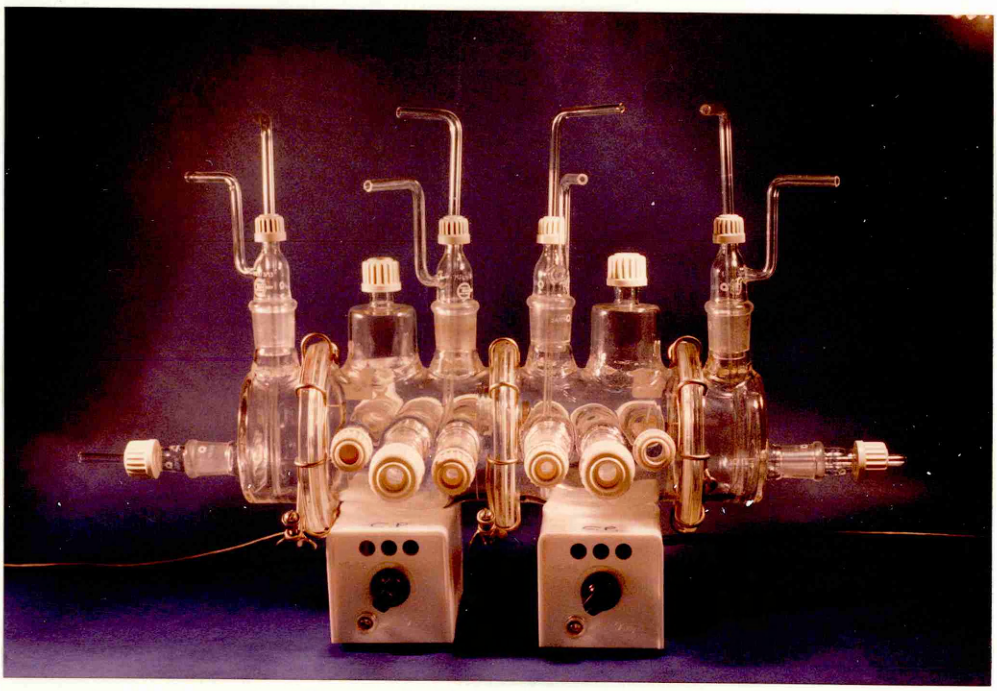

H detector chamber
Gas head space
SECTION
SRB/Test chamber
END
Recessed magnetic
stirrer housing
Ground glass cone joint
SIDE
Ground glass flange joint
Threaded screw-cap joint
PLAN

Plate 4.2 Double chamber bioreactor apparatus

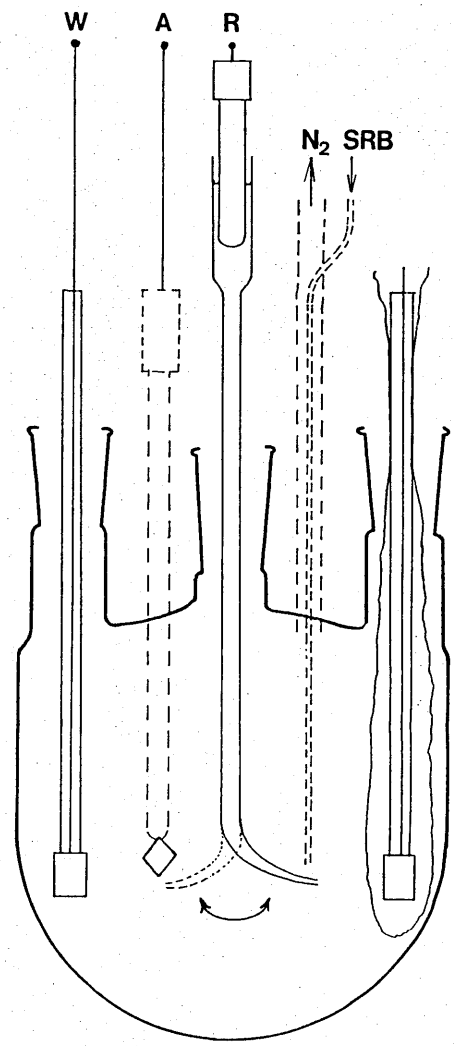


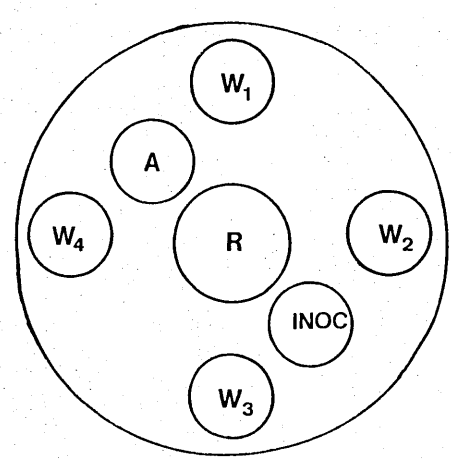
6mm, 8mm and 11mm. Liquid and gas sampling facilities were provided by separate screw-capped ports, each incorporating a pair of three-layer sandwich septa (Hamilton Septa, Greyhound Chromatography Ltd). The remaining screw caps were fitted with flexible silicone rubber inserts and PTFE washers.

4.2.3 Polarisation Cell Apparatus

Studies of the polarisation behaviour of cathodically protected steel employed a spherical, six-limb 'Quickfit' reaction vessel of 1 litre capacity (Figure 4.5). The three central ports were occupied by a rotatable luggin capillary probe, a platinum spade electrode and a combined vent/inoculation port (shown in figure as 'R', 'A' and 'Inoc' respectively). Each of the four remaining ports were occupied by mild steel working electrodes attached to insulated steel studding, and encased in glass tubing (section 4.3.6, "Test specimen support"). Two of the four steel electrodes were also encased in a sleeve of dialysing tubing ('Cuprophan' tubular membrane, Medicell International Ltd). All six electrodes were located and supported by 'QuickFit' cone/screw-thread receiver adapters, each containing flexible silicone rubber inserts and PTFE washers with an internal diameter of 8mm.

Figure 4.5 Polarisation cell apparatus, showing the relative positions of mild steel working electrodes with and without visking tubing sleeve (W1, W2 & W3, W4 resp), reference electrode and rotatable luggin capillary tube (R), platinum auxiliary electrode (A) and a combined vent/inoculation port (Inoc).


W
A
R
N_2
SRB


W_1
A
W_4
R
W_2
INOC
W_3

4.3 ACCESSORY BIOREACTOR APPARATUS

4.3.1 Hydrogen Permeation Detector

The diffusion of cathodic hydrogen through steel membranes was measured in terms of the rate of hydrogen oxidation at the exit surface, and its subsequent reduction at a cathode of anodised nickel. The hydrogen permeation current thus generated was measured using an instrument essentially comprising a sensitive digital ammeter, placed in series with a constant voltage DC supply (plate 4.1).

When in use, a potential of +150mV was applied to the steel specimen with respect to the nickel cathode, using a 9 volt DC supply (battery). Two later versions of the instrument employed a stepped-down mains supply (Sinclair 'ZX', 9V adaptor, Sinclair Corporation), and incorporated a buffer amplifier, giving an output voltage corresponding to the hydrogen permeation current measured. Three current detection ranges were normally used; from 0 to 2.000μA, 0 to 20.00μA and 0 to 200.0μA; each range giving a maximum output voltage of 2.0V. (In practice, a chart recorder sensitivity of 1.0V full scale deflection required the selection of successive current detection ranges beyond current values of 1.000μA, 10.00μA and 100.0μA, according

to the range in use.) Hydrogen permeation studies using single and double chamber bioreactors (section 4.2) correspondingly employed single and double channel chart recorders ('Euroscribe', Gallenkamp Ltd & CR400 Recorder, J.J. Instruments respectively). Data were also acquired manually (section 5.3.1).

Hydrogen detector cathodes were prepared from nickel sheet (Commercial '200 Grade', 99.6% Ni, 1.5mm thick), with a surface area approximately 1/5 the area of steel to be used in the bioreactor. The required size of nickel sheet was sawn off, filed, abraded to a uniform finish (silicon carbide grade 600 paper), and sonically cleaned in acetone. Nickel electrodes were anodised in a solution of 10% sulphuric acid, using a potentiostatically controlled current and variable load output power supply circuit (Greene, Moebus & Baldwin, 1973). An anodising current of 15mA/cm^2 was applied for 30 minutes ('Ministat' Precision Potentiostat, Thompson Electrochem Ltd), having first cleaned the metal by cathodic charging for 1 minute. Anodisation was judged to be successful after the development of a rough but uniform matt grey surface. For use with the double-chamber bioreactor (section 4.2.2), anodised nickel electrodes were assembled as shown in figure 4.6, whereas single chamber bioreactors employed simple sheets of the anodised metal clamped onto the hydrogen detector assembly.

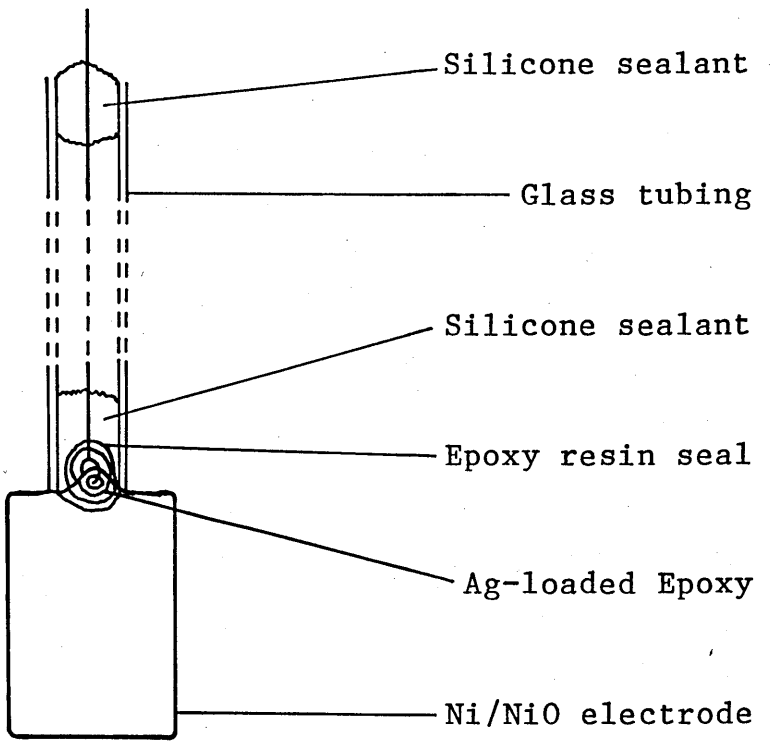


Figure 4.6 Hydrogen detector electrode.

4.3.2 Reference and auxiliary electrodes

Glass calomel reference electrodes (11mm diameter, Russel pH Ltd) were held in specially adapted 'Quickfit' components (figure 4.7, overleaf). The electrode reservoir was filled by drawing up culture media through a 'luggin' capillary tube using a rubber pipette filler. In the later experiments employing cathodically charged steel specimens (section 5.4), the reservoir was filled with saturated potassium chloride solution (285g/l), and luggin probes contained potassium chloride impregnated acrylamide gel, prepared as follows: Using a dreschel bottle (250ml capacity) and nitrogen gas (OFN), a mixture of acrylamide

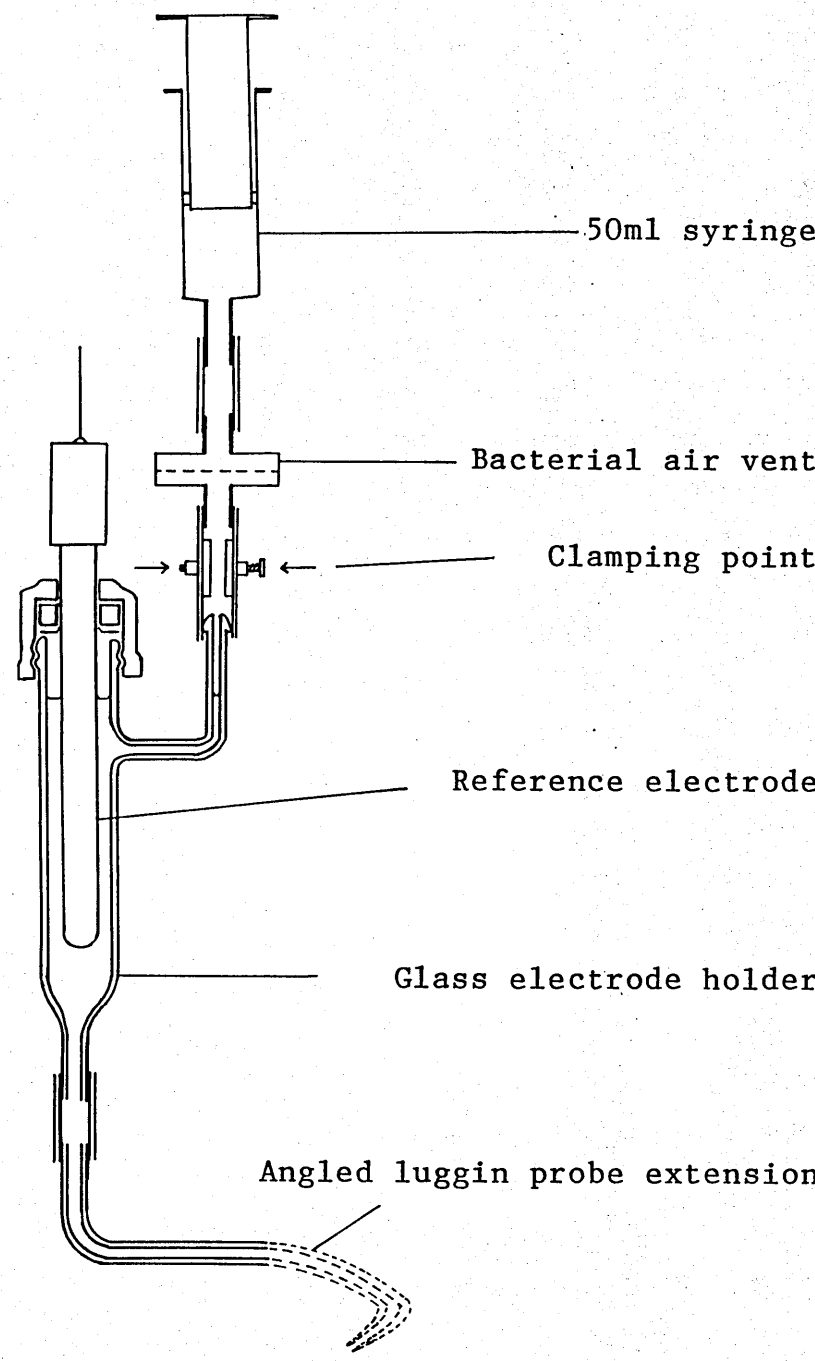


Figure 4.7 Reference electrode housing and luggin probe

(48ml of 30% solution, Sigma corporation), bisacrylamide (2ml of 2% solution, Sigma), distilled water (10ml) and potassium chloride (14 g) was dissolved and deaerated for 30 minutes at 30 C. Freshly prepared ammonium persulphate (0.2ml of 10% solution) and tetramethylethylene diamine catalyst (20µl, Sigma) were added to and mixed with the deaerated solution. With a glass and silicone rubber extension tube attached, the luggin probe was filled with acrylamide solution, and then clamped off and left to harden for 1 hour. The hardened gel was trimmed until just protruding from the pointed end of the luggin probe.

Similar gel-filled glass salt bridges were prepared for use with platinum auxiliary electrodes, except that these employed wider bore tubing (4mm internal diameter each end), and incorporated a flexible central portion (silicone rubber tubing), enabling the bridge to be immersed downwards into a bottle of saturated potassium chloride solution, together with the platinum electrode. Luggin capillary probes and salt bridge tubes were formed by hand, from 6mm diameter soda glass (Gallenkamp).

4.3.3 Stirrers

Experimental cultures and culture media were magnetically stirred throughout (Toyo Kaguku Mini Stirrer, Model MS16B, supplied by ChemLab Instruments Ltd), using non-vortexing

PTFE encased, circular magnetic followers (45mm thick, Gallenkamp Ltd). An insulating layer of expanded polystyrene (1.5cm thick) was applied to the stirring surface, which otherwise tended to heat up. The most reliable stirring performance was found when adjusting for the slowest possible rate of rotation.

4.3.4 Tubing and inoculating ports

Culture media and inocula were delivered through neoprene tubing (6mm or 8mm internal diameter, and 1.5mm or 2mm thick, Gallenkamp). Single chamber bioreactor studies (section 4.2.1) employed a stainless steel tubing and rubber bung arrangement (Figure 4.8). Sterile culture media were introduced into the double chamber bioreactor by a pair of 'QuickFit' dreschel bottle components (ground glass cone fitting size 24mm to 29mm, plate 4.2), and inoculation was performed through rubber septa, using a 50ml syringe. For both sets of apparatus, in order to prevent the wetting of air vents during autoclaving, the neoprene tubing was sealed off by screw clamps. However, to avoid any heat-sealing effects of autoclaving on the clamped tubing, lengths of lightly-greased silicone rubber tubing (20mm x 6mm o.d.) were inserted at clamping points along the neoprene tubing (Figure 4.8, overleaf).

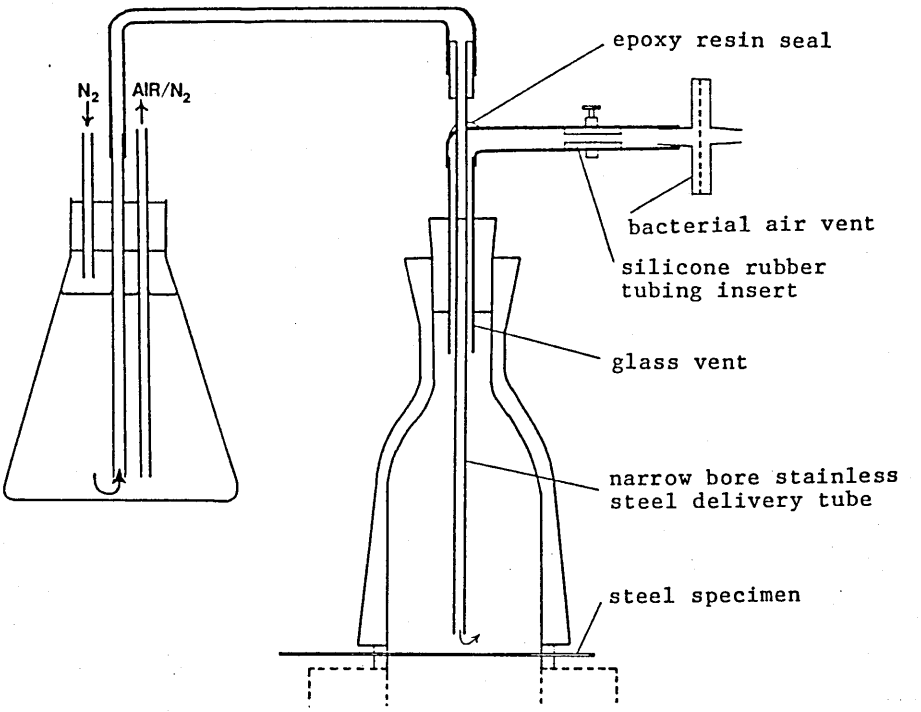


Figure 4.8 Single chamber bioreactor inoculating ports and bacterial air vent clamping arrangement

4.3.5 Test Material Specification

Bioreactor studies using freshwater SRB employed 1.5mm thick mild steel sheet (0.03% Carbon) and shim steel foils with thicknesses of 0.125mm and 0.100mm (0.07% Carbon). Carbon contents were determined accurately by N.E.I. International Combustion Ltd, Derby, using the 'Leco' process. Specimens used in double chamber bioreactor studies were all of 50µm thick (+/- 5µm) 'CS4 Grade Coil' steel, supplied by Knight Strip Metals Ltd, Potters Bar, and containing up to 0.12% carbon, 0.50% manganese, 0.05% phosphorus and 0.05% sulphur.

It was recognised that for a full comparison between hydrogen permeation results obtained under different experimental conditions, test materials with identical composition, microstructure and dimensions would be required. Furthermore, in 'ideal' circumstances, the materials employed would have reflected those used in

industry, such as 50-D steel. However, for specimens of only around 50um thick, the practical difficulties of attaining a fine tolerance of metal thickness precluded the use of such materials. Therefore for the majority of hydrogen permeation tests conducted (Runs J to O), rather than using a constructional steel, a single coil of shim steel served as a useful source of material exhibiting both a uniform composition and thickness. In the results obtained using this material, an allowance was made for the influences of a possible 10% variation in specimen thickness, and the possible irregular abundance and distribution of factors such as hydrogen trapping sites within the metal.

The preliminary single chamber bioreactor experiments conducted with shim steel (Runs C to I) were subject to the same limitations as described above, but with the additional variable of a varying specimen thickness (0.075-0.125mm). In these cases, comparisons were made between the nature and scale of hydrogen permeation results obtained, rather than between absolute values. Similarly, for preliminary experiments using mild steel sheet (Runs A and B), the nature of results obtained was of most significance.

Polarisation studies in SRB culture employed machined cylinders of mild steel measuring approximately 10mm long by 5mm diameter, and each with a central screw thread at one end, machined to accept 8 'BA' steel studding. Although the weight loss measurements conducted with the mild steel

4.3.6 Test specimen support

Flat plates or foils of steel were supported between ground glass flange or coupling joints (section 4.2). For polarisation experiments though, steel cylinders were attached to steel studding, the latter of which was insulated and jacketed with 5mm internal diameter glass tubing (figure 4.9) using a combination of 'Lacomit' sealing compound (Jencons Ltd), PTFE tape and silicone sealant (Dow Corning Ltd).

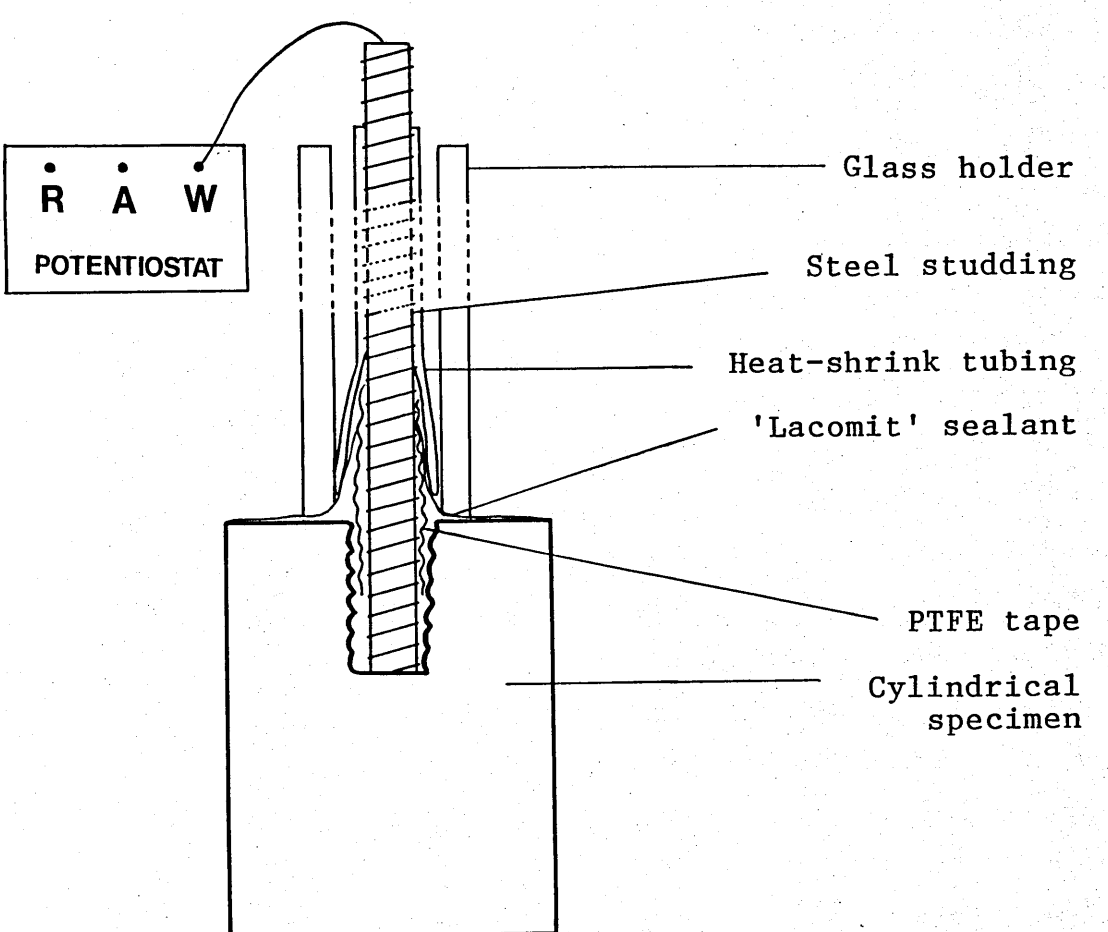


Figure 4.9 Polarisation test specimen arrangement

5. EXPERIMENTAL METHODS

5.1 INTRODUCTION

While the strictly anaerobic habit of SRB places a constraint on the types of apparatus suitable for corrosion experimentation using pure, active cultures, the consequence of sulphate reduction to hydrogen sulphide can serve as an aid to the researcher. By the production of H_2S, SRB activity places a limit on the growth of other contaminant species present. An additional asset is afforded by the tolerance of SRB to aeration, and their continued viablility following dormancy in oxidised media. In practice, these factors can help to ensure the growth and development of pure and active SRB culture, provided that culture media remain sterile and are poised at a suitable redox potential prior to inoculation. It is for these reasons that the following descriptions of SRB handling techniques include little reference to the more sophisticated or time-consuming procedures described elsewhere (Hungate, 1969).

The overall investigation (Chapter 6) was conducted by a series of SRB bioreactor experiments, which in addition to measuring hydrogen absorption, involved the simultaneous monitoring and analysis of the range of physical and biological parameters described below. The detailed specifications for instrumentation specifically associated with bioreactor apparatus are given in Chapter 4.

5.2 MEDIA AND CULTIVATION

5.2.1 Culture Origins

Two SRB species were used. Hydrogen permeation studies conducted with a single chamber bioreactor (section 4.2.1) employed the freshwater organism Desulfovibrio vulgaris (Woolwich), obtained in Postgate's Medium 'B' (Postgate, 1984) from Department of Biological Sciences, City of London Polytechnic, London on 3 May 1985. Studies of hydrogen permeation using a double chamber bioreactor (section 4.2.2) and polarisation experiments (section 4.2.5) employed the marine organism Desulfovibrio desulfuricans (Norway-4), obtained in freeze-dried form (Catalogue No. 8310, National Collections of Industrial and Marine Bacteria Ltd).

5.2.2 Preparation of Culture Media

Stocks of SRB cells to remain viable for at least 6 months were maintained in Postgate's Medium 'B' (Postgate 1984; hereafter referred to as 'PMB', Appendix 2) at 30 C, with the addition of 25g/l NaCl for marine SRB cultures. The ascorbic acid, thioglycollic acid and yeast extract were added last before finally adjusting to pH 8.0 (conc NaOH).

The stirred, precipitated mixture was dispensed carefully to approximately 1.5cm from the top of 20ml screw-capped bottles with butyl rubber inserts (Gallenkamp Ltd; hereafter referred to as 'culture bottles'). After steam sterilisation (121 C/15 minutes), the lids were tightened up, and after cooling to around 30 C, the pH of a single bottle was checked. If above pH 7.8, the whole batch was discarded and a further one prepared. (In practice this was found to be unnecessary, and the fall in pH during sterilisation due to the dissociation of lactic acid dimers could be offset by an initial media adjustment to pH 8.0, giving a final value of pH 7.5 (+/- 0.2) after cooling to 30 C). The greatest degree of success in using PMB was found when minimising the preparation time after adding the reducing agents, and by taking care to ensure a homogenous distribution of the precipitate while dispensing into culture bottles.

For use in bioreactor experiments, SRB were routinely cultivated in Postgate's Medium 'C' (Postgate, 1984; hereafter referred to as 'PMC', Appendix 2), with the addition of 25g/l NaCl for cultures of the marine organism. The sodium tricitrate and yeast extract were added last, before adjusting the complete medium to pH 8.0. Culture bottles were filled, sterilised and then handled in a similar way as described above.

Viable counts were made using test-tubes or plates of Postgate's Medium 'E' (Postgate, 1984; hereafter referred to as 'PME', Appendix 2), prepared as for PMB, except that before adding the reducing agents and yeast extract, the mixture was made up in 500ml distilled water at approximately 50 C. Melted agar (15g/500ml distilled water, steamed at 100 C/5 minutes) and distilled water were added to the stirred mixture, to give a total volume of 1 litre. The remaining components, including 25g/l NaCl for marine SRB, were added and the pH adjusted as above (while compensating for the increased temperature). This method was found to be both more convenient and more successful at supporting SRB growth than the boiling method suggested elsewhere (Postgate, 1984).

Aerobic purity checks were performed on agar plates containing 10g/l glucose, 10g/l peptone and 15g/l agar in distilled water (25g/l NaCl, marine strains). Anaerobe purity was checked using the same media, but dispensed into 20ml test tubes, and containing 0.5g/l $FeSO_4.7H_2O$ for the indication of anaerobiosis by black SRB colony growth.

Chemical supplies used in the above media were as follows: D/L Sodium Lactate (60% Na Salt) and D Glucose, Sigma Chemical Company Ltd; $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, NH_4Cl, $MgCl_2.6H_2O$, KH_2PO_4, Sodium Tricitrate and L Ascorbic Acid (all 'AnalR' grade), British Drug Houses Ltd; $CaCl_2.2H_2O$

('AnalR'), East Anglia Chemicals Ltd; Sodium Tetrathionate (Mercaptoacetic Acid, 95% Na salt) and $CaSO_4$ ('AnalR'), Aldrich Chemical Co.; Agar No.2, Balanced Peptone No.1 and powdered Yeast Extract, Lab M.

5.2.3 Routine Cultivation

Each of the two newly-acquired organisms were subcultured into 20ml bottles of freshly prepared PMB. With great care and using a pasteur pipette, the freeze-dried cells of Desulfovibrio desulfuricans (Norway-4) contained in the ampoule were resuspended in PMB, and then slowly deposited as approximately 1/3 aliquots within the precipitate layer of each of three PMB culture bottles. Subcultures of Desulfovibrio vulgaris (Woolwich) were prepared in a similar way, after resuspending culture and precipitate by gentle swirling, and delivering 1ml aliquots by pasteur pipette into fresh PMB medium. Following incubation (30 C for 4 days), stocks of at least ten 20ml subcultures of each organism were prepared in PMB.

For use in bioreactor experiments, cultures were maintained in PMC medium. Inocula of 1ml were delivered gently beneath the media surface, and caps were tightened straight away prior to incubation at 30 C. Further subcultures were made, typically between 3-4 days of

inoculation, by which time cultures had become highly turbid, and dark olive green in colour. From older cultures, inocula contained a small proportion of the mucilaginous growth which by that time had agglomerated into a single body. (Despite repeated attempts, neither species would grow as successfully while shaken, irrespective of vessel volume, shake amplitude/frequency).

In view of the only temporary effectiveness of the redox poising agents used, successful subculture depended at least partly upon using freshly prepared media. However, when prepared as above and stored in tightly sealed bottles as described, then PMB and PMC media of up to 4 weeks old were sometimes used. The success of subculture into older media was improved considerably by increasing the size of inoculum (up to 3ml), or by the addition of a flamed (Isopropyl alcohol) and heated 1 inch steel nail to the sterile media prior to inoculation (in most cases, rapid growth resulted once a visible build up of growth had developed along the length of the nail).

5.2.4 Inoculum Preparation and Inoculation

In the experiment conducted using the simple bioreactor shown in figure 4.2, a crude unwashed SRB culture was poured aseptically straight into the bioreactor chamber,

and filled to a level just below the base of the stopper when replaced. In the second such experiment, unwashed cells were transferred under OFN, using the inoculation port arrangement shown in figure 4.3. Further experiments employed inocula consisting of washed SRB cells in freshly prepared PMC medium, prepared thus. Approximately three day old cultures were washed by centrifugation at 4000 x g/10mins, and resuspension in sterile phosphate buffer at pH 7.5. Phosphate buffer was added to the pellet by gentle pouring (other, more sophisticated methods of transferring solutions, for example during venting with sterile nitrogen to avoid aeration of the cells, were found to be unnecessary), and culture bottles were filled to within 5mm of the top prior to resuspension by 'Vibromixer'. After the third pellet stage, cells were resuspended in freshly prepared PMC medium.

In single chamber bioreactor experiments employing washed SRB cultures (Runs C,D & E), resuspended cells were poured aseptically into an OFN-vented conical flask containing fresh PMC medium. After gently mixing the washed cells and culture media, the bioreactor was inoculated immediately, using the tubing arrangement shown in figure 4.3.

Washed cells (using saline phosphate buffer, pH 7.5 and 2.5%NaCl) in double chamber bioreactor experiments were delivered by 50ml syringe, through rubber sandwich septa.

5.2.5 SRB Purity Checks

Routine cultures used in bioreactor experiments were streaked onto three glucose/peptone agar plates (section 5.2.3) and incubated aerobically at 30 C, checking daily for contaminant growth. Anaerobe purity was checked using plugged (cotton wool) 20ml test tubes containing molten glucose, peptone and iron agar (section 5.2.2). A separate wire loopful of the test culture was dipped into each of three such tubes, and was mixed by hand rolling. After solidifying in cold water, the agar tubes were incubated at 30 C and checked daily for contaminant growth.

5.2.6 SRB Enumeration

Using stock solutions containing 31.2g/l $NaH_2PO_4.2H_2O$ and 71.7g/l $Na_2HPO_4.12H_2O$ in distilled water (13 volumes to each 87 volumes respectively), serial dilutions of the test culture were prepared in freshly sterilised (121 C/15 minutes) phosphate buffer at pH 7.5 and 30 C, with a maximum SRB culture dilution factor of 10^{-7}. In 1ml aliquots, the serial dilutions were added to separate culture bottles, filled to about 1cm from the top with freshly prepared molten PME medium (section 2.1.2), held at 40 C. The capped bottles were inverted and rolled twice, and the contents poured immediately into sterile

plastic 100mm diameter petri dishes (Sterilin Ltd). One such plate was prepared for sample dilutions 10^{-1} to 10^{-4} cells/ml, and three duplicate plates for dilutions of 10^{-5} to 10^{-7} cells/ml. Immediately after pouring, plates were stacked into a GasPak 150 anaerobic jar (BBL Microbiology Systems), having previously added fresh palladium catalyst, gas generating envelopes and two redox indicator strips. Having activated the three gas generating envelopes (10ml water by syringe), the jar was incubated at 30 C, and from the outside, plates were inspected for the appearance of black colonies. Once visible, colonies were counted for the three similar dilution plates containing between 10 and 100 colonies.

Total SRB cell counts (per ml) were performed using either a Thoma Cell (grid volume, $5 \times 10^{-5} mm^3$; multiplication factor, $2 \times 10^{7)}$, or a conventional counting chamber (volume of $2.5 \times 10^{-3} mm^3$; multiplication factor, 4×10^6), depending upon the approximate cell density. Prior to use, the cell and special cover slip were soaked in alcohol (95% ethanol), and dried in air. When using the Thoma cell, a single drop of bioreactor sample obtained by syringe, was placed on the Thoma cell grid, and the cover slip was applied by pressing firmly around the gridded area using a paper towel. Average counts were deduced from ten diagonal grid squares, and from two separate samples, washing and drying the cell in between.

5.3 ROUTINE ANALYSIS AND MEASUREMENT

5.3.1 Specimen Analysis

Steel specimens were examined immediately after removal from bioreactor apparatus, before and after washing and sonic cleaning, noting the distribution of microbial growth and any areas of bare metal. Cleaned and dried steel surfaces were examined visually for pitting, and for the first of the hydrogen permeation experiments employing mild steel sheet, pit profiles were measured from three traverses of a 'Tallysurf' probe. Metal weight losses were otherwise obtained by weighing to the nearest 0.1 mg.

5.3.2 Hydrogen Permeation Measurement

Total hydrogen permeation current was read off manually or by chart recorder. In general, manual readings were taken every one to three hours for up to two thirds of each day (but with a reduced frequency at night), and where possible, two-hourly values were read off from the traces obtained by chart recorder. A higher recording frequency was maintained just prior to, and for up to 2 hours after the introduction of media and inocula, and also after setting a new specimen potential (experiments involving

cathodically charged foils). Details regarding the operation and design of apparatus for the measurement of hydrogen permeation are given in Chapter 4.

The equilibrium hydrogen permeation current density, J_∞ ($\mu A/cm^2$) was represented by the peak current density attained, and the concentration of hydrogen present within the steel surface, Co (mol H/cm^3) was calculated thus:

$$Co = J_\infty L / F D$$

where F is the Faraday constant (96480 C/mol), L is the specimen thickness (cm) and D is the apparent diffusion coefficient of the steel material (6.3×10^{-7} cm^2/sec).

5.3.3 Determination of Percentage Diffused Hydrogen

The mass of hydrogen (Hdiff) which was discharged prior to diffusion through steel (in grammes, and equivalent to mols atomic H) was determined by integrating hydrogen permeation transients, so that:

$$Hdiff = C / F$$

where C is the total charge (coulombs), and F is Faraday's constant (96480 C/mol). The mass of diffused hydrogen was

then expressed as a percentage of the total mass of hydrogen discharged during corrosion. The mass ratio of iron oxidised to hydrogen reduced is indicated by:

$$Fe + 2H^{+} \longrightarrow Fe^{2+} + 2H$$
$$56g = 2g$$

and therefore the mass of total discharged hydrogen, Htot is deduced from the metal weight loss, WL as follows:

$$Htot = WL \times 2/56$$

The percentage of total discharged hydrogen which diffused through the metal, %Hdiff is then given by:

$$\%Hdiff = Hdiff/Htot \times 100$$

5.3.4 Hydrogen Sulphide Determination

Sulphide present in bioreactor gas samples was analysed by gas chromatography and flame photometry (Pye Unicam PU4500 Chromatograph). Gas flow rates were set by bubble flowmeter at 30ml/min for each of the carrier gas (Oxygen Free Nitrogen, min 99.995%, hereafter referred to as 'OFN'), Hydrogen (min 99.9%) and Air (dry/filtered), all supplied by Special Gases, British Oxygen Corporation Ltd.

Injector, column and detector temperatures were set at 60 C, 60 C and 140 C respectively.

Gas samples were transferred using luer-tipped gas-tight syringes (Greyhound 1700 series, 1ml). The sample volumes required were determined by the magnitude of previous chromatograph peak heights, and syringes were flushed out twice, prior to the analysis of a third sample volume.

The instrument was calibrated using a hydrogen sulphide 'Primary Gas Standard' (Spex Industries Inc; as supplied by Varian Chromatography, USA), produced by an irreversible heat-induced chemical reaction (150 C/15 minutes) within a silicone septum and aluminium crimp-sealed glass bottle, and yeilding a concentration of 10µg/ml (6590ppm) hydrogen sulphide gas in argon.

Standard dilutions were made by delivering volumes of gas by syringe into thoroughly cleaned, dried and nitrogen-purged (OFN) screw-capped and septum-sealed vessels of 1041ml, 44ml, and 31.2ml capacity, giving final H_2S concentrations of 6.3ppm, 75ppm and 421ppm of the gas in nitrogen. Gas concentrations were corrected for successive 0.5ml withdrawals, and converted into peak heights at an equivalent detector gain setting of 1 x 16 and a recorder full scale deflection of 10mV. Peak heights (divisions) and H_2S concentrations (ppm) were plotted logarithmically.

5.3.5 Polarisation Scanning

Polarisation current was determined potentiostatically ('Ministat' Precision Potentiostat, Thompson Electrochem Ltd), using a conventional potentiostatic polarisation circuit (Green et al, 1973). Scanning was performed either manually with voltages measured across a counting resistance of 1000ohms, or automatically (BBC 'Master Series' microcomputer, and Thompson 'Autostat', Thompson Electrochem Ltd).

Scanning was performed at 20mV/minute, from -300mV to +300mV either side of the rest potential. For manual scans, in order to avoid an excessive alteration of the corroding metal surface, anodic scanning ceased when current density exceeded $1.5mA/cm^2$. For each specimen, rest potentials were noted before scanning (one hour after disconnection from cathodic charging) and after scanning (after a 30 minute period of stabilisation).

The results of manual and 'Autostat' scans were presented on logarithmic scales (the results of manually conducted scans were also plotted on linear axes), and where possible, a corrosion rate was determined by intercepting the Tafel region of anodic and cathodic curves, and then reading off the corrosion current along the horizontal axis. (When the anodic Tafel region was less clear,

extrapolations of cathodic curves were intercepted with the rest potential value.) The corrosion current density, I (uA/cm^2) was converted into a rate of metal weight loss (mdd) thus:

$$\mathbf{mdd} = \frac{\mathbf{M\ I\ t}}{\mathbf{F}} \times 100$$

where **M** is the mass of one mole of Fe atoms (56g), **F** is Faraday's Constant (96480 C mol^{-1}), t is the length of one day (86400 seconds), and **x 100** converts dm^2 to cm^2.

5.3.6 Electrode Measurements

Bioreactor pH was monitored either in situ, or from 2ml syringe samples delivered into a thoroughly cleaned and dried 5ml beaker. Using buffer solutions (Russel pH Ltd), pH electrodes were calibrated before and after in situ use, and values were read off as mV and converted using the equation determined from the calibration curve.

Bioreactor redox potentials were measured in situ, using a combination of calomel and platinum spade electrodes. Voltages (mV, SCE) were converted into mV, SHE thus:

$$\mathbf{mV\ (SHE) = mV\ (SCE) + 241\ mV}$$

Corrosion potentials were measured against the same reference electrode as above, and plotted as mV (SCE).

