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The Effect Of Marine Fouling On The Corrosion
Of Steel In The Marine Environment

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NOTATION

C.P.	Cathodic protection
cfu	Colony forming unit
C.P.M.	Counts per minute
D.O.C.	Dissolved organic carbon
D.P.M.	Disintegrations per minutes
E_{corr}	Free corrosion potential
I_{corr}	Free corrosion current density
H.I.C.	Hydrogen induced cracking
L.P.R.	Linear polarisation resistance
pH	Hydrogen ion concentration
S.E.M.	Scanning electron microscope
S.C.E	Standard calomel electrode
S.R.B.	Sulphate reducing bacteria
U.V.	Ultra violet

ABSTRACT

An investigation was made into the corrosion of steel immersed in seawater by electrochemical (LPR scans) and weight loss techniques. Steel samples in the form of studs or a specially designed corrosion probe were immersed in a re-circulating laboratory based flow loop inoculated with marine microorganisms. A parallel biological study into biofilm development on corroding steel was made using viable counts, scanning electron microscopy and radio-respirometric assays. The development of a biofilm on steel studs cathodically protected at -850 mV S.C.E. and -950 mV S.C.E. was studied using these three indices of biological activity.

The corrosion of steel in the real marine environment and in a bacteria-free system were monitored.

Results indicate that steel corrosion followed similar trends in all these investigations. Corrosion rates were initially very high, decreased, stabilised and rose slightly. Attempts to correlate results from weight losses and LPR scans were made. Biofilms on the steel surface were very similar, and not influenced by the use or level of cathodic protection nor period of exposure. Viable counts indicate higher levels of Sulphate Reducing Bacteria were present on corroding than on cathodically protected steel. The development of a biofilm could not be correlated with trends in corrosion rates.

1.0 INTRODUCTION

Some studies have shown that when steel is immersed in seawater it corrodes very rapidly at first, corrosion rates then decrease and subsequently stabilise. There is some evidence that later on corrosion rates increase significantly. These trends may be linked to the activity of a microfouling layer on the steel surface. Microorganisms in the sea rapidly adhere to steel, multiply and soon form a complex biofilm. Some bacteria within the biofilm utilise oxygen at a faster rate than it can diffuse through the accumulated corrosion product and bacterial cells, this results in an oxygen-free zone developing adjacent to the steel surface. Sulphate Reducing Bacteria (SRB) grow only in anaerobic conditions and may proliferate on the steel surface. During steel corrosion hydrogen is produced from the cathodic reaction, which SRB are able to use as an energy source. The consumption of cathodic hydrogen by these obligate anaerobes is believed to accelerate the overall corrosion rate which is limited by the cathodic reaction. Another important metabolic activity of SRB, as their name implies is the reduction of sulphate to sulphide. As various chemical forms of iron sulphide are cathodic to steel they further accelerate corrosion by forming a galvanic cell.

Steel corrosion of offshore structures is often controlled by the use of cathodic protection. This manipulation of the potential, usually to -850 mV S.C.E., promotes the cathodic reaction and stops the anodic reaction in which iron dissolves. SRB are able to grow on cathodically protected steel. The iron sulphide deposited may necessitate the control potential to be lowered by another 100 mV to combat the cathodic nature of it. Cathodic protection results in the formation of hydroxyl ions raising the pH locally. Additionally, much cathodic hydrogen is produced which is a potential energy source for SRB. The interaction of both these factors on the growth of SRB and other microorganisms in the biofilm is not well documented.

This investigation had several aims: to monitor the corrosion of steel immersed in seawater; to study the development of a biofilm on the corroding steel; and to investigate if different levels of cathodic protection influence biofilm formation. To achieve these aims various techniques were employed. Firstly the corrosion of steel in seawater was monitored using electrochemical means and by determining rates of weight loss. The corrosion of small steel studs and of a specially designed corrosion probe were studied. Steel samples were immersed in seawater flowing in a re-circulating flow loop in the laboratory. The artificial seawater contained marine microorganisms grown in fermenters. A parallel study of the biofilm development on the

steel samples was conducted by using viable counts, scanning electron microscopy and radio-respirometric assay. Biofilm development on freely corroding steel samples was compared with that on samples cathodically protected at -850 mV S.C.E. and -950 mV S.C.E.. Additionally the corrosion of steel in the real marine environment and a bacteria-free system were studied.

LITERATURE SURVEY

2.1 CONDITIONING FILM FORMATION

2.1.1 Adsorption Of Macromolecules To The Solid-liquid Interface To Form A Conditioning Film

Metal surfaces (or most other solids) immersed in natural seawater are rapidly covered in a 'conditioning film' as macromolecules spontaneously adsorb to it (Fletcher & Marshall, 1982; Niehof & Loeb, 1974). This conditioning film is essential for the later development of a biofilm. The macromolecules forming the conditioning film are thought to originate from two main sources (Baier, 1984):- Firstly from the spontaneous adsorption of macromolecules to the surface from the bulk liquid; secondly from the bacterial secretion of protein-polysaccharide extracellular material by colonising bacteria. There is some dispute as to whether the conditioning film is composed of glycoproteins (Baier, 1984) or of humic substances (Loeb & Niehof, 1975).

Baier (1984) took infra red spectra of conditioning films in situ on germanium prisms and found them to be composed mainly of glycoproteins and proteoglycans. These macromolecules with

polysaccharides and proteins occur in low concentrations in natural seawater (Fletcher & Marshall, 1982).

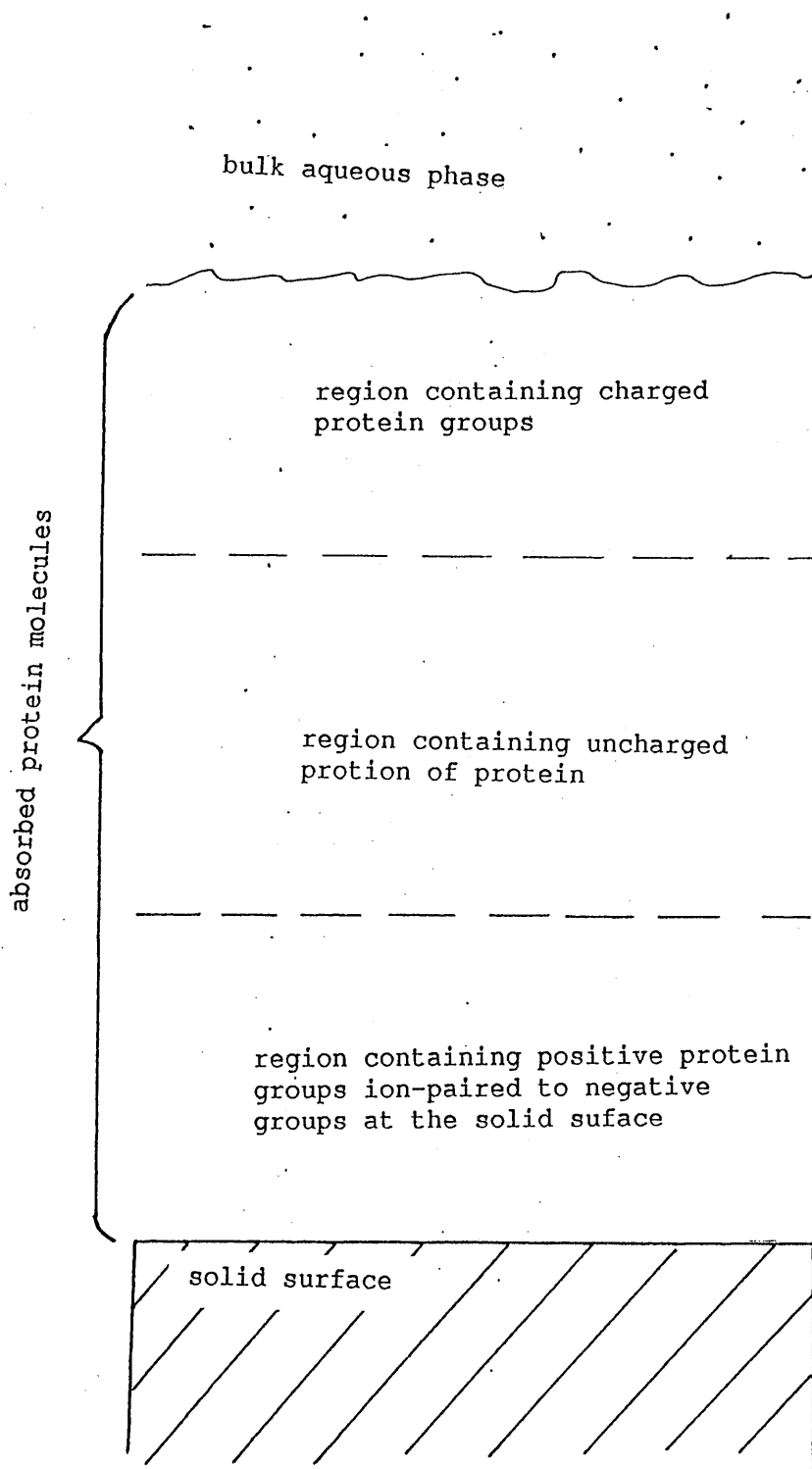
Norde and Lykelema (1978) presented a model for the formation of a proteinaceous conditioning film at the solid liquid interface. The film is composed of three distinct regions (refer to FIGURE 2.1.1). The one closest to the solid surface consists of positive protein groups ion-paired to the negative groups at the solid surface. The middle region contains the uncharged portion of the protein. The region adjacent to the liquid phase contains negative charges. This will result in the surface charge of the solid being altered by the conditioning film as it assumes the charge characteristic of the outermost region of the conditioning film.

Niehof & Loeb (1974) used microelectrophoresis to show that the surface charge of test particles immersed in seawater is altered by an adsorbed organic film. When immersed in natural seawater test particles of quartz, germanium and an anion-exchange resin (with negative, zero and positive surface charges respectively) all assumed moderately negative values. Then the test particles were immersed in organic-free seawater they assumed their expected negative, zero or positive surface charges. Exposing water to ultraviolet radiation destroys the organic matter in it by photo-oxidation (Niehof & Loeb, 1974);

FIGURE 2.1.1

Model Of A Proteinaceous Conditioning Film

(Adapted from Norde and Lyklema, 1978.)



Armstrong, Williams & Strickland, 1966). It was concluded that the surface charge of solids in seawater was due to an adsorbed film of organic molecules, particularly polymers, proteins and humic acids.

2.1.2 The Effect Of Conditioning Film Formation On Surface Free Energy And Its Implications For Bacterial Attachment

The adsorption of macromolecules to an immersed substratum alters the free energy of the surface as well as the surface charge. The free energy of a surface is the available energy resulting from surface groups, molecules or atoms which are able to interact with similar groups molecules or atoms belonging to adjacent phases. Adjacent phases capable of interacting with the surface may be the seawater or a bacterial surface.

The free surface energy of a substratum will be reduced by the adsorption of bacterial surface polymer to it. This reduction in the surface free energy favours the adhesion of the bacterium (Fletcher & Marshall, 1982). The amount of surface free energy indicates the tendency for the substratum to take part in spontaneous interactions and therefore its suitability for bacterial attachment.

Bacterial attachment cannot be regarded in thermodynamic terms alone. The decrease in surface free energy is applicable to the spontaneous attachment of bacteria, but it is not relevant to bacterial attachment mechanisms which consume metabolic energy (Fletcher & Marshall, 1982).

Once a bacterium is adsorbed to the conditioning film on the substratum it may become reversibly attached to it. In this first phase of attachment the bacterium still exhibits Brownian motion and can be washed off the surface (Marshall, Stout & Mitchell, 1971A). Bacterial attachment involves forces of electrostatic attraction and repulsion between the substratum and the bacterium. Most bacteria have a net negative surface charge as does the conditioned substratum (Niehof & Loeb, 1974). Forces between the conditioned substratum and a bacterium will tend to be repulsive. The degree of repulsion between a bacterium and the substratum depends not only on their net charge but also on their separation distance, radii of curvature and the electrolyte concentration (Fletcher & Marshall 1982; Marshall, Stout & Mitchell, 1971A).

The repulsive forces between a bacterium and the substratum are balanced by Van der Waal's forces of attraction. The interaction of repulsive and attractive forces may result in overlapping ionic atmospheres (diffuse double layers) between

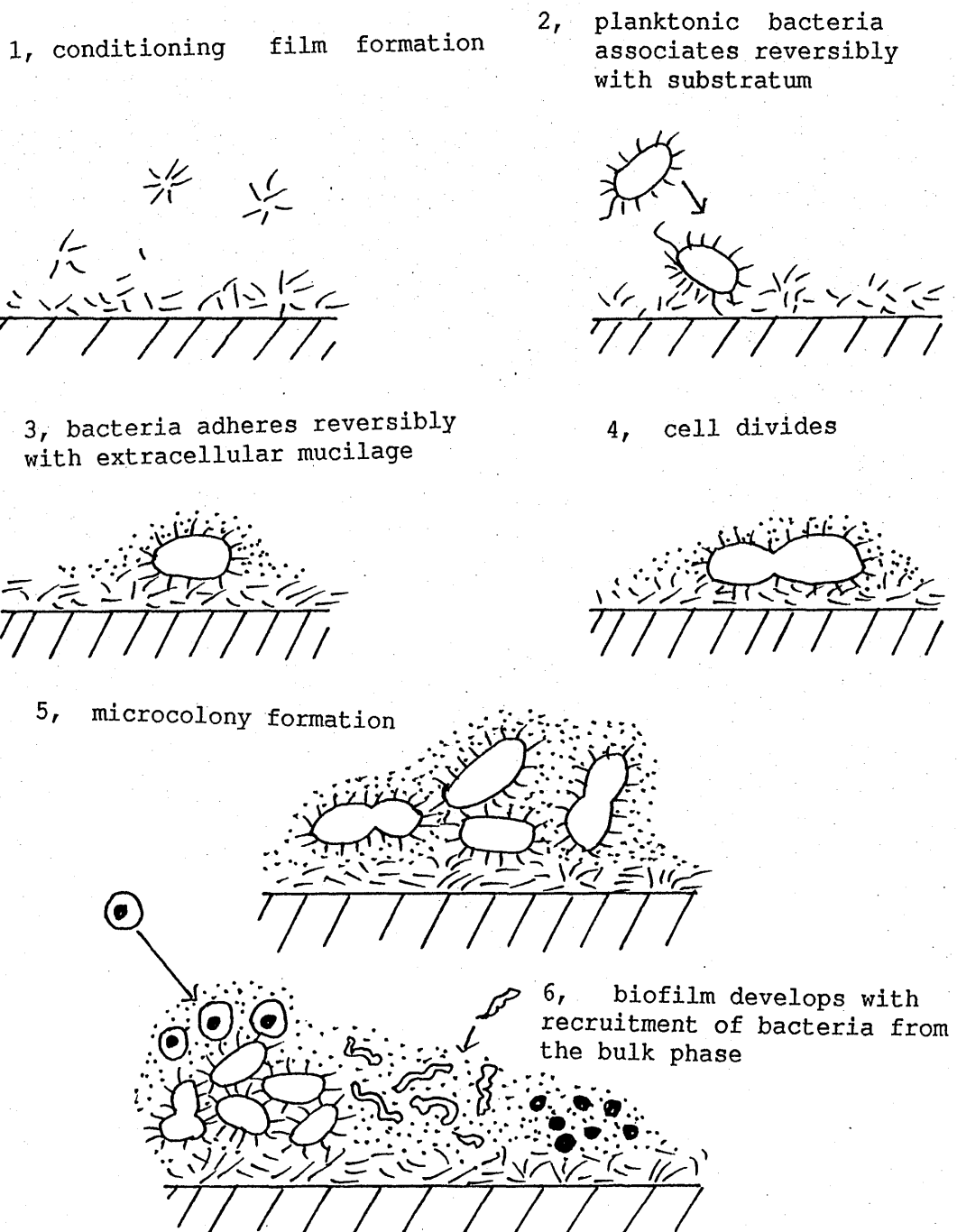
the bacterium and the substratum leading to irreversible attachment (Marshall, Stout & Mitchell, 1971A).

The first reversible phase of bacterial attachment is followed by an irreversible phase in which they adhere firmly to the substratum, no longer exhibiting Brownian motion and are impervious to washing (Marshall, Stout & Mitchell, 1971A). Bacteria produce extracellular polymeric substances which may overcome electrostatic repulsion, sticking the cell to the substratum (Marshall, Stout & Mitchell, 1971A; Fletcher & Marshall, 1982). These events are summarised in FIGURE 2.1.2.

Electronmicroscopy has demonstrated the production of extracellular polymer fibrils by small rod bacteria firmly attached to a nickel grid within 24 hours of immersion (Marshall, Stout & Mitchell, 1971B). Some bacteria produce specialised attachment structures (eg. holdfasts or pilli) but within the short span of the study, 24 hours, such structures were uncommon. The extracellular polymer which gram negative bacteria produce (most aquatic bacteria are gram negative) is lipopolysaccharide, this is produced with proteins and lipoproteins.

It is thought that calcium and magnesium cations may be involved in irreversible attachment. Marine Pseudomonads did not attach irreversibly and polymeric fibrils were not produced in

FIGURE 2.1.2

Diagram Summarising Early Biofilm Formation

seawater lacking these cations (Marshall, Stout & Mitchell, 1971A). These cations may bridge negative charges on the bacterium and substratum surfaces so reducing electrostatic repulsion between them.

2.1.3 Ecological Advantages of Bacterial Attachment to A Substratum

Bacteria attached to a substratum have a nutritional advantage over those remaining in the bulk phase. Most natural seawater is oligotrophic, containing low levels of dissolved organic carbon (D.O.C.) and low concentrations of nutrients. A 'typical' microlitre of seawater contains about 1 ng of D.O.C., of which 50 pg is in a form bacteria can utilise (Fletcher, Gray & Jones, 1987). The conditioning film of organic macromolecules represents a nutrient 'sink' to which bacteria are attracted. Marshall, Stout & Mitchell (1971A) found that very low levels of D.O.C. (7 mg l^{-1} of glucose) stimulated the irreversible attachment of marine Pseudomonads, whereas higher DOC concentrations inhibited it. In an environment of flowing water, static, attached bacteria are surrounded by a continually renewed source of nutrients and oxygen.

2.2 BIOFILMS IN THE MARINE ENVIRONMENT

2.2.1 Microbial Succession And Early Biofilm Development

The development of a biofilm depends on environmental parameters, especially nutrient availability (Hamilton, 1987). Maximal biofilm development may take several days to several weeks. The ecological succession of different bacterial types in the biofilm to a climax microbial community has been studied extensively (Little, 1984; Mitchell & Kirchman, 1984; Fletcher & Marshall, 1982; Marshall, Stout & Mitchell, 1971B)

Marshall, Stout & Mitchell (1971B) detected a sequence of adsorption of bacterial types to glass slides. Some rod bacteria attached within an hour; spiral and coccoidal bacteria attached after 6-8 hours; lastly, stalked bacteria started to attach after 24 hours. Small cocci bacteria were the primary colonisers on nucleopore filters immersed in a lake rich in organic metal complexes in Nova Scotia. After 16.9 - 23 hours larger rod bacteria became dominant (Kepkay, Schwinghammer, Willar & Bowen, 1986). They proposed that the cocci attached to the substratum, utilised nutrients in the conditioning film and grew into larger rod shaped cells. Microelectrodes were used to measure microgradients of oxygen and carbon dioxide above the biofilm surface. Analysis of these microgradients revealed that

the succession of cocci to rods was concurrent with a peak in respiration.

In another study the colonisation of aluminium, copper-nickel, stainless steel and titanium foils immersed in an estuary were investigated (Little, 1984). Small rod bacteria were the first colonisers in all cases, attaching to stainless steel within four hours. The corrosion product which rapidly formed on aluminium and copper-nickel tended to blanket the rod bacteria. Small rods may have an advantage over other bacterial groups as primary colonisers as, they are able to reproduce at low nutrient concentrations and can produce extracellular polymeric fibrils for attachment. Secondary colonisers are in most instances filamentous or stalked bacteria. After 48 hours the biofilm on stainless steel exposed to flowing conditions was colonised by rods and filamentous bacteria.

2.2.2 Biofilm Development And Structure

The early biofilm matures into a dense, complex layer of bacteria intermeshed with extracellular polysaccharide mucilage which they secrete. The time span biofilm development takes is dependent on environmental parameters, principally nutrient availability (Hamilton, 1987). An abundant nutrient supply can lead to a mature biofilm developing in 1-3 days. Generally in laboratory

experiments simulating marine conditions biofilm development reaches a plateau after 10-15 days.

The development of a mature biofilm can be broken down into several sub-stages. The permanent adhesion of primary colonising bacteria to the steel surface is soon followed by their division. Replication produces microcolonies of sister cells (Costerton, Cheng, Geesey & Ladd, 1987). Microcolonies may remain monospecific or may develop into more complex consortia of several species (Costerton, Geesey & Jones, 1987). The biofilm also grows by the recruitment of cells from the bulk water phase (Costerton, Cheng, Geesey & Ladd, 1987) which may be attracted to metabolic products produced by the primary colonisers (Costerton, Geesey & Jones). Fouling of surfaces coated in a variety of anti-fouling paints or left untreated followed this pattern (Jackson & Jones, 1988). Their SEM study revealed that isolated bacterial cells grew into microcolonies, these increased in size and coalesced to form a bacterial film on the substratum. The mature biofilm was a thick mat of bacteria and extracellular polysaccharide.