5.4 PREPARATION OF TEST MATERIAL

Prior to use, the mild steel specimens were cut, filed and abraded to a smooth surface finish with grade 1200 silicon carbide paper, rinsed and cleaned under running water, degreased in acetone, dried and weighed to the nearest 0.1 mg (Oertling model R41 balance). Steel foils were treated similarly, but abraded to a grade 600 finish.

To prevent crevice corrosion around the 'O' ring sealing zone of bireactor flanges, foils used in cathodic protection experiments were painted on each side with 'Lacomit' sealing compound (Canning Solvents Ltd). Using a metal template, the precise zone to be covered on both sides of each foil was marked very lightly in pencil, giving internal unpainted areas of $30cm^2$(later experiments avoided such marking out, and instead Lacomit compound was painted around a template). Three coats of the compound were applied in each case in between drying periods of approximately two hours.

Mild steel cylinders used in polarisation experiments were abraded, cleaned and degreased as for steel foils. The exact mounting arrangements used, including means of insulation were described in section 4.3.6.

5.5 CATHODIC CHARGING

The cathodic charging of individual steel foil specimens employed separate 'Ministat' potentiostats. In the constant voltage/variable load mode, steel specimens were maintained at potentials of either -850mV (SCE) or -1000mV (SCE), making periodic checks on potentials by digital voltmeter. Simultaneously charged steel specimens were connected together in series, but employing separate circuits and potentiostats for each of the two chambers in double chamber bioreactor experiments.

5.6 STERILISATION OF APPARATUS

Apparatus used for initial bioreactor experiments (Runs A, B & C, Chapter 6) were sterilised by dry heat (155 C/90 minutes) with steel specimens *in situ*. In successive single chamber bioreactor experiments (Runs D-I), apparatus was steam sterilised (121 C/20 minutes), either with steel foils *in situ*, or added aseptically after flaming in isopropyl alcohol (70% solution).

The two central chambers of the double chamber bioreactor apparatus (figure 4.4) were steam sterilised (121 C/20 minutes). Depending upon the experimental parameters to be measured, various accessory apparatus were sterilised *in situ*, including the central dialysis membrane, reference electrode housing/luggin probe extensions,

magnetic stirring followers, septum-sealed screw caps and ground glass cone joint adaptors for the later addition of electrodes or salt bridges. Vacant cone joints were filled with glass stoppers, and the end openings of cone joint adaptors were sealed with a combination of autoclave tape and aluminium foil. To avoid disrupting the carefully greased neoprene seals, the end flanges of the main bioreactor chambers were covered using specially prepared 'dishes' of pressed aluminium foil, which could be tightly wedged onto the flanges, and held in place by autoclave tape. A second wrapping of aluminium foil was taped over each end. Finally, the apparatus was sterilised within a disposable autoclave bag (Sterilin).

Steel foils and cylinders, glass electrodes and salt bridges were soaked in a 70% solution of isopropyl alcohol for 40 minutes, and then dried gently in warm, sterile air, prior to aseptic insertion into the bioreactor. When used with salt bridges, platinum auxiliary and calomel reference electrodes were not sterilised.

In double chamber bioreactor experiments, the pair of delivery tubes (vented dreschel bottle heads) and the medium flask were steam sterilised as one unit (121 C/20 minutes), and were attached aseptically to the bioreactor apparatus just prior to filling. The clamping arrangement for tubing leading to bacterial air vents has been shown in the previous chapter (figure 4.8).

6. EXPERIMENTAL INVESTIGATION

6.1 INTRODUCTION

Relatively little quantitative information exists in the literature regarding levels of hydrogen absorption by microbially corroding steel. As an initial level of investigation, it was therefore considered necessary to define the magnitude of hydrogen absorption, and to establish a testing regime which enabled accurate measurements of hydrogen flux to be made in an environment containing an active SRB population. Once this had been achieved, more complex analyses could be undertaken; thereby gradually dissecting out and quantifying the individual aspects of SRB activity which have most influence on microbial corrosion and hydrogen absorption. The characterisation of the corrosive SRB environment in terms of physical corrosion processes would then serve as a final thrust to the investigation, and the combination of results generated might provide some additional insight into the role of SRB in hydrogen absorption during the microbial corrosion of steel.

The goals of the investigation can be summarised thus:

1. To define the levels of hydrogen generated within anaerobically corroding steel exposed to an active SRB population, and to deduce the importance of SRB films and the influence of cathodic protection on hydrogen absorption.

2. To characterise the corrosive SRB environment, in terms of biological and chemical parameters, and also in terms of the polarisation behaviour of anaerobically corroding steel; and to relate these processes to changes in hydrogen absorption.

The previous two chapters have described the apparatus and experimental techniques developed for achieving the above goals. The present chapter describes how various combinations of these techniques were employed in a series of experiments, each making up part of an overall investigation. As a simple guide to the different types of experiment conducted, Appendix 3 lists the various test regimes employed and the variables monitored, and Appendix 4 illustrates the various configurations of bioreactor apparatus used in each of Runs J to O.

6.2 HYDROGEN ABSORPTION DURING MICROBIAL CORROSION

6.2.1 Unwashed SRB Cultures

Hydrogen permeation current densities were monitored during Runs A and B for a minimum of one hour prior to, and 232 hours after the continued exposure of steel to crude, unwashed 3 day old cultures of Desulfovibrio vulgaris (Woolwich). The bioreactor apparatus employed in Runs A and B were as shown in figures 4.2 and 4.3 respectively. Following their removal from the bioreactor assembly, the corroded specimens were examined visually, and after cleaning the respective weight losses were determined. SRB samples were observed microscopically before and after Runs A and B, and a combination of initial and final viable and total cell counts were determined from the culture used in Run B.

6.2.2 Washed SRB Cultures

Using the single chamber bioreactor apparatus shown in figure 4.3, hydrogen permeation transients were obtained in Runs C, D and E for shim steel foils corroding in the presence of a suspension of washed cells from a 3 day old batch culture of Desulfovibrio vulgaris (Woolwich) in

freshly prepared PMC medium. The shim steel test specimens used ranged in thickness from 0.100mm to 0.125mm (Appendix 3). Also, a range of specimen and apparatus sterilisation methods were employed (section 5.6). Following sterilisation, washed cell suspensions were delivered into the bioreactor under nitrogen, and initial cell densities were determined from total cell counts of the unused inoculum. Final cell densities were also determined, and samples of surface SRB films were examined microscopically. Steel specimen weight losses were deduced for each specimen, and the nature and distribution of any corrosion products or apparently corroded areas of metal were examined visually.

6.2.3 The Effect of Test Media on Hydrogen Absorption

A series of four experiments was conducted to deduce the influence of sterile media on hydrogen absorption by steel. Using the same single chamber bioreactor apparatus as in 6.2.2 above, hydrogen permeation transients were obtained for shim steel foils exposed to sterile PMC medium (Runs F & G), sterile PMC medium lacking yeast extract (Run H) and distilled water (Run I). Before and after each test, media were examined for contaminant organisms, and the surfaces of corroded specimens were examined after removal from the apparatus.

6.3 THE INFLUENCE OF SRB FILMS ON HYDROGEN ABSORPTION

6.3.1 Investigations Using the Double Chamber Bioreactor

The apparatus shown in figure 4.4 was set up in a variety of conformations, according to the parameters measured in each separate experimental trial. A generalised representation of the different arrangements of apparatus is shown in figure 6.1 below, but more detailed illustrations of the specific arrangements used in each individual experiment are given in Appendix 4.

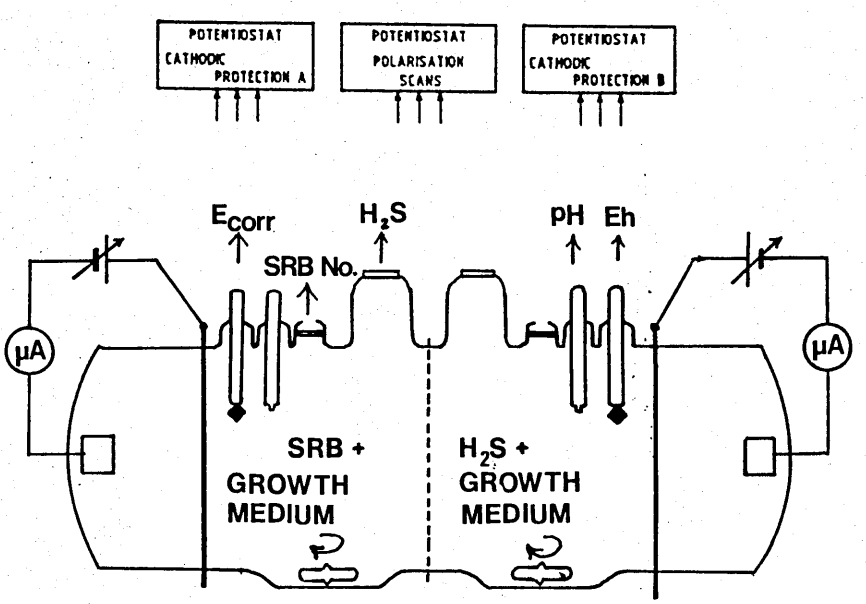


Figure 6.1 Generalised schematic arrangement of double chamber bioreactor for the application of cathodic protection to steel foils, and the measurement of hydrogen permeation current, corrosion potential and polarisation behaviour of steel, in addition to the bulk measurement of redox potential (Eh), pH, gas head space H_2S concentration and SRB numbers.

6.3.2 Test of System Reproducibility

A preliminary investigation was made to gain a measure of the inherent variability in the hydrogen permeation technique when using identical SRB culture conditions. Using the double chamber bioreactor apparatus (figure 4.4), set up according to the arrangement shown in Appendix 4a, two similar steel foils cut from adjacent areas of metal stock were exposed to the same triple-washed batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC medium (containing 2.5% NaCl), for a period of up to 250 hours, following an initial exposure of steel to culture media alone for 170 hours. Hydrogen permeation currents were monitored intermittently for each of the two steel specimens, and the change in SRB cell density was determined from samples just following inoculation, and then after dismantling of the apparatus. Weight losses were deduced for each specimen from measurements taken before and after the experiment.

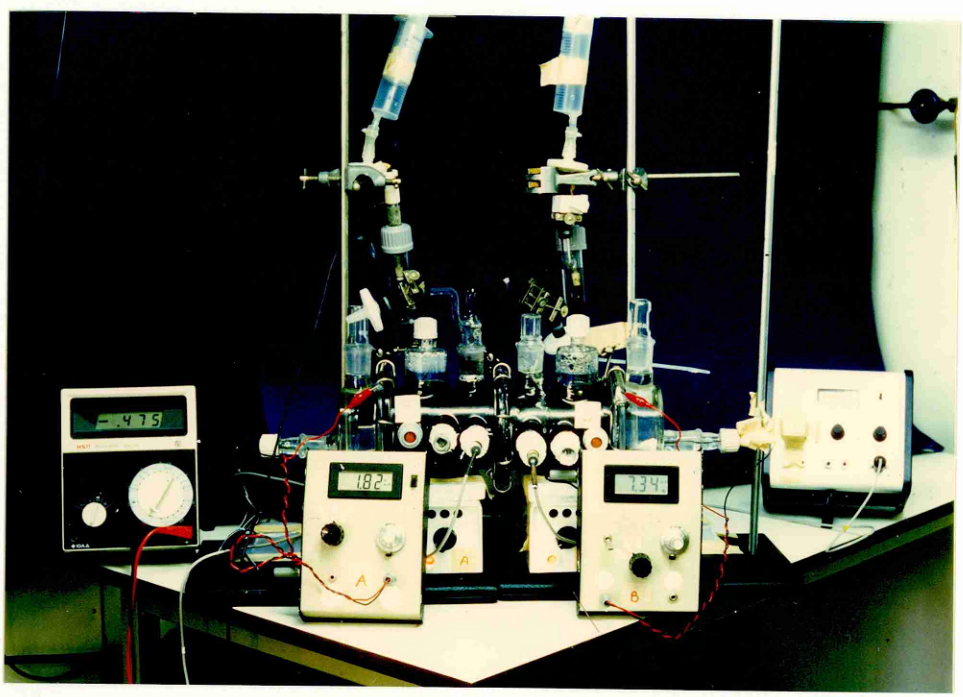
6.3.3 The Influence of SRB Films on Hydrogen Absorption

Using similar culture conditions and media to those used in Run J, two separate experiments (Runs K and L) were conducted to investigate the relative influences of surface SRB films and the dissolved metabolites resulting

from SRB growth on hydrogen absorption by freely corroding steel. Transients were obtained for a period of 370 hours and 270 hours (Runs K and L), and the electrode and sampling port configurations used were as shown in Appendix 4 b and c. The apparatus used in these experiments is also shown during operation, in plate 6.1.

In addition to measuring fluctuations in hydrogen permeation current, transients were obtained which showed changes in the corrosion potential (Ecorr) of foil specimens, the redox potential (Eh) and pH of the culture medium, and changes in gas head space H_2S concentrations. The latter measurements were taken simultaneously for both bioreactor chambers (on each side of the dialysis membrane), except in Run K, in which pH changes were monitored only for the chamber containing SRB cells. Also in Run K, a total of eight viable SRB counts were taken over a period of 175 hours following inoculation of culture media. Weight losses were determined for each of the four specimens in Runs K and L.

Plate 6.1 Double-chamber bioreactor apparatus in operation during hydrogen permeation test Run K.


-.475
18.2
7.34
A
A
B

6.4 THE EFFECT OF SRB FILMS ON HYDROGEN ABSORPTION BY CATHODICALLY POLARISED STEEL

Using similar culture conditions and inocula to those used in Runs K and L (section 6.3.3), two similar experiments were conducted to investigate the relative influence of SRB films and the dissolved products of SRB growth on hydrogen absorption by cathodically protected steel foils (-850mV SCE). In a further experiment (Run O) steel foils were charged to a potential of -1000mV (SCE).

Hydrogen permeation transients were obtained for each of Runs M, N and O, which ran for durations of 404, 324 and 310 hours respectively (due to the discovery of a fault which occurred in the hydrogen detector apparatus, data obtained in Run O were plotted up to 238 hours). Also, in Run N the hydrogen permeation experiment ran concurrently with a determination of the polarisation behaviour of cathodically charged mild steel specimens (section 6.5). The pH of media from which SRB were excluded was monitored in each of Runs M and N. Culture redox potential was monitored in both chambers during Run N; and during Run M, SRB cell densities were determined from three viable counts; made at 2 hours, 20 hours and 65 hours following inoculation. The bioreactor port configurations used for the measurement of the various parameters in Runs M, N and O are as shown Appendix 4d-f.

6.5 INFLUENCES OF THE SRB ON THE POLARISATION OF STEEL

Two separate experiments together constituted an investigation into the influence of SRB films on the polarisation behaviour of steel maintained at a potential of -850mV (SCE).

The first experiment utilised the same SRB culture conditions as were present in double-chamber bioreactor Run N (section 6.4 above), where two pairs of mild steel specimens (section 4.3.6), were electrically connected to the cathodically polarised steel foils which formed part of the hydrogen permeation investigation. Three potentiostatic polarisation scans were performed for each of a single pair of specimens (one on each side of the dialysis membrane), several hours prior to inoculation, and then 96 hours and 236 hours after SRB were added. At approximately 296 hours following inoculation and just before termination of the experiment, scans were also performed using the 'spare' pair of specimens (one on each side of the dialysis membrane), which had not previously been scanned. Using this order of scanning, any possible adverse effects of anodic or cathodic scanning potentials on the properties of surface films on steel would be evident from comparison with scans obtained from the pair of specimens left unscanned until the end of the experiment.

A second such experiment was conducted within a standard commercially available corrosion test vessel (section 4.2.3 and figure 4.5), where similar pairs of test specimens to those used above were suspended in a culture of Desulfovibrio desulfuricans (Norway-4) in PMC medium, and were connected together and polarised to -850 mV (SCE) from a single potentiostat. The screening of two of the specimens was achieved using visking dialysis tubing, and potentiostatic scans were performed on one pair of specimens (one of which was enclosed by visking tubing) 2 hours prior to inoculation, and then 24 hours and approximately 360 hours after the introduction of SRB. A similar 'spare' pair of specimens to those used in the experiment described above was also scanned after approximately 360 hours.

For each polarisation experiment, specimen potential was plotted against corrosion current, from which changes in anodic and cathodic behaviour were interpreted. Where possible, corrosion rates were deduced from logarithmic plots of current versus potential, using the method described in section 5.3.5.

7. RESULTS

7.1 INTRODUCTION

Results from the investigations described in the previous chapter are shown as a series of X/T plots, using equal time scales for ease of cross-referencing, and including some or all of the following variables:

Hydrogen Permeation Current Density

Corrosion Potential

Culture pH

Culture Redox Potential

SRB Cell Numbers

H_2S Gas Concentration

In addition, X/Y plots of the first two of the above variables have been shown, and descriptions of bacterial cultures and corroded steel specimens are given. As a means of reference, bioreactor experiments are each designated as individual 'Runs' as set out in Chapter 6, and a summary showing test Run conditions, the parameters measured and data produced is set out in Appendix 3.

Results of the two separate polarisation experiments are shown as a series of plots of potential versus current.

7.2 HYDROGEN ABSORPTION DURING MICROBIAL CORROSION

7.2.1 The Influence of Unwashed SRB Cultures

The introduction of crude, unwashed batch cultures of Desulfovibrio vulgaris to mild steel specimens gave rise to double-peaked transients of hydrogen permeation current density (Runs A & B, figure 7.1a, p130). Higher maxima were attained in Run A (two peaks at 0.115μA/cm^2) than in Run B (peaks at 0.068 & 0.050μA/cm^2), and peaks were obtained earlier in Run A than those produced in Run B (after 2 & 24 hours and 4.5 & 83 hours respectively). Both transients exhibited similar declines in current density for an experimental duration of up to 178 hours. During the course of each experiment, the bulk phase of SRB cultures darkened, but changed little in either colour or turbidity. An examination of samples from both cultures revealed similar, highly motile populations of the test organism, containing approximately equal proportions of normal and pleiomorphic forms of cell morphology (figure 7.2 a & b below).

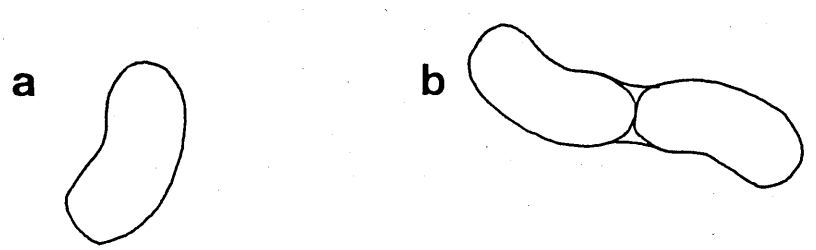
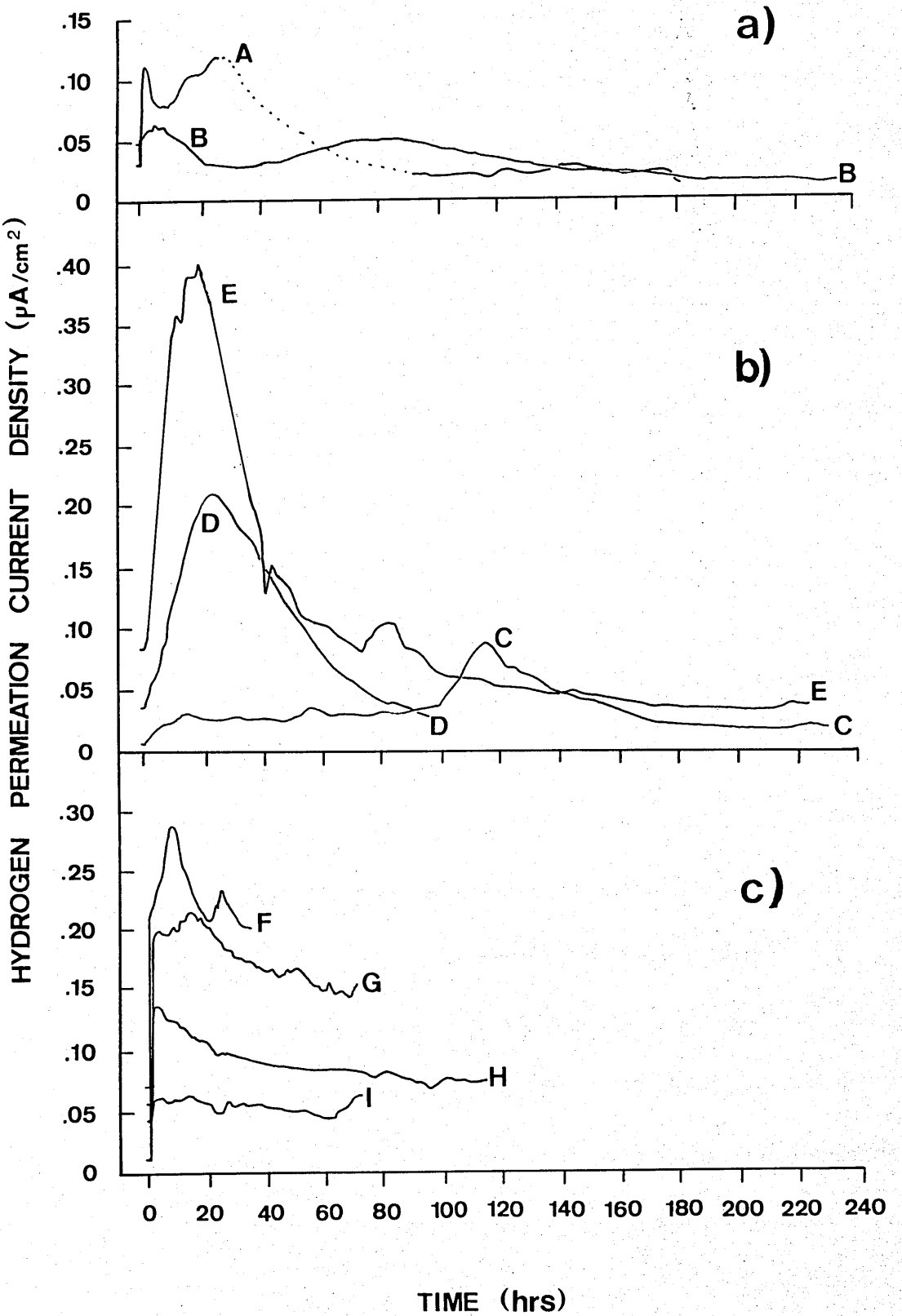


Figure 7.2 Normal (a) and pleiomorphic (b) variants of Desulfovibrio vulgaris (Woolwich) in bioreactor samples.

Figure 7.1 Hydrogen Permeation Transients at 30 C, using a) 1.5mm thick mild steel sheet in unwashed 3-7 day old batch cultures of Desulfovibrio vulgaris (Woolwich) in PMC medium (Runs A & B); b) Runs C,D & E with steel foils (0.1, 0.125 & 0.125mm thick) in similar but washed cultures in fresh PMC medium; c) Runs F,G & H using steel foils with respective thicknesses of 0.125mm, 0.125mm and 0.075mm in Sterile PMC medium (Yeast Extract omitted in Run H), and Run I with 0.125mm thick steel foils in distilled water.


a)
b)
c)
A
B
C
D
E
F
G
H
I
.15
.10
.05
0
.40
.35
.30
.25
.20
.15
.10
.05
0
.30
.25
.20
.15
.10
.05
0
0 20 40 60 80 100 120 140 160 180 200 220 240
HYDROGEN PERMEATION CURRENT DENSITY (μA/cm²)
TIME (hrs)

The initial and final cell counts derived from bulk phase samples in Run B are given in table 7.1 below.

Table 7.1 Total and viable bulk phase cell counts of Desulfovibrio vulgaris (Woolwich) in LSY-C Medium exposed to mild steel for 177 hours at 30 C (Run B).

	Average count cells/ml
Initial total count	5.6×10^9
Initial viable count	1.1×10^9
Final viable count	8.2×10^7

Following Runs A & B, examination of steel surfaces revealed identical loose, black and viscous films, which when observed microscopically, consisted of highly motile cells of the type shown in figure 7.2a, and loose agglomerations of particulate matter (up to 10μm diameter) to which many cells were attached. The gentle removal of surface microbial growth exposed a uniform black film which covered the area of steel previously exposed to SRB culture. Removal of the film material revealed a uniformly tarnished metal surface, which in Run A contained two distinct areas of pitting. Profile analysis

of pitted regions, and also the total corroded area of metal indicated an average pit depth of approximately 12μm, in addition to a uniform depth of corrosion of approximately 6μm. The total volume of corroded material was calculated to be $1.33 \times 10^{-3} cm^3$, with an equivalent average daily metal weight loss of 54.2mdd over the 9.7 day duration of exposure to SRB culture. This compared to an equivalent metal weight loss during Run B of 135.4mdd.

7.2.2 The Influence of Washed SRB Cultures

Run C exhibited a biphasic increase in hydrogen permeation current density (figure 7.1b, p130), with an initial plateau value of approximately $0.03 \mu A/cm^2$, subsequently rising to a peak value of approximately $0.075 \mu A/cm^2$ after between 110 and 120 hours. Culture blackening coincided with the second increase in current density. Following the introduction of washed cell suspensions in Runs D and E, initial and sustained increases in hydrogen permeation current density were obtained after 40 minutes and 78 minutes respectively. Respective peak current density values of $0.206 \mu A/cm^2$ and $0.404 \mu A/cm^2$ were attained after 24 hours and 19 hours, by which time the cultures had become blackened. Runs C, D and E demonstrated similar rates of increase and decline in current density following the attainment of peak values.

Surface hydrogen concentrations (ppm) were derived from peak hydrogen permeation current density values obtained in experimental Runs C, D and E (table 7.2), and using a diffusion coefficient of $6.3 \times 10^{-7} cm^2 sec^{-1}$ (Miodownic, 1972).

Table 7.2 Peak hydrogen permeation current density and surface hydrogen concentrations obtained from shimm steel foils exposed to washed cultures of Desulfovibrio vulgaris (Woolwich) in single chamber bioreactor Runs C, D & E.

Run	Foil Thickness (cm)	Peak H permeation current density ($\mu A/cm^2$)	Surface H concentration (ppm)
C	0.0100	0.100	0.0021
D	0.0125	0.202	0.0053
E	0.0125	0.404	0.0106

Bulk phase samples taken at the end of each of Runs C, D and E contained predominantly senescent, non-motile cells, and a variety of pleiomorphic forms of the organism were observed, with cell lengths varying from approximately 5μm to 20μm. Samples of surface microbial films, however exhibited exclusively motile and morphologically 'normal' cells, similar to those contained in the inoculum. The

high viscosity of the film material and the high cell densities present precluded an accurate enumeration of bacteria, and approximations of minimum cell densities were made. Total cell counts are given in table 7.3.

Table 7.3 Initial and final cell counts of Desulfovibrio vulgaris (Woolwich) from bulk and surface film samples during single chamber bioreactor Runs C, D & E.

Run	Initial Count (bulk phase) cells/ml	Final count (bulk phase) cells/ml	Final count (surface film) cells/ml
C	1.0×10^6	3.7×10^8	4×10^9
D	1.0×10^6	3.9×10^8	2×10^{10}
E	7.3×10^7	8.0×10^7	2×10^{10}

Following the termination of Runs C, D and E, an examination of the steel specimens revealed similar loose, viscous microbial films and tenacious black surface films to those found in unwashed culture experiments. No pitting at the corroded steel surface had occurred, except for a single pit in Run D of approximately 0.5mm diameter. While Run D specimen registered a weight loss of 2.26mdd, the weight loss in Run E was negligible.

7.2.3 The Effect of Culture Media on Hydrogen Absorption

The introduction of sterile PMC media to shim steel foils in Runs F & G gave rise to rapid increases in current density to maximum values of 0.295μA/cm^2 and 0.214μA/cm^2 respectively (figure 7.1c, p130 & table 7.4, below).

Table 7.4 Hydrogen permeation current densities and surface hydrogen concentrations obtained for shim steel exposed to sterile PMC medium (Runs F & G), PMC excluding yeast extract (-Y/E, Run H) and distilled water (Run I), for up to 120 hours at 30 C.

Run	Test Medium	Foil Specimen Thickness (cm)	Background Current Density (μA/cm^2)	Maximum Current Density (μA/cm^2)	Surface Hydrogen Conc'n (ppm H)
F	PMC	0.0125	0.013	0.295	0.0077
G	PMC	0.0125	0.010	0.214	0.0056
H	PMC-Y/E	0.0075	0.003	0.136	0.0021
I	Dist H_2O	0.0125	0.045	0.065	0.0017

The omission of yeast extract in Run H produced similarly rapid increases in current density, but with a lower maximum of 0.14μA/cm^2. The use of distilled water gave

rise to a consistently low current density value of approximately 0.06μA/cm^2. Each of the four experiments demonstrated a faster rate of increase and a slower rate of decrease in hydrogen permeation current density than in experiments involving SRB culture. The initial background current densities, the peak values attained and equivalent surface hydrogen concentrations are given in table 7.4.

A final examination of foils exposed to complete PMC media revealed a tenacious yellow film. The odour and consistency of the film suggested that it derived from components of the yeast extract present in the PMC medium, and on microscopic analysis, the material was found not to consist of a microbial film. Exposure of steel foils to PMC media from which yeast extract was excluded (Run H) resulted in the development of a uniform but rough and grey surface, but specimens exposed to distilled water exhibited no such surface changes. In each of the control experiments, the media under test remained clear, and final microscopic examination revealed only very low densities of contaminant organisms (predominantly motile rods). A final pH of 7.1 was recorded for culture media in each of Runs F and G.

7.3 THE INFLUENCE OF SRB FILMS ON HYDROGEN ABSORPTION

7.3.1 Test of System Reproducibility

Simultaneous changes in current density were recorded for two similar steel foils exposed to a single culture of Desulfovibrio desulfuricans (Norway-4) (Run J, figure 7.3, p139). However, the rise in current density due to the introduction of sterile culture media differed between the two foils by up to 0.08μA/cm^2, while the maximum values attained differed by 0.05μA/cm^2. Both transients exhibited a higher overall rate of increase than decrease in current density, and culture blackening coincided with increasing current density directly after inoculation.

Average total cell counts from bulk phase samples increased from 2.2 x 10^7 cells/ml at inoculation, to 1.2 x 10^8 cells/ml after 252 hours (10½ days). Examination of unwashed steel foils revealed an uneven distribution of microbial growth, which had accumulated especially around the inner circumference of the corroded area of the specimen (where in contact with the bioreactor flanges), although some growth was visible over the entire specimen surface. An underlying, mostly uniform layer of black adherent corrosion product was observed on both specimens. The film material was mostly removed by wiping with a

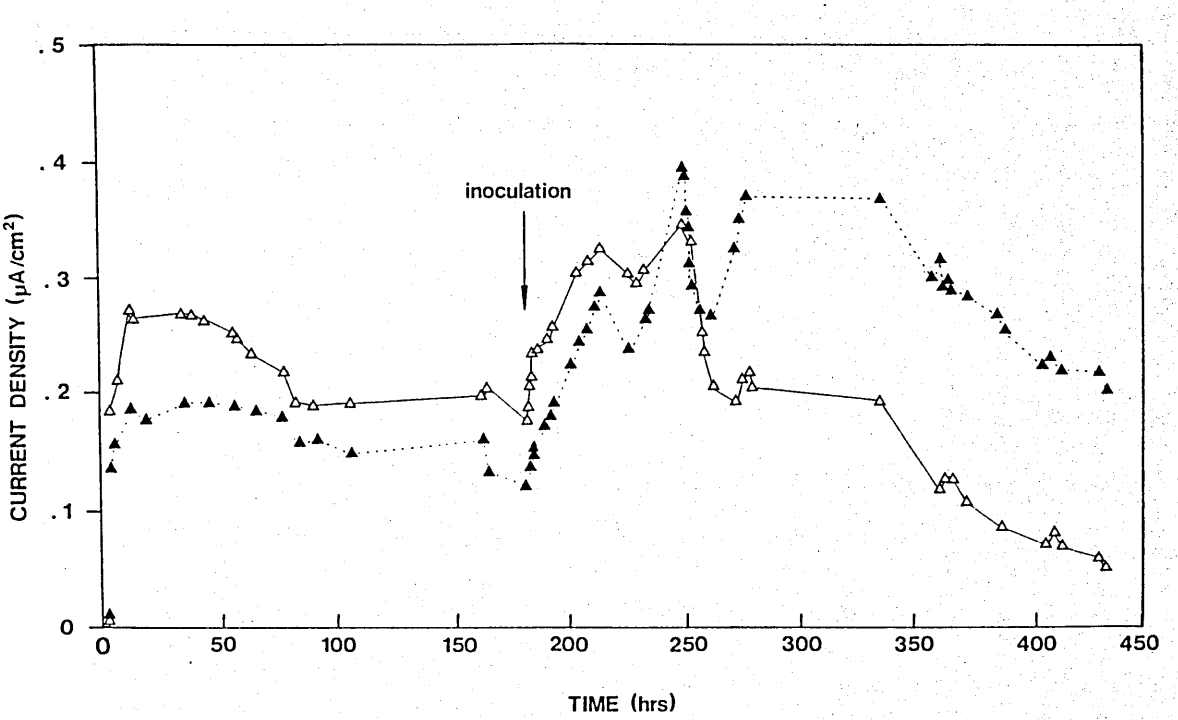
tissue wetted in alcohol (IPA). This revealed a uniform surface tarnish which covered most of the specimen surface except for some brighter areas of metal around the inner circumference of the foil. The development of pits did not appear to have taken place, and metal weight losses of 5.85mdd and 6.07mdd were recorded for the two foils.

The total quantities of hydrogen which diffused through the steel foils as determined by integrating the hydrogen permeation transients in Run J are shown in table 7.5. The two foils were found to have generated both similar total masses of cathodic hydrogen, and also similar proportions of diffused cathodic hydrogen.

Table 7.5 Total diffused hydrogen and total cathodic hydrogen generated during Run J by a similar pair of anaerobically corroding steel foils in SRB culture.

Total Fe Wt Loss mg/cm^2	Total H Cathodic mg/cm^2	H Perm Charge C/cm^2	Mass Diff H mg/cm^2	% Diff H
1.053	0.0376	0.311	0.0032	8.5
1.093	0.0390	0.368	0.0038	9.7

Figure 7.3 Transients of hydrogen permeation current density for a pair of steel foils exposed to a single batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C (Run J).


.5
.4
.3
.2
.1
0
CURRENT DENSITY (μA/cm²)
inoculation
0
50
100
150
200
250
300
350
400
450
TIME (hrs)

7.3.2 The Effects of SRB Screening on Hydrogen Absorption

During Runs K and L, steel foils in contact with SRB gave rise to similar changes in hydrogen permeation current density to the foils which were screened from the bacteria by means of a dialysis membrane (figure 7.5a, p148 & figure 7.6a, p152). From similar initial background current densities, and similar values obtained after the introduction of sterile culture media, almost equal peak current densities and surface hydrogen concentrations were obtained following inoculation with SRB. This was true whether the SRB were in contact with, or were excluded from the steel surface (table 7.6).

Table 7.6 Hydrogen permeation current densities obtained at various phases of Runs K and L, for foils with a thickness of 0.005cm and a hydrogen diffusion coefficient of 6.3×10^{-7} cm/sec, in the presence (SRB) and absence (-) of SRB at the steel surface.

Run	Hydrogen Permeation Current Density ($\mu A/cm^2$)			Peak Surface Hydrogen Conc'n (ppm H)
	Initial Background	After Media Introduced	After SRB Inoculation	
K (SRB)	0.0064	0.083	0.357	0.0037
K (-)	0.0069	0.137	0.371	0.0039
L (SRB)	0.0099	0.051	0.360	0.0037
L (-)	0.0066	0.092	0.374	0.0039

Gas chromatograph peak heights obtained for dilutions of the primary H_2S gas standard are shown in figure 7.4 (overleaf).

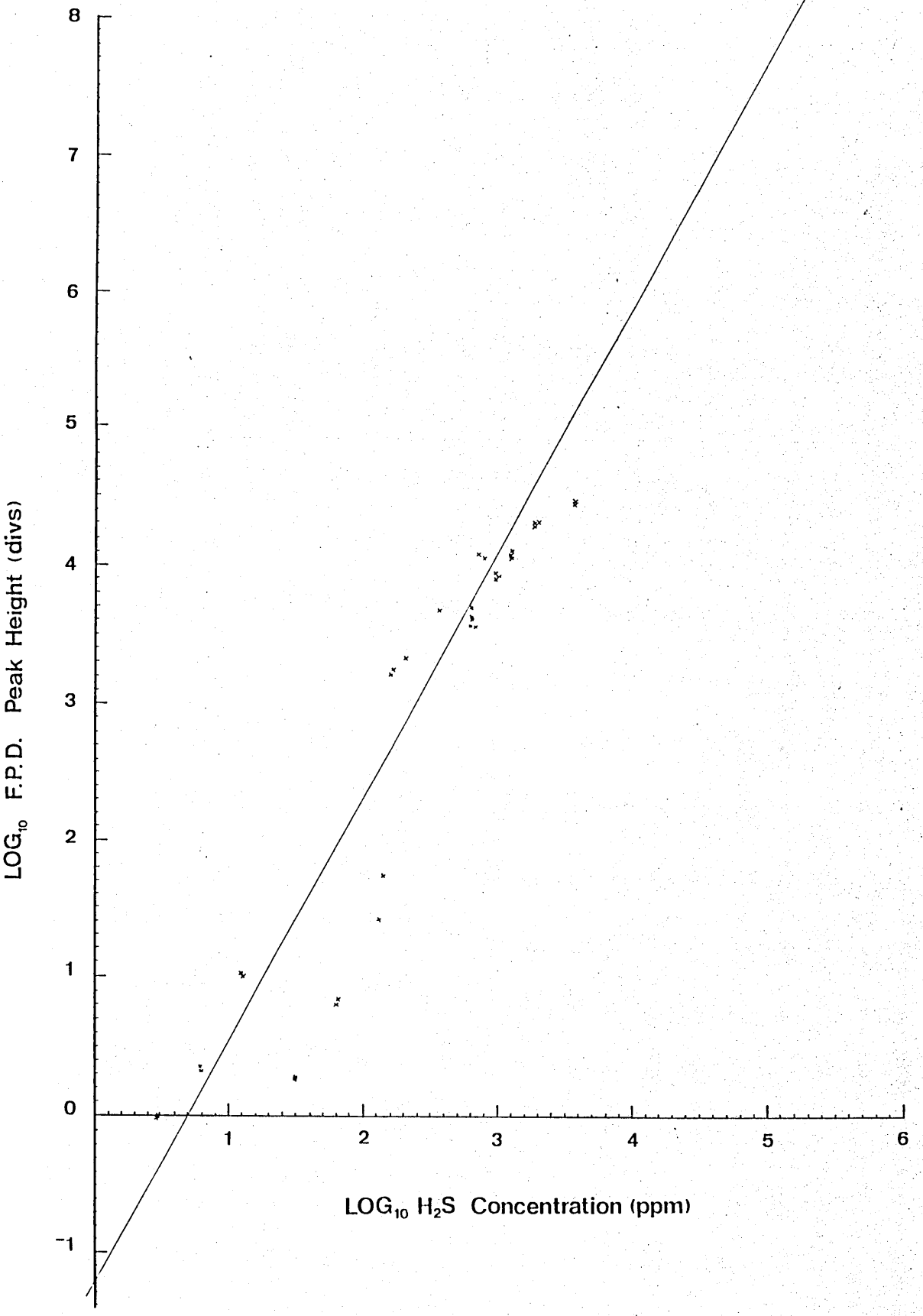


Figure 7.4 Graph used for determining bioreactor gas H_2S concentrations by flame photometry detection (FPD).

Following SRB inoculation, the current density peaks obtained for steel foils in the presence of SRB preceded the peaks obtained in the absence of bacteria by approximately 10 hours and 20 hours (Runs K and L respectively). While a high and sustained level of current was obtained in Run K, the sharply declining values in Run L resembled those shown previously, in experimental Runs C, D and E (figure 7.1b, p130).

During exposure to sterile media and prior to inoculation, the steel specimens established a corrosion potential of between -710mV and -740mV (SCE). Approximately 40 hours after inoculation, and coincident with a blackening of the culture medium, corrosion potentials of the colonised and SRB-screened specimens shifted upwards by approximately 100mV. This newly established potential then persisted throughout the remainder of both Runs K and L (figure 7.5b, p148 & figure 7.6b, p152). When plotted versus hydrogen permeation current, the most marked shifts in corrosion potential during Run K coincided with periods during which rates of cathodic hydrogen absorption changed little (figure 7.5c, p149). When the experiment was repeated under the same experimental conditions (Run L, figure 7.6c, p153), the latter relationship was less clear. The corrosion potential of the SRB colonised steel

foil shifted positively with increasing hydrogen absorption, whereas that of the SRB screened foil temporarily shifted by +110mV without any net increase in hydrogen permeation current density.

Following their removal from the dismantled bioreactor, and after draining off excess liquid or loose microbial growth, corroded steel foils resulting from Runs K and L revealed similar tenacious black films as before, but with marked regions of bare metal. An indication of the distribution of filmed and unfilmed regions of metal is given in plate 7.1a (p144). Such regions were either spread evenly over the specimen surface and typically of 1-2mm diameter, or covered larger areas, including a band of up to 5mm around the circumference of the corroded specimen area, but no definite pits were observed.

Removal of the bulk of tenacious black corrosion product using alcohol-soaked (IPA) tissue revealed a tarnished steel surface, which remained after sonication and cleaning in alcohol (plate 7.1b, p144). While in Run K, the SRB-screened specimen indicated a limited degree of aerobic crevice corrosion, no such corrosion was indicated in other cases, nor on the hydrogen detector face of any foils in Runs K and L. Higher weight losses were recorded for foils exposed to SRB than for screened specimens (table 7.7, p145).

Plate 7.1a Appearance prior to cleaning of steel foils screened (A) and unscreened (B) from SRB cells during an 11 day period of exposure at 30 C to Desulfovibrio desulfuricans (Norway-4) in PMC Medium.