The extracellular polysaccharide mucilage secreted by bacteria is one of the most striking features of the biofilm when examined with the SEM. Indeed, it may compose 50-90 % of the biofilm (Characklis & Cooksey, 1983). SEM studies show that an

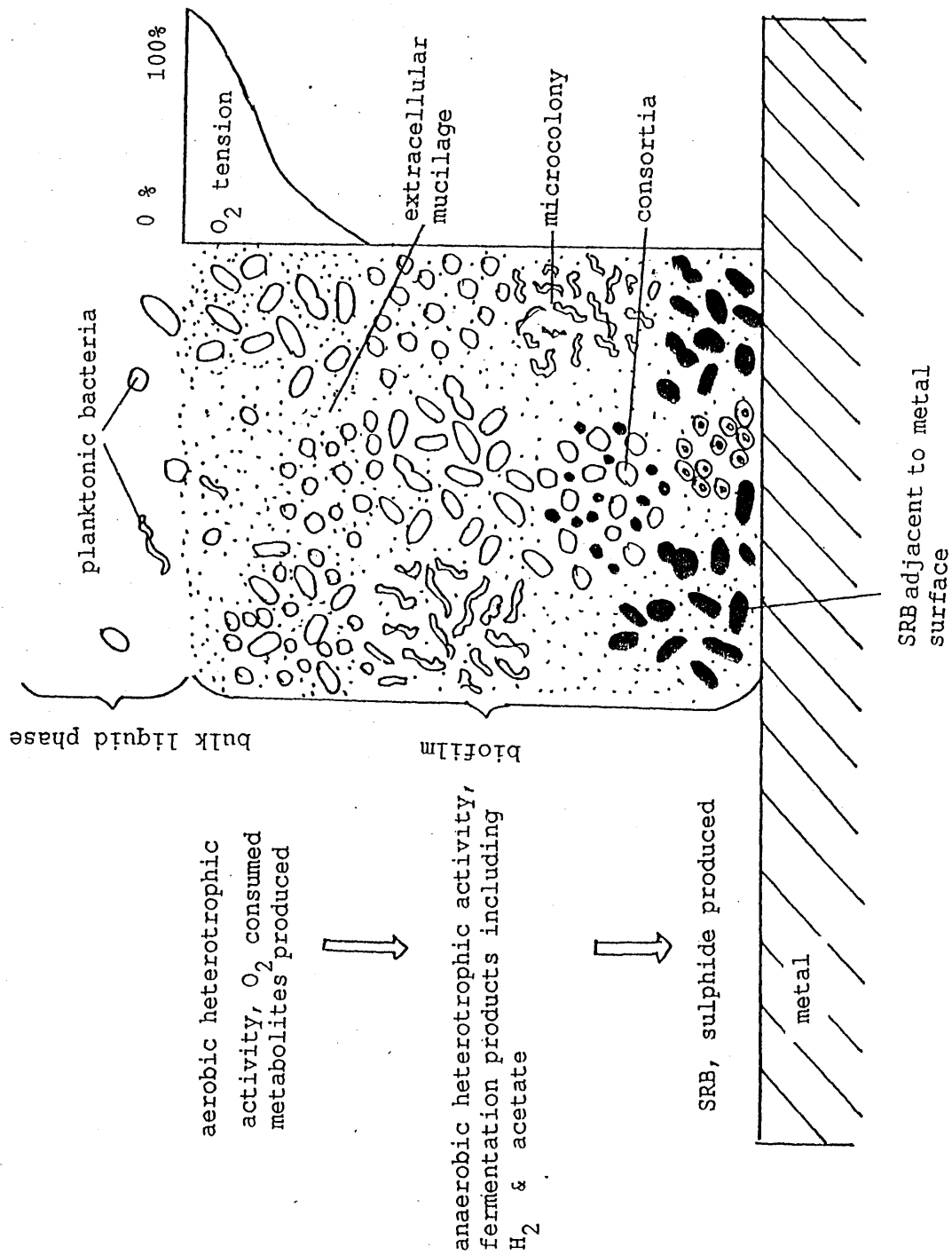
elaborate 3-D network of mucilage extends throughout the biofilm (Dempsey, 1981; Jackson & Jones, 1988), although some of its features may be artefacts of fixation techniques. The mucilage is mainly composed of different polysaccharides and has a variety of functions in addition to its adhesive role in binding primary and secondary colonisers to the substratum (Dempsey, 1981; Hamilton, 1987). It can act as an ion exchange matrix and concentrate organic nutrients (Costerton & Lashen 1984). Another role of the mucilage is protective against predators (eg. phagocytic amoebae) and agressins (eg. antifoulants) (Dempsey, 1981).

Within a mature biofilm complex nutritional relationships occur between bacterial microcolonies (refer to FIGURE 2.2.2). Organisms present in the biofilm adjacent to the bulk liquid metabolise hydrocarbons and general organic detritus in it. These microorganisms are mainly phototrophic or heterotrophic and may be aerobic or facultative. Their metabolic activities produce fatty acids and sugars (Hamilton, 1985). Nutrients from the bulk liquid phase diffuse into the biofilm, resulting in increased cell numbers and extracellular polysaccharide production (Characklis, 1984). An oxygen gradient occurs as it diffuses from the bulk liquid phase into the biofilm. Nearer to the substratum surface where the rate of oxygen diffusion into the biofilm is less than its rate of utilisation by aerobic

FIGURE 2.2.2

Diagram Representing A Mature Biofilm On A Metal Surface

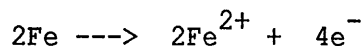
(Adapted from Costerton, Geesey & Jones, 1987; Maxwell & Hamilton, 1985)



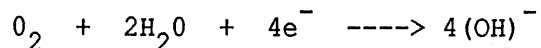
organisms an anaerobic zone forms (Hamilton, 1985 & 1987). Here anaerobic bacteria such as sulphate reducing bacteria (SRB) proliferate and metabolise end products from the metabolism of aerobic organisms.

2.3 STEEL CORROSION IN THE MARINE ENVIRONMENT

Corrosion of steel in seawater involves electrochemical reactions in which an anodic oxidation reaction occurs simultaneously with a cathodic reaction at adjacent sites. The anodic reaction of iron in neutral seawater is:



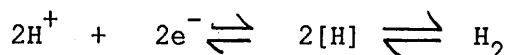
The metal ions are either released into solution or react forming an insoluble corrosion product. For the anodic reaction to proceed it must be accompanied by a simultaneous cathodic (reduction) reaction. In aerated seawater this is commonly the reduction of oxygen:



Electrons produced by the anodic reaction are taken up at the cathode, so a corrosion current flows from the anode to the

cathode. In order for the electrical circuit to be completed ions diffuse through the seawater (electrolyte). The anodic and cathodic reactions may occur over the entire metal surface resulting in fairly uniform corrosion. The anodic and cathodic reaction sites exchange places at intervals. The corrosion rate is proportional to the flow of current (I) from the anode to the cathode and the potential (E) of the iron at equilibrium with its own ions in seawater.

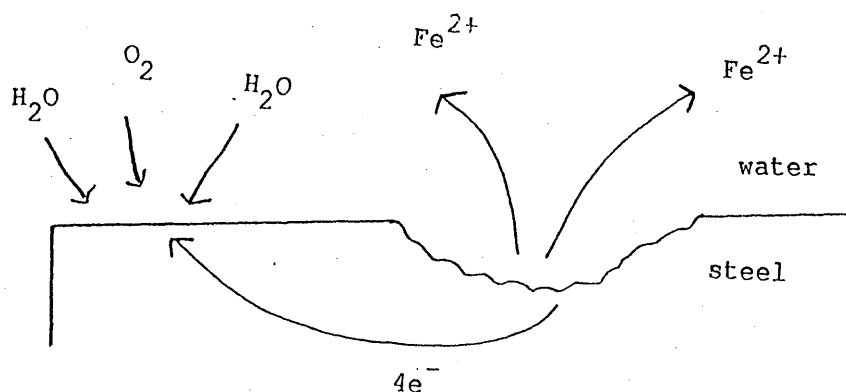
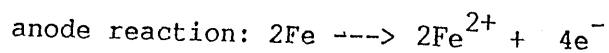
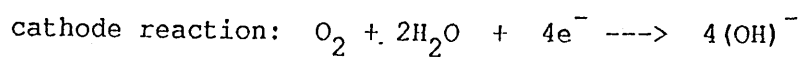
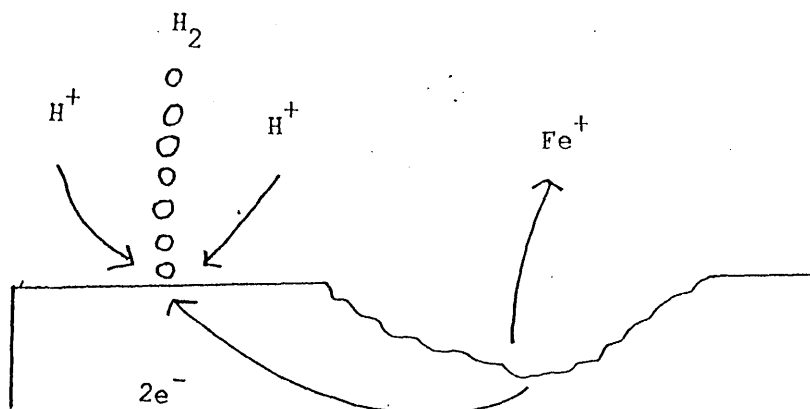
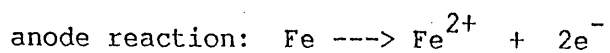
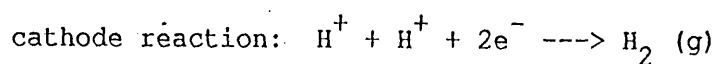
In the absence of oxygen the cathodic reaction results in the formation of hydrogen. Protons take over the role of accepting electrons in the following reaction:



The basic corrosion processes under aerobic and anaerobic conditions are summarised in the FIGURE 2.3.A. Removal of the end products of the cathodic reactions (ie. hydroxyl ions under aerobic conditions and hydrogen under anaerobic conditons) accelerate the corrosion rate.

The overall rate of steel corrosion in seawater tends to be under cathodic control, so it is determined by the rate of the cathodic reaction. This depends on the rate at which oxygen can diffuse to the steel surface. Physical barriers hindering the

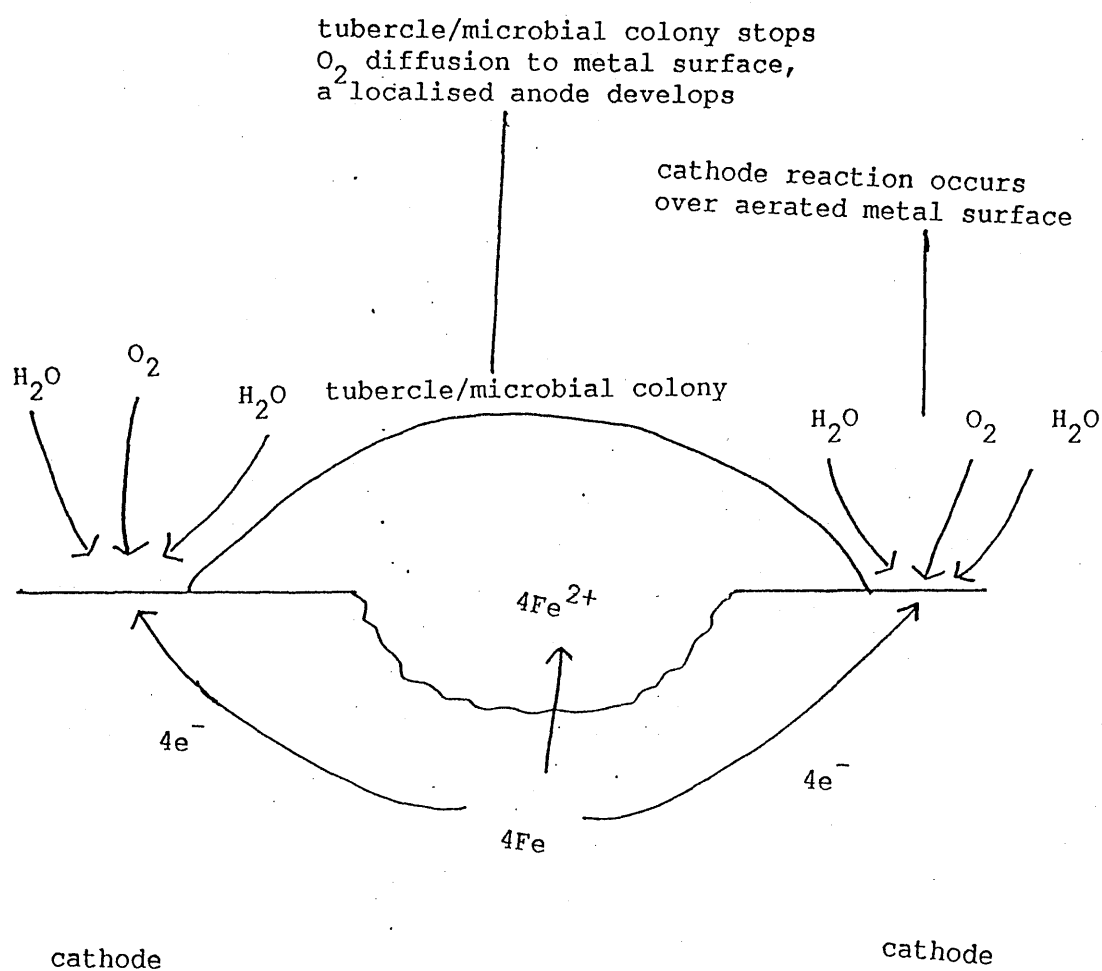
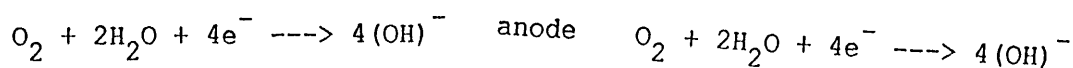
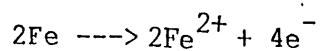
FIGURE 2.3.A

Steel Corrosion In Aerated, Neutral SeawaterSteel Corrosion In Deaerated Seawater

rate of diffusion to the steel surface will decrease the corrosion rate. The non-uniform accumulation of corrosion product or colonies of fouling organisms on the steel surface may result in the formation of differential aeration cells. In these circumstances corrosion is not uniform over the metal surface site for the cathodic reaction. The mechanism of corrosion by a differential aeration cell is shown in FIGURE 2.3.B.

FIGURE 2.3.B

Mechanism Of Differential Aeration Cell Under A Tubercle Or Microbial Colony



2.4 MICROBIAL CORROSION

Microbial corrosion can be defined as metal loss caused or accelerated by microbial action at the anode, cathode or both these sites (Miller, 1981). Important groups of microfouling organisms associated with corrosion are aerobic and anaerobic bacteria and algae. Marine fouling organisms influence steel corrosion by several different methods:

- a, The physical presence of fouling organisms creating differential aeration, pH cells or other cells.
- b, End products of metabolism by fouling organisms which accelerate corrosion such as acids and sulphur-containing compounds.
- c, By the stimulation of cathodic (reduction) reactions.
- d, By the stimulation of anodic (oxidation) reactions.

2.4.1 Differential Aeration and Other Cells

The non-uniform growth of fouling organisms may result in the formation of a corrosion cell due to the differential aeration at the steel surface (Gerchachov & Udey, 1984). Aerobic respiration by fouling organisms forms a locally anoxic region beneath the microbial colony (Tiller, 1983). The anoxic region becomes the anode, localised metal dissolution can lead to severe pitting. Pits may initiate fatigue cracks in the steel (Edyvean, 1987). The aerated steel surface surrounding the microbial colony becomes the site for the cathodic reaction. A variety of microorganisms including bacteria, yeasts, filamentous fungi and algae can grow into a coherent colony or mat and set up a concentration cell (Miller, 1981). The formation of differential aeration cells by micro or macro fouling is an important corrosion mechanism in well-oxygenated seawater (Characklis, Zilver & Roe, 1986).

Steel corrosion by differential aeration is encouraged by the development of large tubercles through the activities of iron bacteria. These bacteria are chemolithic autotrophs, obtaining energy from the oxidation of ferrous to ferric ions (Miller, 1981). Ferric hydroxide produced by Gallionella spp., Sphaerotilus spp., and Crenothrix spp. is precipitated in a

slimy sheath around the cells (Edyvean, 1987) forming large tubercles. The following diagram (FIGURE 2.4.1) shows the mechanism of anode formation by differential aeration cells under a tubercle (and compares it to sulphur concentration cells), the situation may equally well occur under a microbial colony

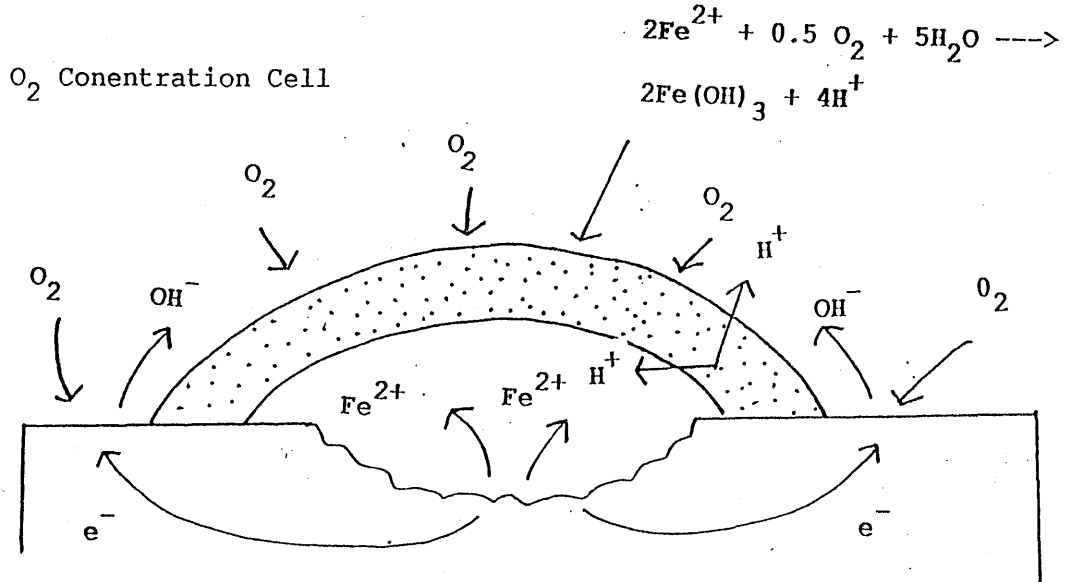
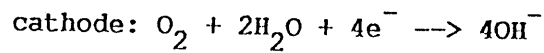
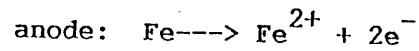
Microalgae readily settle on steel surfaces forming a dense mat, particularly in the photic zone. The pH under a microalgal mat exhibits a diurnal pattern, becoming more acidic at night and more basic during the day due to their photosynthesis. The pH under Oscillatoria sp. (Edyvean & Terry, 1983) and colonial diatoms and Enteromorpha sp. (Terry & Edyvean, 1981) was raised to 9.8 - 10.1 during the day. The steel may become 'passivated' due to the high pH, and corrosion rates decrease as a protective oxide layer forms (Terry & Edyvean, 1981). In practice this is not likely to occur since the pH drops each night as photosynthesis stops. Differential pH cells may develop on adjacent areas of steel surface as a consequence of the non-uniform coverage by algal mats. Terry and Edyvean (1983), found that very low pH (as low as 2) were generated by decaying colonial diatoms. Differences in pH may set up corrosion cells and enhance pitting corrosion.

The extracellular mucilage produced by different bacterial microcolonies may accelerate corrosion rates. Extracellular

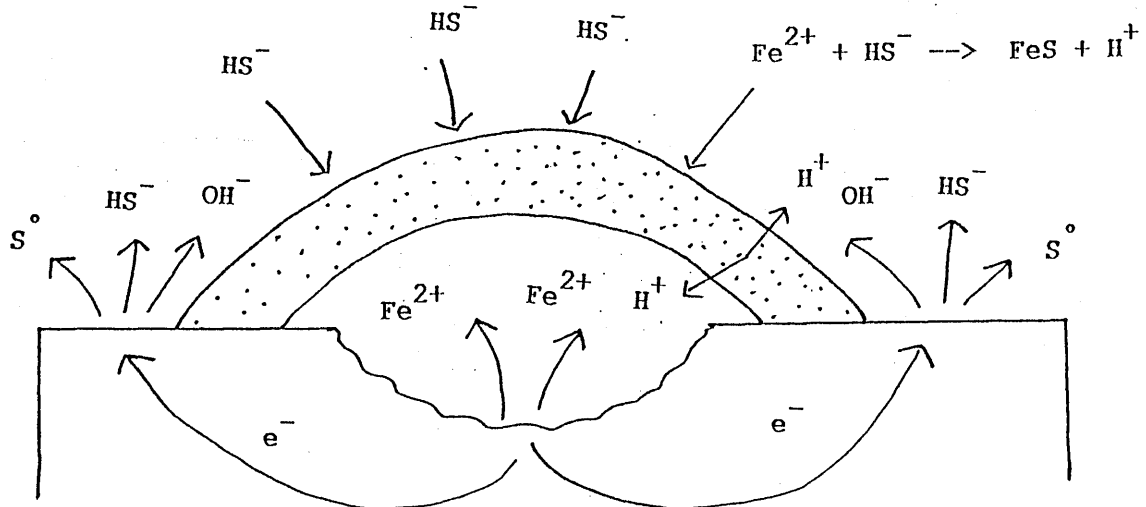
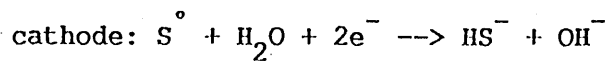
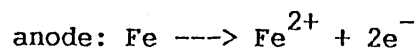
FIGURE 2.4.1

Diagram Comparing Oxygen Concentration And Sulphur Concentration Cells

(After Schaschl, 1980)



sulphur concentration cell

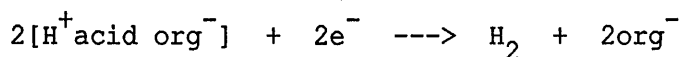


mucilage produced by different microcolonies of a freshwater bacterium have been isolated (Geesey, Mittleman, Iwaoka & Griffiths, 1985) and shown to possess differing abilities to bind copper. Microcolonies of the bacteria when attached to a copper surface formed copper concentration cells with adjacent areas in a biofilm containing less reactive mucilage. Indeed, copper corrosion was enhanced by the presence of two mucilages with different metal binding capacities in the absence of living bacteria. Natural biofilms that develop on submerged steel contain a mixed population of bacteria. Their extracellular mucilages will have different metal binding capacities and may contribute to concentration cell formation resulting in pitting corrosion.