Plate 7.1b Appearance of steel foils (A) & (B) after the removal of loose surface film material by ultrasonic cleaning.

a

b

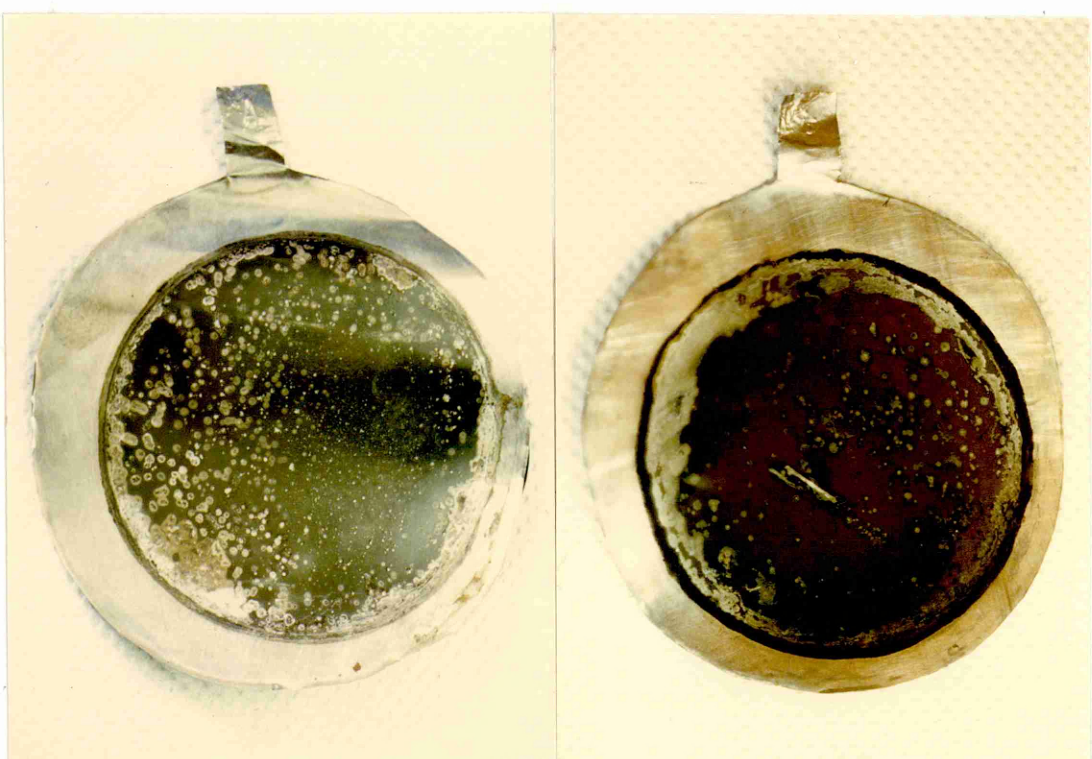


Table 7.7 Weight losses recorded in Runs K and L for steel foils corroding in the presence (SRB) and absence (-) of SRB over 15.3 days (Run K) and 11.2 days (Run L)

Run	Total Wt Loss (mg/cm^2)	Average Wt Loss (mdd)
K (SRB)	1.98	12.9
K (-)	1.18	7.7
L (SRB)	4.41	39.4
L (-)	1.84	16.4

Using the total weight loss values shown in table 7.6 (p140) and by integrating transients of hydrogen permeation from Run K in figure 7.5a (p148) the equivalent masses of discharged hydrogen and diffused hydrogen were obtained (section 5.3.3), thus giving the percentage diffused hydrogen (table 7.8).

Table 7.8 Determination of total discharged hydrogen and diffused hydrogen during Runs K and L.

Run	Total Fe Wt Loss (mg/cm^2)	Total H Cathodic (mg/cm^2)	H Perm Charge (C/cm^2)	Mass Diff H (mg/cm^2)	% Diff H
K (SRB)	1.98	0.071	0.290	0.0030	4.2
K (-)	1.18	0.042	0.217	0.0025	5.9
L (SRB)	4.41	0.157	0.126	0.0013	0.83
L (-)	1.84	0.066	0.156	0.0016	2.4

Of the total mass of cathodic hydrogen generated during corrosion, between 0.83% and 5.9% entered and diffused through the steel foils in Runs K and L. While the specimen exposed to SRB in Run L registered the highest weight loss, it absorbed the least amount of hydrogen, and therefore also the lowest percentage diffused hydrogen. Conversely, the specimen screened from SRB in Run K registered the lowest weight loss, the highest mass of absorbed hydrogen, and the greatest proportion of diffused cathodic hydrogen.

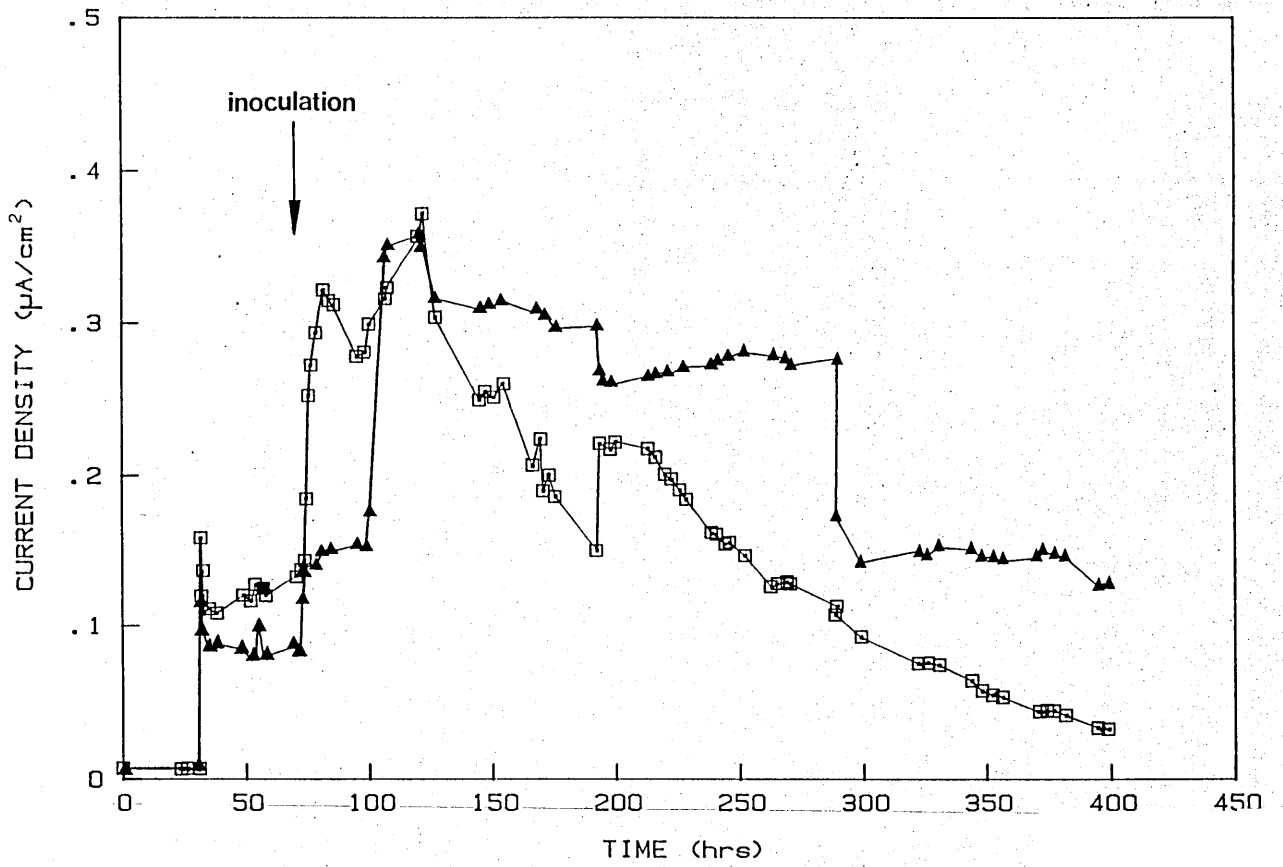
Decreases in media redox potential of between 200-300mV were recorded following SRB inoculation in experimental Runs K and L (figure 7.5d, p150 & figure 7.6d, p154). Minimum values of -310mV and -340mV (SHE) were attained 31 & 50 hours after inoculation (Runs K and L respectively), and in both experiments, in the presence and absence of SRB, the redox potential stabilised at a value of between -220mV and -240mV (SHE). The most marked overall drops in redox potential coincided with rapid H_2S evolution (figure 7.5e, p150 & figure 7.6e, p154) and an increase in cell numbers (figure 7.6d, p154). The initial rates of increase in gas head space H_2S concentration during Runs K and L are shown in figure 7.5f (p151) and figure 7.6f (p155). In both experiments, a concentration of approximately 1000ppm H_2S was attained 25 hours after SRB inoculation, and levels of approximately

10^4 and 10^5 ppm H_2S were detected in Runs K and L respectively before sampling ceased (due to the corrosion of gas sampling needles). A period of approximately 10 hours separated an initial increase in viable cell numbers following inoculation and the first recorded drop in redox potential. Subsequent changes in cell growth and redox potential followed a similar pattern.

The pH of media in both Runs K and L decreased following inoculation with SRB (figure 7.5e, p150 & figure 7.6e, p154). In Run L, the decline continued for 90 hours following inoculation, by which time the pH had fallen from initial values of 7.9 (SRB screened) and 7.7 (SRB present), to a single value of 6.9 units. Following inoculation in Run K though, the pH of the SRB culture chamber declined from only 7.1 to 6.7 units, over a period of approximately 35 hours. Approximately 110 hours after inoculation, there followed a further pH drop, to a final value of 5.8 units. No such pH drop was recorded for either bacterially screened or SRB culture chambers in Run L, during which pH levels stabilised between 6.9 and 7.0 units at 74 hours after inoculation, and then persisted for a further 100 hours, until the end of the experiment. When tested against standard buffer solutions, the glass pH electrode used in Run K was found to operate normally.

Figure 7.5a Transients of hydrogen permeation current density for anaerobically corroding shim steel foils, in direct contact with (▲) and excluded from (□) cells in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C (Run K).

Figure 7.5b Changes in the corrosion potential of a pair of steel foils; one in direct contact with a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC medium (▲), and the other screened from cells by dialysis membrane (□).


inoculation
CURRENT DENSITY (μA/cm²)
.5
.4
.3
.2
.1
0
0
50
100
150
200
250
300
350
400
450
TIME (hrs)

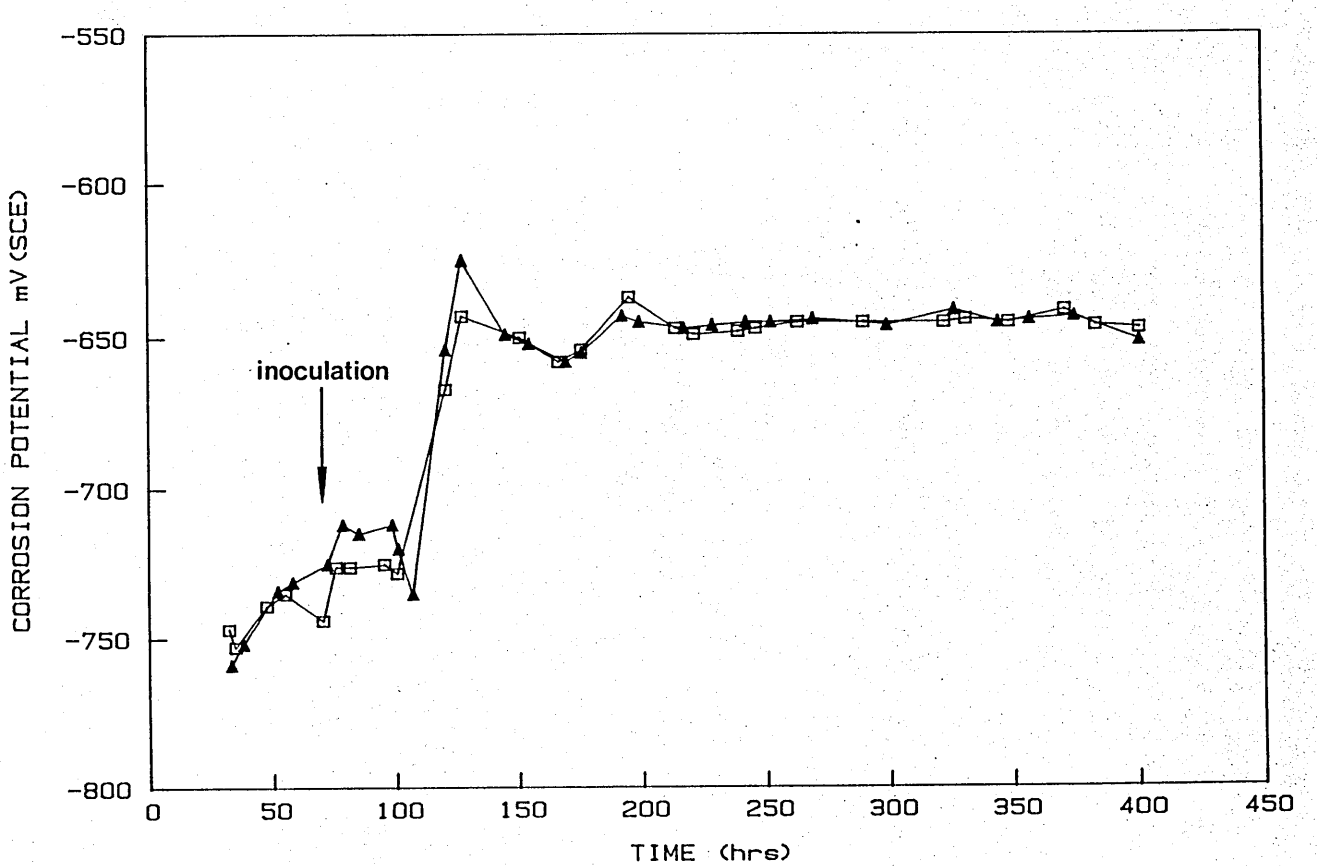
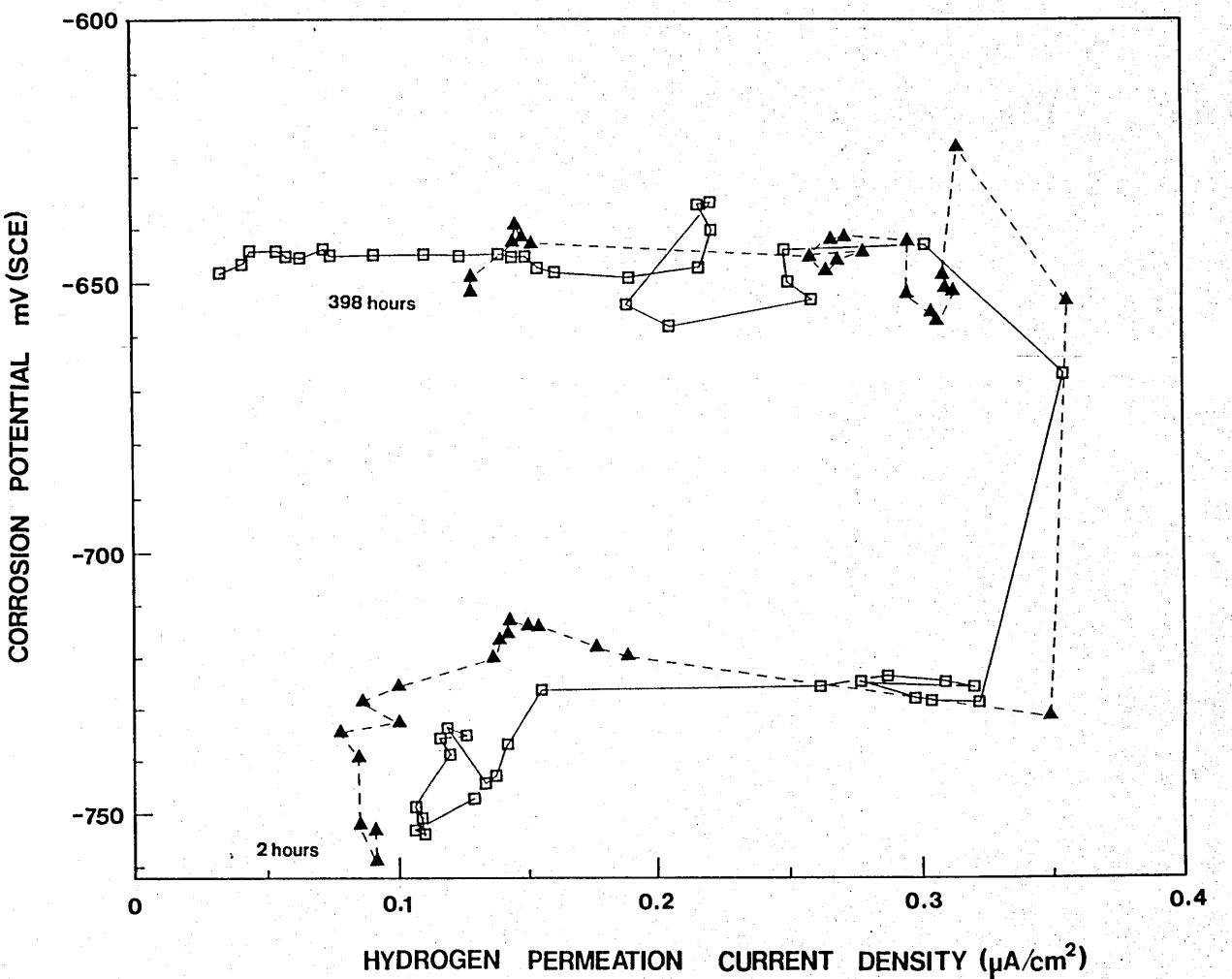
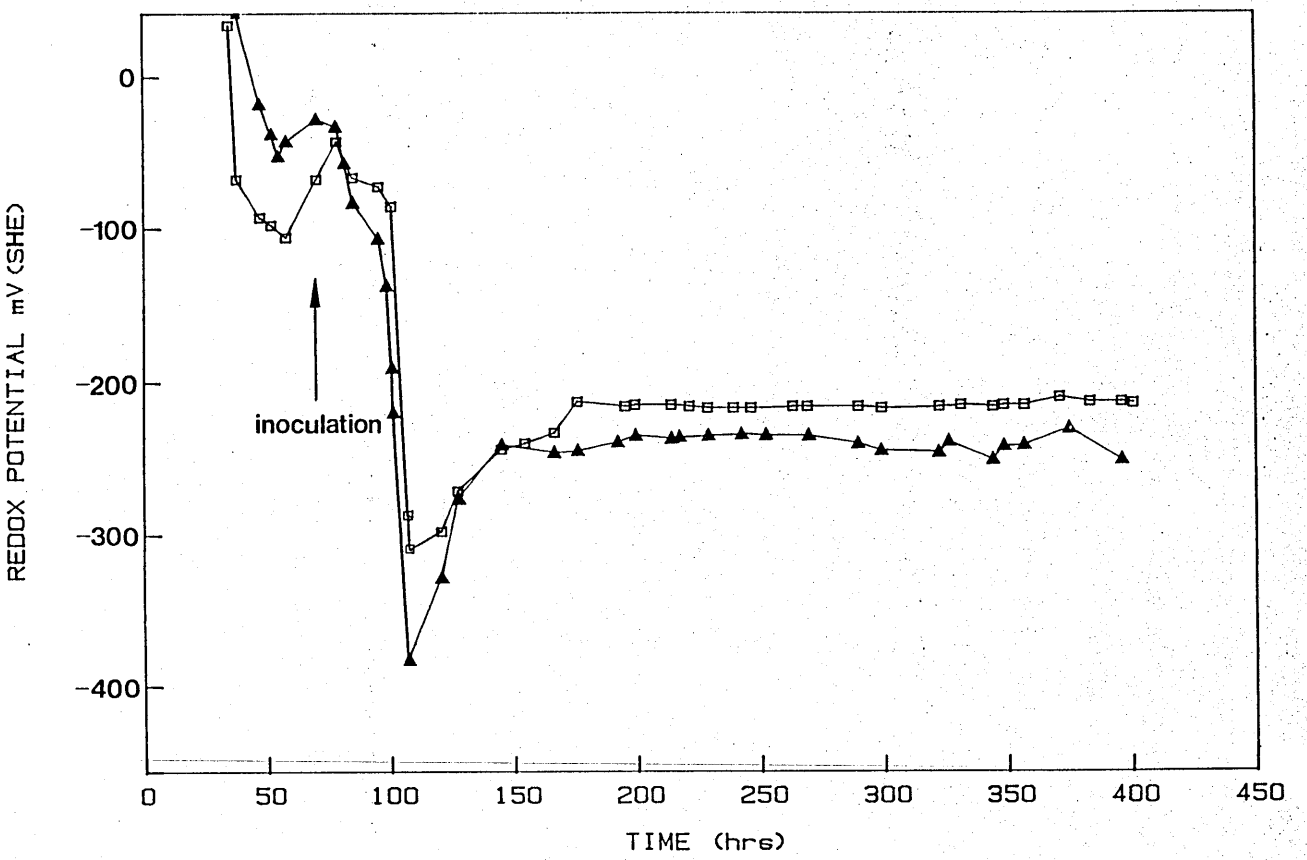

inoculation
CORROSION POTENTIAL mV(SCE)
-550
-600
-650
-700
-750
-800
0
50
100
150
200
250
300
350
400
450
TIME (hrs)

Figure 7.5c Changes in hydrogen permeation current density with corrosion potential of corroding steel foils in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C in the presence (▲) and absence (□) of SRB.


-600
-650
-700
-750
CORROSION POTENTIAL mV (SCE)
398 hours
2 hours
0
0.1
0.2
0.3
0.4
HYDROGEN PERMEATION CURRENT DENSITY (μA/cm²)

'igure 7.5d Variations in the redox potential of a atch culture of Desulfovibrio desulfuricans (Norway-4) in MC Medium at 30 C, during the anaerobic corrosion of teel in the presence (▲) and absence (□) of SRB cells.

'igure 7.5e Changes in pH during anaerobic corrosion of teel in a batch culture of Desulfovibrio desulfuricans Norway-4) in PMC Medium at 30 C (▲), and gas head space H_2S concentrations in the presence (△) and absence (□) of SRB.


REDOX POTENTIAL mV (SHE)
0
-100
-200
-300
-400
inoculation
0
50
100
150
200
250
300
350
400
450
TIME (hrs)

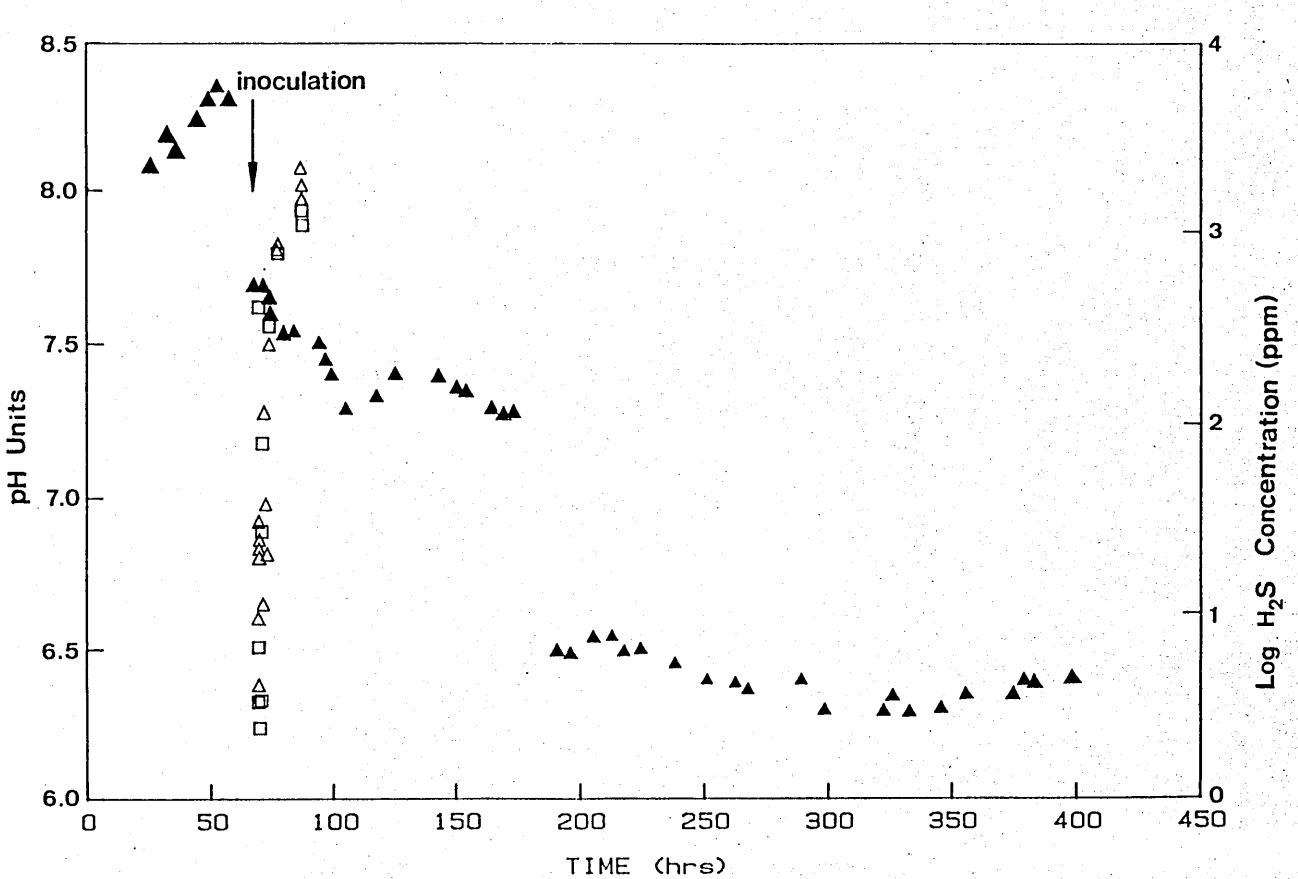

pH Units
8.5
8.0
7.5
7.0
6.5
6.0
inoculation
0
50
100
150
200
250
300
350
400
450
TIME (hrs)
Log H_2S Concentration (ppm)
4
3
2
1
0

Figure 7.5f Increases in gas head space H_2S concentration (parts per million) after the inoculation of PMC Medium with Desulfovibrio desulfuricans (Norway-4) during anaerobic corrosion of steel foils, in the presence (▲) and absence (□) of SRB cells (Run K).

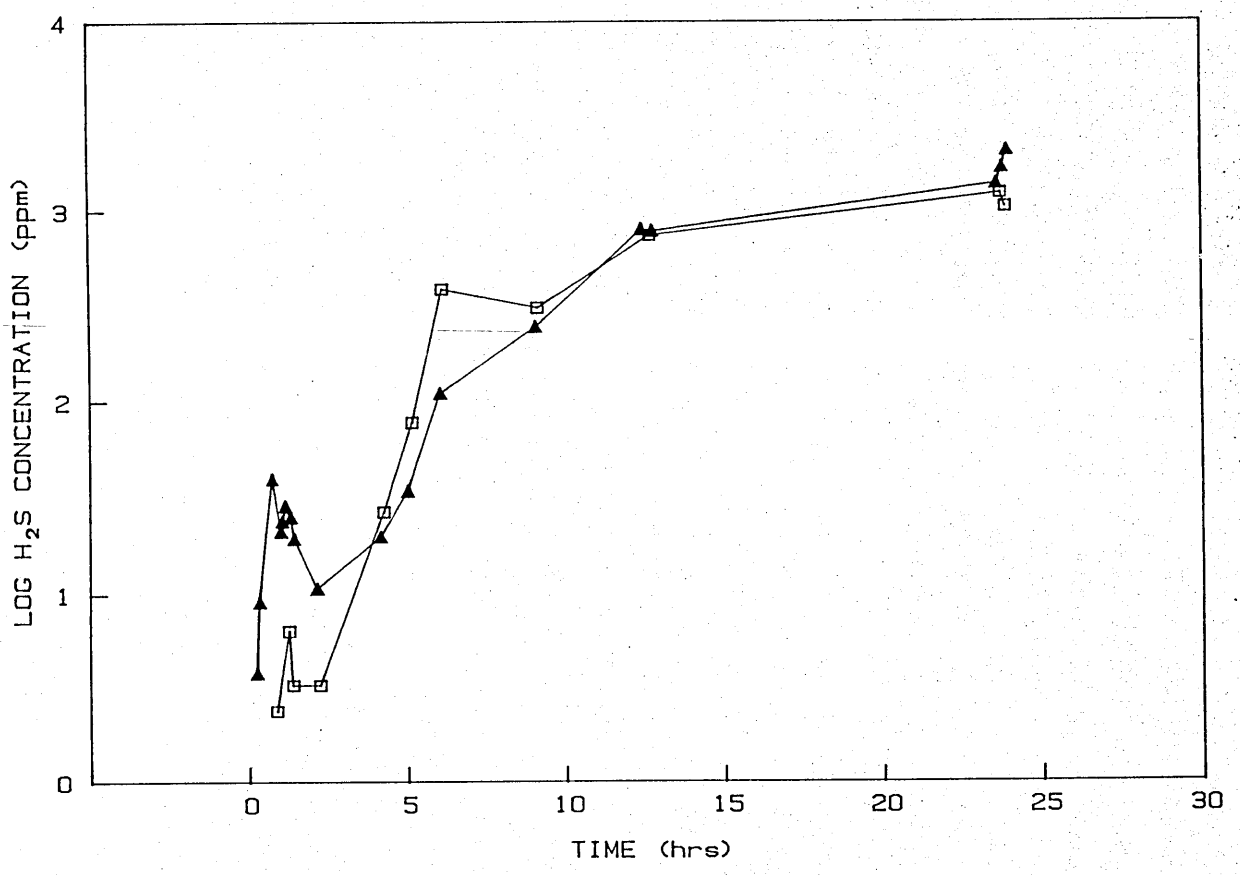
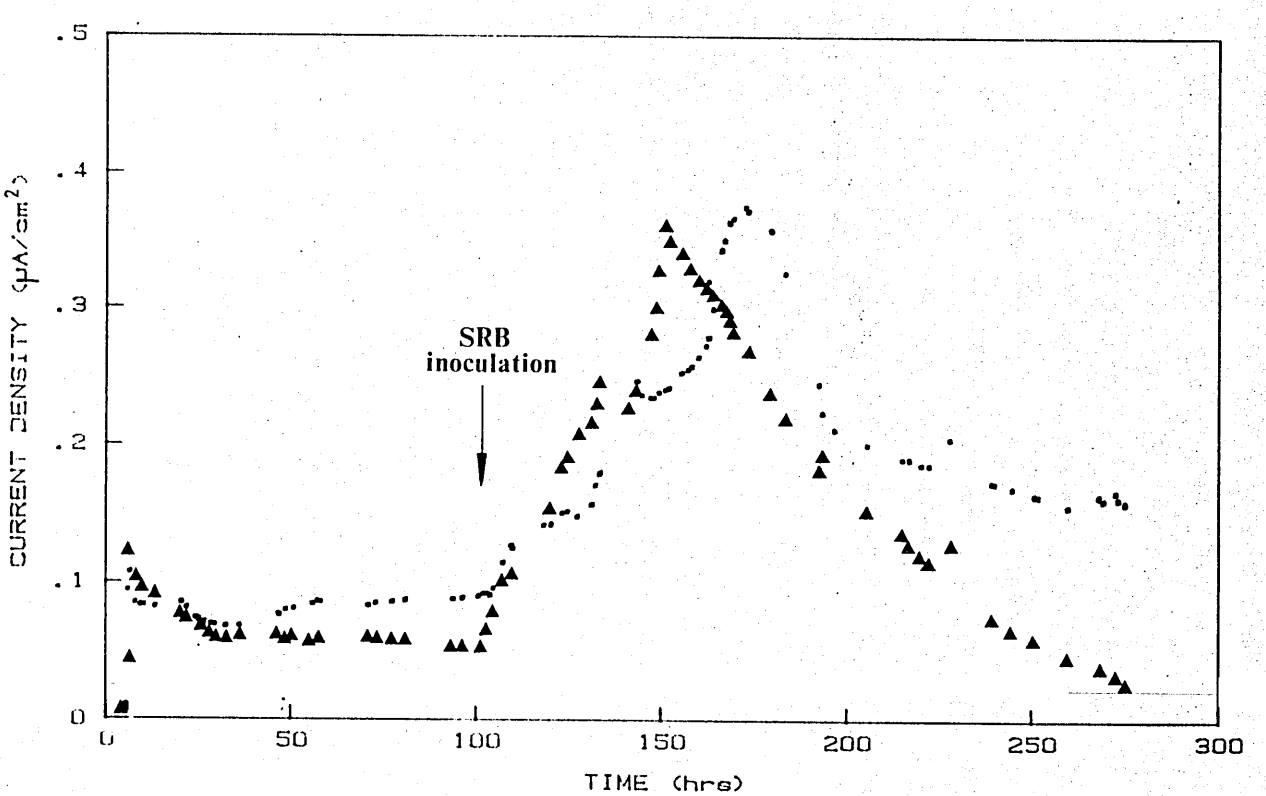

LOG H_2S CONCENTRATION (ppm)
4
3
2
1
0
0
5
10
15
20
25
30
TIME (hrs)

Figure 7.6a Transients of Hydrogen Permeation Current for anaerobically corroding shim steel in contact with (▲) and excluded from (▪) SRB cells in a batch culture of Desulfovibrio desulfuricans (Norway-4)/PMC Medium at 30 C (Run L).

Figure 7.6b Changes in the corrosion potential of anaerobically corroding steel foils in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C in the presence (▲) and absence (▪) of SRB cells.


CURRENT DENSITY (μA/cm²)
.5
.4
.3
.2
.1
0
SRB
inoculation
0
50
100
150
200
250
300
TIME (hrs)

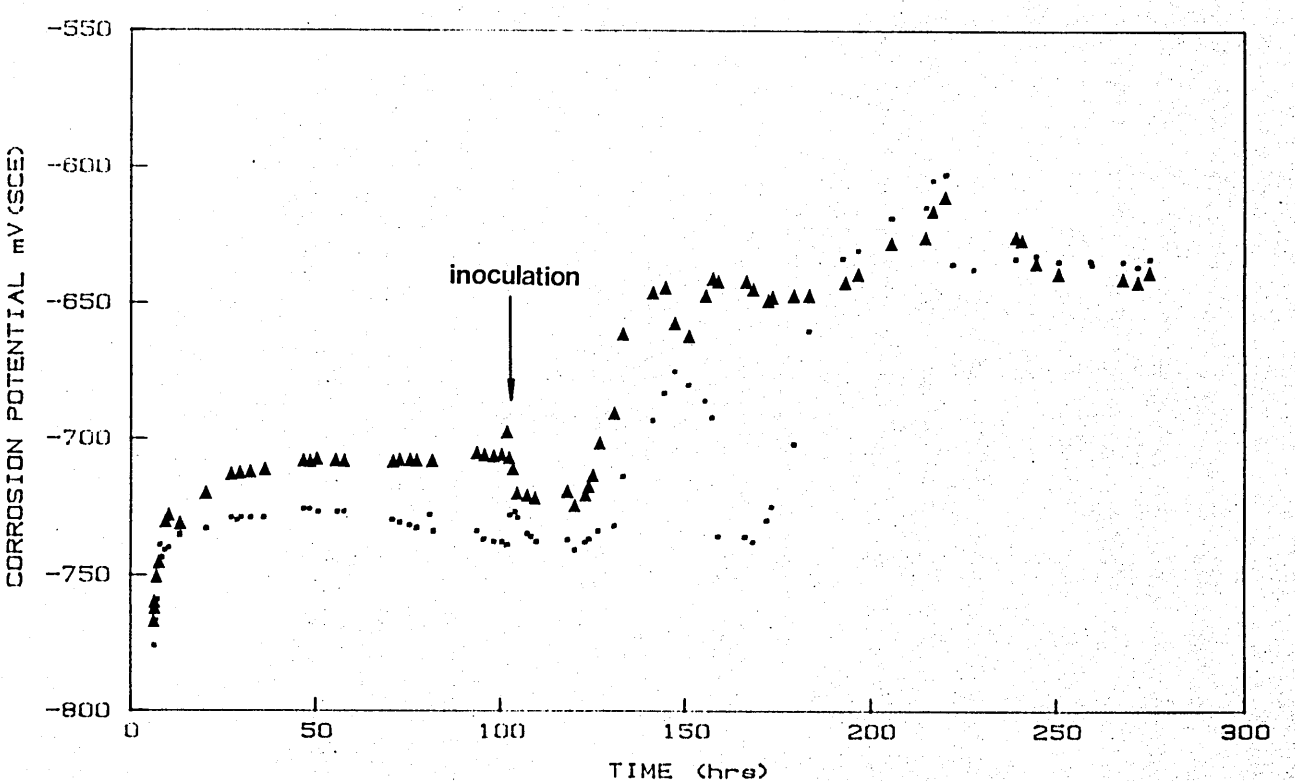

CORROSION POTENTIAL mV(SCE)
-550
-600
-650
-700
-750
-800
inoculation
0
50
100
150
200
250
300
TIME (hrs)

Figure 7.6c Changes in corrosion potential with hydrogen permeation current for corroding steel foils in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium/30 C, in the presence (▲) and absence (□) of SRB.

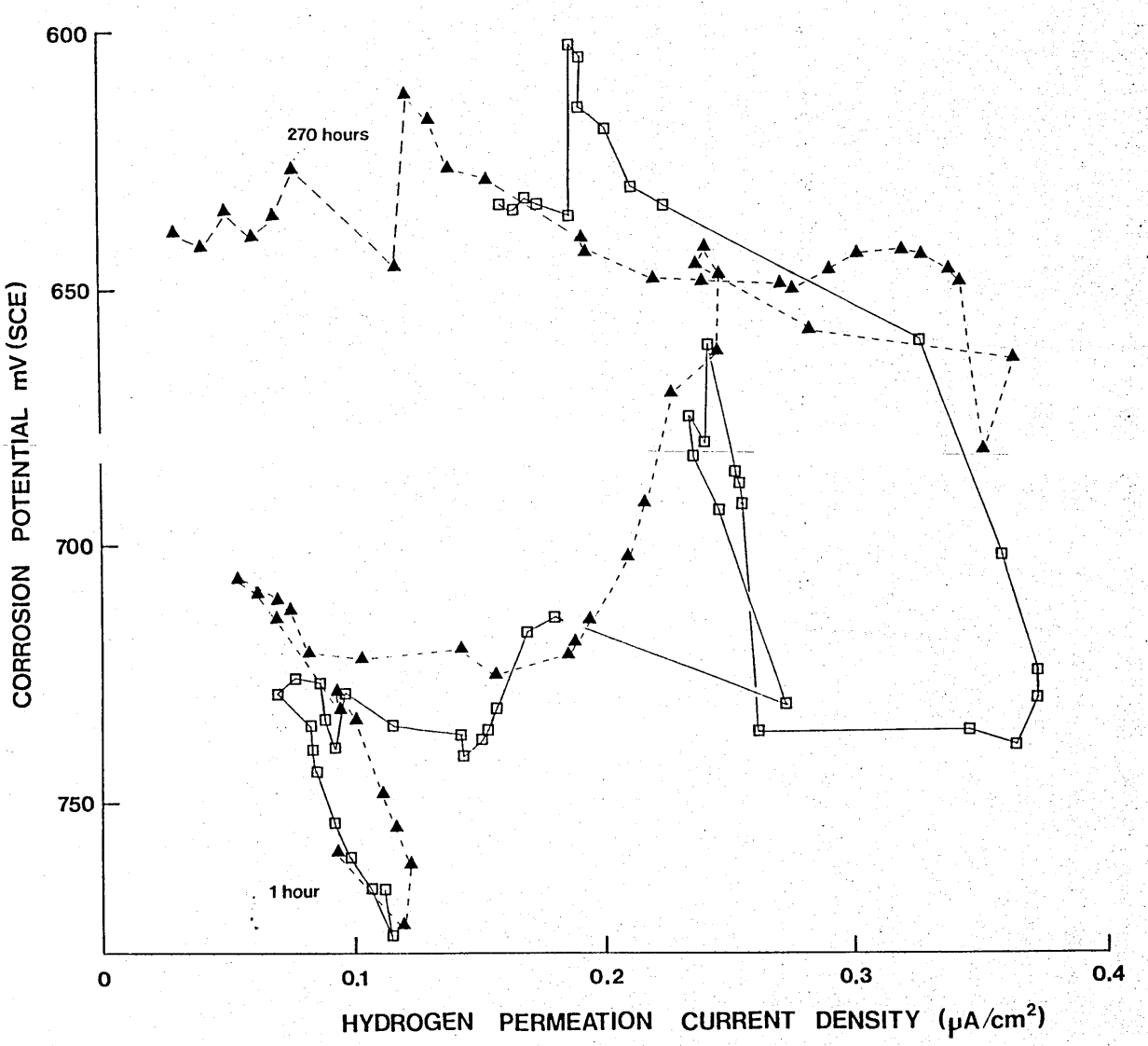
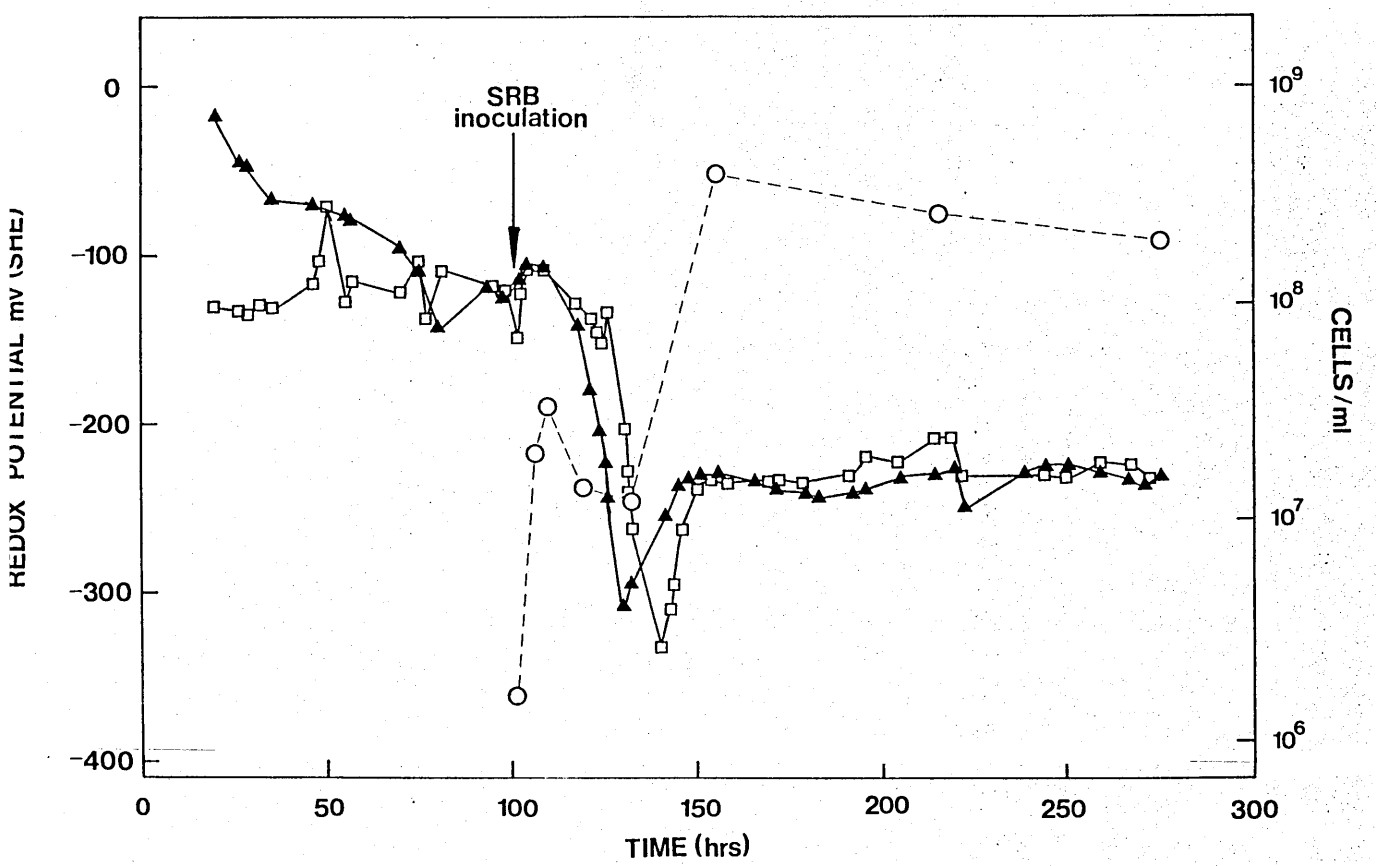

600
650
700
750
CORROSION POTENTIAL mV (SCE)
270 hours
1 hour
0
0.1
0.2
0.3
0.4
HYDROGEN PERMEATION CURRENT DENSITY (μA/cm²)

Figure 7.6d Variations in bioreactor redox potential during the anaerobic corrosion of steel foils in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium, in the presence (▲) and absence (□) of SRB, and viable cell numbers present in the bioreactor culture chamber (○).