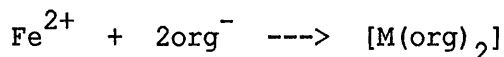
Under certain conditions elemental sulphur may be deposited on steel surfaces. Sulphur is thought to promote corrosion in anoxic conditions by forming a concentration cell in a similar manner to differential aeration cells (Schaschl, 1980). An anode develops under a tubercle or microbial colony which shields the steel from dissolved sulphur. In the surrounding cathodic region hydrogen sulphide is generated. The mechanism of sulphur concentration cells is compared to differential aeration cells in FIGURE 2.4.1.

2.4.2 Production of Corrosion promoting Substances

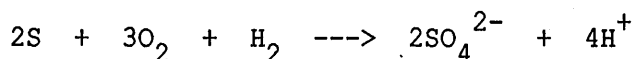
Some microorganisms secrete organic or inorganic acids as end products of their metabolism which enhance corrosion. Organic acids such as acetic, succinic and lactic are produced by bacteria during the Krebs cycle. Metals such as steel which evolve hydrogen whilst corroding encourage the reduction of organic acids (Calderon, Stratfeld & Coleman, 1968):

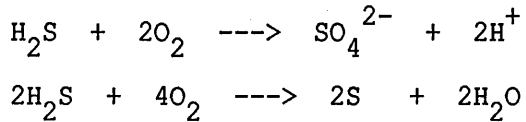


This reaction is followed by the precipitation of an organic salt of iron:



Sulphur oxidising bacteria are capable of deriving energy for growth from the oxidation of a wide range of inorganic sulphur compounds (Craginalino & Tuovinen, 1984). Thiobacilli are a group of aerobic sulphur oxidising bacteria able to produce sulphuric acid from sulphide or elemental sulphur (Purkiss, 1971)., Some of the oxidation reactions that Thiobacilli are capable of include:





Sulphuric acid is a much stronger acid than weak organic acids and so is likely to promote corrosion more.

Under anaerobic conditions SRB can produce highly corrosive, volatile phosphorus compounds (Iverson & Olsen, 1984). Iron phosphide has been identified as an important corrosion product in addition to iron sulphide.

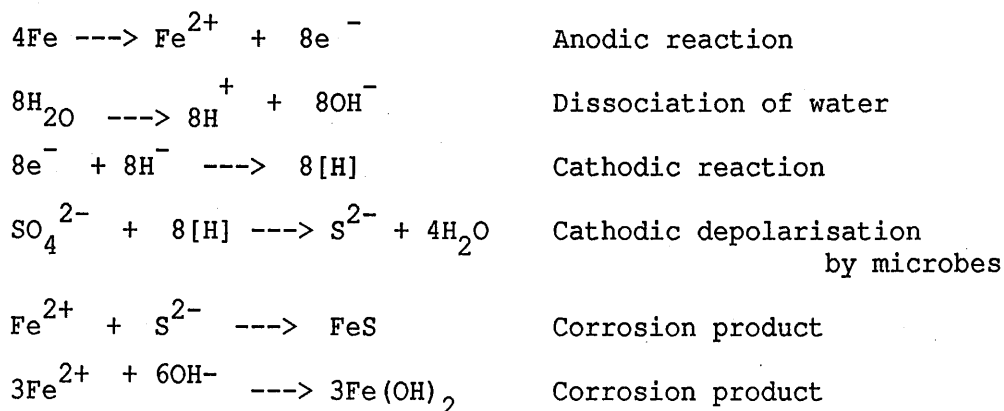
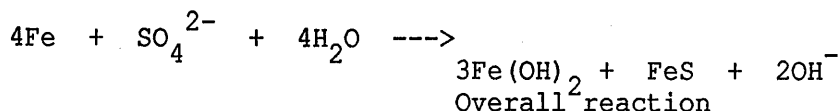
2.4.3 Stimulation of Cathodic (Reduction) Reactions

Steel corrosion promoted by the stimulation of cathodic (reduction) reactions is mostly due to the activities of SRB under anaerobic conditions. In anoxic conditions the reduction of oxygen as the cathodic reaction can not occur. Instead protons take over the role of oxygen by accepting electrons, thus hydrogen is formed. The combination of atomic to molecular hydrogen is slow and often the rate limiting step to the cathodic corrosion reaction. In practice observed corrosion rates are frequently much higher than expected; an enhancement of the cathodic reaction must be occurring.

The role that SRB have in enhancing steel corrosion is controversial. SRB may accelerate the cathodic reaction by utilising cathodic hydrogen for the dissimilatory reduction of sulphate. In this process they produce sulphide which is itself corrosive. Firstly the hydrogen metabolism of SRB and its role in anaerobic corrosion will be considered.

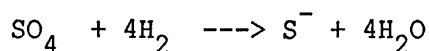
Hydrogen Metabolism in Sulphate Reducing Bacteria and Its Role in Anaerobic Corrosion

Wolzogen Kuhr and van der Vlugt (1934) studied severe corrosion of pipes buried in water-logged soil in Holland. Iron sulphide was present on the pipes and in surrounding wet soil with SRB. They attempted to explain this type of corrosion in terms of chemical reactions:

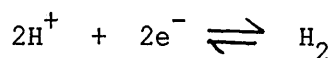


If this occurred a molar ratio of corroded iron to iron sulphide of 4:1 would be produced. This is not commonly observed (Miller, 1981).

SRB produce two or three enzymes in a sulphate reductase system (Tiller, 1983) with which they conduct dissimilatory sulphate reduction. In this process the sulphate ions act as an oxidising agent for the dissimilation of organic matter. Concurrent with sulphate reduction SRB are able to take up molecular hydrogen:



Many SRB are able to oxidise molecular hydrogen as well as other organic electron donors such as lactate and pyruvate (Pankhania, 1988). SRB can also produce hydrogen which may contribute to hydrogen induced cracking and hydrogen embrittlement of steels. Since SRB can both produce and utilise molecular hydrogen they have the enzyme hydrogenase. This enzyme catalyses the reversible reactions:



Evidence for the classic depolarisation theory has centred on the possession of hydrogenase enzymes by some SRB and its

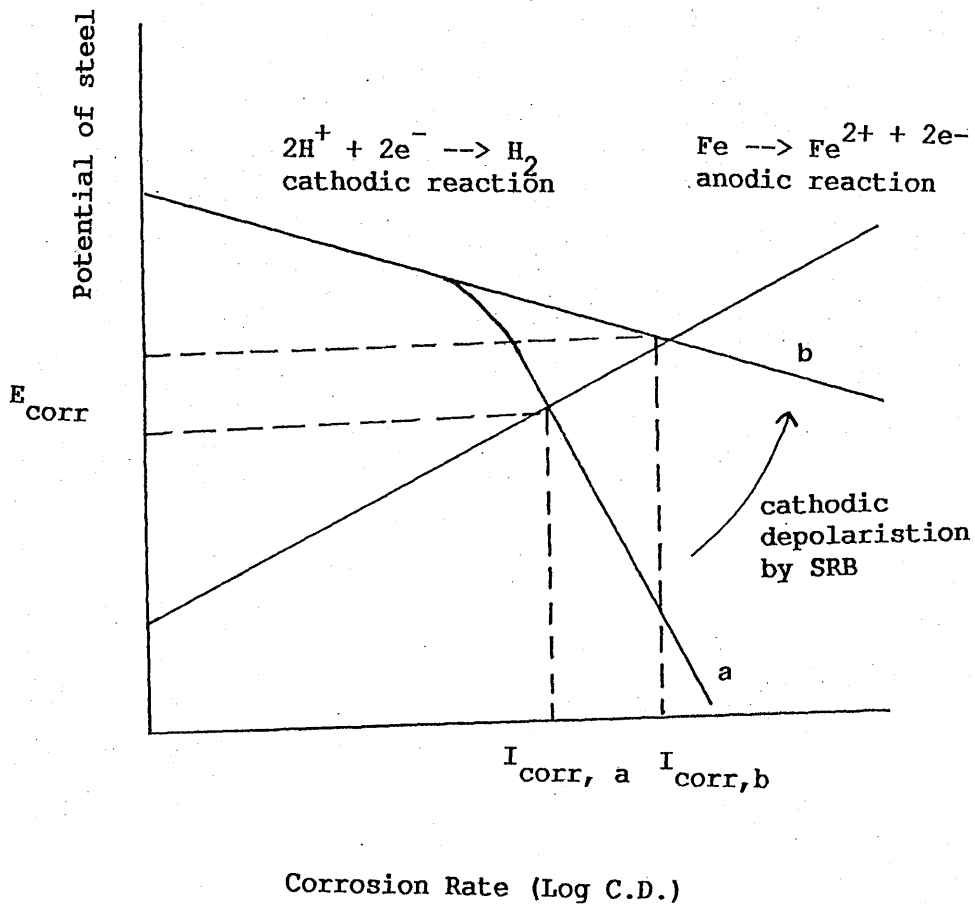
absence in hydrogenase negative strains. Booth and Tiller (1960) constructed polarisation curves for mild steel immersed in cultures of SRB (refer to FIGURE 2.4.3.A). Hydrogenase positive Desulfovibrio vulgaris progressively depolarised the cathodic reaction. A hydrogenase negative strain Desulfotomaculum orientis was not able to depolarise the cathode. It was concluded that the depolarising capacity of SRB correlated with their ability to produce hydrogenase.

In a subsequent study (Booth & Tiller 1962) the hydrogenase positive Desulfovibrio salexigens (strain California 43:63) rapidly depolarised the cathode (refer to FIGURE 2.4.3.B). This correlated strongly with its hydrogenase coefficient for a redox dye (acting as an electron acceptor), but not with its hydrogenase coefficient for sulphate. It was concluded by Booth and Tiller that the ability of SRB to use hydrogen (for whatever purpose) is the criterion for anaerobic microbial corrosion of steel, the early equating of corrosion with sulphate reduction was unwarranted.

Iverson (1966) also did experiments using a redox dye (benzyl viologen) as an electron acceptor. He found that cathodic hydrogen could be utilised by hydrogenase positive SRB to reduce the redox dye. Booth and Tiller (1968) used washed resting cells in an inert buffer, again with benzyl viologen as the electron

FIGURE 2.4.3.A

Evans Diagram Showing Effect Of Cathodic Depolarisation On The Corrosion Current



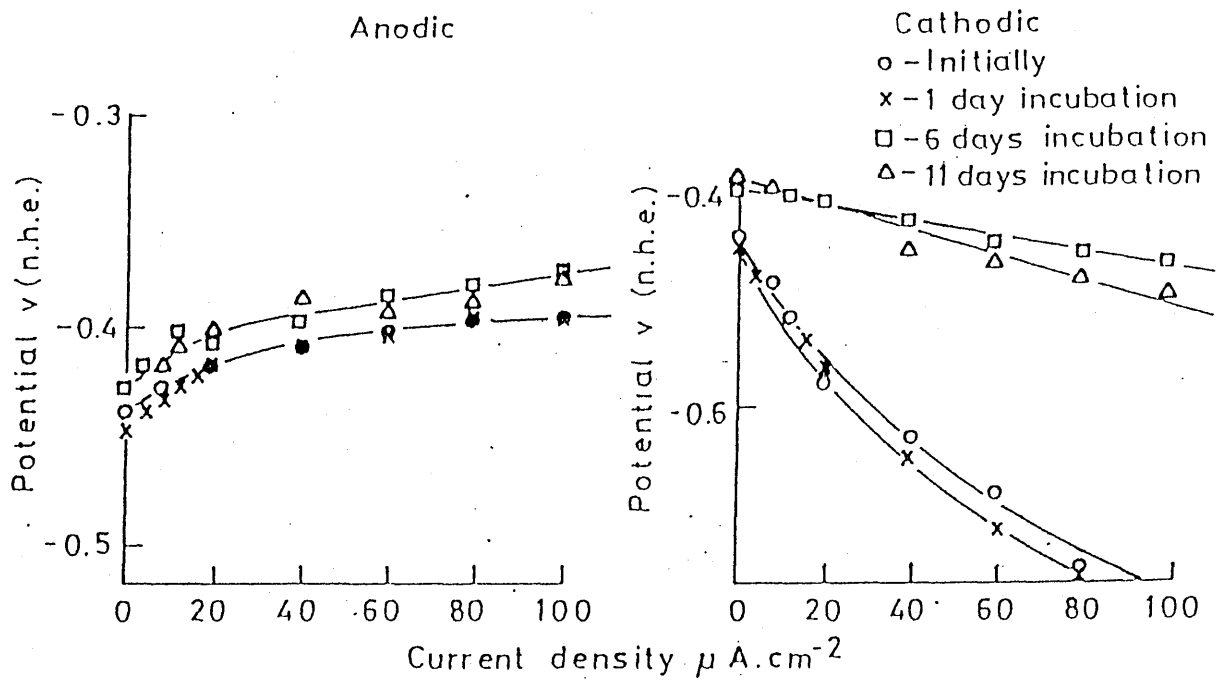
Steel corrosion is usually under cathodic control (line a), limited by the accumulation of H_2 at the surface. SRB remove H_2 from the cathode (line b) depolarising it, thus increasing the overall corrosion rate (from $I_{\text{corr}, a}$ to $I_{\text{corr}, b}$)

FIGURE 2.4.3.B

Polarisation Curves For Mild Steel In Cultures Of Sulphate Reducing Bacteria

(After Booth & Tiller, 1962)

SRB was Dv salexigens (Strain California 43:63 - Hydrogenase positive and was cultured at 30°C.

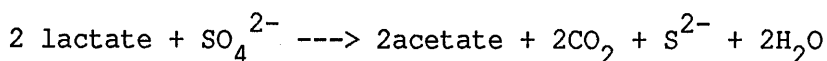


acceptor. Polarisation studies showed that when a reducible substrate was not present the polarisation of mild steel was not related to the hydrogenase activity of SRB. But when the redox dye was present, cathodic depolarisation was increased by the hydrogenase activity. Indeed, the rate of reduction of the redox dye was a function of the hydrogenase activity.

Some controversy as to the validity of using artificial dyes as electron acceptors exists. Costello (1974) showed that the oxidised form of benzyl viologen could depolarise mild steel, invalidating some previous studies. SRB produce sulphide which often remains attached to their cell wall in the form of iron sulphide (Miller & King, 1975). This may have also contributed to depolarisation of the cathode.

More recently, Hardy (1983) demonstrated that cathodic hydrogen on X-65 steel was oxidised by hydrogenase positive Desulfovibrio vulgaris concurrent with the reduction of sulphate to hydrogen sulphide. In this study a radio-respirometric assay (Rosser & Hamilton, 1983; Maxwell & Hamilton, 1986) was used to measure the reduction of S-35 sulphate to S-35 labelled hydrogen sulphide by a cell suspension of D. vulgaris. It was also shown that when a steel electrode was not present almost no labelled hydrogen sulphide was produced.

A study by Cord-Ruwisch and Widdel (1986) also used a radio-respirometric assay to demonstrate hydrogen sulphide production by Desulfovibrio (hydrogenase positive strain) and Desulfovibrio sapovorans (hydrogenase negative strain) in the presence and absence of corroding steel wool. An extra organic electron donor, lactate was also supplied. It was found that hydrogenase positive Desulfovibrio produced more hydrogen sulphide than was expected from theoretical calculations based on the stoichiometry of lactate oxidation:



The extra sulphide produced was thought to be due to the utilisation of cathodic hydrogen from the steel wool. In contrast the hydrogenase negative D. sapovorans could not use cathodic hydrogen for sulphate reduction. It was also shown that the utilisation of cathodic hydrogen by the hydrogenase positive strain was related to environmental parameters (pH, temperature and salinity) as well as the surface area of the steel wool.

Recent work by Pankhania, Moosavi and Hamilton (1986) has demonstrated with electrochemical and physiological evidence that SRB are able to oxidise cathodic hydrogen. Desulfovibrio vulgaris (Hildenborough) is able to grow on acetate and carbon dioxide as carbon sources only if hydrogen is provided as the

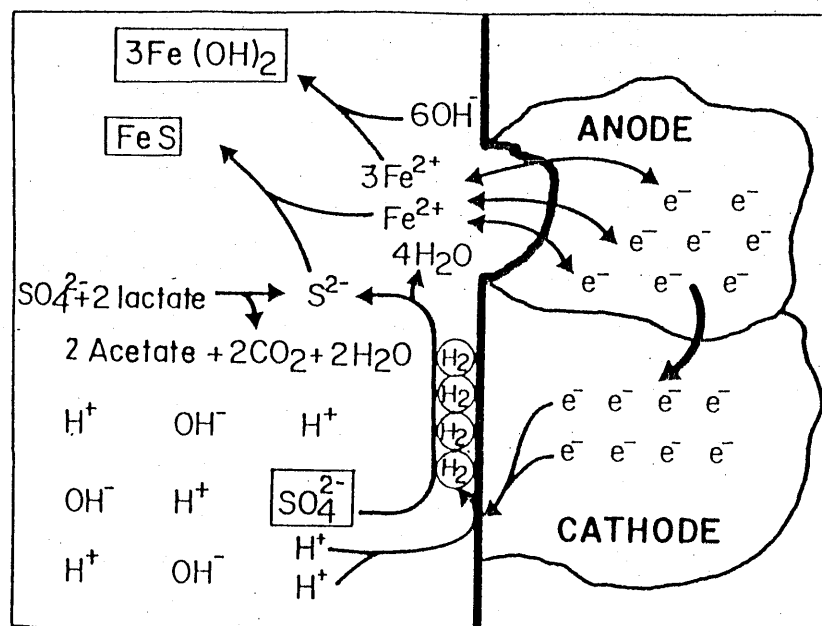
sole electron donor. The rest potential of a mild steel electrode was measured; when D.vulgaris was added to the media it became depolarised. The corrosion rate of the mild steel electrode was calculated from polarisation data to be 0.21 mm yr^{-1} under anaerobic conditions. When 0.276 mM sulphide was added it increased slightly to 0.28 mm yr^{-1} but upon the addition of D.vulgaris cells it nearly doubled to 0.5 mm yr^{-1} . Further evidence for the utilisation of cathodic hydrogen was obtained by studying the growth of D.vulgaris by measuring the increase in cell numbers, optical density, hydrogenase activity, and by sulphate and acetate utilisation. When the potential of the steel electrode was held at -100 mV (in the passive range so cathodic hydrogen is not formed) D.vulgaris did not grow. Subsequently the potential was changed to -1400 mV and rapid increase in all of the growth criteria were observed. This demonstrates that D.vulgaris consumed cathodic hydrogen. Eventually growth stopped. This was attributed to the formation of an FeS film which can poison the cathodic reaction.

These studies demonstrate that SRB are capable of using cathodic hydrogen as an electron donor. The role of SRB in corrosion is complex, the presence of FeS also significantly promotes steel corrosion. FIGURE 2.4.3.C represents the proposed reactions of anaerobic corrosion in the presence of SRB on an iron surface (After Pankhania, 1988).

FIGURE 2.4.3.C

Proposed Reactions Of Anaerobic Corrosion In The Presence Of Sulphate Reducing Bacteria On An Iron Surface

(After Pankhania, 1988)



Booth, Elford and Wakerley (1968) studied corrosion of mild steel in suspensions of SRB growing without sulphate metabolism but in the presence and absence of chemically prepared suspension of ferrous sulphide. Polarisation experiments and weight loss measurements provided evidence that polarisation of the cathode occurred by two distinct mechanisms. Firstly, the SRB hydrogenase positive Desulfovibrio desulfuricans utilised cathodic hydrogen. Secondly, the accumulation of solid ferrous sulphide on the mild steel also depolarised the cathode. Differences in corrosion rate in systems with and without added ferrous sulphate indicated that the iron sulphide film was of major significance in depolarising the cathode.

Polarisation studies of iron in the presence of five different iron sulphide compounds (mackinawite, greigite, pyrrhotite, marcasite, and pyrite) show that these compounds affect both anodic and cathodic depolarisation (Mara & Williams, 1972). It is thought that initially a thin film of mackinawite (sulphur deficient sulphide) forms on iron in the presence of SRB (Miller, 1981; King, Dittmer & Miller 1972). Sulphide produced by SRB under anaerobic conditions helps to transform mackinawite to greigite and smithite (Mara & Williams, 1972). Greigite and smithite enhance corrosion by stimulating the anodic reaction as they are cathodic to steel.