Figure 7.6e Changes in pH during anaerobic corrosion of steel in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium in the presence (▲) and absence (▪) of SRB, and increases in the gas head space H_2S concentration in the presence (△) and absence (■) of SRB


SRB
inoculation
0
-100
-200
-300
-400
10^9
10^8
10^7
10^6
CELLS/ml
0
50
100
150
200
250
300
TIME (hrs)

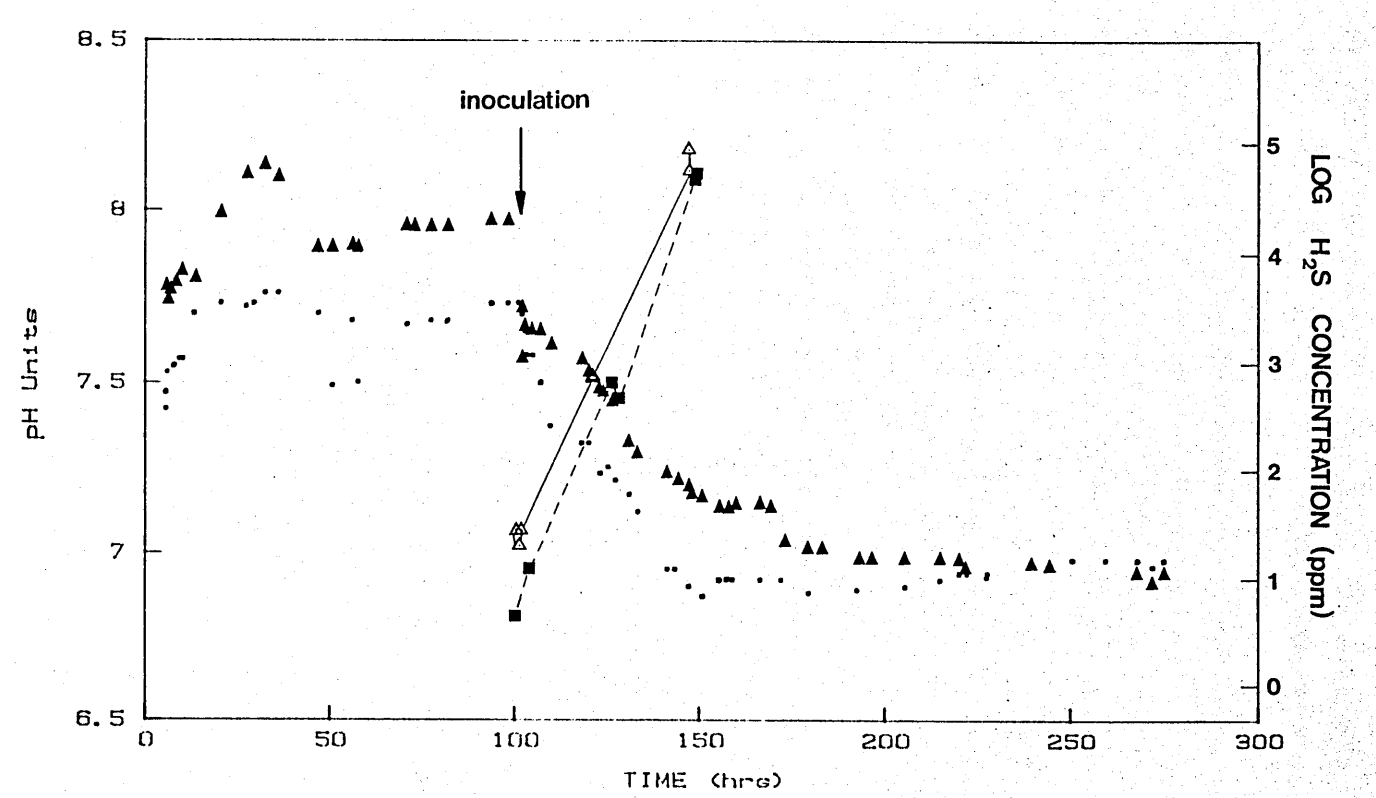
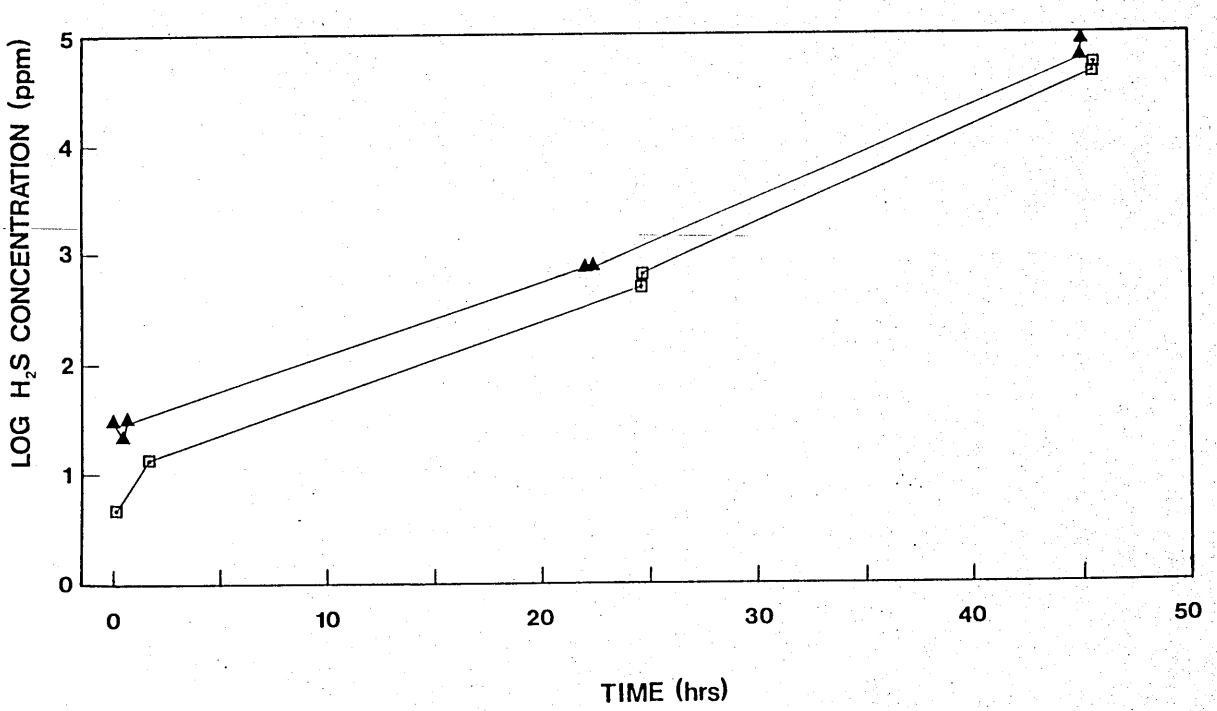

inoculation
8.5
8
7.5
7
6.5
pH Units
5
4
3
2
1
0
LOG H_2S CONCENTRATION (ppm)
0
50
100
150
200
250
300
TIME (hrs)

Figure 7.6f Increases in gas head space H_2S concentration (parts per million) after the inoculation of PMC Medium with Desulfovibrio desulfuricans (Norway-4) during the anaerobic corrosion of steel foils, in the presence (▲) and absence (□) of SRB cells (Run L).


LOG H_2S CONCENTRATION (ppm)
5
4
3
2
1
0
0
10
20
30
40
50
TIME (hrs)

7.4 THE INFLUENCE OF SRB FILMS ON HYDROGEN ABSORPTION BY CATHODICALLY PROTECTED STEEL

7.4.1 Hydrogen Permeation Transients

Following inoculation with SRB, the rate of hydrogen permeation through cathodically charged foils in Runs M & N rose sharply, to between 7 and 16 times pre-inoculation values (figure 7.7a, p157 & figure 7.8a, p158). While the maximum current densities attained during the two experiments were significantly different (table 7.9, below), there was little difference in the peak values obtained within each experiment, betweeen steel specimens which were screened and those which were exposed directly to SRB cells.

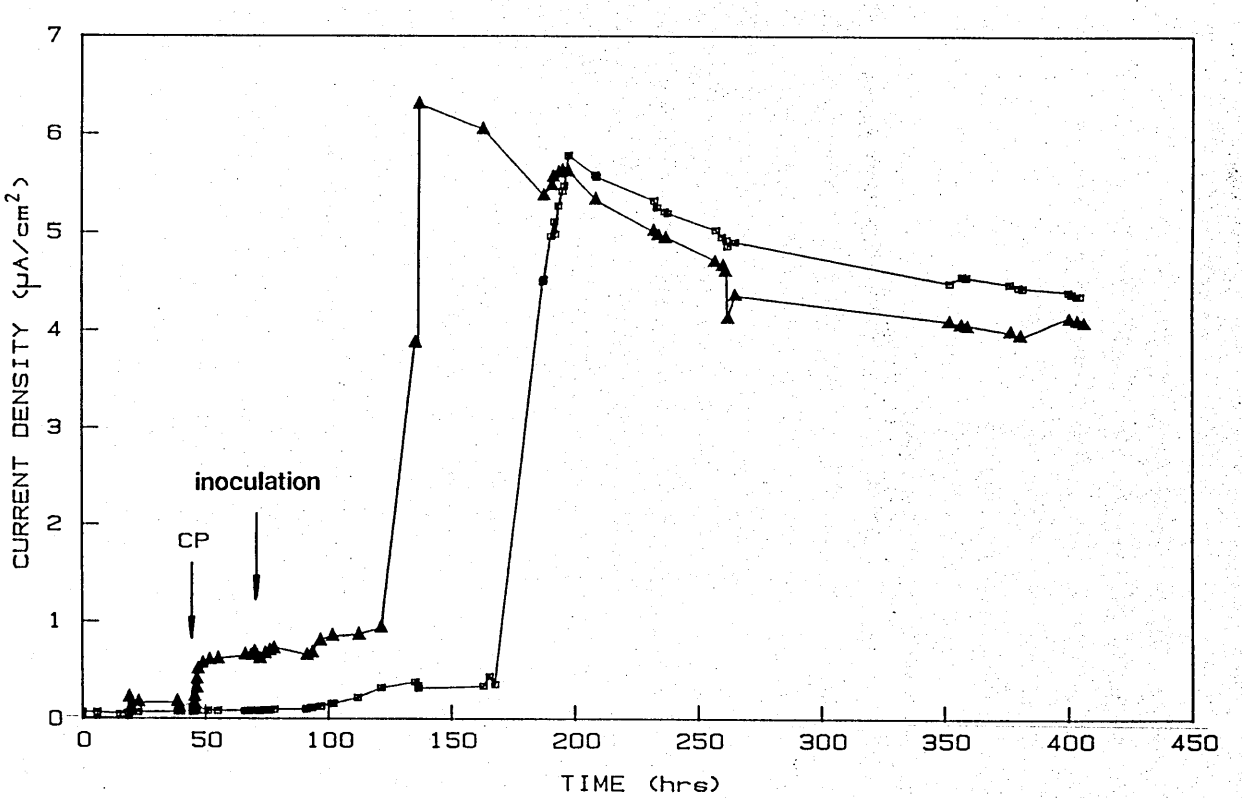
Table 7.9 **Peak hydrogen permeation current density and surface hydrogen concentrations obtained at various phases of Runs M, N and O, for cathodically charged steel foils, with a thickness of 0.005cm and a hydrogen diffusion coefficient of $6.3 \times 10^{-7} cm^{-2}$, in the presence (SRB) and absence (-) of SRB.**

Run	Hydrogen Permeation Current Density ($\mu A/cm^2$)				Peak surface hydrogen conc'n (ppm H)
	Initial Background	After Media Introduced	CP on	After SRB Inoculation	
M (SRB)	0.009	0.23	0.68	6.30	0.066
M (-)	0.047	0.066	0.074	5.78	0.060
N (SRB)	0.059	0.10	0.15	3.21	0.033
N (-)	0.026	0.12	0.43	3.73	0.039
O (SRB)	0.044	0.203	0.59	3.57 *	0.037
O (-)	0.010	0.094	0.34	2.95 *	0.031

* Maximum current density attained following inoculation and after cathodic charging was increased to -1000mV (SCE).

Figure 7.7a Transients of hydrogen permeation current density for steel foils cathodically charged to -850mV (SCE) and in direct contact with (△) or screened from (▫) cells in a culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C (Run M).

Figure 7.7b Changes in bulk pH (□) and total cell numbers (○) determined from SRB-screened and SRB culture bioreactor chambers respectively (Run M), before and after inoculation with Desulfovibrio desulfuricans (Norway-4).


CURRENT DENSITY (μA/cm²)
TIME (hrs)
inoculation
CP
0
1
2
3
4
5
6
7
0
50
100
150
200
250
300
350
400
450

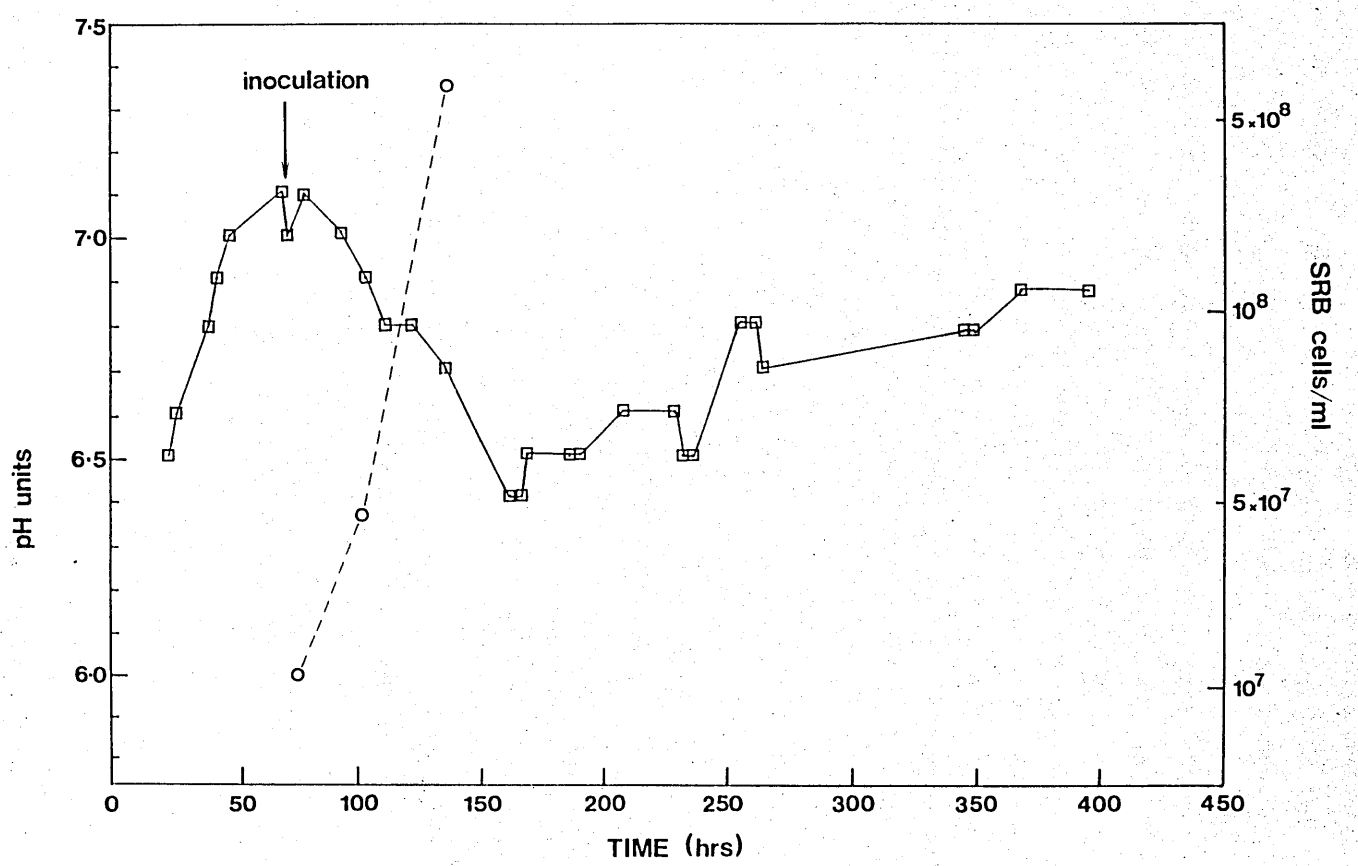
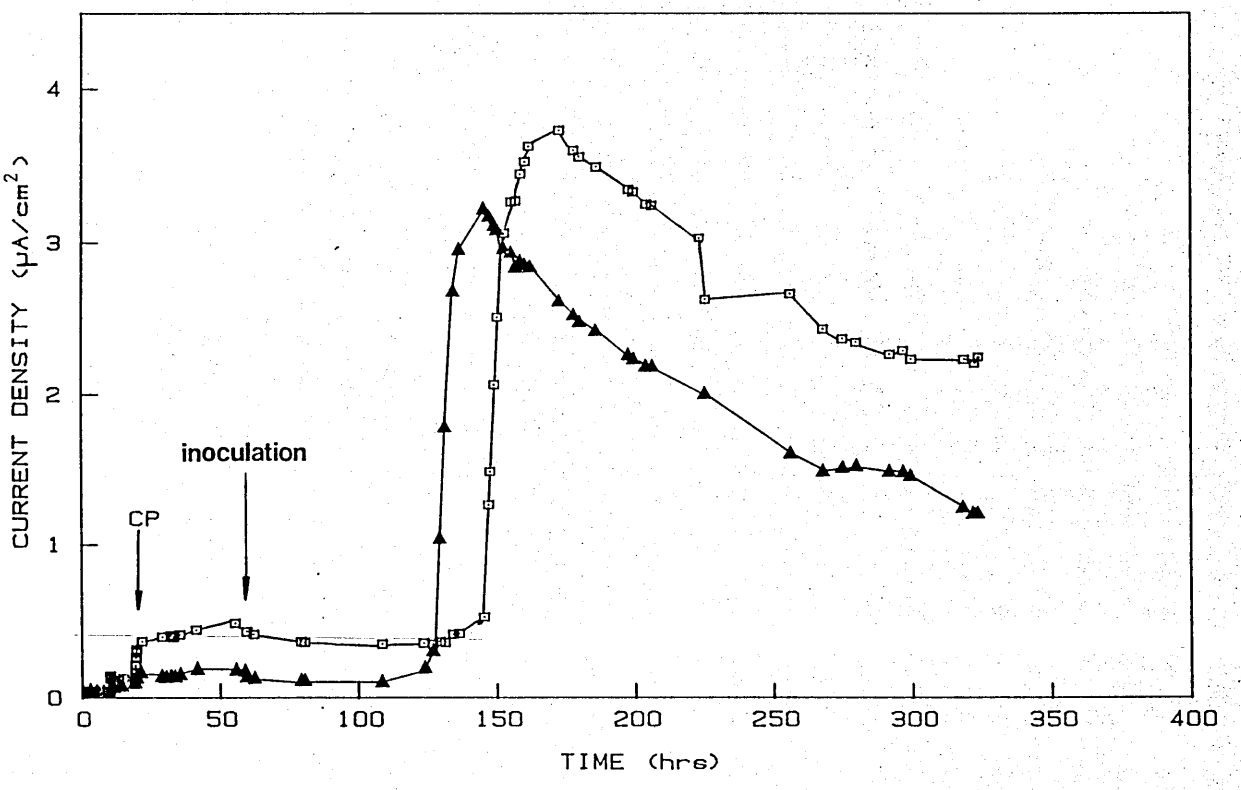

pH units
SRB cells/ml
TIME (hrs)
inoculation
7·5
7·0
6·5
6·0
5×10⁸
10⁸
5×10⁷
10⁷
0
50
100
150
200
250
300
350
400
450

Figure 7.8a Transients of hydrogen permeation current density for steel foils cathodically charged to -850mV (SCE) and in direct contact with (▲) or screened from (□) cells in a culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C (Run N).

Figure 7.8b Changes in redox potential in the presence of SRB cells (▲), and changes in both redox potential (□) and bulk pH (◇) in the absence of cells from a culture of Desulfovibrio desulfuricans (Norway-4)/PMC medium at 30 C.


CURRENT DENSITY (μA/cm²)
TIME (hrs)
inoculation
CP
0
1
2
3
4
0
50
100
150
200
250
300
350
400

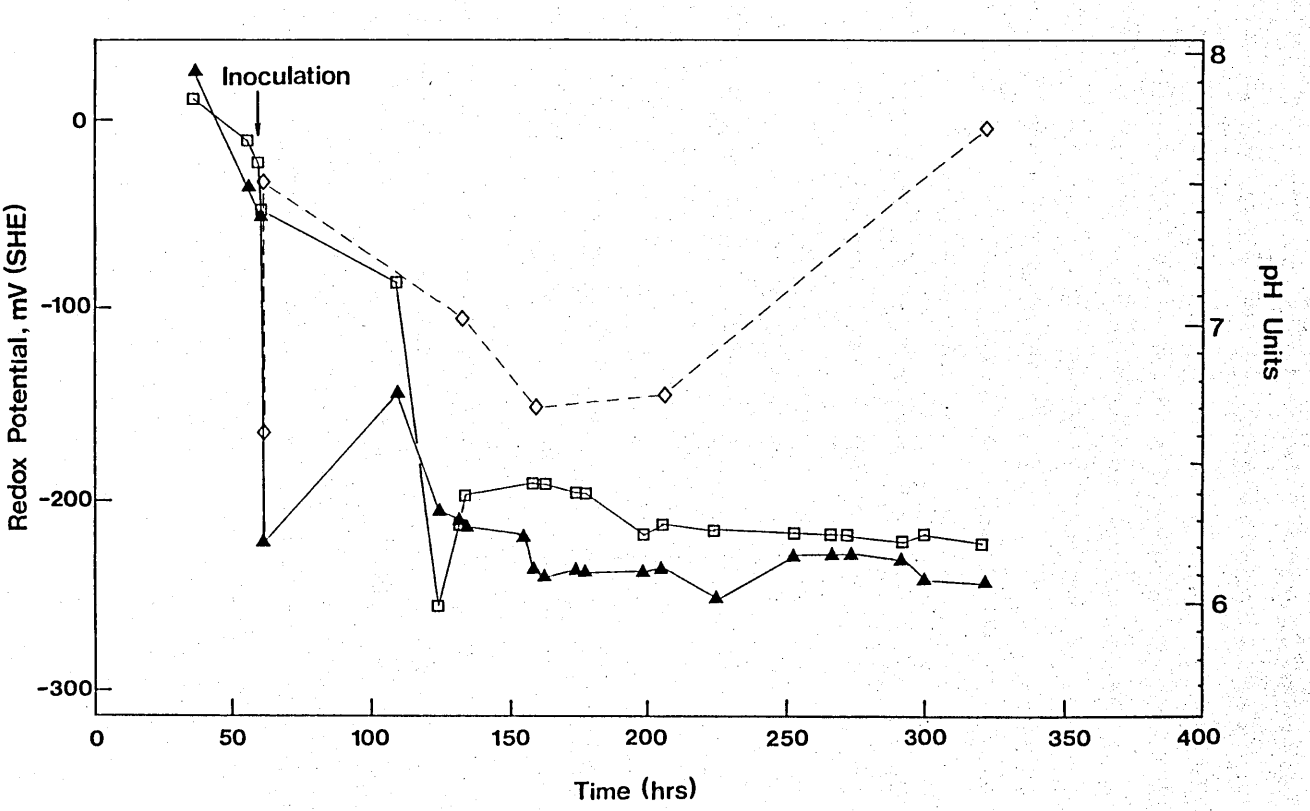
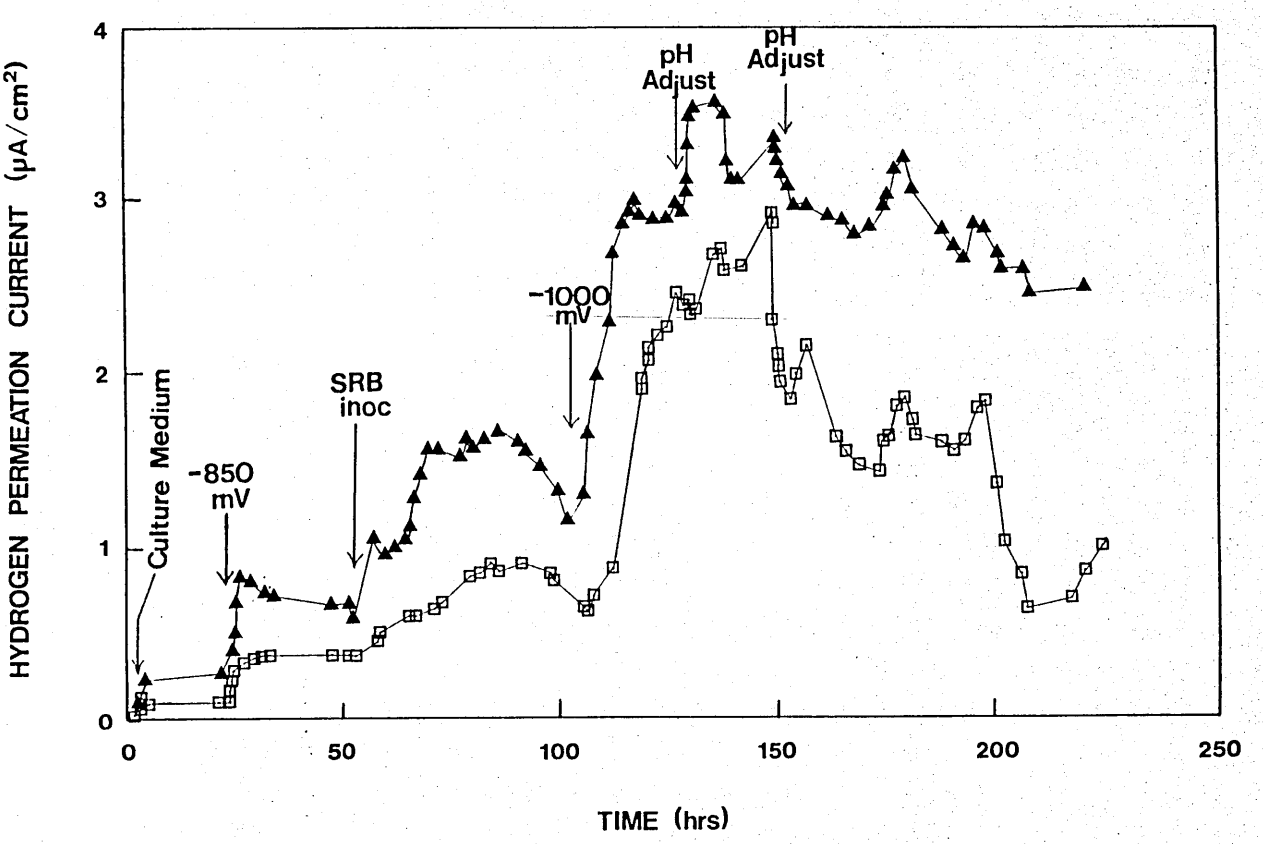

Redox Potential, mV (SHE)
pH Units
Time (hrs)
Inoculation
0
-100
-200
-300
6
7
8
0
50
100
150
200
250
300
350
400

Figure 7.9 Transient of hydrogen permeation current density generated during Run O in shim steel foils in direct contact with (▲) and screened from cells (□) in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C, while charged cathodically at -850mV (SCE) prior to SRB inoculation, and -1000mV (SCE) 50 hours thereafter.


HYDROGEN PERMEATION CURRENT (μA/cm²)
TIME (hrs)
Culture Medium
-850 mV
SRB inoc
-1000 mV
pH Adjust
pH Adjust
0
1
2
3
4
0
50
100
150
200
250

In contrast to the characteristic declines in hydrogen permeation current obtained in experiments conducted at the free corrosion potential (Runs A to E, J, K & L; figures 7.1, 7.3, 7.5a & 7.6a; pages 130, 139, 148 & 152) for the cathodically protected foils, the overall rates of increase in current density greatly exceeded subsequent rates of decline after the attainment of peak values (Runs M, N & O; figures 7.7a, 7.8a & 7.9; pages 157, 158 & 159).

Three consecutive increases in hydrogen permeation current were detected after the introduction of culture media in Run O, corresponding to respective changes in potential of the steel specimens (from the corrosion potential to -850mV, SCE), followed by inoculation, and an increase in cathodic charging of steel from -850mV to -1000mV (SCE). As in all other experiments employing dialysis membranes, changes in hydrogen permeation current in the presence of SRB reflected those obtained in the absence of bacteria (allowing for a time delay between changes in current on either side of the membrane, which was supposed to be related to the rate of diffusion of corrosive factors from one bioreactor chamber to the other).

There was some variation in levels of hydrogen permeation current following the application of cathodic protection (table 7.9, p156). During Runs M and N though, this variation became less significant as a proportion of

total current density, and following SRB growth and the attainment of peak values, similar current densities were observed within pairs of foils. In Run O though, the difference between current generated in screened and unscreened foils at -850mV (SCE) persisted during SRB growth. At this potential and after SRB growth, the highest values attained in Run O were considerably lower than comparable peaks obtained at -850mV in the two other experiments. The subsequent increase in cathodic potential to -1000mV (SCE) resulted in a two fold and three fold increase in hydrogen permeation current for unscreened and screened foils respectively. However, the overall peak values at -1000mV (SCE) were similar to those obtained previously at -850mV (SCE) (Runs M and N).

7.4.2 Corrosion of Cathodically Charged Specimens.

The first of the two experiments employing cathodic protection at -850mV (SCE) ended following the perforation of the steel foil screened from SRB (Run M), which was found to have corroded preferentially around the outer circumference of the exposed specimen area. A similar localised pattern of corrosion was observed in specimens resulting from Run N, and the foils examined from each of the two experiments were filmed unevenly with a non-uniform black sulphide-containing material. Weight losses recorded for specimens at -850mV (SCE) in the presence and

absence of SRB were 2.84mdd and 3.11mdd respectively Run M). Accurate weight loss determinations from the other experiments (Runs M and O) were precluded however, owing to a combination of factors including perforation of the foil specimen (Run M) and the development of an air leak in the apparatus (Run O).

7.4.3 Changes in pH and Redox Potential During SRB Growth

During SRB growth and H_2S production (as indicated by culture blackening and a decline in culture redox potential), the pH in each of Runs M and N declined by 0.7 and 0.8 pH units respectively (figure 7.7b, p157 & figure 7.8b, p158). Approximately 100 hours after inoculation in each case, the pH began to increase back towards the pre-inoculation values. In Run O though, the increase in alkalinity was more rapid. Consequently, adjustments were made to the pH, from 8.2 units to 7.5 units at 132 hours, and a further adustment from 8.3 to 7.5 units after 151 hours (figure 7.9, p159). Small but temporary increases in hydrogen permeation current were effected by these pH adjustments.

Following inoculation, SRB growth (as indicated by either an increase in cell numbers or by a blackening of the culture medium) was noted after 22 hours (Run M) and 5 hours (Run O). However, 30 hours into experimental Run N,

and before SRB inoculation, contaminant motile rods (1.2 x 12μm) were detected within one of the two bioreactor chambers. These attained a maximum cell density of 5 x 10^6/ml just prior to the introduction of SRB (culture blackening and H_2S evolution had not occurred at this stage). Although the numbers of contaminants declined thereafter, the SRB inoculum failed to grow, and it was found that the pH had declined from an initial value of 7.5 to to 6.6 units during the period of contaminant growth. The pH was therefore adjusted to 7.5 units (concentrated NaOH), and a further SRB inoculum was introduced into the contaminated bioreactor chamber. From repeated microscopic examinations of the culture medium, SRB growth was seen to become established within 48 hours of inoculation, and after a further 12 hours, the contaminant organism was found to have disappeared. At 94 hours after inoculation, the SRB cell density had increased to 10^8/ml , and viable counts made at 138 hours and 260 hours indicated cell densities of 7.5 x 10^8/ml and 10^9/ml respectively. In the same experiment, the 'sterile' chamber also became contaminated, (vibrios, 0.6 x 0.8μm; and also rods, 0.8 x 4μm; maximum total density of approximately 10^6/ml), though the numbers of contaminants was seen to decline following SRB growth.

In the presence of steel protected to -1000mV (SCE), contaminant rods (at approximately 10^4/ml) were detected

in the chamber screened from the test organism. Whilst vigorous SRB growth was seen in previous cathodic protection experiments, in Run 0, SRB cell numbers at -850mV (prior to increasing to -1000mV, SCE) did not increase above 10^7/ml, the culture exhibited little motility and pleiomorphic cell forms predominated (contaminant growth was thought to have depleted the carbon source, which would account for the reduced SRB growth and H_2S evolution, and also the relatively low hydrogen permeation currents generated). Although an equivalent quantity of lactate (4.5ml of 50% solution) to that present at the start of the experiment was injected into each bioreactor chamber (whilst adjusting to pH 7.5), no further increase in growth was observed before the experiment was terminated.

7.5 THE INFLUENCE OF SRB ON THE POLARISATION OF STEEL

The potentiostatic polarisation curves obtained from the two series of polarisation experiments conducted are shown in figures 7.10a-g and 7.11a-g, on pages 167-170 and 171-174 (the way in which results were treated and presented was explained in section 6.5, p126). In general, the potential versus current plots for each individual steel specimen indicated an overall depolarisation of the cathodic reaction (figures 7.10a & b, p167 and figures

7.11a & b, p171). Changes in anodic activity were less clear though. While the overall rate of change of anodic current decreased in each case, at lower potentials the actual anodic current density increased with exposure to the corrosive media. The rest potential became more cathodic in each case, by between -20mV and -80mV of the initial value obtained in sterile culture medium.

Prior to inoculation, the duplicate specimens exposed to culture medium exhibited little variation in polarisation behaviour (figure 7.10c, p168 and figure 7.11c, p172). However, such variation increased over the total duration of each experiment (190 hours and 360 hours after inoculation and SRB growth; figures 7.10d/e, p168/169; and figures 7.11d/g, p172 & p174 respectively). In the first experiment, SRB growth was delayed (section 7.4.2), and the results shown (figures 7.10a-g, pages 167-170) refer to the time after onset of growth (48 hours after inoculation). Specimens exposed to SRB were generally less active anodically and cathodically than screened specimens, although in one of the 'spare' pair of specimens (used in final scan only), this effect was not apparent (figure 7.11g, p174).

The polarisation characteristics of the two similar pairs of screened and unscreened specimens 360 hours after SRB inoculation are shown in figures 7.11e and 7.11f (p173). While there was a small difference in cathodic curves for

specimens exposed to SRB (figure 7.11e), the positions of anodic and cathodic curves differed substantially for the two specimens screened from the bacteria (figure 7.11f). However, there was little overall variation in the final slopes of anodic and cathodic curves obtained.

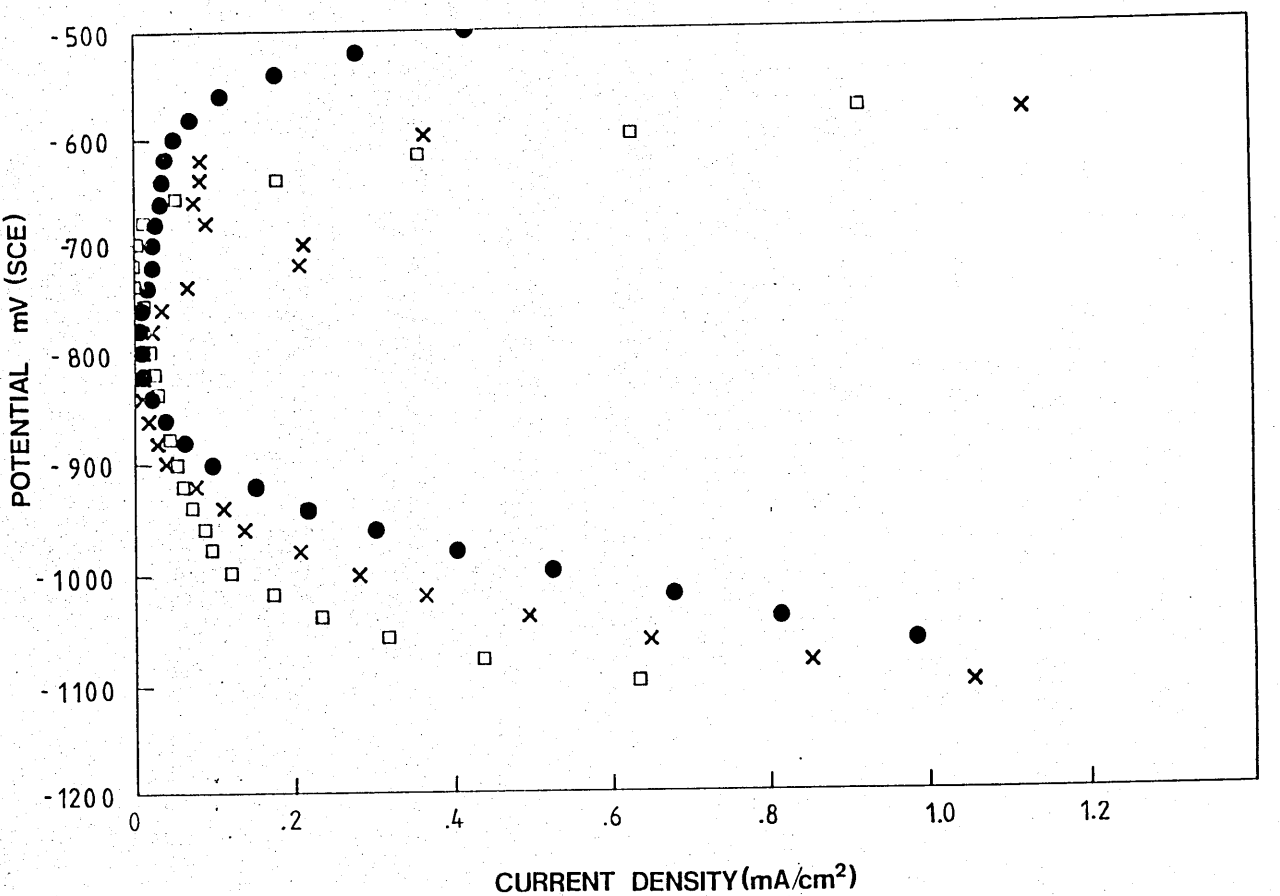
Using the method of calculation described previously (section 6.5), corrosion rates were deduced from the second series of polarisation diagrams (shown in figures 7.11a, 7.11b and 7.11g on pages 171 & 174; and table 7.10, below). Tafel regions of individual plots were less clearly defined in the first experiment, and were therefore not used for this purpose.

Table 7.10 Corrosion rates determined from potentiostatic polarisation diagrams for mild steel in the presence (SRB) and absence (-) of a batch culture of Desulfovibrio desulfuricans (Norway-4) in Postgate's Medium 'C' at 30 C, for up to 360 hours.

Figure	Specimen	Exposure time (hours)	Log Icorr (nA/cm^2)	Corr rate (mdd)
7.11a	(SRB)	0	3.5	16
7.11a	(SRB)	24	4.6	200
7.11a	(SRB)	360	3.8	32
7.11b	(-)	0	3.3	10
7.11b	(-)	24	3.5	16
7.11b	(-)	360	4.3	100
7.11g	(SRB)	360	3.8	32
7.11g	(-)	360	3.8	32

Figure 7.10a Polarisation curves obtained during the exposure of steel to a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C, before inoculation of sterile medium (□), and at 48 hours (×) and 190 hours (•) after the onset of SRB growth.

Figure 7.10b Polarisation curves obtained for steel specimens screened from bacterial cells by dialysis membrane, during the growth of a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C, before inoculation of sterile medium (□), and at 48 hours (×) and 190 hours (•) after the onset of SRB growth.


-500
-600
-700
-800
-900
-1000
-1100
-1200
POTENTIAL mV (SCE)
0
.2
.4
.6
.8
1.0
1.2
CURRENT DENSITY (mA/cm²)

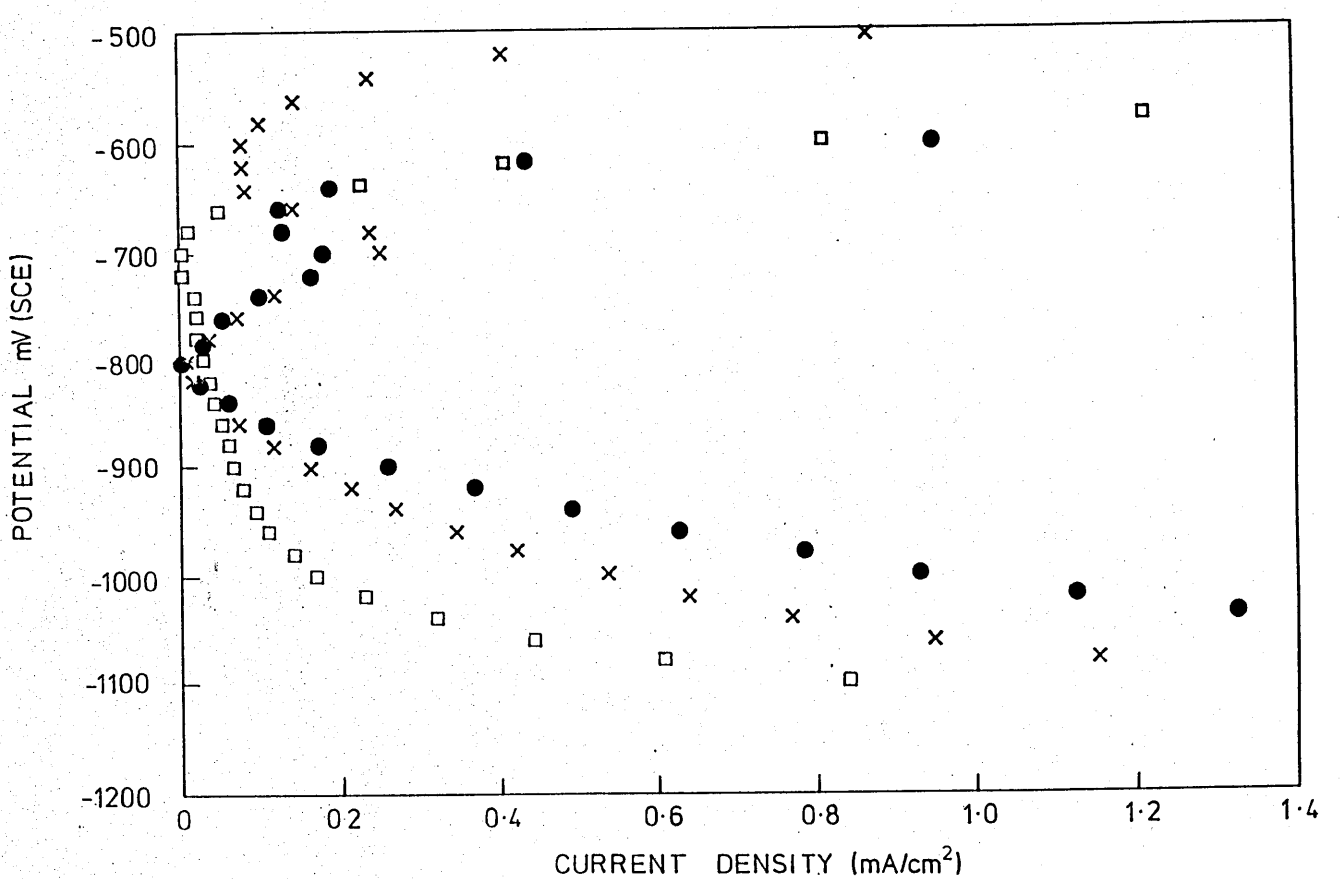
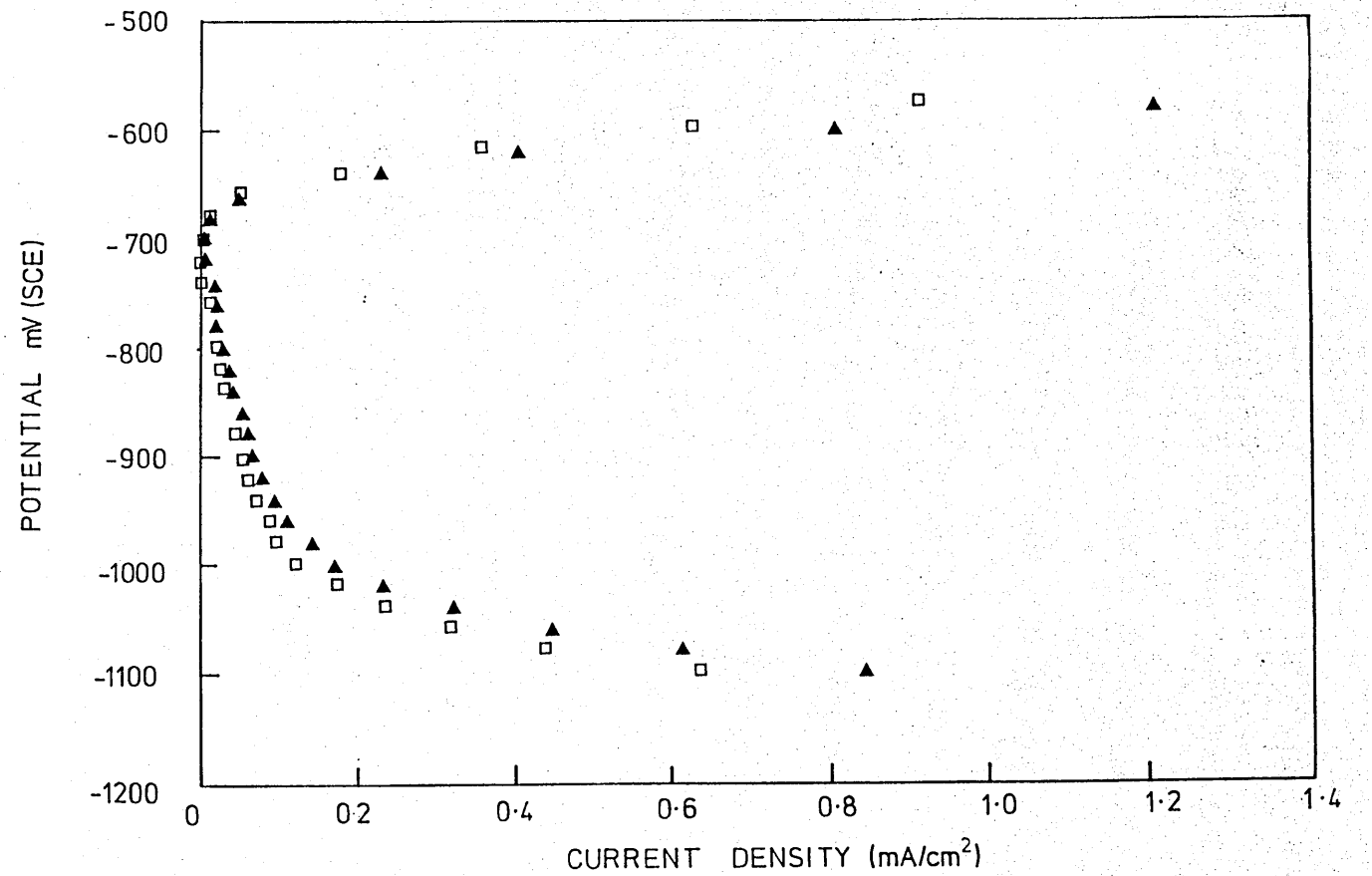

-500
-600
-700
-800
-900
-1000
-1100
-1200
POTENTIAL mV (SCE)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
CURRENT DENSITY (mA/cm²)

Figure 7.10c Comparison between polarisation curves obtained prior to inoculation, for steel specimens exposed to sterile PMC Medium.

Figure 7.10d Comparison between polarisation curves obtained 190 hours after the onset of SRB growth in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C, for the same pair of specimens as in figure 7.10c, when exposed to SRB cells (▲) and when screened from SRB cells by dialysis membrane (□).


-500
-600
-700
-800
-900
-1000
-1100
-1200
POTENTIAL mV (SCE)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
CURRENT DENSITY (mA/cm²)

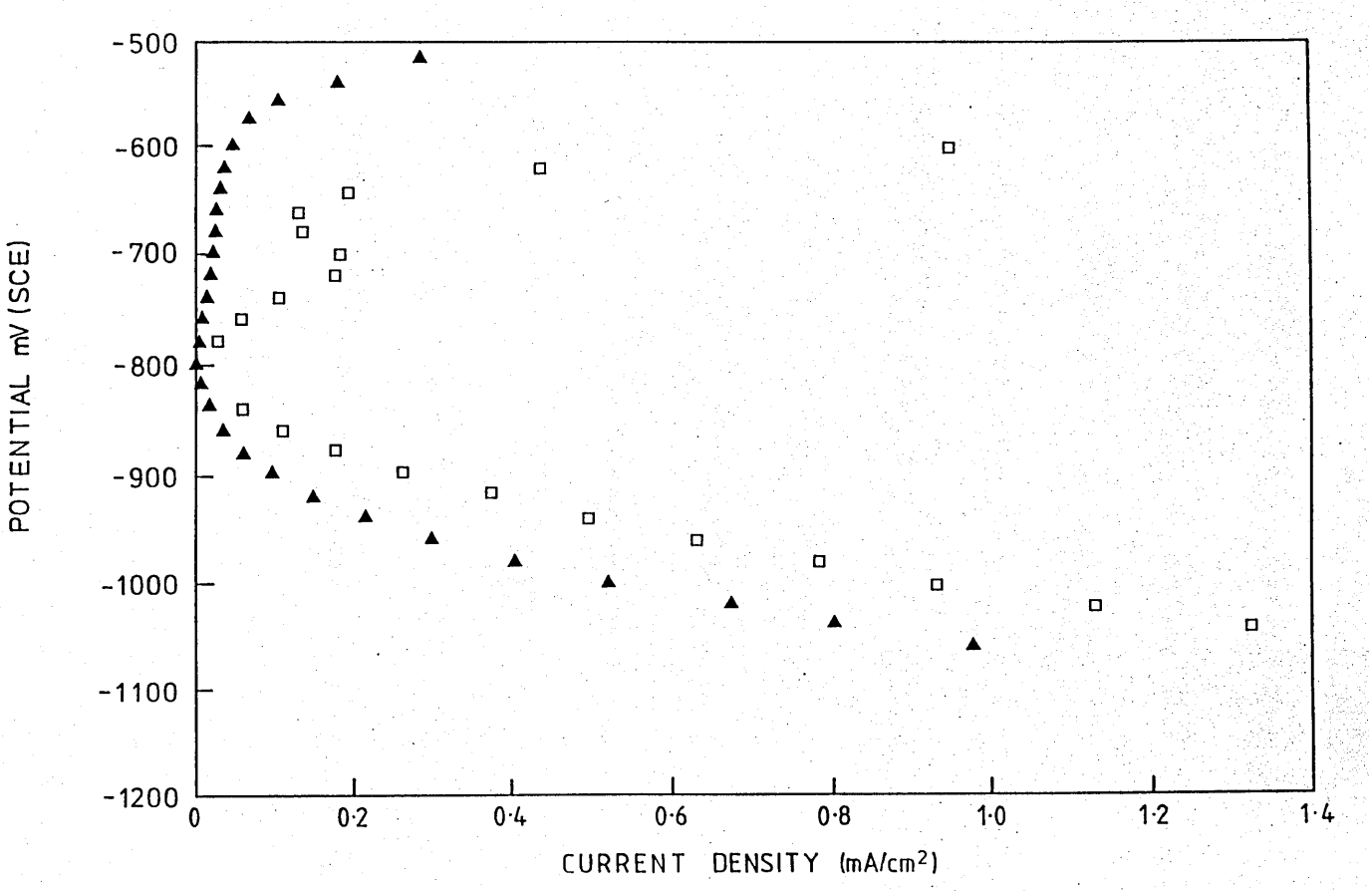

-500
-600
-700
-800
-900
-1000
-1100
-1200
POTENTIAL mV (SCE)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
CURRENT DENSITY (mA/cm²)

Figure 7.10e Polarisation curves obtained for a replicate pair of specimens to those used in figures 7.10a-d, obtained 190 hours after the onset of SRB growth in a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C, for steel exposed to SRB cells (▲) and screened from cells by dialysis membrane (□)

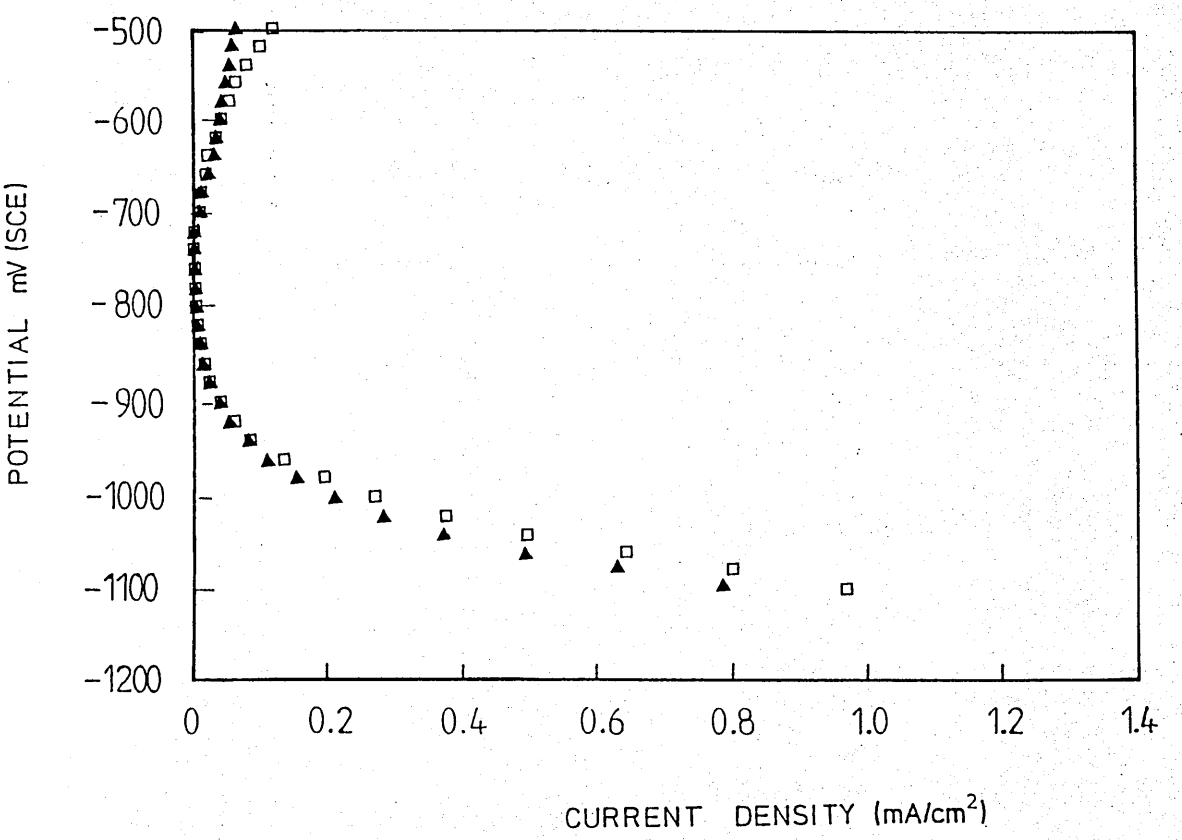
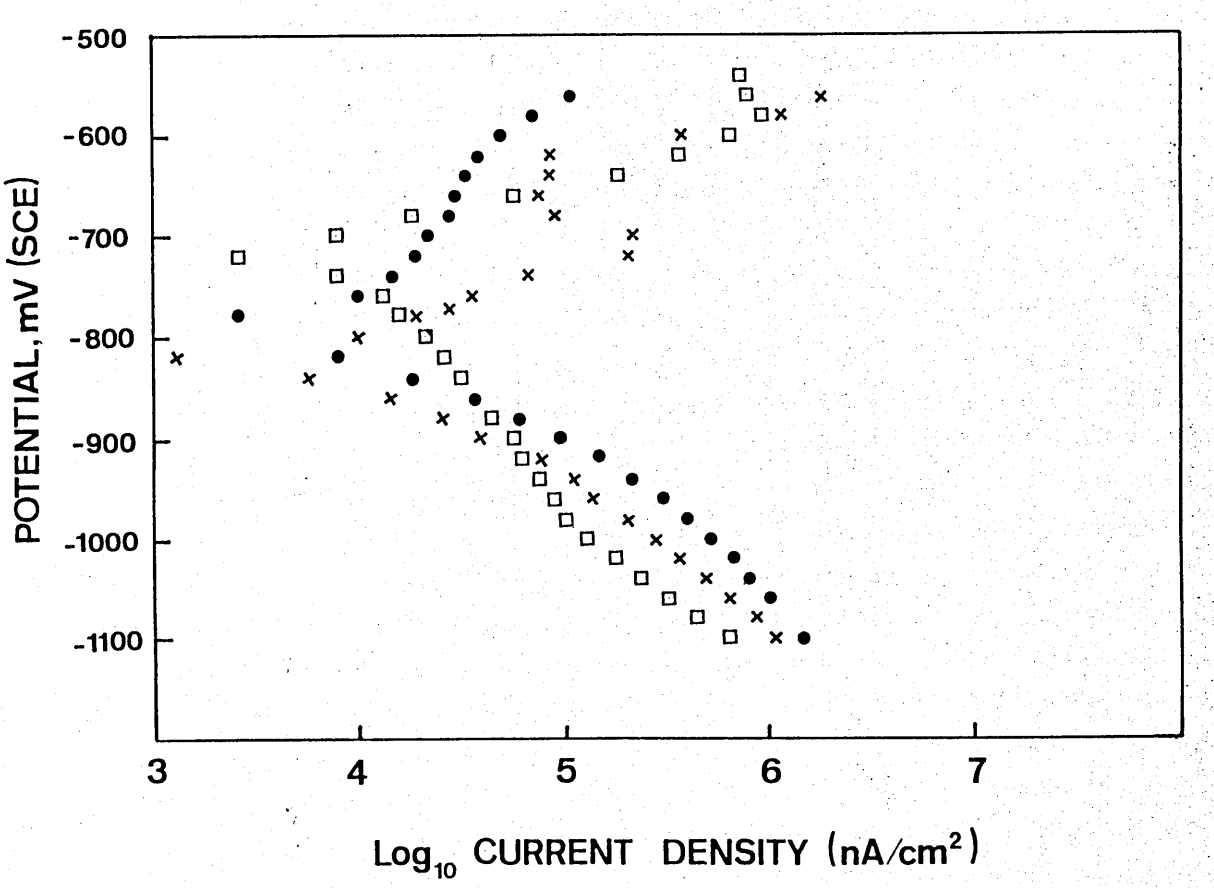

-500
-600
-700
-800
-900
-1000
-1100
-1200
POTENTIAL mV (SCE)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
CURRENT DENSITY (mA/cm²)

Figure 7.10f Polarisation curves obtained during the exposure of steel to a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C, before inoculation of sterile medium (□), and 48 hours (×) and 190 hours (●) after the onset of SRB growth.

Figure 7.10g Polarisation curves obtained for steel specimens screened from bacterial cells by a dialysis membrane during the growth of a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium/30 C, before inoculation of sterile medium (□), and at 48 hours (×) and 190 hours (●) after the onset of SRB growth.


-500
-600
-700
-800
-900
-1000
-1100
POTENTIAL, mV (SCE)
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

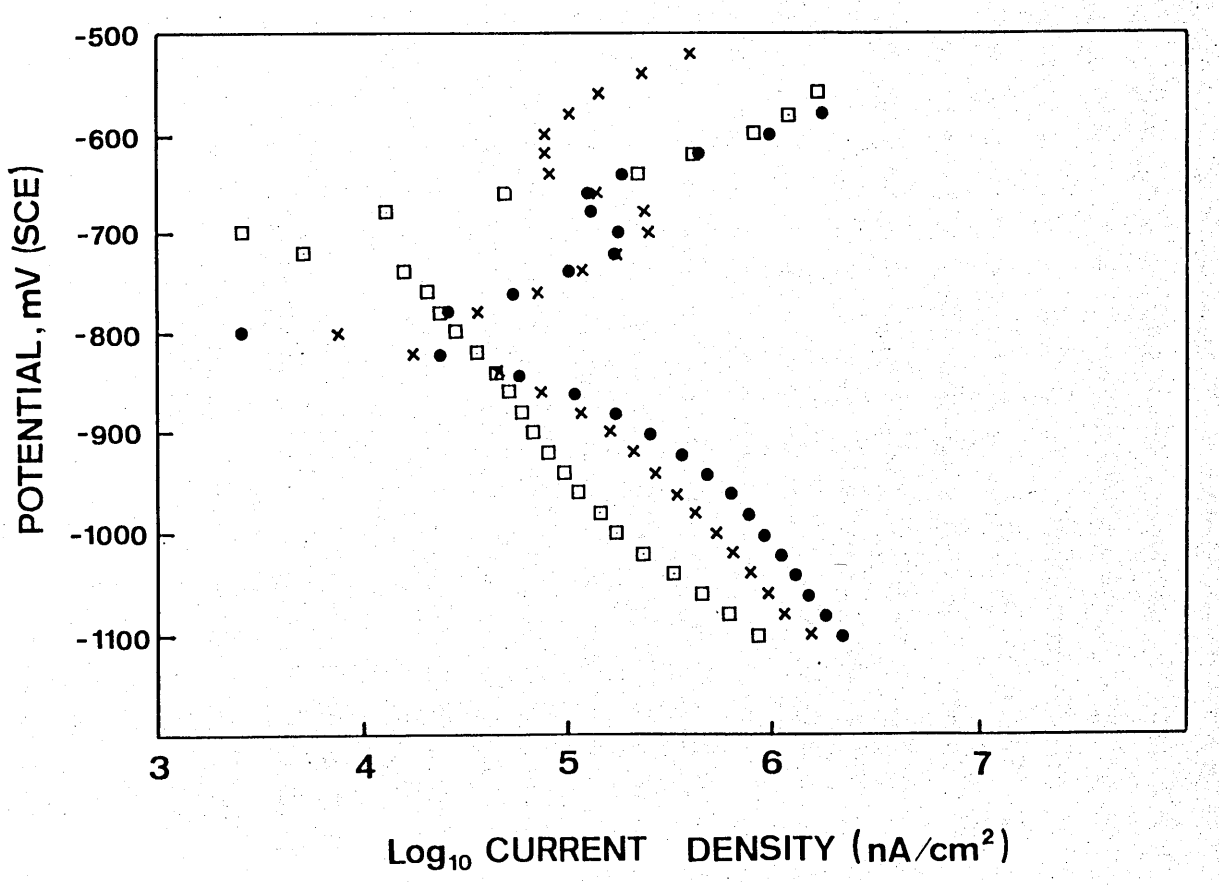
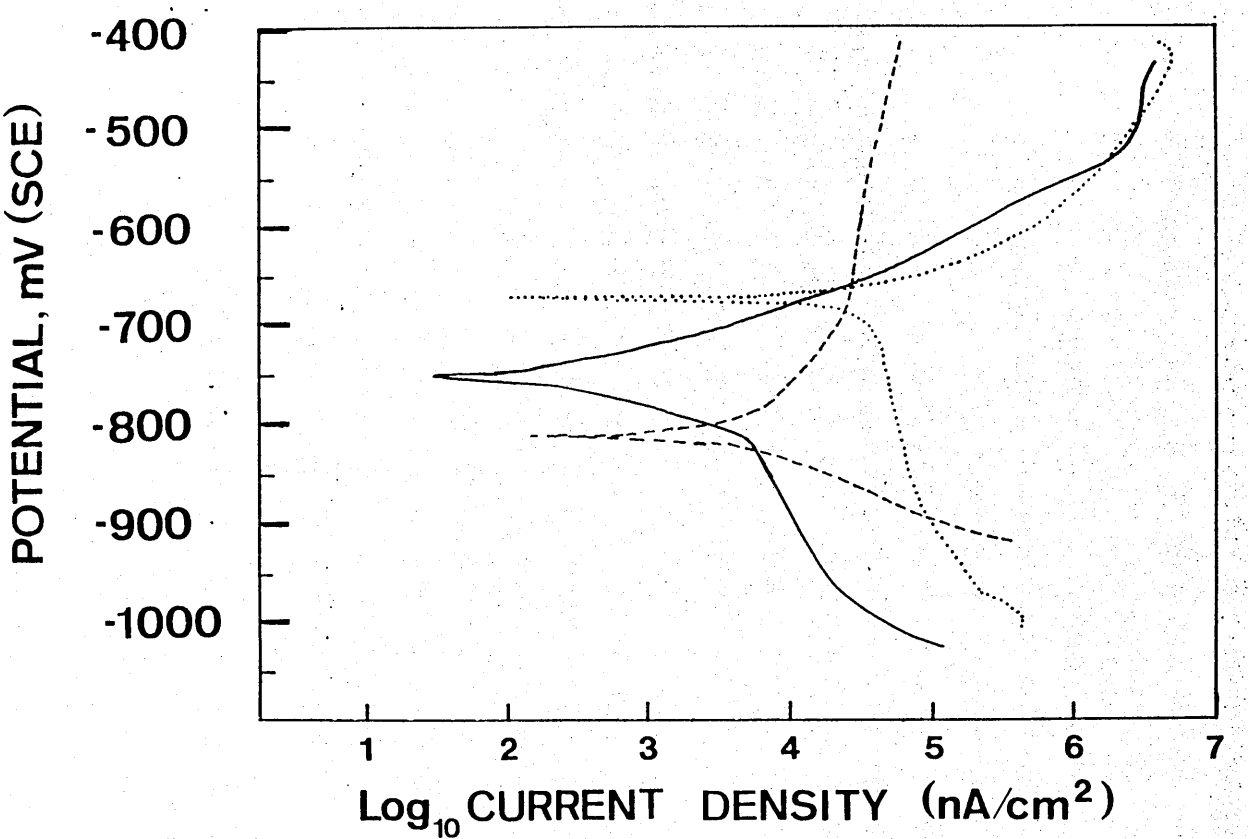

-500
-600
-700
-800
-900
-1000
-1100
POTENTIAL, mV (SCE)
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

Figure 7.11a Polarisation curves obtained during the exposure of steel to a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C, before inoculation of sterile medium (——), and at 24 hours (····) and 360 hours (- - -) after inoculation.

Figure 7.11b Polarisation curves obtained for steel specimens screened from bacterial cells by dialysis membrane, during the growth of a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium/30 C, prior to the inoculation of sterile medium (——), and at 25 hours (····) and 360 hours (- - -) after inoculation.


-400
-500
-600
-700
-800
-900
-1000
POTENTIAL, mV (SCE)
1
2
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

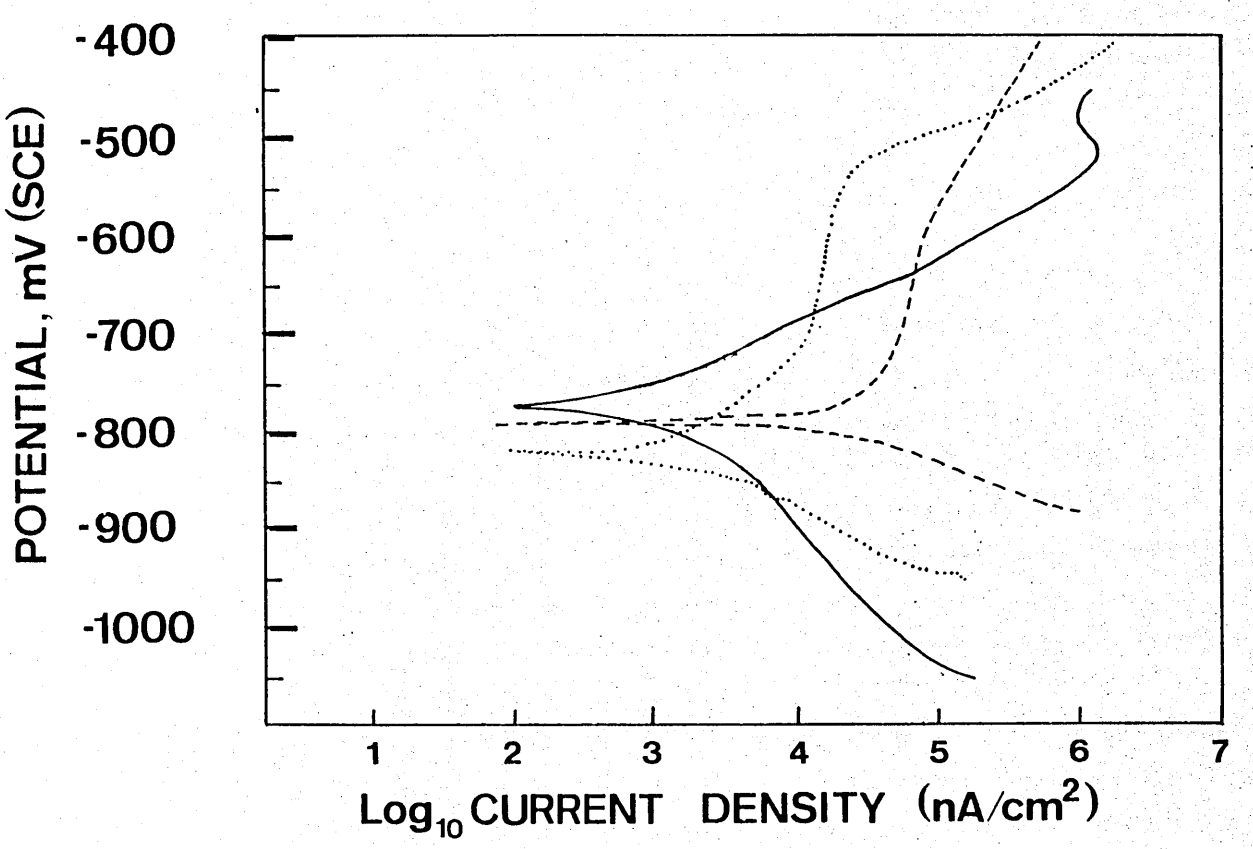
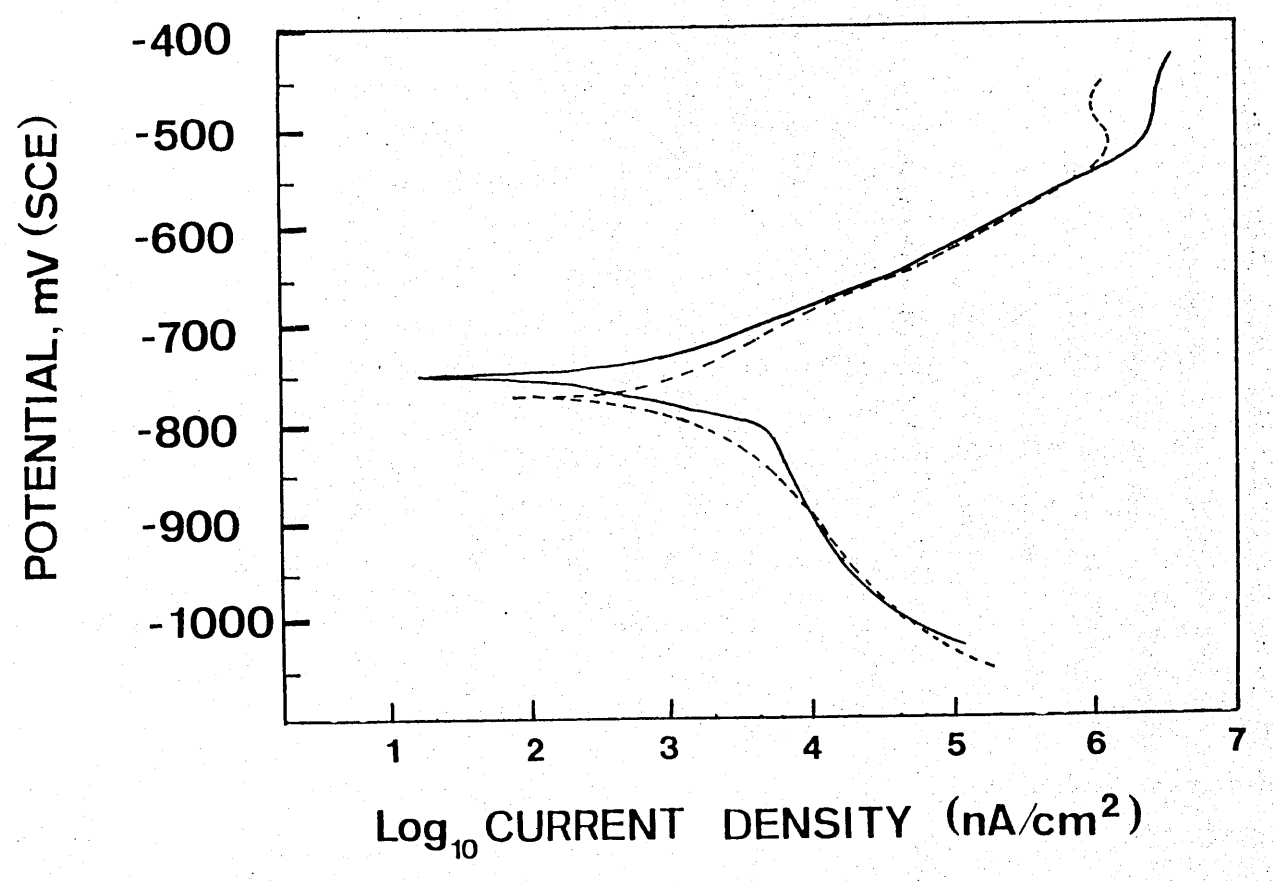

-400
-500
-600
-700
-800
-900
-1000
POTENTIAL, mV (SCE)
1
2
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

Figure 7.11c Comparison between polarisation curves obtained prior to inoculation, for a pair of steel specimens exposed to sterile PMC Medium.

Figure 7.11d Comparison between polarisation curves obtained approximately 360 hours after inoculation of a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C, for the same pair of steel specimens as in figure 7.11c, in the presence of SRB cells (—) and screened from cells by dialysis tubing (- - -).


-400
-500
-600
-700
-800
-900
-1000
POTENTIAL, mV (SCE)
1
2
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

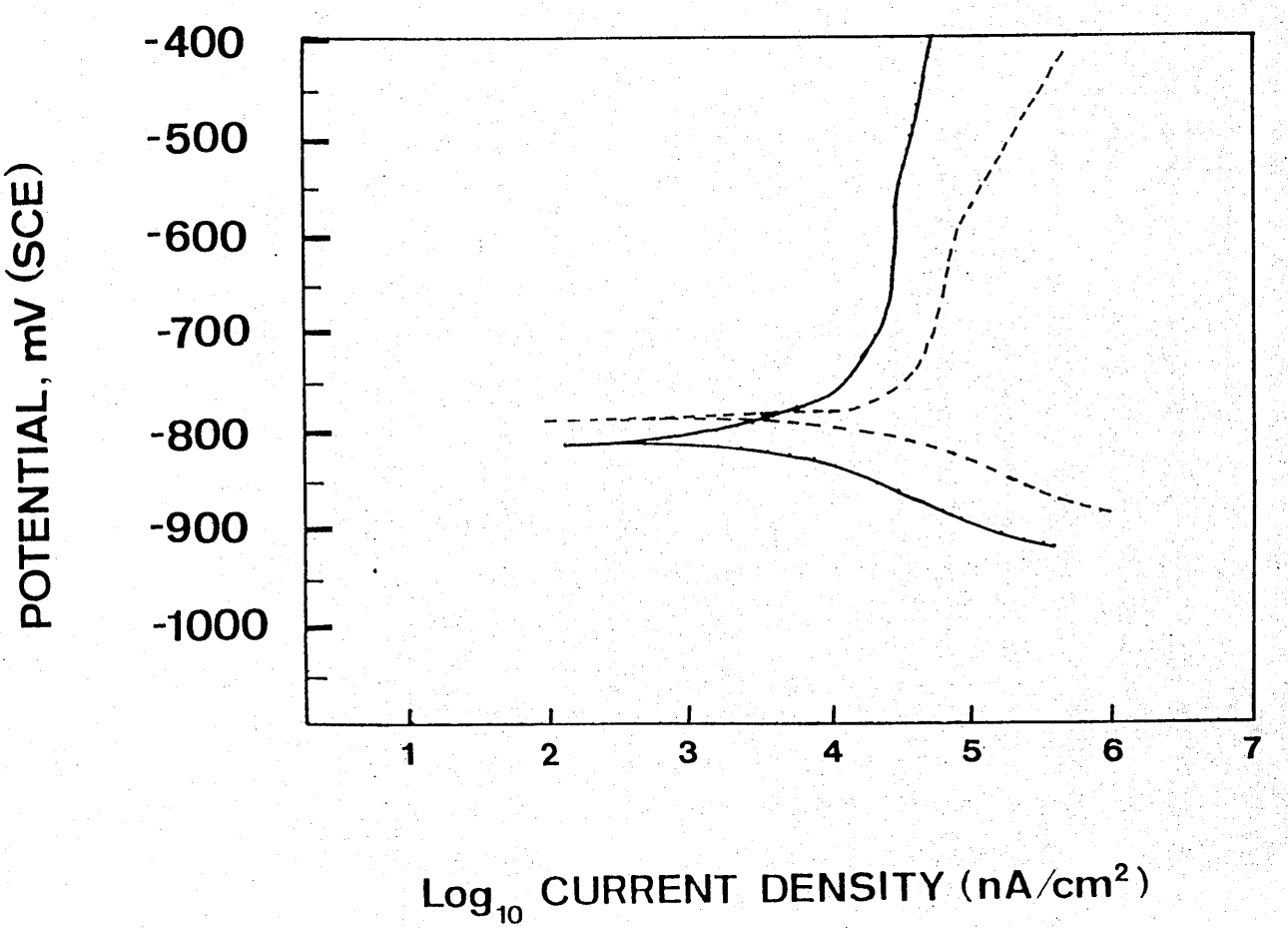
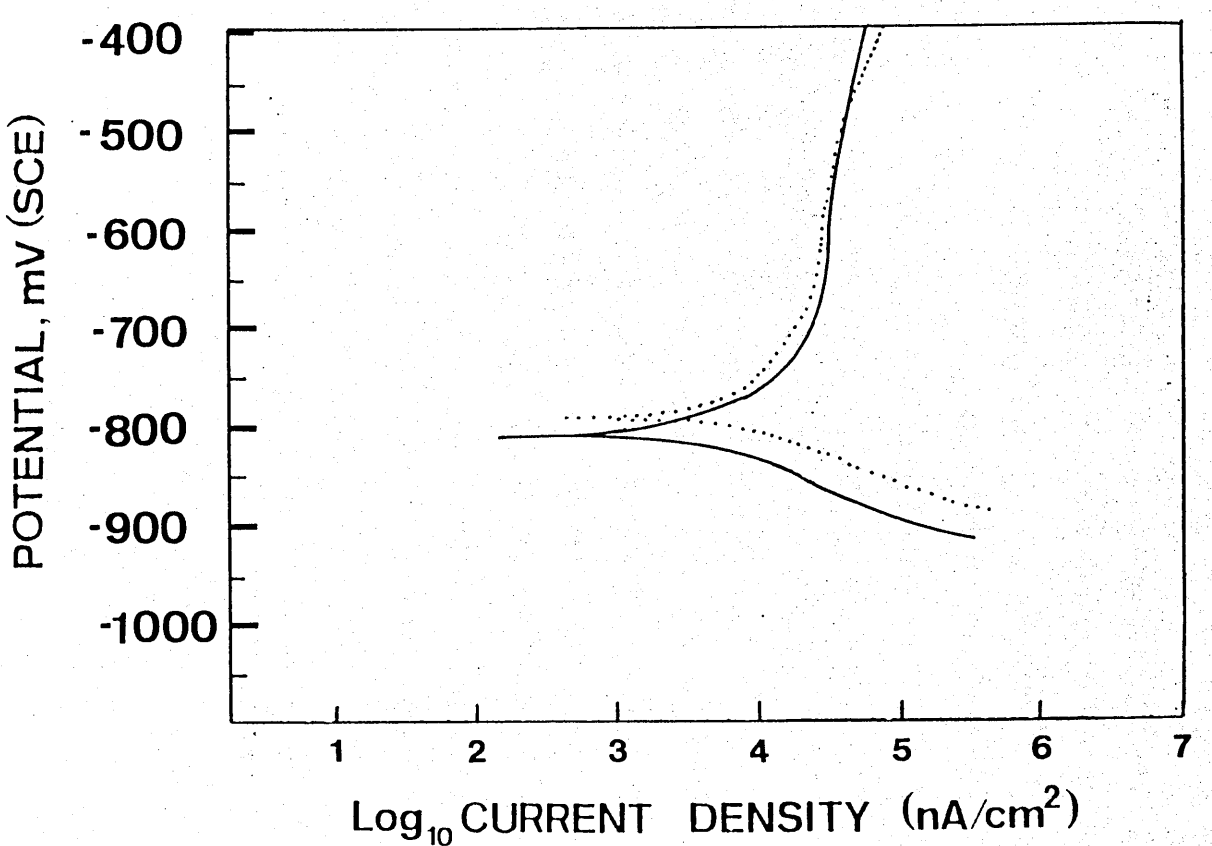

-400
-500
-600
-700
-800
-900
-1000
POTENTIAL, mV (SCE)
1
2
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

Figure 7.11e The difference between polarisation curves obtained for two similar steel specimens exposed to a SRB cells for approximately 360 hours, within the same batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C.