King and Miller (1971) suggested that SRB depolarise the cathode by utilising cathodic hydrogen, but iron sulphide produced by their activities were more important quantitatively. In a subsequent study (King, Miller & Smith, 1973) it was demonstrated that the corrosive nature of chemically prepared sulphide correlated with the sulphur stoichiometry. In bacteria-free systems the stimulation of corrosion by sulphide decreased with time. High corrosion rates in sterile systems were only maintained if iron sulphide was continually renewed. It was thought (Mara & Williams, 1972), that iron sulphides at adjacent sites depolarise the cathode by absorbing hydrogen in proportion to the cationic defects in the sulphide lattice.

Gilbert, Attwood, David, Morgan and Herbert (1987) used a laboratory based rig to simulate conditions in a seawater injection system. Corrosion rates measured by impedance measurements showed that corrosion rates were enhanced by the presence of an aggressive chemical film, probably of iron sulphide. This film was associated with the formation of a mature biofilm containing SRB on the steel surface. Biofilm coated steel electrodes were autoclaved to kill the bacteria but leaving the biofilm intact. This treatment resulted in a relatively small decrease in the corrosion rates. The sulphide in the dead biofilm was still able to depolarise the cathode significantly, probably due to the non-biological reduction of

chemical species within the dead biofilm.

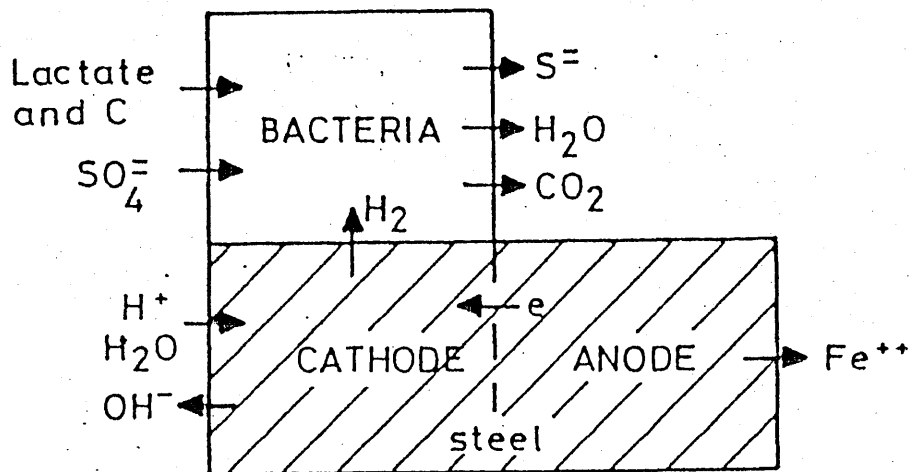
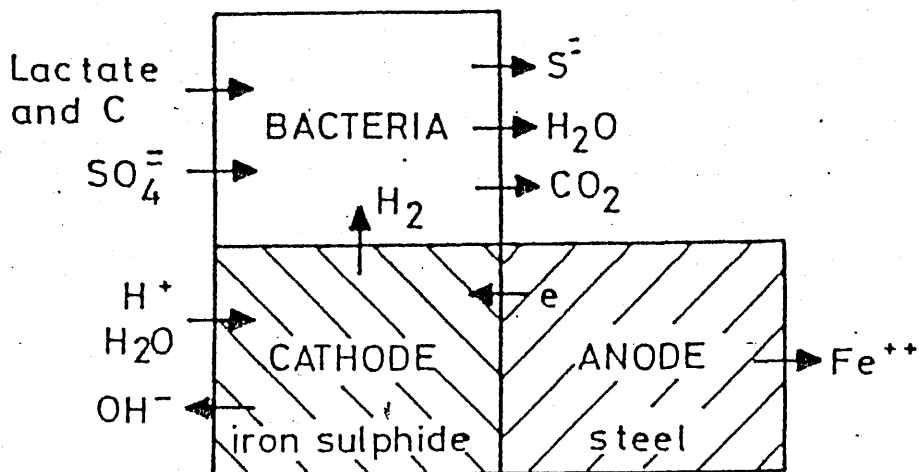
Miller (1981) proposed that all iron sulphides are cathodic towards iron but in bacteria-free systems they do not act as a permanent cathode. He suggested that SRB keep iron sulphide cathodically active by 'regenerating' (or depolarising) it, or producing 'fresh' iron sulphide by their growth, or bring new iron sulphide surfaces constantly into contact with the steel by cell movement. Bacteria grown in iron-rich media are frequently covered in iron sulphide particles (Miller & King, 1975).

Miller and King (1975) illustrated the fundamental differences between the classical depolarisation theory and the alternative theory based on the cathodic nature of iron sulphide in the following diagram (FIGURE 2.4.3.D).

FIGURE 1.4.3.D

Schematic Representation Of The Classical Mechanism (top) And Alternative Mechanism (bottom) Of Microbial Corrosion Under Anaerobic Conditions

(After Miller & King, 1975)



Differing from these opinions Costello (1974) believed that dissolved hydrogen sulphide produced by SRB was responsible for depolarisation of the cathode. A study the following year (Togano, Sasak, Nakahara, Kanda & Osato, 1975) provided further evidence by demonstration that the corrosion rate of mild steel was proportional to the concentration of hydrogen sulphide produced by SRB. Togano et al suggested that dissolved hydrogen sulphide accelerated the anodic as well as the cathodic corrosion reactions.

2.4.4 Stimulation of Anodic (Oxidation) Reactions

Bacteria involved in nitrogen cycling may be involved in promoting the anodic dissolution of iron. Pseudomonas spp. are anaerobic, nitrate reducing bacteria which use nitrate as a terminal electron acceptor for anaerobic respiration. Nitrosomonas spp. are aerobes which oxidise more complex nitrogenous compounds to nitrate and nitrite (Edyvean 1987). It has been demonstrated that consortia of these various bacteria stimulate anodic dissolution (Obuekwe, Westlake, Plambeck & Cook, 1981).

2.5 CORROSION CONTROL OF OFFSHORE STEEL STRUCTURES

Structures and pipelines offshore are exposed to a wide range of environments from burial in benthic sediments, continual submersion in water, to intermittent wetting in the splash zone at the surface (West, 1986). Very high corrosion rates occur in the splash zone which is exposed to high levels of aeration and intermittent wetting from waves and tide. Thicker steel sections and coatings are commonly employed to balance and control the effects of corrosion in the splash zone. Steel structures submerged or buried in the benthic sediments corrode at slower rates. In these areas cathodic protection and coatings are used to counter corrosion.

2.5.1 Cathodic Protection

Steel corrosion in seawater can be lessened or prevented by passing a direct electrical current through it of the correct polarity (Wilkins, 1982). The current suppresses the anodic iron dissolution and promotes the cathodic reaction over the entire metal surface (Edyvean, 1984). Corrosion is inhibited since the anodic and cathodic reactions must occur simultaneously in order for electrons to flow from the anode to the cathode.

Cathodic protection of steel is supplied via impressed current systems or sacrificial anodes. With the former system the potential of the steel is controlled at between -750 and -850 mV Ag/AgCl. This suppresses the anodic reaction (Wilkins, 1982). The same result can be achieved by reducing the potential of the protected steel by 150-200 mV more negative than an insulated reference piece of steel (La Que, 1975). The impressed current anode is based on a corrosion resistant metal (Eg. Titanium covered with platinum cladding), to increase its length of service.

Impressed current protection systems are used on some production platforms due to the abundant supply of cheap electricity (West, 1986). Any metal that is substantially more anodic (electronegative) than steel when coupled to it will become the site for the anodic reaction, whilst the more electropositive steel becomes the site for the cathodic reaction forming a galvanic cell. Zinc and aluminium are commonly used as sacrificial anodes (Wilkins, 1982), corroding preferentially to steel when coupled to it.

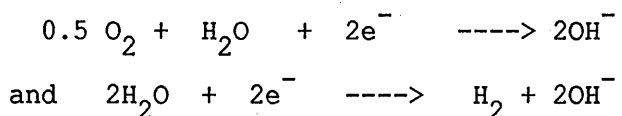
2.5.2 Involvement Of SRB In Hydrogen Induced Cracking

Steel is usually cathodically protected at a potential of -850 mV S.C.E., but when SRB are present it should be reduced to -950 mV S.C.E. to protect the steel from the sulphides produced by bacterial activity (Maxwell & Hamilton, 1985). Cathodic protection suppresses the anodic reaction and favors the cathodic one, thus more hydrogen will be produced. Hydrogen atoms may be absorbed into the steel rendering it more susceptible to cracking under stress (Edyvean, 1987). This form of steel failure, facilitated by the embrittlement of steel through hydrogen absorption, is often termed Hydrogen Induced Cracking (H.I.C). Studies have shown that high degrees of overprotection lead to the more rapid cracking of high strength steels due to increased hydrogen embrittlement (Bath & Trioano, 1972).

Sulphide, which may be biogenic in origin, poisons the combination of hydrogen atoms to the gaseous molecule (Edyvean, 1987) and so encourages the likelihood of steels to suffer hydrogen embrittlement (Parker, Seal & Robinson, 1986). Parker et al (1986) measured absorption of hydrogen into freely corroding steel foils in the presence of SRB using an elaborate 'double chamber bioreactor'. They showed that hydrogen embrittlement of freely corroding steel was enhanced by SRB due to their production of sulphide.

2.5.3 Formation Of Calcareous Scale

Both the cathodic protection systems generate a high pH at the cathodic steel surface due to the reduction of oxygen:



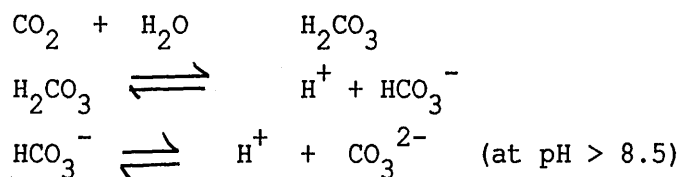
(Hartt, Culberson & Smith, 1984; Hartt, 1984; Wolfson & Hartt, 1981; Edyvean, 1984).

The pH at the metal/electrolyte interface is determined by the rate of hydroxyl ion production and its rate of removal by convection and diffusion. A pH profile at the surface of a cathodically polarised metal surface shows that pH decreases with increasing distance from the cathodic surface (Hartt, Culberson & Smith, 1984).