Figure 7.11f The difference between polarisation curves obtained for similar steel specimens screened from SRB by dialysis tubing, approximately 360 hours after inoculation of a batch culture of Desulfovibrio desulfuricans (Norway-4) in PMC Medium at 30 C.


-400
-500
-600
-700
-800
-900
-1000
POTENTIAL, mV (SCE)
1
2
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

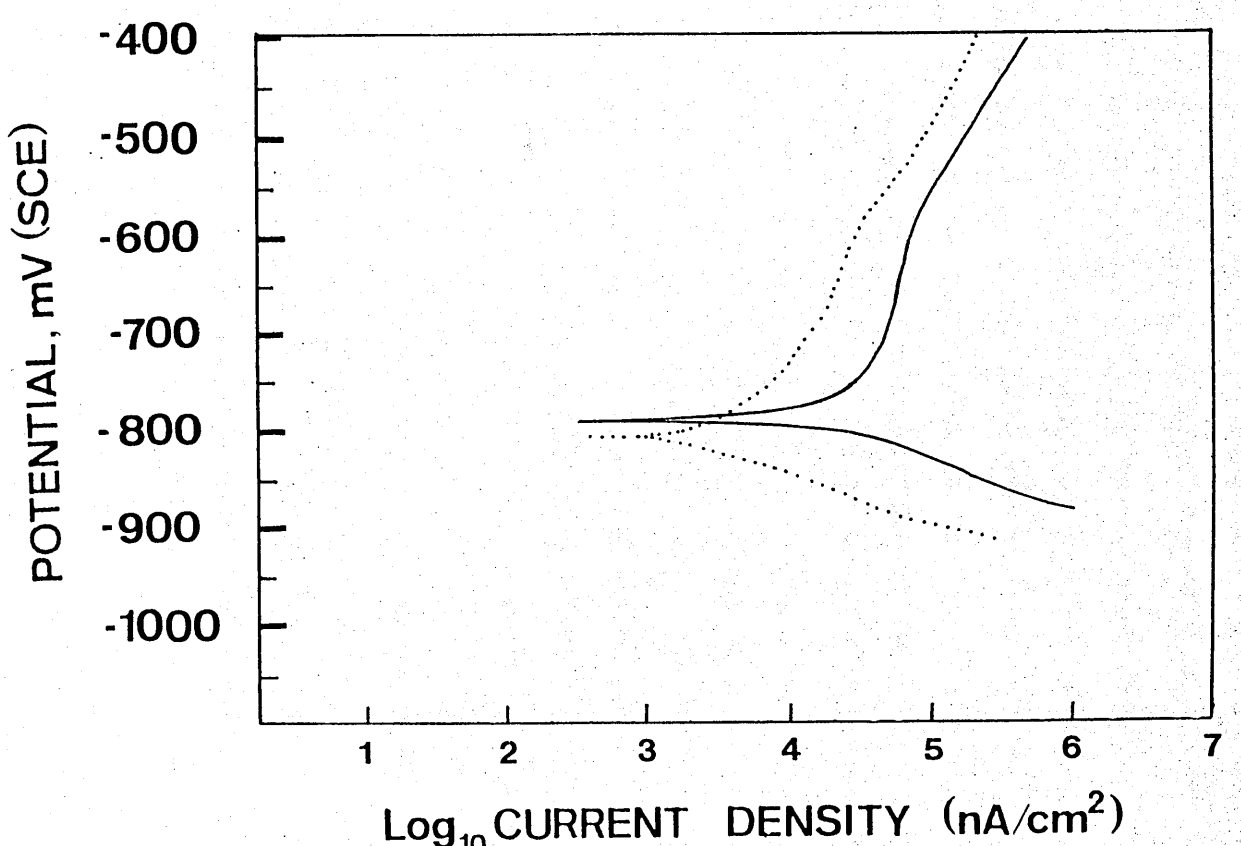
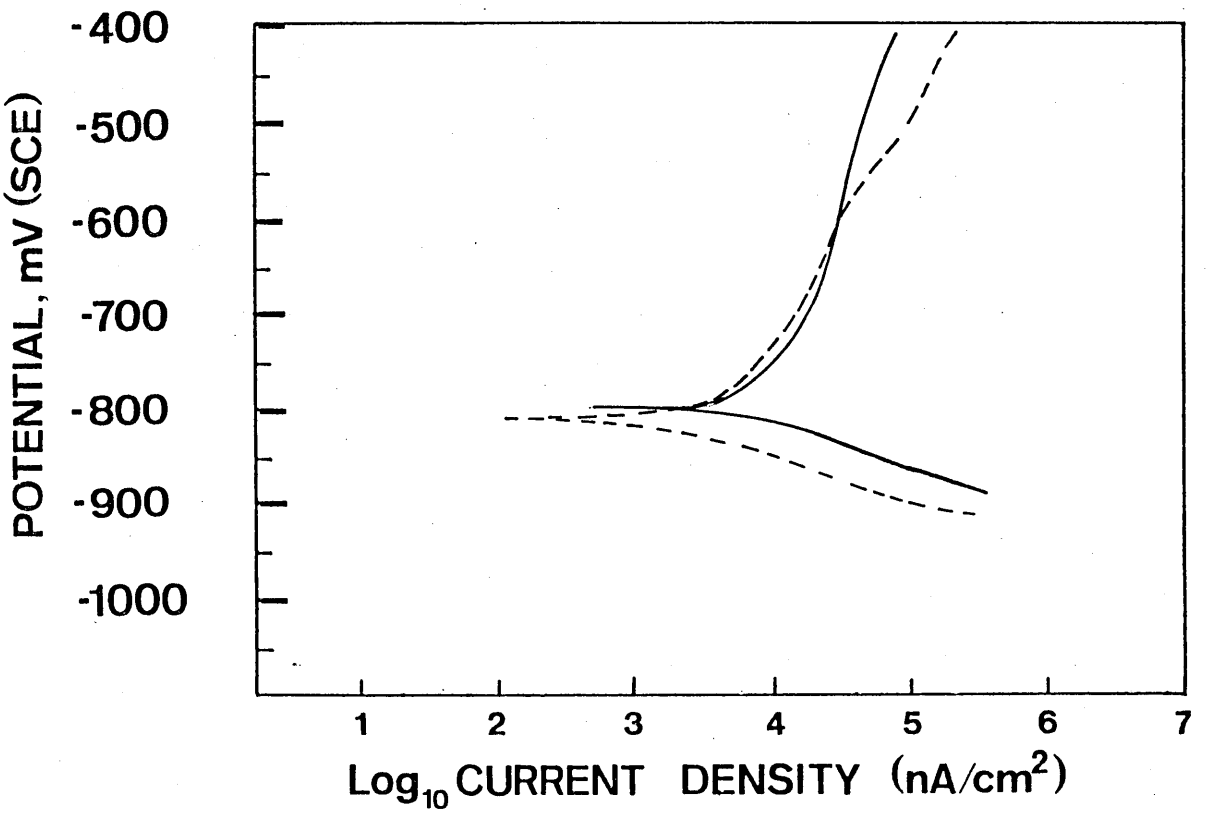

-400
-500
-600
-700
-800
-900
-1000
POTENTIAL, mV (SCE)
1
2
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

Figure 7.11g Polarisation curves obtained for a replicate pair of steel specimens to those shown in figure 7.11b, when screened from (- - -) and exposed to (——) cells in a batch culture of Desulfovibrio desulfuricans (Norway-4) for approximately 360 hours in PMC Medium/30 C.


-400
-500
-600
-700
-800
-900
-1000
POTENTIAL, mV (SCE)
1
2
3
4
5
6
7
Log10 CURRENT DENSITY (nA/cm2)

8 DISCUSSION

8.1 INTRODUCTION

8.1.1 Relationships Between Hydrogen Absorption and SRB Activity.

During SRB growth, dissimilatory sulphate reduction proceeds with the development of a reactive and chemically complex mixture of dissolved metabolites. A number of volatile reactive hydrocarbons may also be produced, together with the evolution of a range of gases, essentially comprising CO_2 and H_2S (Hatchikian et al, 1975). Hydrogen may also be evolved, the proportion of which depends to a large extent upon the types and concentrations of carbon and energy sources available .

It is principally the reducing power of biogenic H_2S which accounts for the success of SRB in beneficially altering their surrounding environment and promoting further growth, by lowering the redox potential and establishing more widespread anaerobiosis (Postgate 1984).

In some freshwater environments in which ferrous ions occur in abundance, the quantity of sulphide ions in solution may be substantially reduced, owing to the formation of only sparingly soluble iron sulphides (Nriagu

& Hem, 1980). However, the converse situation is most likely in marine environments. Dissolved iron typically exists in sea water at only sub micromolar concentrations, and ferrous ions would be quickly mopped up by reaction with the H_2S produced by SRB, which in a variety of marine situations has been reported to occur at millimolar concentrations or more (Edyvean, 1985). The consequent excess dissolved H_2S generated within active SRB populations may then have a considerable influence on the redox potential, owing to the reducing properties of the HS^- ion (Nriagu and Hem, 1980). This would apply especially where microbial communities were shielded from the bulk ocean environment, for example beneath a layer of macrofouling organisms.

In addition to lowering the redox potential, H_2S evolution also reduces the pH, owing to its dissociation thus:

$$H_2S = HS^- + H^+$$

which links inextricably the factors of SRB growth, H_2S evolution, redox potential and pH, the latter two factors of which are partly interdependent. Therefore when interpreting the effects of a dynamic SRB culture system on corroding steel, it is clearly very useful to be able to observe changes in all four variables simultaneously.

When an SRB population is introduced to corroding steel, the inhibitory effects of H_2S may become reduced, owing to the formation of insoluble iron sulphides. The changes in pH and redox potential (Eh) which occur in the absence of metal may thus be more conducive to SRB growth. In addition though, cathodic hydrogen generated at the corroding metal surface is thought to serve as an energy source for the bacteria, thus promoting further H_2S evolution and increasing the aggressiveness of the environment towards the metal (figure 8.1, below). While promoting corrosion and cathodic hydrogen evolution, biogenic H_2S also serves to promote hydrogen absorption by poisoning the hydrogen combination reaction. The rate of such hydrogen absorption is thus ultimately dependent upon all of the interactive factors which control SRB activity and H_2S evolution. With this complex situation in mind, the influences of SRB growth on hydrogen absorption will be discussed in the following sections.

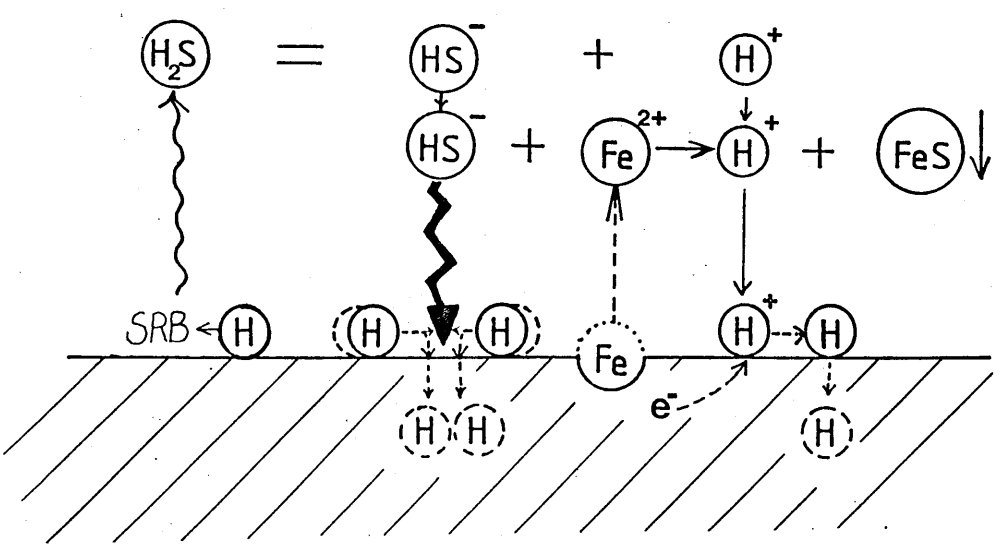


Figure 8.1 Some of the relationships between H_2S dissociation, pH, Eh, SRB growth and corrosion.

8.1.2 Some Initial Considerations of Hydrogen Permeation Transients Generated During SRB Growth.

Figure 7.5a (Run K, p148) and figure 7.6a (Run L, p152) illustrate the separate components of typical hydrogen permeation transients generated by consecutive additions of culture media and SRB inoculum to hydrogen depleted steel. In figure 7.1a & b though (Runs A to E, p130), the influences of culture media and sulphate-reduction (as indicated by culture blackening) have become superimposed, except in Run C where culture blackening only became evident some 4 days following initial exposure of the steel to SRB cells. The delayed activity of cells probably arose from an excessive aeration of inoculated media during transfer into the bioreactor chamber, wherein a redox potential favouring SRB growth will have been attained following the removal of dissolved oxygen by aerobic corrosion occurring thus:

$$O_2 + Fe + 2H_2O \longrightarrow 4OH^- + Fe^{2+} + 6e^-$$

The deaeration of media might also have been caused by the growth of aerobic contaminant organisms, although no such evidence was revealed from microscopic examination of either freshly inoculated or final soured media.

A possible separate influence of SRB over and above the corrosiveness of culture media is suggested from the initial gradients of hydrogen permeation transients (Runs A to E, figures 7.1a and 7.1b, p130) and is illustrated schematically in figure 8.2, below).

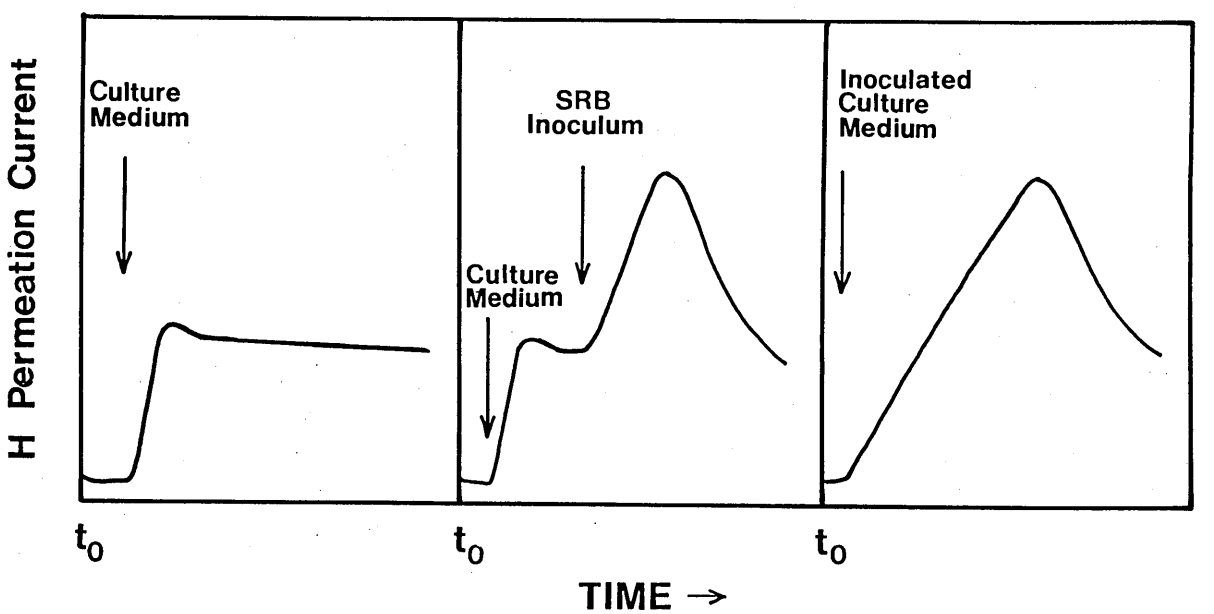


Figure 8.2 Components of hydrogen permeation transients arising from the separate and combined influences of SRB and culture media.

The rate of increase in hydrogen permeation current is lower following the introduction of inoculated culture media than it is when steel is exposed to culture media alone, suggesting that the presence or activity of SRB in some way moderates the rate of hydrogen absorption due to

culture media. The subsequently generated hydrogen permeation peaks and declines indicate that any such depressive influences on hydrogen absorption @persisted throughout the remainder of the experiments conducted.

If the primary source of diffusible hydrogen is generated due to the cathodic anaerobic corrosion reaction, then the above effects of SRB culture on hydrogen permeation could be explained in two possible ways. Either the corrosion rate decreased, and less hydrogen became available for pick-up by steel, or the rate of corrosion continued unabated, but the proportion of hydrogen entering steel declined.

A reduction in corrosion rate could be effected by either an alteration in the aggressiveness of the corrosive environment, for example due to a decline in SRB activity and the rate of H_2S evolution, or due to the development of a surface film which confers a degree of protection to the steel. However, the former hypothesis is inconsistent with the observed coincidence between rising hydrogen permeation current and rapid SRB growth (Run L, figures 7.6a and 7.6d, pages 152 & 154). More likely is a mechanism involving the formation of protective films, which would account for the hydrogen permeation peaks and declines generated subsequent to SRB growth. The absence of any subsequent resurgence in hydrogen

permeation current would suggest that such films were persistent, although there was some variation in the coverage of films on corroded specimens, and also in the stability of current generated during the decline phase of hydrogen permeation transients.

Alternatively, the corrosion rate and therefore the rate of proton discharge may be maintained, but where the accessibility of atomic hydrogen atoms to the steel surface is lowered. This situation might be envisaged where a film of corrosion product, such as ferrous sulphide acts as a cathode on which hydrogen discharge may occur. According to the thickness and properties of the film material, the proportion of diffusible atomic hydrogen present would decrease in the time taken to reach the steel surface and then gain access to the metal lattice. This hypothesis would depend upon the presence of distinct anodic zones in between filmed regions of metal, and also on a means of cathodic depolarisation. In this work, these latter conditions may to some extent have been satisfied simultaneously, as suggested by the uneven distribution of film material and SRB growth observed on some corroded specimens.

In addition to any changes in the rate of hydrogen absorption caused by a changing corrosion rate, a reduction in the accessibility of hydrogen to the steel

surface might also be effected by the presence of a hydrogen 'sink'. For instance, an important destination for cathodic hydrogen might lie within voids or 'traps' occurring within corrosion product material, or perhaps within the steel itself. However, any such forms of hydrogen sink could only be sustained by a continual removal of hydrogen, for example by means of SRB hydrogenase (King & Wakerley, 1973). Although the corollary of cathodic depolarisation is an increase in the total amount of hydrogen generated, it has been suggested that the removal of cathodic hydrogen by SRB might be sufficiently vigorous to actually cause a reduction in the quantity of hydrogen available for absorption by the metal (Walch and Mitchell, 1985).

Some consideration also needs to be given to possible alternative sources of hydrogen to that generated cathodically. Additional sources of hydrogen might be provided either by chemical reactions occurring within the bioreactor, or perhaps as a byproduct of SRB metabolism. Alterations in the rates of these latter processes may then be related to the shape of the hydrogen permeation transients generated by corroding steel in SRB culture.

Finally, there needs to be a consideration of other, non-biological influences on hydrogen permeation. For instance some variability in results may be attributable to changes in bioreactor temperature, sources of electrical noise

and physical disturbance; or perhaps due to the properties inherent in the steel itself, such as the dimensions, composition and microstructure of the metal.

While a range of factors may be involved in generating the forms of hydrogen permeation peaks observed using SRB cultures, their influence on steel is further complicated by the dynamic nature of SRB cultures. Following inoculation, the corrosiveness of such batch cultures changes constantly, due to nutrient depletion, the accumulation of metabolic byproducts (most notably, H_2S), and also due to a changing cell density. Even if a steady state aqueous environment were established by the use of continuous cultures, then the nature of the steel surface may still have undergone some kind of dynamic change, due to the accumulation or formation at the steel surface of biologically or chemically derived products of metabolism or corrosion. Therefore the nature of hydrogen permeation transients generated in batch cultures of SRB needs to be considered in terms of both changes in the bulk medium and alterations in the environment immediately adjacent to the steel surface. Furthermore, there needs to be a consideration of the different factors which govern the quantity of hydrogen present at the steel surface (whether generated cathodically or biologically), as well as those factors which control the proportion of total surface hydrogen entering the metal lattice.

As an aid to resolving these inter-related influences on hydrogen absorption, the observed changes in hydrogen permeation current for steel in SRB culture will be discussed in terms of:

1 SRB influences on the anodic dissolution reaction

2 SRB influences on cathodic hydrogen evolution

3 Changes in the fate of hydrogen at the steel surface

4 Possible influences of biogenic hydrogen.

whilst also examining the possible separate physical influence of SRB films on the steel surface.

8.2 THE RELATIVE ROLES OF SRB AND SULPHIDE FILMS IN HYDROGEN ABSORPTION

8.2.1 SRB Growth and H_2S Evolution.

The clear demonstration of SRB growth from cell densities of approximately 10^6/ml up to 5 x 10^8/ml, and the successful use of dialysis membranes to partition bioreactor chambers satisfies two important preconditions for interpreting hydrogen permeation results. Thus any observed differences between the hydrogen permeation transients obtained for steel foils in the presence and absence of SRB cells (Run K, figure 7.5a, p148 and Run L,

figure 7.6a, p152) may be accountable, at least in part, to the differential action on steel of whole cells and the products of SRB metabolism (with a maximum molecular mass of 10 000 daltons). Conversely, any specific features of hydrogen transients which are shared by screened and unscreened foils cannot be attributable to the activities of SRB cells present at the steel surface.

It is equally important that, unlike the transients produced by steel foils which were exposed simultaneously to inoculum and culture media (Runs C to E, figure 7.1b, p130), the separate addition of these factors to steel causes an increase in hydrogen permeation current which is clearly well in excess of any separate influences of culture media alone (Runs J to L; figures 7.3, 7.5a and 7.6a; pages 139, 148 and 152). An interpretation of the results is aided further by the general similarities between hydrogen permeation transients generated using Desulfovibrio desulfuricans (Norway-4) in saline (3.5% NaCl) media and those employing the freshwater system and inocula of Desulfovibrio vulgaris (Woolwich).

Where dialysis membranes were employed, the peak hydrogen permeation currents attained were independent of the presence of SRB, suggesting that if the generation of peaks and subsequent declines were due to protective film formation, then such film development was not significantly affected by the presence of SRB on the steel

surface. As explained earlier (section 8.1.2, p178), hydrogen permeation peaks could be attributable to either a reduction in the aggressiveness of the corrosive environment, or due to some protective influence on the steel. The possible involvement of the SRB in either or both types of process can be further examined by cross-referencing observed changes in corrosion potential and hydrogen permeation current (Run K, figures 7.5a/b/c; p148/149 and Run L, figures 7.6a/b/c; p152/153).

8.2.2 The Establishment of Protective Films.

Following SRB growth and H_2S evolution, the potential of freely corroding specimens shifted in the noble direction (Run K, figure 7.5b, p148 and Run L, figure 7.6b, p152). This may be indicative of cathodic depolarisation, as represented in figure 8.3a (overleaf). Following a shift in the cathodic curve from a to a', the anodic and cathodic reactions become balanced at the more noble potential Ecorr', and at an increased corrosion current Icorr'. Conversely though, the observed shift in Ecorr may equally be an indication of anodic polarisation, where the anodic curve moves from b to b' (figure 8.3b, overleaf).

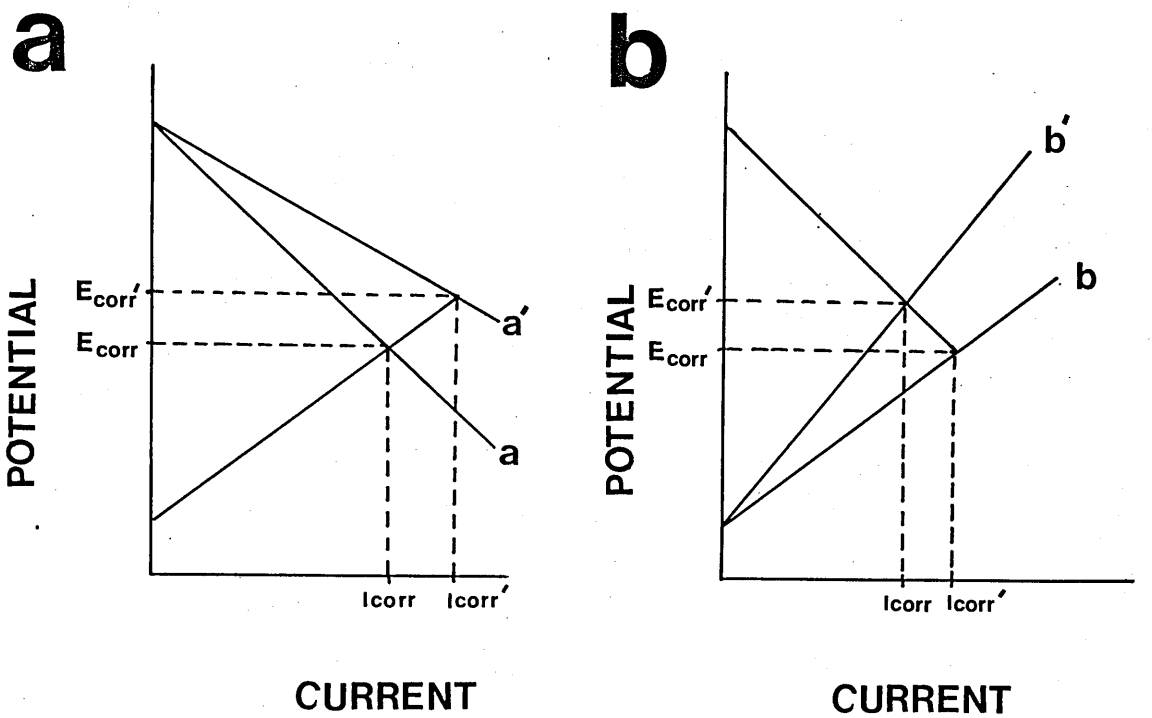


Figure 8.3 Evans diagrams illustrating how a positive shift in the corrosion potential (Ecorr) may arise from a shift in curves representing either the cathodic (a), or anodic (b) reactions.

However, these shifts in Ecorr have been shown to coincide with hydrogen permeation current peaks (Run K, figure 7.5c, p149 and Run L, figure 7.6c, p153), which in addition to the subsequent declines in permeation current are consistent with neither cathodic depolarisation nor an increased rate of hydrogen evolution. It is more

likely that the anodic reaction has become stifled, following the reaction of dissolved H_2S with the steel surface and the establishment of a protective corrosion product film. The possible protectiveness of such films was suggested by the tarnished appearance of the metal surface and the thick black overlaying layer of relatively adherent corrosion product, both of which were distributed widely across the exposed test area of steel (plates 7.1a and 7.1b, p144).

The positive shifts in Ecorr and the blackening of culture media in Runs K and L coincided with the onset of rapid H_2S evolution and SRB growth. Although no chemical analysis of the film material was undertaken, this strongly suggested that the films generated were in fact largely composed of some form of iron sulphide, especially in view of the widespread occurrence of primary sulphide film formation in SRB culture reported in the literature (Booth & Tiller, 1960; King et al, 1973a; King et al, 1976; Tiller, 1983). Furthermore, it has long been established that following SRB growth and biogenic H_2S production, steel undergoes an overall shift in Ecorr towards a more noble value, and that such a shift is associated with the formation of a protective film (Wanklyn and Spruit, 1952; Booth and Tiller, 1960). For the films established during this work, it is possible that due to the sustained evolution of CO_2 associated

with SRB activity (Hatchikian et al, 1975), a certain proportion of the corrosion product material could develop in the form of siderite ($FeCO_3$), which has been reported to occur on steel either as a loose bulky film (Booth et al, 1967), or as a particularly adherent grey corrosion product (King et al, 1973a).

The exact structure of corrosion product films generated in SRB culture has been shown elsewhere to depend upon a range of factors (Smith and Miller, 1975) but most notably upon the concentration of dissolved ferrous ions (King et al, 1976). The latter workers demonstrated a positive correlation between corrosion rate and the concentration of added Fe^{2+}, wherein media Fe^{2+} concentrations far exceeding those thought to be applicable in this work (Fe^{2+} only present as impurities in the reagents used and that resulting from metal dissolution) gave rise to the formation of tenacious, and protective iron sulphide (Mackinawite and Greigite) films, similar in description to those reported here. Therefore while it is somewhat uncertain as to the exact structure and composition of the films formed in this work, the evidence presented would strongly suggest that it was both protective towards the underlying metal, and consisting largely of iron sulphide.

It follows that if the overall shifts in Ecorr observed (Run K, figures 7.5a/c, p148/149 and Run L,figures 7.6a/c, p152/153) were generally related to the formation of

sulphide films, then the subsequent fluctuations in Ecorr observed may be due to a transitory alteration in the distribution, uniformity or structure of the film material. The factors which may cause the latter changes have been reviewed by Smith and Miller (1975), and for the ranges of pH, temperature and redox potential applicable in this work, the most notable influences on film structure are probably the concentration of dissolved Fe^{2+} and H_2S present in the culture, either of which may determine the stoichiometry of iron and sulphur present within the corrosion product, and therefore may also determine the final product's crystalline structure. Using chemically-prepared sulphides, King et al (1973b) demonstrated a general correlation between the corrosion rate and the sulphur content of sulphide species. Moreover, it was shown by King et al (1976) that on addition of excess Fe^{2+} to corroding steel in SRB culture, the primary sulphide films consisting of mackinawite ($Fe_{(1+x)}S$), were transformed into more sulphur rich and corrosive secondary films, either in the form of pyrrhotite (Fe_7S_8) or as smythite ($Fe(3+x)S_4$). Although H_2S samples were taken only up to 50 hours following inoculation (owing to the corrosion of sampling syringe needles), it is reasonable to assume that rates of H_2S evolution will have declined in step with a decline in the limiting concentration of either lactate or sulphate (Postgate, 1984) if not due to sulphide inhibition of

growth (Groves, 1972), with a resultant increase in the ratio of ferrous ion to dissolved sulphide. Therefore after an initial period during which H_2S levels greatly exceeded the Fe^{2+} concentration (thus promoting protective film formation), a ratio of these species may gradually have become established which was more favourable towards the formation of the types of non-protective sulphide precipitates which are normally associated with high corrosion rates (King et al, 1976). The uneven films formed on foil specimens and metal weight losses measured suggested that metal dissolution did persist following initial film formation. The overall stability in Ecorr values observed in Run K (figure 7.5b, p148) and in Run L (figure 7.6b, p152) might be explained by the continual tendency for these areas of corroding metal to react with residual H_2S present in the medium, thereby acquiring a protective layer of corrosion product. An alternative hypothesis might suggest that protective sulphide films gradually and irreversibly deteriorated, with a consequent increase in the area of bare metal exposed to the culture medium. However, this would not account for the very marked overall declines in hydrogen permeation current shown to occur over the 10 day period following the onset of SRB growth in each experiment (Run K, figure 7.5a, p148 and Run L, figure 7.6a, p152).

Whichever of the above mechanisms were applicable, there should be some consideration of other external influences, such as vibration, on the possible integrity of sulphide films. Indeed, it has been assumed that the simultaneous fluctuations in Ecorr are due to the effects of diffusible species (especially H_2S) between bioreactor chambers, rather than other external physical factors (temperature was controlled thermostatically).

In addition to the effect of Fe^{2+} concentration, Booth et al (1965) showed that the structure and protectiveness of sulphide films depended upon the time scale of development. After the exposure of steel for between 30 and 40 weeks to cultures of Desulfovibrio desulfuricans, the rate of metal weight loss approximately quadrupled. This acceleration of corrosion was related to breakdown of protective sulphide-containing coatings, the formation of which had occurred during early SRB growth, and in the presence of a minimal Fe^{2+} concentration (approximately 10^{-5}M). It would certainly be useful to investigate the possible influence of longer timescales in terms of the effect of film formation on hydrogen absorption.

8.2.3 The Influence of SRB Films on Protective Film Formation and Hydrogen Absorption

Even if the precise nature of the protective films is not certain, it has been firmly established that the magnitude and direction of the overall shifts in free corrosion potential is not dependent upon the presence of SRB at the steel surface (Run K, figure 7.5b, p148 and Run L, figure 7.6b, p152). It was notable that in common with transients of hydrogen permeation current (figure 7.5a, p148 and figure 7.6a, p152), overall changes in Ecorr were similar between specimens in the presence and absence of SRB cells, and also between specimens used in separate experiments.

In addition to the similarities between Ecorr transients, there were no general differences between the nature and distribution of corrosion product on screened and unscreened steel foils (plate 7.1, p144), either within or between separate experiments. Other workers (Dumas et al, 1988) who employed cultures of Desulfovibrio desulfuricans in lactate sulphate-based media have also noticed broad similarities between the nature of sulphide films generated on screened and unscreened steel foils. Such observations have not been made universally though, and corroding mild steel coupons in a culture of Desulfovibrio sp. in Postgate's Medium 'C' were seen by

Gaylarde and Johnston (1980) to acquire "thicker, sulphide rich precipitates" when in contact with SRB cells,compared to specimens which were sequestered within dialysis membranes. Moreover, it is still unclear as to what extent the deposition of sulphide films (and the consequent changes in Ecorr) is influenced by the presence of SRB films at the steel surface, and to date the subject might benefit from additional work in this area.

A possible indication of separate SRB influences upon hydrogen absorption is given by the fact that the metal weight losses incurred by steel foils exposed to SRB cells were higher (approximately double) than those from which SRB were screened (table 7.6, p140). Since hydrogen permeation occurs directly in proportion to the corrosion rate, and having established that the overall rate of corrosion appears to be under anodic control, then the inference is that the SRB might somehow interfere with or perhaps modify the development of protective films. It was considered that the positive shift in Ecorr was more likely to be a consequence of anodic polarisation rather than increased cathodic activity, in view of the sharp reduction in hydrogen absorption observed. However, the adherent sulphide films which formed in each experiment would have afforded the steel with a more efficient cathodic surface on which hydrogen ion discharge could occur (the different cathodic properties of sulphides

reviewed by Smith & Miller, 1975). Thus any enhancement of the cathodic reaction may have been superimposed with the positive shift in Ecorr mentioned earlier.

Of the traditional theories of cathodic depolarisation mechanisms for steel exposed to SRB (Chapter 3), the process whereby hydrogen is taken up by loose, mobile forms of iron sulphide precipitate, and transported away from sites of cathodic activity (King and Wakerley, 1973) may not be applicable in this work, due to the formation of protective films. However, it has been suggested that in a similar process, the efficiency of the cathodic reaction may be enhanced by the matrix of extracellular polysacharide substance (EPS) produced by the SRB. In this process, iron sulphide particles present within an SRB film may act as cathodes, which are mobile and may become electrically connected to sites of localised corrosion on the steel surface (Herbert and Stott, 1984). Characklis and Cooksey (1983) went further, to suggest that the EPS may itself act as an electron sink. A further increase in the efficacy of SRB biofilm as an electron sink in localised corrosion might be achieved by motility of the cells themselves. In a process akin to a depolarisation mechanism proposed by Miller (1981), SRB cells to which iron sulphide particles were attached might serve as motile cathodes, bringing "fresh" (non-polarised with hydrogen) sulphide into contact with corroding steel.

Most attention regarding the role of SRB in corrosion has traditionally been given to the possible utilisation of cathodic hydrogen as an energy source during sulphate reduction, thereby depolarising the cathodic reaction. Certainly in this work, if the corrosion rate (and therefore the rate of hydrogen absorption), as indicated by metal weight loss were influenced by the hydrogenase enzyme, then the effect would be evident only for steel exposed to SRB cells. Any such effects of hydrogen uptake would not be evident in specimens from which SRB cells were excluded, since the maximum sized molecule which is diffusible through the dialysis membrane (10 000 daltons) is exceeded by even the smallest hydrogenase subunit of Desulfovibrio desulfuricans (Norway 4), which has a molecular mass of 27 100 Da (Rieder et al, 1984). If hydrogenase-mediated uptake of cathodic hydrogen were important during the bioreactor tests conducted, then its influence upon the corrosion rate (and hydrogen absorption) would be most apparent while corrosion was under cathodic control, prior to the formation of protective sulphide films. Furthermore, it has been demonstrated that cathodic hydrogen utilisation during growth is most important when it proceeds in concert with the oxidation of lactate (Cord-Ruwisch and Widdel, 1986), which is present in abundance just after introduction of the SRB inoculum. However, the increase in SRB numbers was seen to reach a maximum after approximately 50 hours

of inoculation, by which time both lactate and sulphate (which are present in the medium in stoichiometric proportions, at 30mM and 60mM respectively) are likely to have become depleted. Therefore it is to be expected that hydrogenase activity as a mode of cathodic depolarisation is unlikely to be important after the initial 50 hours following inoculation, since the terminal electron acceptor (SO_4^{2-}) has become depleted. Moreover, limiting sulphate concentrations have elsewhere been shown to be one of the conditions in which SRB may produce rather that utilise hydrogen (Lespinat et al, 1987), where protons can serve as an alternative electron acceptor to the sulphate ion. In addition, during the initial exposure of steel to SRB cells, the reverse of hydrogen uptake might be applicable. In this case, due to an initially limited energy supply in the form of adenosine triphosphate (ATP), insufficient activated sulphate would be available for reduction to sulphide, and the excess electrons generated from lactate oxidation would instead be used for the reduction of protons, thus liberating hydrogen (Tsuji and Yagi, 1980). Thus at different stages of bioreactor tests, the steel surface might have become subjected to alternate influences upon the quantity of hydrogen available for absorption, but due to the limited period during which hydrogen metabolism is likely to have occurred, it would seem that these changes would be unlikely to account for any significant or prolonged

differences between rates of hydrogen permeation through corroding foils in the presence and absence of SRB cells in Runs K and L.

In terms of hydrogen absorption, as an additional measure of the aggressiveness of conditions at the steel surface, the proportions of cathodically generated hydrogen which diffused through corroding steel foils were found to vary considerably between experiments. Comparable values of percentage diffused hydrogen were though obtained for pairs of specimens within each test (table 7.5, p138 and table 7.8, p145). Of the six values obtained, there appears to be no relationship between percentage diffused hydrogen and the presence or absence of SRB. However, in view of the formation of protective sulphide films and the pattern of corrosion observed, it is interesting that such a significant percentage of cathodic hydrogen actually enters the metal. It is unclear though how these protective films would affect the proportion of hydrogen entering the metal, since the film could either serve to enhance hydrogen absorption, due to the cathodic properties of the sulphide material, or it could act as a partial barrier to the diffusion of hydrogen into the underlying metal. While accounting for the preferential absorption of hydrogen by steel when generated by corrosion rather than by cathodic polarisation, Nobe (1980) suggested that fresh, continuously renewed iron

surfaces offered less resistance to adsorbed hydrogen than did non-renewed ones, which in this case would include steel surfaces covered in a protective film. The inference is, that if the film were removed, which for example might be effected by a higher Fe^{2+} concentration, or due to sulphidation as discussed earlier, then higher overall proportions of cathodic hydrogen might enter the steel than the levels observed here. Moreover, if protective film formation were prevented altogether, then the more rapid corrosion rates possible at the bare steel surface could serve to enhance SRB growth by providing a more sustained energy source in the form of cathodic hydrogen. It is doubted though that the SRB could partake in any competition for cathodic hydrogen with the steel as suggested elsewhere (Walch and Mitchell, 1984), since the diffusible cathodic hydrogen is adsorbed in atomic form at the steel surface, and is therefore unavailable to bacterial cells.

Observations of the hydrogen permeation data acquired in this work have so far been made under an important assumption; that the experimental conditions to which pairs of steel specimens were subjected were similar in every way except for their exposure to SRB cells. Despite the various precautions taken during selection of test material samples, it is inevitable that at least some variation was observed in the transients of hydrogen

permeation. Such variation may be attributed to differences in the properties of the steel specimens themselves, such as slight variations in thickness and also in microstructure. For instance the distribution of hydrogen trapping sites, such as manganese sulphide inclusions, impurities, deformities and defects within the crystal lattice of the metal can all be important influences upon hydrogen permeation (McCright, 1977). The influence of these latter factors might be further enhanced due to the rolling of the steel during manufacture, thus increasing their surface area (the effects of using different material thicknesses and specifications on hydrogen permeation were most notable when comparing transients obtained from mild steel (0.15cm, 0.03% C; Runs A and B, p130) with those obtained using the thinner shim type steel foils (0.005cm, 0.17% C; Runs J to O, pages 139, 148, 152 and 157-159).

In summary, it has been established thus far that in minimal iron medium, SRB growth and H_2S evolution results in rapid corrosion and a high rate of hydrogen absorption. The formation of a protective sulphide-containing film however causes a subsequent irreversible decline in hydrogen permeation. The SRB do not appear to have any other influence on hydrogen absorption other than by their production of H_2S.

8.2.4 The Influence of Culture Media

While the most notable characteristic of hydrogen permeation transients is the generation of peaks shortly following the introduction and subsequent activity of SRB, similar peaks are not displayed using complete sterile culture medium (Runs F and G, figure 7.1c, p130). In addition, the steady state hydrogen permeation rates attained in sterile medium prior to inoculation were very much lower than the peak permeation currents obtained following SRB growth (Runs K to N; figures 7.5a, 7.6a, 7.7a and 7.8a; pages 148, 152, 157 and 158). Separate experiments using sterile media however demonstrated that culture media exerted a significant and sustained influence on hydrogen absorption (Runs F to H, figure 7.1c, p130). This may complicate an interpretation of the possible separate role played by SRB activity when steel was exposed simultaneously to SRB cells and culture media.

The levels of hydrogen absorption demonstrated in figure 7.1c (Runs F to H, p130) suggested that at pH 7.5, some media components may be corrosive. The steep rises in permeation current obtained in sterile medium are consistent with the introduction of a new source of dissolved protons at a bare, dry steel surface, and in the absence of oxygen or a cathodic poison such as H_2S, the generation of a polarising hydrogen film would be

expected to eventually retard the corrosion rate. The more persistent increase in permeation current seen in figure 7.1c (Run F, p130) though may indicate that sterile medium had a corrosive influence on the metal. An implication of the lower levels of hydrogen permeation current observed in Run H (figure 7.1c, p130) is that to some extent the presence of yeast extract determines the overall corrosiveness of the culture medium used (Postgate's Medium 'C'). While accounting for the different thicknesses of test materials used, the surface hydrogen concentration, Co attained in the steel exposed to PMC medium lacking yeast extract (Run H, 0.075cm thick, Co = 0.0021ppm hydrogen) was approximately one third of the Co value attained in steel exposed to complete medium (0.0125cm, Runs F and G, 0.0077ppm and 0.0056ppm hydrogen respectively). However, it should be noted that such an order of variation in Co values could be a consequence not only of different specimen thicknesses, but also of variations in material composition or microstructure. While a final examination of the specimen concerned revealed what appeared to be constituents of yeast extract adhered onto the steel surface, no evidence of localised corrosion resulting from the distribution of the material was seen.

It has been suggested elsewhere that the corrosion rates of steel may vary according to the presence of yeast

extract (King et al, 1976). However, there appears to be little substantiation in the literature that yeast extract may in itself be corrosive towards steel (of greater importance may be the possible nutritional role of yeast extract in determining SRB activity). Gaylarde and Videla (1987) demonstrated that certain components of PMC medium, including lactate, citrate and especially phosphate ions, had the reverse effect, and gave rise to passive film formation on steel. The same workers however pointed out that this effect was counteracted completely by the depassivating influence of chloride ions, which would be present in cultures of marine SRB strains. However, the relatively large hydrogen permeation currents generated employing culture media (which lacked added salt) in this work would suggest that such a passivating mechanism were not applicable, assuming all other conditions were equal. Moreover, it is apparent from the initial hydrogen permeation plateaux shown in figures 7.3, 7.5a and 7.6a (bioreactor Runs J, K and L; pages 139, 148 and 152), that even in the presence of added salt, the culture medium is no more aggressive towards steel than it is when formulated for use with freshwater cultures.

8.3 THE INFLUENCE OF SRB FILMS ON HYDROGEN ABSORPTION DURING CATHODIC PROTECTION

8.3.1 Hydrogen Absorption During Cathodic Protection

From the three experiments involving cathodically polarised specimens, it was clear that the quantity of hydrogen entering the metal increased considerably following SRB growth and H_2S evolution. The magnitudinal increase in hydrogen permeation current generated by steel at -850mV (SCE) compared to that obtained at the free corrosion potential is consistent with the increased quantity of cathodic hydrogen generated at the lower potential, coupled with the poisoning effect of sulphide on the hydrogen combination reaction. Whereas in freely corroding foils, subsequent hydrogen permeation currents declined markedly (for example, Runs C to E, figure 7.1b, p130 and Run L, figure 7.6a, p152), there was some variation in the nature of permeation transients in cathodically protected specimens. In Run M (figure 7.7a, p157), the decline was less steep than that seen in Run N (figure 7.8a, p158) the latter of which resembled in form (although not in magnitude) the transients obtained by freely corroding steel in Run L (figure 7.6a, p152). This variation between decline phases of hydrogen permeation transients may be a function of the types of films formed on the steel surface, which may serve as a partial barrier

to cathodically generated hydrogen, in the same way as suggested for freely corroding specimens. The few specimens examined after cathodic protection did not posses the uniform surface 'mats' of black sulphide-containing corrosion product which were characteristic of steel which had corroded freely, suggesting that the cathodic protection potential somehow interfered with film development.

In view of the observed effects of cathodic charging at -850mV (SCE) (Run O, figure 7.9, p159), the increases in hydrogen permeation current detected for specimens polarised to -1000 mV (SCE) was lower than expected. In fact, the levels of hydrogen permeation current established at -850mV (SCE) in Run O were between three and six times less than the levels attained in Runs M and N, even though the initial experimental parameters were similar. The observed growth of a contaminant organism prior to the introduction of SRB cells does though clearly complicate an interpretation of the levels of hydrogen permeation current attained, since SRB growth and H_2S evolution which largely determine the aggressiveness of the corrosive medium, depend upon the presence of an oxidisable substrate, in this case lactate. Even though under similar conditions in an earlier experiment (Run N), the growth of a contaminant organism did not appear to influence the overall SRB cell density or the magnitude of

hydrogen permeation current, it is difficult to deduce exactly why only rather poor and capricious SRB growth was achieved in Run O, because of the great variety of effects of contamination possible, not only on the depletion of nutrients, but also on other growth parameters, such as pH and Eh. Clearly, in order to determine absolute rates of hydrogen absorption at -1000mV (SCE), there may be much benefit in repeating the experiment, but whilst ensuring the maintenance of sterile conditions prior to the introduction of SRB. Under these conditions, cathodic charging would only be increased to -1000mV once vigorous SRB growth had become established, and where the levels of hydrogen permeation current generated at -850mV (SCE) were similar to those detected in previous experiments (Runs M and N).

Although the maximum levels of hydrogen permeation current attained at -1000mV (SCE)did not exceed the levels attained in separate experiments at -850mV (SCE), hydrogen permeation currents did increase up to three times initial values following the transition from -850mV to -1000mV (SCE). Starting from the levels attained at -850mV in previous experiments, a similar such increase would give a hypothetical maximum current density of up to 19$\mu A/cm^2$, and a surface hydrogen concentration of 0.2ppm. The possible significance of such rates of hydrogen absorption are discussed in section 8.5 (p218).

8.3.2 The Influence of SRB Films

The apparently synergistic effect of SRB growth and cathodic polarisation on hydrogen absorption was not dependent on the presence of SRB films at the steel surface, and the relative degree of variation between peak hydrogen permeation currents attained in the presence and absence of SRB cells was similar to that encountered when using freely corroding steel foils. If SRB were active in the removal of cathodic hydrogen from steel held at -850mV (SCE), then in order to maintain this potential, extra current would need to be supplied to the metal, thus increasing both the rate of hydrogen discharge and the quantity of hydrogen available for absorption. However, although no record was made of the current supplied to the steel specimens, the hydrogen permeation transients obtained gave no indication that the rate of hydrogen discharge was increased in the presence of SRB.

For the specimens polarised to -1000mV (SCE), no clear relationship could be deduced between the presence of SRB at the steel surface and the rate of hydrogen absorption. The permeation currents detected for screened foils were consistently lower than for foils exposed to media inoculated with SRB. However, this situation had clearly become established well before SRB were introduced, while steel foils were still being exposed to sterile culture media. It is suggested that such variation in permeation

currents may be at least partly attributable to a difference in the physical properties of the steel specimens, as was suggested previously in relation to the differences between transients obtained for the pair of similar freely corroding specimens in Run J (figure 7.3, p139). It is significant though, that changes in hydrogen permeation current detected from screened foils did reflect the fluctuations observed on foils exposed to SRB culture, and that the peak values attained by both foils were similar, suggesting that the presence of SRB cells had little, if any significant influence on the rate of hydrogen absorption at this potential.

The difficulty encountered in establishing good SRB growth in the presence of steel polarised to -1000mV (SCE) was probably related to the constantly increasing pH, due to the cathodic reduction of hydrogen ions thus:

$$2H_2O + 2e^- = H_2 + 2OH^-$$

Despite the several attempts made to maintain pH levels at or near an optimum for SRB growth, corresponding to between 7.2 and 7.6 units (Postgate, 1984), the types of cells present continued to exhibit the morphological characteristics of a stressed, senescent culture. It was shown by Wolfson and Hartt (1981) that for steel exposed to natural sea water, and polarised to potentials of between -780mV and -1030mV (SCE) using different stirring

rates and durations of exposure, the pH levels established 0.1mm from the metal surface lay between 10 and 11.5 units. Such an order of alkalinity can lead to cell death, and the dangers of allowing SRB cultures to become excessively alkaline are well known (Postgate, 1984). It is therefore not surprising that SRB growth, in addition to sulphate-reduction was absent on specimens polarised to -1000mV (SCE), even though bulk pH values (at a maximum of 8.5 units) may have been more favourable to growth.

In addition to the possible influence of pH upon biogenic H_2S evolution, the high pH levels associated with the surfaces of specimens polarised to -1000mV (SCE) may have encouraged the precipitation and deposition on the steel surface of calcium and magnesium carbonates in the form of calcareous films. The progressive development of such films at increasingly lowered potentials has been shown to depress the rate of hydrogen permeation through steel exposed to artificial sea water (Lucas and Robinson, 1986). In the present work though, it was unclear how important an effect the development of calcareous films might have had upon hydrogen absorption, since the concentrations of calcium and magnesium ions in the culture medium used (2.7μM and 2.4μM respectively) were very much lower than those present in commonly used formulations of artificial sea water (26mM and 10mM respectively: ASTM standard D1141-75; ASTM, 1980 and BDH

Artificial Sea Water mixture, BDH Ltd). Moreover, in a field situation, where prolonged periods of cathodic protection result in the establishment of calcareous deposits on steel, the current demand for protection from corrosion may become reduced (LaQue, 1976), thus lowering the quantity of hydrogen generated. In view of the combined effects of a lower overall rate of hydrogen evolution and the displacement of hydrogen discharge away from the steel surface, it would seem less likely that the SRB films could have a significant influence on H absorption once calcareous films have become established.

A common factor in experiments conducted with cathodically polarised steel was the extra time taken for the establishment of SRB growth, compared to the rapid onset of growth and H_2S evolution when using freely corroding specimens. While delayed growth may have been related to local increases in pH at the steel surface, the successful establishment of an active SRB biofilm may also have been influenced by the limited availability of certain nutrients in the environment immediately adjacent to the steel. A requirement for calcium ions in the process of bacterial adhesion and the formation of EPS has been reported by a number of workers (Characklis and Cooksey, 1983), but the locally high pH levels associated with the cathodic steel surface would encourage the precipitation of Ca^{2+} ions, which would therefore become less available

in the biofilm development process. Perhaps of more immediate consequence for SRB activity at the bare steel surface would be a limiting concentration of Fe^{2+} ions, which are required by the bacteria in relatively large quantities (Postgate, 1984) for the synthesis of the redox proteins (such as cytochromes) involved in sulphate-reduction. It was suggested earlier (section 8.2.3, p192) that an initial excess of H_2S over dissolved Fe^{2+} would serve as an iron sink, until such a time that due to the depletion of sulphate ion and the reaction of sulphide with the steel surface, the relative concentration of Fe^{2+} began to increase. However, in experiments where steel foils were cathodically polarised, the availability of dissolved Fe^{2+} for SRB growth and respiration would also be limited by the much reduced tendency for anodic dissolution to occur at the reduced potential. A similar effect on iron concentration would be expected due to the tendency for the formation of passive $Fe(OH)_2$ films at raised pH levels (Wranglen, 1972).

Despite the possible influences of pH, protective film formation and a limited iron concentration on SRB growth, thick SRB films did eventually become established on steel foils which were polarised to -850mV (SCE). It is suggested that the delay in cell growth may be related to a period of physiological adjustment of the culture to the surface environment of the polarised steel. Once growth

had become established, with the consequent evolution of H_2S and an increase in corrosion rate, then dissolved Fe^{2+} ions could have become available to SRB cells by the dissolution of the metal. Clear evidence of corrosion was evident from observations of pitting and metal weight loss. It is not suggested though that SRB iron uptake caused a stimulation of the anodic reaction, since a similar localised pattern and extent of corrosion was found on screened and unscreened specimens.

The localised nature of corrosion on cathodically polarised specimens, in the form of circumferentially distributed pits, was probably related to a stage of the specimen preparation process. It was thought that by marking out the area of metal to be exposed to the corrosive medium, the slight depression thus formed may have corroded preferentially, owing to a slight change in thickness. Although successive methods of preparation avoided the marking of specimens, similar but less localised patterns of corrosion were observed around the circumference of the exposed area of metal.

As indicated by the observations of pitting and metal weight loss, a potential of -850mV (SCE) clearly afforded an inadequate level of protection to the steel foils, whether in contact with, or screened from SRB (weight losses of 2.84 and 3.11mdd respectively). In view of the effects of H_2S on depressing the reversible potential of

the anodic dissolution reaction (Horvath and Novak, 1964), it is to be expected that a lower potential would be required to maintain immunity from corrosion, compared to that needed in the absence of sulphide. The effect becomes more pronounced as the iron concentration decreases and as the H_2S concentration increases, both of which occur during SRB growth and sulphate reduction. The requirement for a minimum potential of -950mV, $Cu/CuSO_4$ (equivalent to -873mV, SCE) is widely regarded as an acceptable level of protection in environments containing SRB, and has been shown to cause only very low residual corrosion rates, in the order of 0.001mdd (Fischer, 1984). However, the cathodic potential used in this work (which differed from the former value by only +23mV) resulted in significant corrosion, with weight losses of the same order as those obtained for freely corroding specimens in Run J (5.85mdd and 6.07mdd, section 7.4.2, p161). It is unclear though how much the dissolved iron concentration is taken into account in deducing what constitutes a safe level of cathodic protection. Although the values for sea water are likely to vary according to sampling location, an average dissolved Fe^{2+} concentration of approximately 2×10^{-7} M has been quoted (Weast, 1989), which according to a Nernstian response, corresponds to an iron potential of -885mV, SCE (-962mV, $Cu/CuSO_4$). In the presence of biogenic H_2S, an iron concentration lower than this might be expected, in

which case a more active potential of steel would become established. This might account for the marked corrosion observed on steel specimens 'protected to -850mV (SCE).

As an indication of the corrosion rate, the various determinations of hydrogen permeation current and metal weight loss do not suggest that a relationship exists between the level of protection afforded by a cathodic potential of -850mV (SCE) and the presence or absence of SRB at the steel surface. The findings of these experiments may therefore be inconsistent with those of Booth and Tiller (1968), who suggested that the extra current required to maintain the immunity of steel in buffered SRB cell suspensions was a function of cellular hydrogenase activity. Furthermore, an implication of additional decreases in the potential of steel, for example to -1000mV (SCE), is that iron will cease to dissolve and will become unavailable to the bacteria. With the additional effect of rising pH levels at the steel surface, it is uncertain how a bacterial colony could grow and respire sufficiently close to the metal surface to influence the current demand during cathodic protection. Moreover, at the potentials used (-1400mV, SCE) by some workers to demonstrate cathodic hydrogen uptake by SRB (Pankhania, Moosavi and Hamilton, 1986), it would seem more likely that bacterial hydrogen uptake occurred in solution, and some distance from the steel surface.

8.4 THE INFLUENCE OF SRB ACTIVITY ON THE SURFACE HYDROGEN CONCENTRATION

The surface hydrogen concentration (Co) values shown in Appendix 5 give an indication of the aggressiveness of the varying conditions at the steel surface during SRB growth. When comparing the Co values obtained from Runs C, D and E with those deduced from subsequent experiments using the double chamber apparatus, it is important to note that the composition, and particularly the carbon contents of the two batches of steel test material were slightly different (0.15% and 0.12% respectively). The two types of material also differed in thickness (0.01 to 0.0125cm compared to 0.005cm). Therefore considering the possible influences of composition and different manufacturing processes on microstructure, the assumption that the apparent diffusion coefficient was equal for the two materials may not have been entirely accurate. However, what is clear from an overview of the Co values obtained, is the very marked influence of cathodic protection on hydrogen absorption during SRB growth, an effect which was reproduced using separate specimens, and separate experimental conditions.

Although this synergism between cathodic protection and SRB activity appears to be a real effect, a possible difficulty arises when attempting to compare the result with those of other workers. This is not least due to the

dearth of available data in the field, but also because of the variation in methods of hydrogen determination (section 2.3.2, p21), and also the great variation possible in the factors which control hydrogen absorption and diffusion in metals (microstructure, composition, temperature etc). Therefore use of the maximum value obtained (0.066ppm) is undoubtedly of most value when comparing Co values using the same test material.

It was suggested earlier (section 8.3.1, p205) that a hypothetical Co value obtained for foils poised to -1000mV (SCE) could exceed 0.2ppm hydrogen in steel. This was based on the three-fold increase in current density observed when reducing the potential value from -850mV to -1000mV (SCE), but assuming an initial (base level) current density equivalent to that obtained at -850mV in previous experiments. In view of the fact that a charging potential of -850mV (SCE) was clearly insufficient to prevent corrosion, it is obviously of concern that significantly higher levels of protection might lead to a disproportionate increase in hydrogen absorption, possibly leading to an increase in susceptibility of steel to cracking.

While it is well known that in addition to the effects of cathodic charging and microstructure, the levels of H_2S in the corrosive medium have a major influence upon

hydrogen absorption by steel, it is not clear what H_2S concentrations were present during the experiments conducted using cathodically polarised specimens. During bioreactor experiments conducted with freely corroding specimens, changes in the gas phase H_2S partial pressures gave only an indication of relative increases in dissolved sulphide concentration, since there is some difficulty in deducing the absolute dissolved H_2S concentrations from these gas phase values, principally because the gas tends to react with dissolved ions present in the solution (especially Fe^{2+}). The problem is compounded by the fact that at near neutral pH values, small changes in pH cause relatively large alterations in the rate of ionisation of the gas into HS^- ion (Nriagu and Hem, 1978). However, Edyvean (1987) reviewed the limited available values of H_2S concentration in biologically active systems, which ranged from 50ppm to 600ppm, depending upon the nutrient status of the media used. Assuming that dissolved H_2S gas partitions with air at a ratio of 200:1, then the gas phase H_2S concentrations measured in this work (10^5ppm) would suggest a dissolved concentration of approximately 500ppm. Gilbert (1985) reported that in laboratory culture media, H_2S concentrations which were inhibitory to further SRB growth became established at between 600ppm and 750ppm. It would therefore seem reasonable to consider that the H_2S concentrations applicable in this work might be

comparable in scale to the latter values. Certainly, in view of the possible accumulation of high H_2S levels in a closed environment such as an oil pipeline, it would be useful to deduce the Co values applicable during SRB growth at increased levels of cathodic protection.

8.5 THE POLARISATION OF STEEL DURING SRB GROWTH

8.5.1 The Influence of SRB Growth on the Cathodic Reaction

The polarisation scans performed on steel specimens in each separate experiment indicated quite clearly that the growth of SRB and the liberation of hydrogen sulphide resulted in a depolarisation of the cathodic reaction (figures 7.10a/b, p167 and figures 7.11a/b, p171). The effect is illustrated by a comparison between the cathodic currents recorded at a common potential of -900mV (SCE). These were seen to increase from 10-12$\mu A/cm^2$ in sterile growth medium, to values between 100$\mu A/cm^2$ and in excess of 1000$\mu A/cm^2$ (figure 7.11b, p171) following inoculation with SRB. A similar effect is apparent at potentials below -950mV, SCE (figures 7.10a and 7.10b, p167). Such changes in cathodic activity compare favourably with the increases in cathodic currents reported by other workers (Booth and Tiller, 1962) who investigated the effects of SRB growth over a similar range of cathodic potentials to those used in this work, whilst using comparable types of growth media and marine SRB species. For comparison, at a potential of -900mV, SCE (-660mV, NHE) a similar scale of increase in cathodic current density was obtained by the latter workers after the exposure of steel to SRB culture for between 6 and 11 days (figure 3.3, p51).

It is suggested that, in common with the polarisation data produced by other workers (including that shown in figure 3.3, p51), this demonstration of cathodic depolarisation in SRB culture is a consequence of the formation of sulphide containing films. Moreover the presence of such films was indicated from an examination of polarisation specimens, which became coated with rich black deposits of a similar nature to those established upon foil specimens in hydrogen permeation experiments (section 8.2.2, p186 and section 8.2.4, p200). The properties of iron sulphides which are associated with enhanced cathodic activity were reviewed by Smith and Miller (1975), and include a combination of physical and electrochemical factors which serve to both enhance the rate of hydrogen discharge, while also facilitating the diffusion and absorption of discharged hydrogen within the sulphide material itself. The electrochemical effects of iron sulphides were investigated by Mara and Williams (1972), whose work provides a clear demonstration of cathodic depolarisation (over a similar range of potentials to those being considered in this work), resulting from the exposure of iron specimens to a variety of chemically and biologically derived iron sulphide compounds (figure 3.8, p63).

In common with the results cited in figure 3.8, there was some variation in displacement of the cathodic curve with time. In figures 7.10f/g (p170) and figure 7.11b (p171) the active shift in cathodic current was progressive with time, whereas in figure 7.11a (p171), the more active situation became established after 24 hours exposure to SRB culture, compared to the situation obtained 336 hours later. In addition, for the separate pair of specimens in figure 7.10e (p169), the overall shift in cathodic curves was relatively small compared to the longer term changes observed in other specimens. The inference that such an enhancement of the cathodic reaction is only transitory is consistent with the results obtained by King, Miller and Wakerley (1973), and also with the hypothesis put forward by King and Wakerley (1973), which proposed that the initial cathodic properties of sulphide films can only be maintained if polarising hydrogen is continually removed. The latter workers suggested that the iron sulphide films could be depolarised either by:

a) introducing 'fresh' non-polarised sulphide to the steel surface, or

b) by hydrogenase mediated hydrogen uptake, for instance by an SRB population in close contact with the surface of the sulphide material.

Conditions in which mechanism a) could become established might have been applicable in this work, if over a longer

period of time the primary sulphide film (which in minimal iron media has been identified as Mackinawite, FeS_{1-x}; King, Miller and Wakerley, 1973) broke down, and became transformed into looser, and more sulphur rich iron sulphides (such as smythite, Fe_3S_4 and greigite, Fe_3S_4), which have been found to be more corrosive, owing to their greater capacity to absorb cathodic hydrogen (King, Miller and Smith, 1973).

Mechanism b) above would only apply while a reducible substrate, in this case the sulphate ion, was available. It is unclear though in these experiments what concentrations of sulphate remained, and at which rate sulphate reduction occurred by the time the final series of polarisation scans were performed, since cell growth and respiration become inhibited by the accumulation of high sulphide levels (Groves, 1973), probably due to the lack of dissolved Fe^{2+} present (Postgate, 1984).

For the longer terms of exposure of steel to corrosive media it was apparent that the overall shifts in cathodic activity were no greater in the presence of SRB cells than in their absence (after 238 hours, figures 7.10d/e, p168/169 and figures 7.11d/g, p172/174). Moreover, excepting the curves shown in figure 7.11g (p174), the latter comparisons suggest that the presence of SRB might rather serve to reduce cathodic activity, for example by

the accumulation of cathodic hydrogen within bacterial EPS present at the steel surface.

An interpretation of the cathodic curves generated over a shorter term (up to 48 hours following the establishment of growth) is complicated by the variability in results obtained (figures 7.10a/b, p167 and figures 7.11a/b, p171), which need to be considered in terms of the metabolism of the bacteria.

Cord-Ruwisch and Widdel (1986) observed that the uptake of cathodic hydrogen during the corrosion of steel wool occurred only during the simultaneous oxidation of carbon source (lactate). Any effects of bacterial hydrogen uptake in accordance with the 'Classical Theory' of cathodic depolarisation (Chapter 3) would therefore be most evident during SRB growth. In this work, the polarisation curves obtained 48 hours after the onset of SRB growth gave no indication that the SRB cause any additional increase in cathodic activity compared to that caused by the bacterial dialysate. In fact at a potential of -1000mV (SCE), the current density was seen to increase in the absence of the bacterial cells, from 0.28mA/cm^2 (figure 7.10a, p167) to 0.53mA/cm^2 (figure 7.10b, p167). However, the curves obtained in the second of the polarisation experiments suggested that 24 hours following inoculation, a more pronounced shift in the cathodic curve

occurred in the presence of SRB (figure 7.11a, p171) compared to the smaller shift in SRB cell-free conditions (figure 7.11b, p171), perhaps suggesting a bacterial cathodic depolarisation mechanism.

8.5.2 Changes in Anodic Activity Following SRB Growth

It was clear from an examination of the anodic behaviour of specimens shown in figures 7.10f/g (p170) and figures 7.11a/b (p171) that SRB growth effected a considerable polarisation of the anodic reaction. This is illustrated most strikingly by contrasting figure 7.10c (p168) with figure 7.10e (p169), in which the anodic current obtained at a potential of -580mV (SCE) decreased by 24 times, from 1.2mA/cm^2 prior to inoculation to a value of 0.05mA/cm^2 following growth of the bacteria. The nature of this shift in anodic curves is consistent with the results of the polarisation work performed by Booth and Tiller (1960), who measured changes in the anodic and cathodic characteristics of steel over a range of time periods and using a number of Desulfovibrio sp, and similar culture conditions to those used in the work of Booth and Tiller (1962) cited previously (figure 8.4, overleaf).

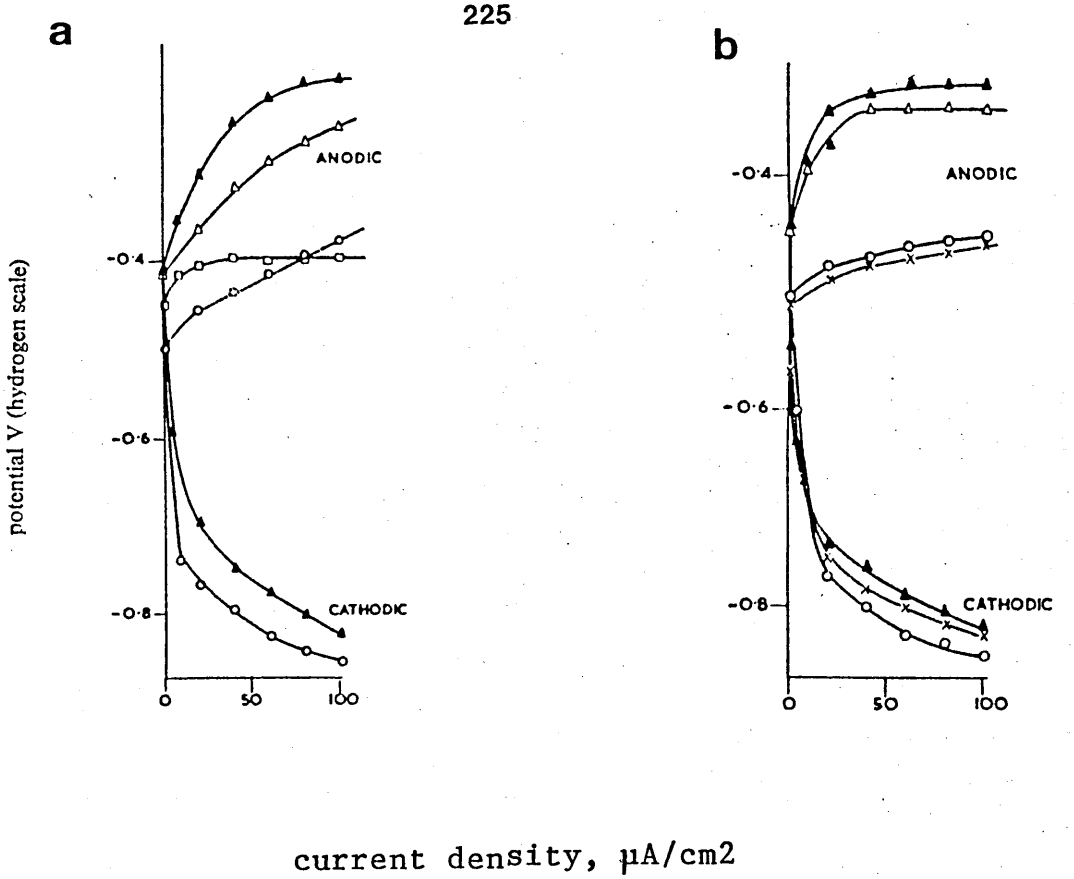


Figure 8.4 Polarisation of the anodic reaction at 30 C for mild steel specimens exposed to an active culture of a) Desulfovibrio desulfuricans (Hildenborough), initially (○) and after 3 days (□), 7 days and 10 days (▲); and b) Desulfovibrio orientis (Singapore), initially (○), after 2 days (X), 7 days (△) and 10 days (▲). After Booth and Tiller (1960).

The decreased anodic activity of polarisation specimens is consistent with the noble shifts in corrosion potential observed during hydrogen permeation experiments (figure 7.5b, p148 and figure 7.6b, p152), and these polarisation data therefore give strong support to the general hypothesis made previously (section 8.2.2, p186) that the sharply declining rates of hydrogen absorption in freely

corroding steel were attributable to the establishment of protective sulphide films. In addition, the similarities in electrochemical behaviour of polarisation specimens and freely corroding foils were reflected by the similar adherent, black sulphide-containing films which became established on both types of specimen.

It was a notable feature of each of the eight polarisation scans that after the longest term of exposure (either 232 hours or 360 hours) of steel to SRB cells or cellular metabolites, a more active rest potential (Ecorr) had become established (figures 7.10a/b/c, p167/168 and figures 7.11c/d/g, pages 172/174). In view of the effect of a combined anodic polarisation and cathodic depolarisation on the corrosion potential (Ecorr' and Ecorr'', figures 8.5 and 8.6), and the important influences of both of these processes identified in this work, a marked shift towards more noble potentials would be expected. This was in sharp contrast to the ubiquitous noble shifts in potential observed for freely corroding steel foils in hydrogen permeation experiments (figure 7.5b, p148 and figure 7.6b, p152), and was also inconsistent with the findings of a number of other workers who also conducted polarisation studies of mild steel in SRB culture (Wanklyn and Spruit, 1952; Booth and Tiller, 1960; Booth, Elford and Wakerley, 1968). The former comparison is made in table 8.1, overleaf.

Table 8.1 Comparison between shifts in Ecorr following SRB growth obtained for freely corroding steel foils (fcp) and cathodically protected specimens (CP).

CP/fcp	figure	Ecorr, mV (SCE)		shift
		initial	final	
fc *	7.5b	-725	-645	+80
fc	7.5b	-725	-645	+80
fc *	7.6b	-710	-630	+80
fc	7.6b	-730	-630	+100
CP *	7.10f	-720	-800	-80
CP	7.10g	-710	-800	-90
CP *	7.11a	-750	-815	-65
CP	7.11b	-770	-790	-20

* Specimens exposed directly to SRB cells

The above results can be most readily accounted for by a part of the experimental technique, where each scan was performed on specimens one hour after the removal of a constant potential of -850mV (SCE), and that this provided insufficient time for the steel to achieve its true rest potential. The residual effects of supplying an external current to the steel were thus still apparent during the scans, by a reduction in rest potential.

8.5.3 Changes in the Corrosion Rate

Despite the clear reductions in anodic activity described in the previous section, over the course of each polarisation experiment the corrosion rates of specimens increased markedly. This is shown most clearly in figures 7.11a and 7.11b (p171), which over a period of 360 hours exposure to SRB cells and cell dialysate, show respectively two-fold and ten-fold increases in current density, corresponding to an increase in the rate of metal weight loss from between 16mdd and 10mdd in sterile medium, to between 32mdd and 100mdd after inoculation with SRB (table 7.10, p166). Similar increases in corrosion current density of up to 10 times initial values are also suggested in figures 7.10f and 7.10g (p170), after approximately 236 hours exposure to corrosive media.

The polarisation data described thus far have indicated that over a period of up to 360 hours exposure of steel to sulphide-containing media, there was an enhancement of the cathodic reaction and a simultaneous reduction in anodic activity, but the corrosion rate increased markedly overall. This situation can be represented schematically as in figure 8.5, overleaf.

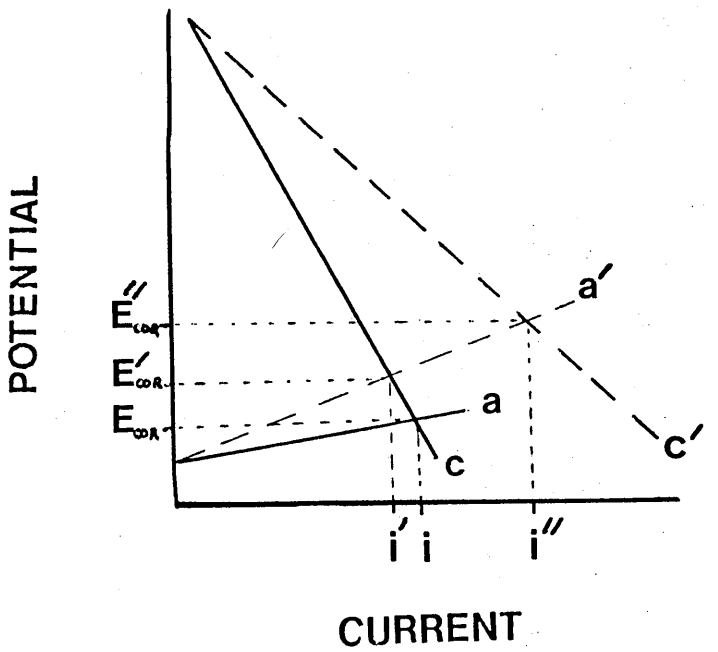


Figure 8.5 Evans diagram representing the establishment of partially protective sulphide films on steel, which cause a reduction in anodic activity from a to a', together with a a more pronounced enhancement of the cathodic reaction, from c to c', thus increasing the overall corrosion rate from i to i'.

In hydrogen permeation experiments, similar adherent sulphide-containing deposits became established on steel foils as on polarisation specimens. This suggested that both types of specimen were afforded with a combination of enhanced cathodic properties, and reduced anodic activity. Moreover, a comparison between both sets of results obtained during active SRB growth and H_2S evolution serves to illustrate that corrosion rates increased during this period.

The rate of hydrogen absorption by freely corroding steel foils (which increases in proportion to the overall rate of hydrogen discharge), was seen to rise progressively for between 50 hours (figure 7.5a, p148 and figure 7.6a, p152) and 70 hours (figure 7.3, p139) following inoculation with bacteria. These periods of rapid hydrogen absorption were associated with the highly corrosive effects of biogenic H_2S on the bare metal surface (section 8.2.1, p184), where both cathodic and anodic processes were thought to have been enhanced.

During equivalent periods of exposure to SRB cells and byproducts to those quoted above, electrochemical measurements made separately also indicated an increase in the corrosion rate of steel. This applied to all of the specimens scanned, each of which generated a higher corrosion current density following SRB growth (that is after either 24 hours of inoculation; figures 7.11a/b (p171), or 48 hours after the onset of SRB growth; figures 7.10f/g (p170), compared to that generated in sterile medium. The most pronounced such increase is illustrated in figure 7.11a (p171) in which a ten-fold increase in corrosion current occurred within the first 24 hours (SRB growth had become established by this time, as indicated by the blackening of culture media, and the increase in turbidity observed).

However, in hydrogen permeation experiments, the association between declining rates of hydrogen absorption and a reduced corrosion rate (section 8.2.2, p186) indicate that in the longer term (from between 50 hours and 70 hours of inoculation, and up to 310 hours thereafter), the anodic effect must have become dominant (the resulting net reduction in the corrosion rate is represented schematically in figure 8.6, below).

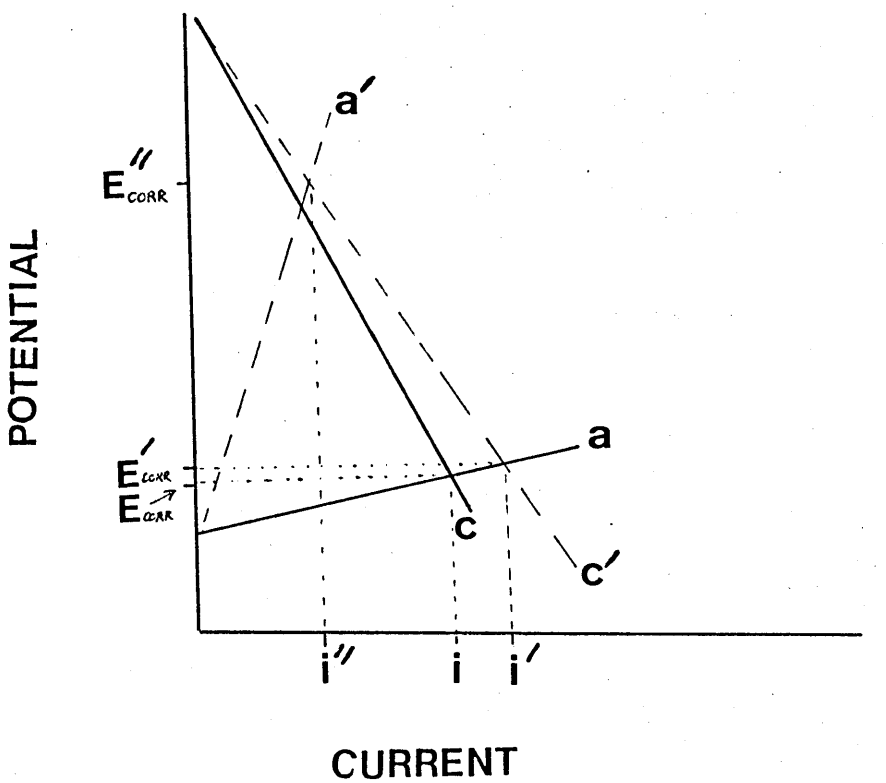


Figure 8.6 Evans diagram showing how the establishment of a protective sulphide film may cause an overall reduction in corrosion rate following an enhancement of the cathodic reaction from c to c', in concert with a more prominent negative shift in the anodic curve from a to a'.

The dominant effect of anodic polarisation would account for the lower overall corrosion rates (weight losses) determined for steel foils (Appendix 3), compared to the higher rates of corrosion deduced electrochemically in polarisation experiments (table 7.8, p145). The rapid decline in hydrogen permeation currents (to levels below those generated by sterile culture medium) observed for freely corroding foils (figure 7.1b, p130 and figure 7.6a, p152) would also be consistent with a dominant polarisation of the anodic reaction.

9. CONCLUSIONS

By exposing steel specimens to growing batch cultures of freshwater and marine SRB species in minimal iron media, the following information has been gained:

1. The rate of hydrogen absorption by freely corroding steel membranes increases rapidly within the first 30-50 hours of SRB growth and H_2S evolution, owing to the effects of H_2S on both the corrosion rate and the hydrogen combination reaction.

2. Approximately 30-50 hours after inoculation, a peak rate of hydrogen absorption is attained which gives a surface hydrogen concentration in steel (Co) of 0.004ppm. A subsequent decline in hydrogen permeation occurred, which was attributed to the formation of a protective sulphide film.

3. When cathodic protection is applied to -850mV (SCE), hydrogen permeation rates are increased by up to 15 times the values attained by freely corroding steel, giving a maximum Co value of approximately 0.06ppm.

4. Lowering of the applied potential from -850mV (SCE) to -1000mV (SCE) resulted in a doubling or tripling in the rate of hydrogen absorption, although initial hydrogen permeation currents were lower than in other similar experiments.

5. Polarisation scans performed in conditions similar to cathodic protection experiments confirmed the establishment of partially protective films, which had enhanced cathodic properties, but where anodic polarisation had a dominant effect on the corrosion rate.

APPENDICES

APPENDIX 1

MATERIALS AND APPARATUS MANUFACTURERS AND SUPPLIERS

BBL Microbiology Systems, P.O. Box No 243, Cockeysville, Maryland 21030 USA

British Drug Houses (BDH), Broom House, Poole, Dorset B12 4NN

British Oxygen Corporation (BOC), Special Gases

W Canning Materials Ltd, Great Flampton, Birmingham

C.A.P.C.I.S., Bainbridge House, UMIST, University of Manchester, Manchester M16

Chemlab Instruments Ltd, London

Ciba-Geigy Plastics & Additives Co (Plastics Division), Duxford, Cambridge CB2 4QA

City of London Polytechnic, Department of Biological Sciences, Old Castle Street, London E1

Gallenkamp Belton Road West, Loughborough, Leics, LE11 OTR.

Gelman Sciences Ltd, 10 Harrowden Road, Northampton NN4 OEZ

Greyhound Chromatography, 80 Grange Road West, Birkenhead, Merseyside L43 4XF

J.J. Lloyd Instruments Ltd, Brook Avenue, Southampton SO3 6HP

Jencons (Scientific Ltd), Cherrycourt Way Industrial Estate, Stanbridge Road, Leighton Buzzard, Beds LU7 8UA.

Knight Strip Metals Ltd, Potters Bar, Hertfordshire

Medicell International Ltd, 239 Liverpool Road, London N1 1LX

National Collection of Industrial Bacteria (NCIB), Torrey Research Station, 135 Abbey Road, Aberdeen AB9 8DG

N.E.I. International Combustion Ltd, Metallurgical Services, Sinfin Lane, Derby

Norton Performance Plastics, Chesterton Works, Loomer Road, Newcastle, Staffordshire ST5 7HR

Pye Unicam Ltd (Phillips), York Street, Cambridge CB1 2PX

Russel pH Ltd, Station Road, Auchtermuchty, Fife, Scotland KY14 7DP

S.J.Gaskets Ltd, Tything Road, Arden Forest Industrial Estate, Kinwarton, Alcester, Warwickshire B49 6EP

Sigma Chemical Company Ltd, Fancy Rd, Poole, Dorset BH17 7NH

Spex Industries Inc, 3880 Park Avenue, Metuchen, New Jersey 08840, USA

Sterilin Ltd, Sterilin House, Clockhouse Lane, Feltham, Middlesex TW14 8QS

Thompson Electrochem Ltd, Newcastle upon Tyne

Wesley Coe (Wingents) Ltd, 115-117 Cambridge Road, Milton, Cambridge CB4 4AY

APPENDIX 2: CULTURE MEDIA (Postgate, 1984)

Postgate's Medium 'B' (PMB)	**g/l**
KH_2PO_4	0.5
NH_4Cl	1.0
$CaSO_4$	1.0
$MgSO_4.7H_2O$	2.0
Sodium Lactate (60% Na Salt)	3.5ml
Yeast Extract	1.0
Ascorbic Acid	0.1
Thioglycollic Acid	0.1
$FeSO_4.7H_2O$	0.5

Postgate's Medium 'C' (PMC)	
KH_2PO_4	0.5
NH_4Cl	1.0
Na_2SO_4	4.5
$CaCl_2.6H_2O$	0.06
$MgSO_4.7H_2O$	0.06
Sodium Lactate (60% Na Salt)	6.0ml
Yeast Extract	1.0
$FeSO_4.7H_2O$	0.004
Tri-sodium Citrate.$2H_2O$	0.3

Postgate's Medium 'E' (PME)	
KH_2PO_4	0.5
NH_4Cl	1.0
Na_2SO_4	1.0
$CaCl_2.6H_2O$	1.0
$MgCl_2.7H_2O$	2.0
Sodium Lactate (60% Na Salt)	3.5ml
Yeast Extract	1.0
Ascorbic Acid	0.1
Thioglycollic Acid	0.1
$FeSO_4.7H_2O$	0.5
Agar	15.0

APPENDIX 3 SUMMARY OF TEST CONDITIONS AND RESULTS

Key to Notation used in Appendix

Test Medium:

PMC = Postgate's Medium 'C'
-Y/E = Yeast Extract omitted
Dis H_2O = Distilled Water
S = Sodium Chloride Supplement Added (3.5%)

Inoculum:

Dv.v = Desulfovibrio vulgaris (Woolwich)
Dv.d = Desulfovibrio desulfuricans (Norway-4)
(U) = Unwashed cell extract

Conditions/Parameters:

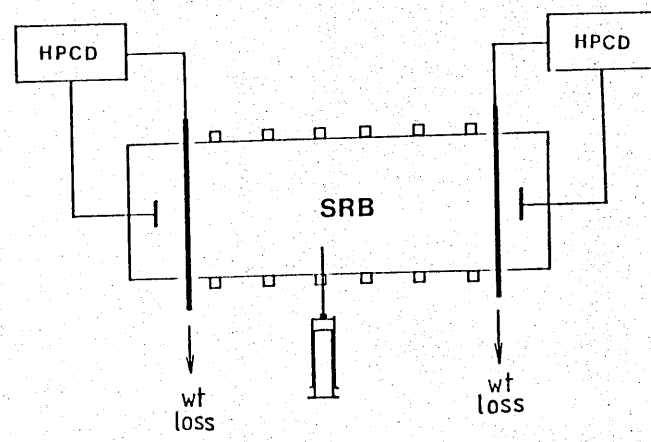
C = Cathodic charging of specimen
E = Redox Potential
F = Free Corrosion Potential
G = H_2S Gas Analysis
H = Hydrogen Permeation Current
N = SRB Cell Count
P = pH
Pol = Polarisation Analysis

Run	Test Medium	Inoculum	Specimen Thickness (cm)	Parameters Measured	Max H Current $\mu A/cm^2$	Surface H Conc'n (ppm) *	Metal Wt Loss (mdd)	Graph Reference	
								Figure	Page
A	PMC	Dv.v(U)	0.15	H	0.115	0.0359	-54.2	7.1a	130
B	PMC	Dv.v(U)	0.15	H,N	0.068	0.0212	-135.4	7.1a	130
C	PMC	Dv.v	0.01	H,N	0.100	0.0021	-	7.1b	130
D	PMC	Dv.v	0.0125	H,N	0.202	0.0053	-2.26	7.1b	130
E	PMC	Dv.v	0.0125	H,N	0.404	0.0105	+0.35	7.1b	130
F	PMC	-	0.0125	H	0.295	0.0077	-	7.1c	130
G	PMC	-	0.0125	H	0.214	0.0056	-	7.1c	130
H	PMC-Y/E	-	0.0075	H	0.136	0.0021	-	7.1c	130
I	Dis H_2O	-	0.0125	H	0.065	0.0017	-	7.1c	130
J	PMC/S	Dv.d	0.005	H,N	0.343	0.0036	-5.85	7.3	139
	"	Dv.d	0.005	H,N	0.392	0.0041	-6.07		
K	PMC/S	Dv.d	0.005	E,F,G,H,P	0.357	0.0037	-12.92	7.5a-f	148-151
	"	-	0.005	E,F,G,H	0.371	0.0039	-7.72		
L	PMC/S	Dv.d	0.005	E,F,G,H,N,P	0.360	0.0037	-39.24	7.6a-f	152-155
	"	-	0.005	E,F,G,H,P	0.374	0.0039	-16.46		
M	PMC/S	Dv.d	0.005	C,H,N	6.30	0.066	-	7.7a/b	157
	"	-	0.005	C,H,P	5.78	0.060	-		
N	PMC/S	Dv.d	0.005	C,E,H,Pol	3.21	0.033	-2.84	7.8a/b	158
	"	-	0.005	C,E,H,P,Pol	3.73	0.039	-3.11		
O	PMC/S	Dv.d	0.005	C,E,H,N,P	3.60	0.037	-	7.9	159
	"	-	0.005	C,H	2.90	0.030	-		

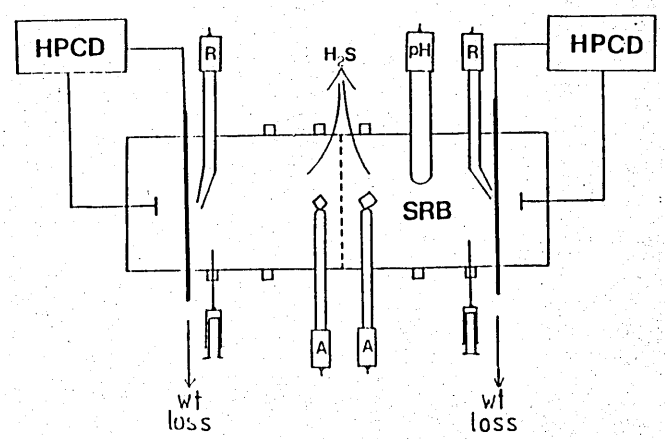
* Diffusion Coefficient, $D = 6.3 \times 10^{-7}$ cm^2 sec^{-1}

APPENDIX 4 Bioreactor Electrode Configurations

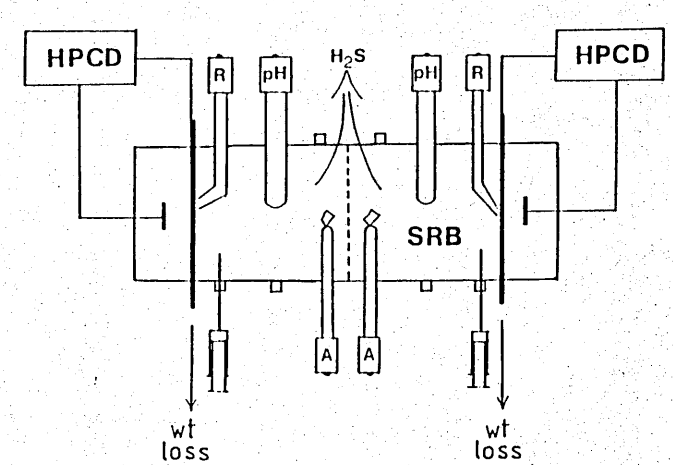
a)



b)

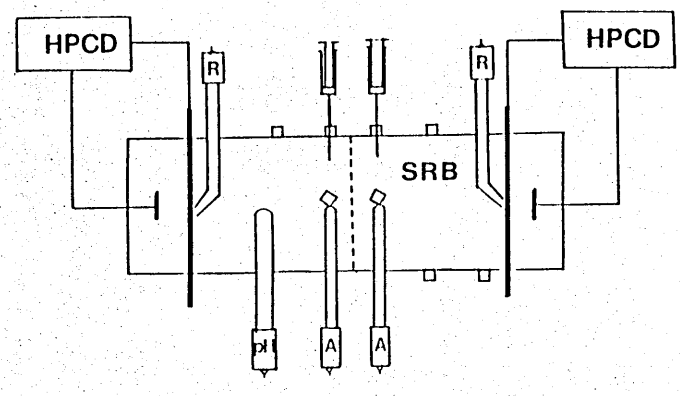


c)

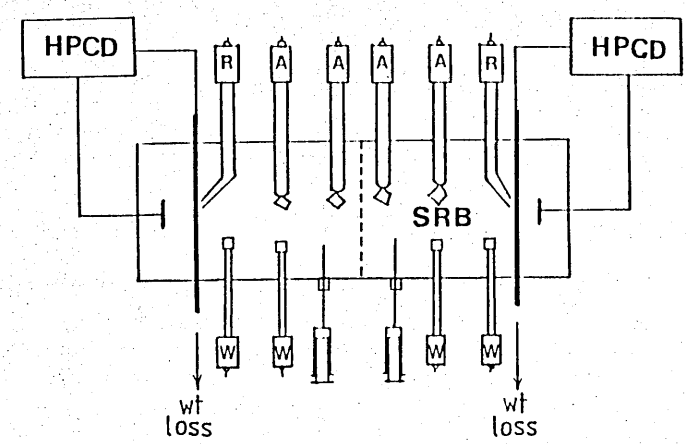


Electrode arrangements used in hydrogen permeation experiments on freely corroding steel foils in Run J (a), Run K (b) and Run L (c).

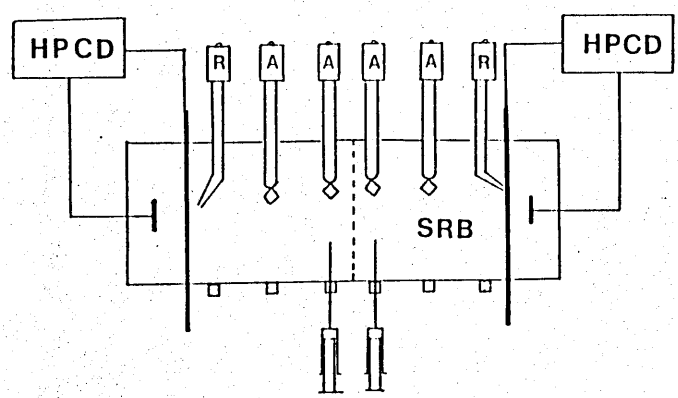
d)



e)



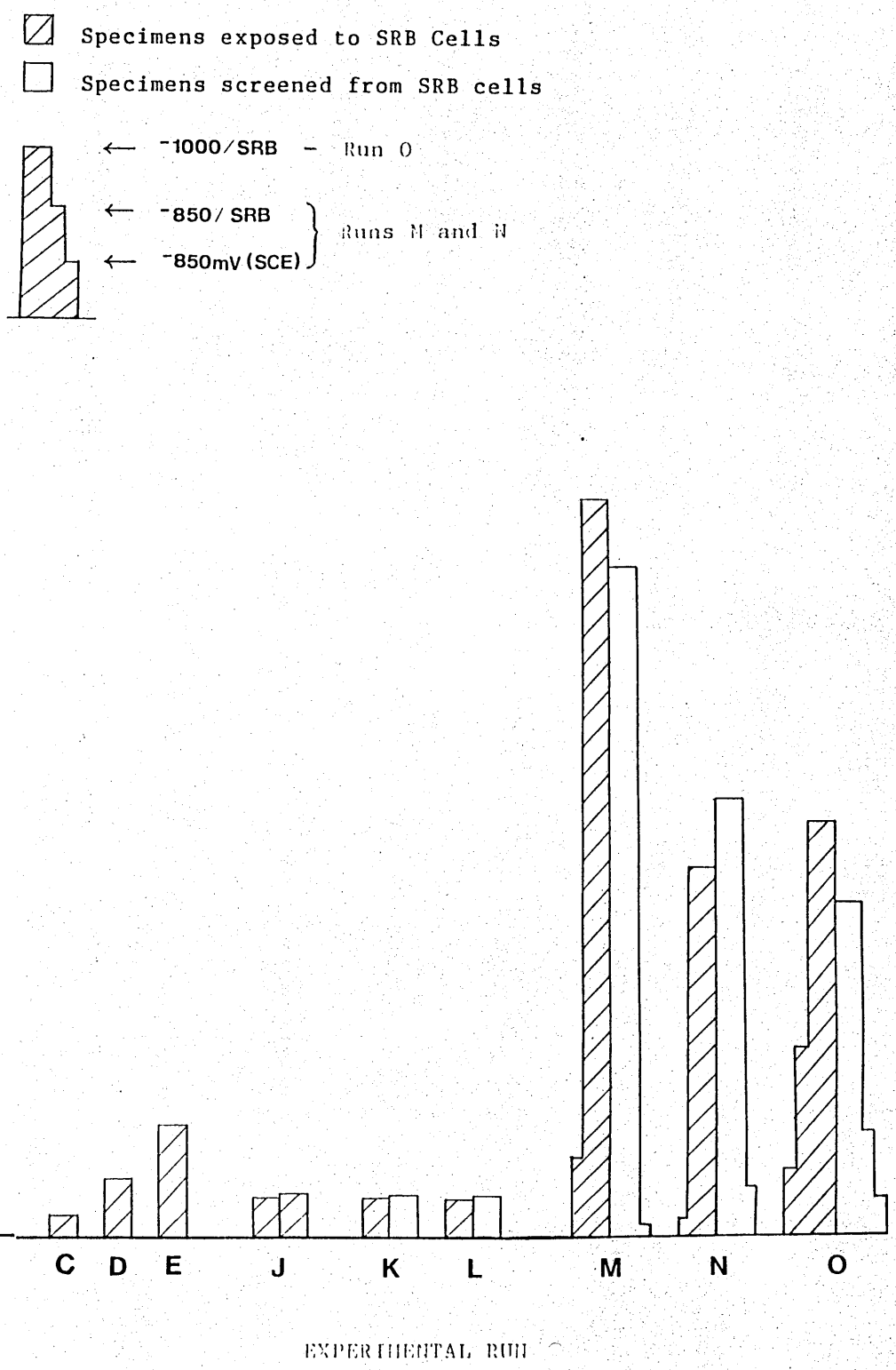
f)



Electrode arrangements used in hydrogen permeation experiments with cathodically protected steel foils in Run M (d), Run N (e) and Run O (f).

APPENDIX 5

Surface Hydrogen Concentrations Established During the Microbial Corrosion of Steel



REFERENCES

Abram, J.W. and Nedwell, D.B. (1978) Hydrogen as a Substrate for Methanogenesis and Sulphate Reduction in Anaerobic Saltmarsh Sediment. Archives of Microbiology, Vol 117, pp93-97.

Baars, J.K. (1930) Over sulfaat reductie door bacterien. Doctoral Thesis. Meinema: Delft.

Badziong, W.; Thauer, R.K. and Zeikus, J.G. (1978) Isolation and Characterisation of Desulfovibrio Growing on Hydrogen plus Sulfate as the Sole Energy Source. Archives of Microbiology, Vol 116, pp41-49.

Badziong, W. and Thauer, R.K. (1978) Growth yields and Growth Rates of Desulfovibrio vulgaris (Marburg) Growing on Hydrogen plus Sulfate and Hydrogen plus Thiosulfate as the Sole Energy Sources. Archives of Microbiology, Vol 117, pp209-214.

Badziong, W. and Thauer, R.K. (1981) Growth of Desulfovibrio species on Hydrogen and Sulphate as Sole Energy Source. Journal of General Microbiology, Vol 126, pp249-252.

Bak, F. and Cypionka, H. (1987) A novel type of energy metabolism involving fermentation of inorganic sulphur compounds. Nature, Vol 326, pp891-892.

Berman, D.A.; DeLuccia, J.J. and Mansfeld, F. (1979) Barnacle Electrode: New Tool for Measuring Hydrogen in High Strength Steels. Metal Progress. May, 1979, pp58-61.

Bockris, J.O'M. (1977) On Hydrogen Damage and the Electrical Properties of Interfaces. From the Proceedings of the Conference on 'Stress Corrosion Cracking and Hydrogen Embrittlement of Iron Base Alloys', Unieux-Firminy, France, June 12-16, 1973, pp286-305. Houston, Texas: NACE.

Bolmer, P.W. (1965) Polarisation of Iron in H_2S-NaHS Buffers. Corrosion-NACE, Vol 21, No 3, pp69-75.

Booth, G.H.; Cooper, P.M. and Wakerley, D.S. (1966) Corrosion of Mild Steel by Actively Growing Cultures of Sulphate-Reducing Bacteria. The Influence of Ferrous Iron. British Corrosion Journal, Vol 1, pp345-349.

Booth, G.H.; Cooper, A.W. and Cooper, P.M. (1967) Rates of Microbial Corrosion in Continuous Culture. Chemistry and Industry, December, pp2085-2086.

Booth, G.H.; Elford, L. and Wakerley, D.S. (1968) Corrosion of Mild Steel by Sulphate-Reducing Bacteria: An Alternative Mechanism. British Corrosion Journal, Vol 3, September, pp242-245.

Booth, G.H.; Shinn, P.M. and Wakerley, D.S. (1965) The Influence of Various Strains of Actively Growing Sulphate-Reducing Bacteria on the Anaerobic Corrosion of Mild Steel. Proceedings of Congres International de la Corrosion Marine et la Salissures, Cannes, pp363-371. Paris: CREO.

Booth, G.H. and Tiller, A.K. (1960) Polarization Studies of Mild Steel in Cultures of Sulphate-Reducing Bacteria. Transactions of the Faraday Society, Vol 56, pp1689-1696.

Booth, G.H. and Tiller, A.K. (1962) Polarisation Studies of Mild Steel in Cultures of Sulphate-Reducing Bacteria. Part 3: Halophilic Organisms. Transactions of the Faraday Society, Vol 58, pp2510-2516.

Booth, G.H. and Tiller, A.K. (1968) Cathodic Characteristics of Mild Steel in Suspensions of Sulphate-Reducing Bacteria. Corrosion Science, Vol 8, pp583-600.

Booth, G.H. and Wormwell, F. (1961) Corrosion of Mild Steel by Sulphate-reducing Bacteria. Effect of Different Strains of Organisms. Proceedings of the First International Congress on Metallic Corrosion, London 1961. pp341-344. London: Butterworth Press.

Brown, B.F. (1971) Stress Corrosion Cracking of High Strength Steels. From 'The Theory of Stress Corrosion Cracking of Alloys'; pp186-204. Edited by J.C. Scully. Brussels: NATO.

Brunt, K.D. (1987) Biocides for the Oil Industry. From 'Microbial Problems in the Offshore Oil Industry', Edited by E.C. Hill; J.L. Shennan and R.J. Watkinson. Proceedings of an International Conference Organised by the Institute of Petroleum Microbiology Committee, held in Aberdeen, April, 1986, pp201-208. Chichester: John Wiley and Sons.

Brysch, K.; Schneider, C.; Fuchs, G. and Widdel, F. (1987) Lithoautotrophic growth of sulfate-reducing bacteria, and description of Desulfobacterium autotrophicum gen. nov., sp. nov. Archives of Microbiology, Vol 148, pp264-274.

Characklis, W. G. and Cooksey, K. E. (1983) Biofilms and Microbial Fouling. From 'Advances in Applied Microbiology', Vol 29, pp93-138. London: Academic Press.

Coe, F.R. (1973) Welding Steels Without Hydrogen Cracking. The Welding Institute.

Cord-Ruwisch, R. and Widdel, F. (1986) Corroding Iron as a Hydrogen Source for Sulphate Reduction in Growing Cultures of Sulphate-Reducing Bacteria. Applied Microbiology and Biotechnology, Vol 25, pp169-174.

Costello, J.A. (1969) The Corrosion of Metals by Micro-Organisms - A Literature Survey. International Biodeterioration Bulletin, Vol 5, No 3, pp101-118.

Costello, J.A. (1974) Cathodic Depolarisation by Sulphate Reducing Bacteria. South African Journal of Science, Vol 70, pp202-204.

Costerton, J.W. and Geesey, G.G. (1978) Microbial Contamination of Surfaces. From the 'Proceedings of a Symposium on Surface Contamination: Its Genesis, Detection and Control', held at the Fourth International Symposium on Contamination Control, Washington D.C., September 10-14, 1978. Edited by K.L. Mittal. Vol 1, pp211-221. New York: Plenum Press.

Cragnolino, G. and Tuovinen, O.H. (1984) The Role of Sulphate-Reducing and Sulphur-Oxidising Bacteria in the Localised Corrosion of Iron-Base Alloys - A Review. International Biodeterioration, Vol 20, No 1, pp9-26.

Costerton, J.W. and Lashen, E.S. (1984) Influence of Biofilm on Efficacy of Biocides on Corrosion-Causing Bacteria. Materials Performance, February 1984, pp17.

Cypionka, H.; Widdel, F. and Pfennig, N. (1985) Survival of sulfate-reducing bacteria after oxygen stress, and growth in sulfate-free oxygen-sulphide gradients. FEMS Microbiology Ecology, Vol 31, pp39-45.

Devanathan, M.A.V. and Stachurski, Z. (1962) The Adsorption and Diffusion of Electrolytic Hydrogen in Palladium. Proceedings of the Royal Society Vol A270, pp90-102.

Devanathan, M.A.V. and Stachurski, Z. (1964) The Mechanism of Hydrogen Evolution on Iron in Acid Solutions by Determination of Permeation Rates. Journal of the Electrochemical Society, Vol 11, No. 5, pp619-623.

Dumas, S.; Massiani, Y. and Crousier, J. (1988) Microbiological Battery Induced by Sulphate-Reducing Bacteria. Corrosion Science, Vol 28, No 11, pp1041-1050.

Edyvean, R.G.J; Thomas, C.J; Brook, R. and Austen, I.M. (1986) The Use of Biologically Active Environments for Testing Corrosion Fatigue Properties of Offshore Structural Steels. From 'Biologically Induced Corrosion', Proceedings of International Conference on Biologically Induced Corrosion, Gaithsburg, Washington D.C., June 1985, Edited by Dexter, S.C., pp254-267. Houston, Texas: NACE.

Edyvean, R.G.J. (1987) Biodeterioration Problems of North Sea Oil and Gas Production - A Review. International Biodeterioration, Vol 23, pp199-231.

Elbeik, S; Tseung, A.C.C. and Mackay, A.L. (1986) The Formation of Calcareous Deposits During the Corrosion of Mild Steel in Sea Water. Corrosion Science, Vol 26, No 9, pp669-680.

Fischer, K.P. (1984) Cathodic Protection in Saline Mud Containing Sulfate-Reducing Bacteria. From 'Petroleum Microbiology', pp110-116; Edited by R.M. Atlas. New York: MacMillan.

Gaines, R.H. (1910) Bacterial Activity as a Corrosive Influence in the Soil. Industrial and Engineering Chemistry, Vol 2, pp128-130.

Gaylarde, C.C. and Johnston, J.M. (1980) The Importance of Microbial Adhesion in Anaerobic Metal Corrosion. A poster contribution in a compilation of corrosion related papers from 'Microbial Adhesion to Surfaces', Edited by R.C.W. Berkeley, J.M.Lynch. Chichester: Ellis Horwood Ltd.

Gaylarde, C.C. and Johnston, J.M. (1982) The Effect of Vibrio anguillarum on the Anaerobic Corrosion of Mild Steel by Desulfovibrio vulgaris. International Biodeterioration Bulletin, Vol 18, No 4, pp111-116.

Gaylarde, C.C and Videla, H.A. (1987) Localised Corrosion Induced by a Marine Vibrio. International Biodeterioration Vol 23, pp91-104.

Gerchakov, S.M.; Little, B.J. and Wagner, P. (1986) Probing Microbiologically Induced Corrosion. Corrosion-NACE, Vol 42, No 11, November, pp689-692.

Gilbert, P. D. (1985) Verbal presentation delivered to the International Biodeterioration Research Group on Microbial Corrosion, Cranfield Institute of Technology, Bedford, 9 September, 1985.

Gilbert, P.D.; Attwood, P.A.; David, T.; Morgan, B. and Herbert, B.N. (1987) Biofilm Associated Corrosion. From 'Corrosion '87', Proceedings of the NACE Conference, Brighton, October, 1987.

Gooch, T.G. (1982) Hardness and Stress Corrosion Cracking of Ferritic Steel. The Welding Institute Research Bulletin, August 1982.

Gooch, T.G. (1984) Cathodic Protection and Steel Properties. From 'Corrosion and Growth on Offshore Structures', pp81-94. Edited by J.R. Lewis and A.D. Mercer. Chichester: Ellis Horwood Ltd.

Greene, N.D.; Moebus, G.A. and Baldwin, M.H. (1973) The Mini Potentiostat: A Versatile Power Source for Electrochemical Studies. Corrosion-NACE, Vol 29, No. 6, pp234-236.

Groves, G.R. (1972) A Study of Bacterial Sulphate Reduction. PhD Thesis, Department of Chemical Engineering, UMIST, August 1972.

Hamilton, W.A. (1985) Sulphate-Reducing Bacteria and Anaerobic Corrosion. Annual Reviews of Microbiology, Vol 39, pp195-217.

Hamzah, R.B. and Robinson, M.J. (1987) Hydrogen Absorption Resulting From the Simulated Pitting Corrosion of Carbon-Manganese Steels. Corrosion Science, Vol 27, No 9, pp971-979.

Hardy, J.A. (1983) Utilisation of Cathodic Hydrogen by Sulphate-Reducing Bacteria. British Corrosion Journal, Vol 18, No 4, pp190-193.

Hardy, J.A. & Bown, J.L. (1984) The Corrosion of Mild Steel by Biogenic Sulfide Films Exposed to Air. Corrosion-NACE, Vol 40, No 12, pp650-654.

Hardy, J.A. and Hamilton, W.A. (1981) The Oxygen Tolerance of Sulfate-Reducing Bacteria Isolated from North Sea Waters. Current Microbiology, Vol 6, pp259-262.

Hatchikian, E.C.; Chaigneau, M. and Le Gall, J. (1975) Analysis of Gas Production by Growing Cultures of Three Species of Sulphate-Reducing Bacteria. Proceedings of Symposium on Microbial Production and Utilisation of Gases, 1975. Gottingen: E.Goltze.

Herbert, B.N. and Gilbert, P.D. (1984) Isolation and Growth of Sulphate-Reducing Bacteria. From 'Microbiological Methods For Environmental Biotechnology', Society of Applied Biotechnology Technical Series, No 19, pp235-257. London: Academic Press.

Herbert, B.N. and Stott, F.D.J. (1983) The Effects of Pressure and Temperature on Bacteria in Oilfield Water Injection Systems. From 'Microbial Corrosion', Proceedings of Conference Organised jointly between The Metals Society and the National Physical Laboratory, and held at the NPL, Teddington, London, 1983. London: Metals Society.

Herbert, B.N. (1987) Reservoir Souring. From 'Microbial Problems in the Offshore Oil Industry', Edited by E.C. Hill; J.L. Shennan and R.J. Watkinson. Proceedings of an International Conference Organised by the Institute of Petroleum Microbiology Committee, held in Aberdeen, April, 1986, pp63-72. Chichester: John Wiley and Sons.

Hoar, T.P. and Farrer, T.W. (1961) The Anodic Characteristics of Mild Steel in Dilute Aqueous Soil Electrolytes. Corrosion Science, Vol 1, pp49-61.

Iverson, W.P. (1966) Direct Evidence for the Cathodic Depolarisation Theory of Bacterial Corrosion. Science, Vol 151, No 3713, pp986.

Iverson, W.P. (1968) Corrosion of Iron and Formation of Iron Phosphide by Desulfovibrio desulfuricans. Nature, Vol 217, No 5153, p1265.

Iverson, W.P. and Olson, G.J. (1982) Problems Related to Sulfate-Reducing Bacteria in the Petroleum Industry. From 'Petroleum Microbiology', edited by Atlas, R.M. Chapter 16, pp620-641.

Iverson, W.P. and Olson, G.J. (1984) Anaerobic Corrosion by Sulfate-Reducing Bacteria due to Highly Reactive Volatile Phosphorous Compound. From 'Microbial Corrosion' Proceedings of Conference Organised jointly between The Metals Society and the National Physical Laboratory, and held at the NPL, Teddington, London, 1983. London: Metals Society.

Khosrovi, B.; MacPherson, R. and Miller, J.D.A. (1971) Some Observations on Growth and Hydrogen Uptake by Desulfovibrio vulgaris. Archives of Microbiology, Vol 80, pp324-337.

King, R.A. and Miller, J.D.A. (1971) Corrosion by the Sulphate-Reducing Bacteria. Nature, Vol 233, pp491-492.

King, R.A.; Dittmer, C.K. and Miller, J.D.A. (1976) Effect of Ferrous Ion Concentration on the Corrosion of Iron in Semi-continuous Cultures of Sulphate-Reducing Bacteria. British Corrosion Journal, Vol 11, No 2, 105-107.

King, R.A.; Miller, J.D.A. and Wakerley, D.S. (1973a) Corrosion of Mild Steel in Cultures of Sulphate-Reducing Bacteria: Effect of Changing the Soluble Iron Concentration During Growth. British Corrosion Journal, Vol 8, March, pp89-93.

King, R.A.; Miller, J.D.A. and Smith, J.S. (1973b) Corrosion of Mild Steel by Iron Sulphides. British Corrosion Journal, Vol 8, pp137-141.

King, R.A. and Wakerley, D.S. (1973) Corrosion of Mild Steel by Ferrous Sulphide. British Corrosion Journal, Vol 8, January, pp41-45.

LaQue, F.L. (1975) Marine Corrosion, Causes and Prevention. New York: John Wiley and Sons.

Lucas, K.A. and Robinson, M.J. (1986) The Influence of Lattice Hydrogen Content on the Hydrogen-Assisted Cracking of High Strength Steel. Corrosion Science, Vol 26, No 9, pp705-717.

Lupton, F.S.; Conrad, R. and Zeikus, J.G. (1984) Physiological Function of Hydrogen Metabolism During Growth of Sulfidogenic Bacteria on Organic Substrates. Journal of Bacteriology, Vol 159, No 3, pp843-849.

Mara, D.D and Williams, D.J.A. (1972a) Polarisation of Pure Iron in the Presence of Iron Sulphide Minerals. British Corrosion Journal, Vol 7, March, pp94-95.

Mara, D.D and Williams, D.J.A. (1972b) The Mechanism of Sulphide Corrosion by Sulphate-Reducing Bacteria. From 'Biodeterioration of Materials', edited by Walters, A.H. and Hueck-van der Plas, Vol 2, p103. London: Applied Science.

Mara, D.D and Williams, D.J.A. (1972c) Influence of the Microstructure of Ferrous Metals on the Rate of Microbial Corrosion. British Corrosion Journal, Vol 7, May, pp139-142.

Maxwell, S. and Hamilton, W.A. (1985) Effect of Cathodic Protection on the Activity of Microbial Biofilms. From 'UK Corrosion 85', Proceedings of the Conference held by NACE, 4-6 November, 1985, Harrogate, pp281-291.

McCright, R.D. (1977) Effects of Environmental Species and Metallurgical Structures on the Hydrogen Entry Into Steel. Proceedings of the Conference on 'Stress Corrosion Cracking and Hydrogen Embrittlement of Iron Base Alloys', Unieux-Firminy, France, June 12-16, 1973, pp306-325. Houston, Texas: NACE.

Mergey, C. (1984) Hydrogen Diffusion through Steel and Tinplate. British Corrosion Journal, Vol 19, No. 3, pp132-138.

Miller, J.D.A. (1971) Microbial Aspects of Metallurgy. Aylesbury: Medical and Technical Publishing Co Ltd.

Miller, J.D.A. (1981) Metals, Chapter 6, 'Microbial Biodeterioration', Edited by Rose, A.H. Economic Microbiology Series, Vol 16, pp149-202. London: Academic Press.

Miller, J.D.A. and King, R.A. (1975) Biodeterioration of Metals. From 'Microbial Aspects of the Deterioration of Materials', Edited D.W. Lovelock and R.J. Gilbert. pp83-103. London: Academic Press.

Miller, J.D.A. and Wakerley, D.S. (1966) Growth of Sulphate-Reducing Bacteria by Fumarate Dismutation. Journal of General Microbiology, Vol 43, pp101-107.

Miodownik, A.P. (1977) The Interaction of Hydrogen with Dislocations, Stacking Faults, and Other Interfaces. Proceedings of a Conference on Stress Corrosion Cracking and Hydrogen Embrittlement of Iron Base Alloys', Unieux-Firminy, France, June 12-16, 1973, pp272-285. Houston, Texas: NACE.

Moosavi, A.N. and Hamilton, W.A. (1987) Microbial Corrosion Studies in a Marine Sulphuretum. From 'Microbial Problems in the Offshore Oil Industry', Edited by E.C. Hill; J.L. Shennan and R.J. Watkinson. Proceedings of an International Conference Organised by the Institute of Petroleum Microbiology Committee, held in Aberdeen, April, 1986, pp13-26. Chichester: John Wiley.

Morgan, T.D.B. and Steele, A.D. (1987) Factors affecting the Durability of Reinforced Concrete under Semi-stagnant Offshore Conditions. From 'Microbial Problems in the Offshore Oil Industry', Edited by E.C. Hill; J.L. Shennan and R.J. Watkinson. Proceedings of an International Conference Organised by the Institute of Petroleum Microbiology Committee, held in Aberdeen, April, 1986, pp39-48. Chichester: John Wiley and Sons.

Mortenson, L.E. and Chen, J. (1974) Hydrogenase. Chapter 11 from 'Microbial Iron Metabolism', pp231-282. New York: Academic Press.

Nriagu, J.O. and Hem, J.D. (1980) Chemistry of Pollutant Sulfur in Natural Waters. From Sulphur in the Environment Part II: Ecological Impacts. Edited by Jerome O. Niagru. New York: John Wiley & Sons.

Odum, J.M. and Peck, H.D. (1981) Hydrogen Cycling as a General Mechanism for Energy Coupling in the Sulfate-Reducing Bacteria. FEMS Microbiology Letters, Vol 12, pp47-50.

Oriani, R.A. (1987) Hydrogen - The Versatile Embrittler. Corrosion-NACE, Vol 43, No 7, July 1987, pp390-397.

Pankhania, I.P. (1988) Hydrogen Metabolism in Sulphate Reducing Bacteria and its Role in Anaerobic Corrosion. Biofouling, Vol 1, pp27-47.

Pankhania, I.P.; Gow, L.A. and Hamilton, W.A. (1986) The Effect of Hydrogen on the Growth of Desulfovibrio vulgaris(Hildenborough) on Lactate. Journal of General Microbiology Vol 132, pp3349-3356.

Pankhania, I.P.; Moosavi, A.N. and Hamilton, W.A. (1986) Utilisation of Cathodic Hydrogen by Desulfovibrio vulgaris (Hildenborough). Journal of General Microbiology Vol 132, pp3357-3365.

Peck, H.D. (1974) The Evolutionary Significance of Inorganic Sulfur Metabolism. Proceedings of the Symposium for the Society of General Microbiology, 1974, Vol 24, pp241-262.

Pfennig, N. and Biebl, H. (1977) Desulfuromonas acetoxidans gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate oxidising bacterium. Archives of Microbiology, Vol 55, pp245-256.

Pfennig, N.; Widdel, F. and Truper, H.G. (1981) The Dissimilatory Sulfate-Reducing Bacteria. From 'The Prokaryotes', Edited by Starr, M.P.; Stolp, H.; Truper, H.G.; Balows, A. and Schlegel, H.G. Chapter 74, pp926-940. Berlin: Springer-Verlag.

Postgate, J.R. (1951) The Reduction of Sulphur Compounds by Desulphovibrio desulphuricans. Journal of General Microbiology, Vol 5, pp725-738.

Postgate, J.R. (1984) The Sulphate Reducing Bacteria, Second Edition. Cambridge: Cambridge University Press.

Pumphrey, P.H. (1980) The Effect of Sulphide Inclusions on the Diffusion of Hydrogen in Steels. From 'Hydrogen Effects in Metals', Proceedings of the Third International Conference on the Effects of Hydrogen on the Behaviour of Materials, Wyoming, USA, August 1980; Edited by Bernstein, I.M. and Thompson, A.W. Metallurgical Society of AIME.

Reider, R.; Cammack, R. and Hall, D.O. (1984) Purification and Properties of the Soluble Hydrogenase from Desulfovibrio desulfuricans (strain Norway 4). European Journal of Biochemistry, Vol 145, pp637-643.

Salvarezza, R.S. and Videla, H.A. (1984) Corrosion of Carbon Steel Induced by Sulfate-Reducing Bacteria. Effect of Chloride and Sulfide Anions. From 'Marine Biology', Proceedings of the Sixth International Congress on Marine Corrosion and Fouling, Athens, Greece, 1984, pp429-441.

Scully, J.C. (1975) The Fundamentals of Corrosion. Second Edition. International Series on Materials Science and Technology, Volume 17. Oxford: Pergamon Press.

Siegel, L.M. (1975) Biochemistry of the Sulphur Cycle. Chapter 7 from 'Metabolism of Sulfur Compounds', Edited by Greenerg, D.M. Metabolic Pathways series, Vol 7, third edition, pp217-286. New York: Academic Press Inc.

Smith, J.S. and Miller, J.D.A. (1975) Nature of Sulphides and Their Corrosive Effect on Ferrous Metals: A Review. British Corrosion Journal, Vol 10, No 3, pp136-143.

Staehle, R.W.; Hochmann, J.; McCright, R.D. and Slater, J.E. (1977) (Editors) Proceedings of the Conference on 'Stress Corrosion Cracking and Hydrogen Embrittlement of Iron Base Alloys', Unieux-Firminy, France, June 12-16, 1973, pp306-325. Houston, Texas: NACE.

Starkey, R.L. (1958) The General Physiology of the Sulfate Reducing Bacteria in Relation to Corrosion. Producers Monthly, No 6, 1958, pp12-30.

Stephenson, M. and Stickland, L.H. (1931) Hydrogenase II. The Reduction of Sulphate to Sulphide by Molecular Hydrogen. Biochemical Journal, Vol 25, pp215-220.

Thauer, R.K.; Jungermann, K. and Decker, K. (1977) Energy Conservation in Chemotrophic Anaerobic Bacteria. Bacteriological Reviews, Vol 41, No 1, pp 100-180.

Thomas, C.J.; Edyvean, R.G.J. and Brook, R (1986) Biologically Enhanced Corrosion Fatigue. Biofouling, Vol 1, pp65-77.

Tiller, A.K. (1982) From 'Corrosion Processes', Edited by Parkins, R.N., pp115-159. London: Applied Science Publishers.

Togano, H.; Sadaki, H.; Nakahara, T.; Nakauchi, H.; Kanda, Y.; Osato, K (1975) The Influence of Sulfate-Reducing Bacteria on the Corrosion of Mild Steel in Artificial Sea Water. Proceedings of the Sixth International Congress on Metallic Corrosion, Victoria, Australia, pp1258-1269. Australian Corrosion Association.

Treseder, R.S. (1977) Oil Industry Experience with Hydrogen Embrittlement and 'Stress Corrosion Cracking. Proceeding of a Conference on Stress Corrosion Cracking and Hydrogen Embrittlement of Iron Base Alloys', Unieux-Firminy, France, June 12-16, 1973, pp147-161. Houston, Texas: NACE.

Tsuji, K. and Yagi, T. (1980) Significance of Hydrogen Burst from Growing Cultures of Desulfovibrio vulgaris, Miyazaki, and the Role of Hydrogenase and Cytochrome c3 in Energy Production System. Archives of Microbiology, Vol 125, pp35-42.

Uhlig, H.H. and Revie, R.W. (1985) Corrosion and Corrosion Control; An introduction to corrosion science and engineering. Third Edition, New York: John Wiley.

van Gelder, K.; Simon Thomas, M.J.J. and Kroese, C.J. (1986) Hydrogen-Induced Cracking: Determination of Maximum Allowed H_2S Partial Pressures. Corrosion-NACE, Vol 42, No 1, January, pp36-43.

von Wolzogen Kuhr, C.A.H. and van der Vlugt, I.S. (1934) The Graphitisation of Cast Iron as an Electrobiochemical Process in Anaerobic Soils. Water, Vol 18, 147-165.

Walch, M. and Mitchell, R. (1984) Hydrogen Uptake by Metals in the Presence of Bacterial Films. From 'Biodeterioration 6', pp228-232, Proceedings of the Sixth International Biodeterioration Symposium, held in Washington D.C., August, 1984. C.A.B. International.

Wanklyn, J.N. and Spruit, C.J.P. (1952) Influence of Sulphate-reducing Bacteria on the Corrosion Potential of Iron. Nature, Vol 169, pp928-929.

Weast, R.C. (1989) Chemical Rubber Company Handbook of Chemistry and Physics. 70th Edition. p F-169. Florida, USA: CRC Press Inc.

White, D.C.; Nivens, D.E.; Nichols, P.D.; Kerger, B.D.; Henson, J.M.; Geesey, G.G. and Clarke, C.K. (1985) The Role of Extracellular Polymers in Microbial Adhesion and Corrosion. From Proceedings of Argentine-USA Workshop on Biodeterioration (Conicet-NSF), April, 1985, La Plata, Argentina, pp73-86.

Wilkinson, T.G. (1984) Biological Mechanisms Leading to Potential Corrosion Problems. From 'Corrosion and Marine Growth on Offshore Structures', Edited by J.R. Lewis and A.D. Mercer, section 1.4, pp31-37. Chichester: Ellis Horwood Ltd.

Wolfson, S.L. and W.H. Hartt (1981) An Initial Investigation of Calcareous Deposits Upon Cathodic Steel Surfaces in Sea Water. Corrosion-NACE, Vol 37, No2, pp70-76.

Wranglen, G. (1972) An Introduction to Corrosion and Protection of Metals. Frome, Dorset: Butler and Tanner.

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