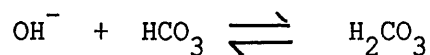
Wolfson and Hartt (1981) calculated local pH concentration using mass transfer analysis at cathodic control potentials from -0.780 V S.C.E. to -1.030 V S.C.E.. They predicted the pH at the cathodic surface to be higher the more negative the control potential, due to a faster rate of hydroxyl ion production. An initial surface pH of 11.25 was predicted for steel protected

at -1.030 V S.C.E.; after 182 hours exposure this decreased to a pH of 10.50

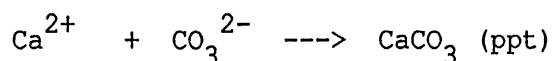
Since the solubility limit of most inorganic compounds decreases with increasing pH (particularly calcium and magnesium carbonates and magnesium hydroxide) they tend to precipitate out onto the polarised metal surface (Hartt, 1984; Edyvean, 1984). The decrease in pH at the metal/electrolyte interface also upsets the carbonic acid equilibrium in the seawater. In the seawater pH is controlled by the following reactions :



If more hydroxyl ions are added to the system more carbonate ions are produced (Hartt, 1984):



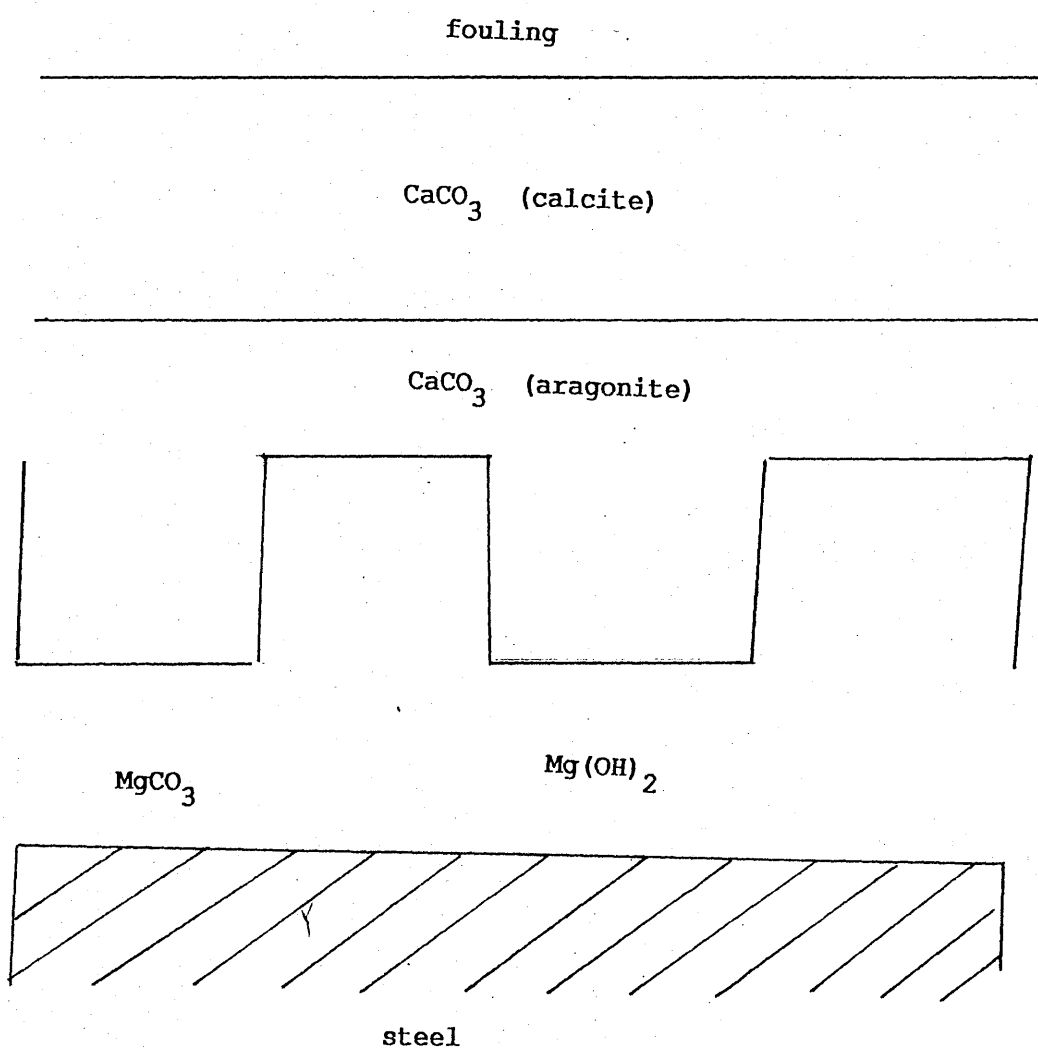
Consequently the precipitation of calcium carbonate is promoted due to the increased availability of the carbonate ions (Wolfson & Hartt, 1981):



Calcium carbonate can be found in two morphological types in the marine environment; calcite and aragonite. Aragonite is the form that precipitates onto the metal surface as the hydroxyl ion concentration increases (Hartt, Culberson & Smith, 1984).

Edyvean (1984) studied the composition of calcareous scale formed on steel protected by sacrificial anodes and impressed current. FIGURE 2.5.3.A shows the composition of scale formed on a protected steel surface. EDAX analysis showed that the scale closest to the metal surface contains mainly magnesium. Less magnesium was detected in the scale further away from the metal surface where it was composed entirely of calcium compounds. X-ray diffraction analysis revealed that magnesium hydroxides and carbonates were deposited first at the metal surface in the region of highest hydroxyl ion concentration. When the pH of seawater is greater than 9.5 magnesium hydroxide is precipitated (Hartt, Culberson & Smith, 1984). EDAX analysis of a transverse section through scale on steel protected by an impressed current showed there was no gradation between the magnesium and the calcium dominated areas but a separation into distinct layers (Edyvean, 1984). The proportion of magnesium in the scale increased with increasing current density of the impressed current (Hartt, Culberson & Smith, 1984).

Composition Of Scale Formed On A Cathodically Protected Steel Surface
(After Edyvean, 1984)



The morphology of calcareous scale was variable in different regions on a steel surface (Wolfson & Hartt, 1981) and under constant experimental conditions (Hartt, 1984). Scale tended to be more developed, denser and thicker the higher the current density used for protection (Wolfson & Hartt, 1981; Wilkins, 1982). Due to hydrogen evolution at very high current densities scale is more porous and adheres loosely to the metal surface. At lower current densities scales were quite porous and coarsely crystalline in structure (Wilkins, 1982).

With increasing water velocity over the metal surface the boundary layer thickness decreases, altering the pH profile. This is illustrated in FIGURE 2.5.3.B (Hart, Culberson & Smith, 1984).

As a consequence of the altered pH profile in the water adjacent to the metal surface thicker calcareous scale deposits were observed at slower water velocities (Wolfson & Hartt, 1981).

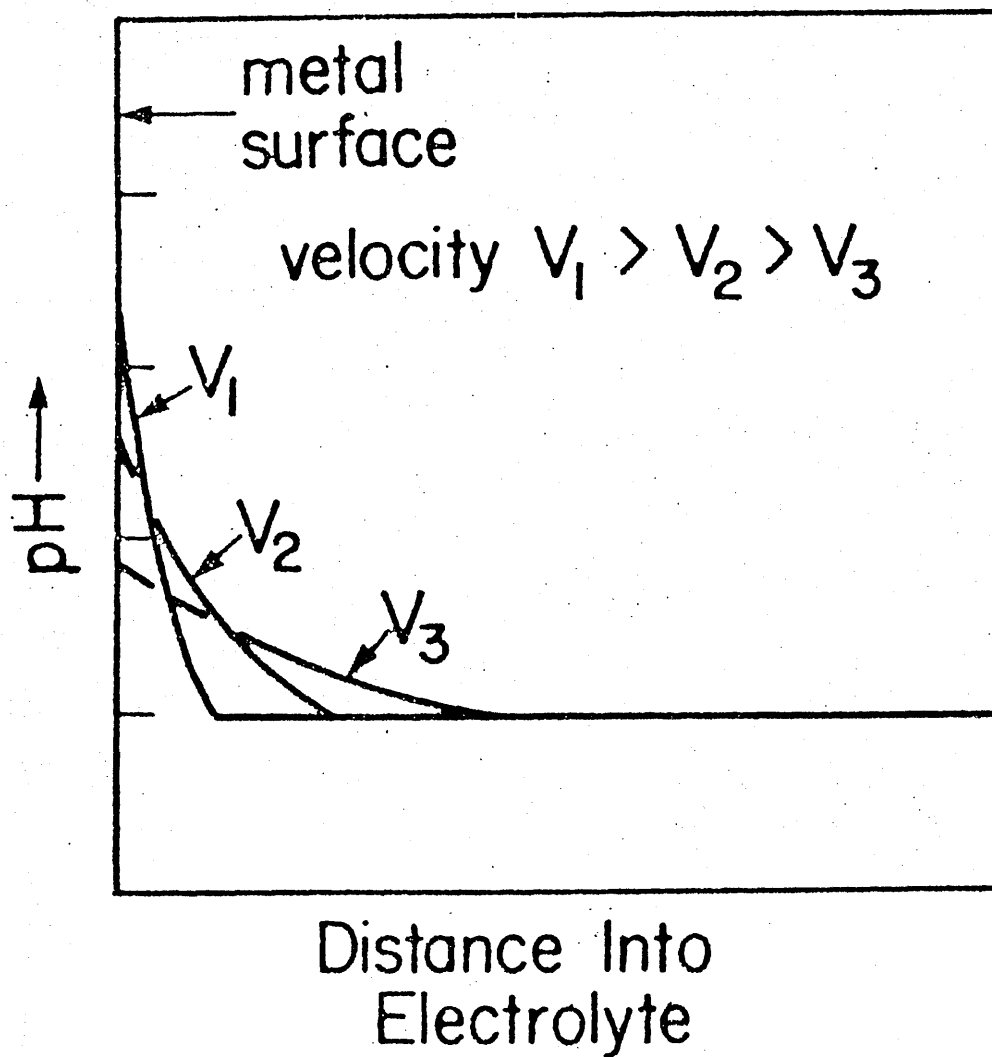
2.5.4 Effect Of Cathodic Protection On Biofilm Formation

The surface of cathodically protected steel provides an environment differing from the surface of freely corroding steel in several ways. The scale on cathodically protected steel initially increases in depth until it reaches a certain

FIGURE 2.5.3.B

Schematic Representation Of pH Profiles Near The Surface
Of A Film-Free, Cathodically Polarised metal For Three
Different Water Velocities

(After Hartt, Culberson & Smith, 1984)



thickness, usually determined by the rate of water flow (Wolfson & Hartt, 1981). The surface of cathodically protected steel can be considered as being relatively stable. In contrast, metal dissolution is constantly happening from the surface of freely corroding steel. The accumulation of corrosion product provides a greater surface area for bacterial attachment and metabolism (Maxwell & Hamilton, 1985).

Edyvean and Terry (Edyvean & Terry, 1983; Terry & Edyvean, 1981) examined steel surfaces which were cathodically protected, freely corroding or painted with epoxy coal tar with SEM. After 100 days immersion in seawater a similar flora of diatoms, blue-green algae and bacteria developed on protected and unprotected steel surfaces. It was found that diatoms readily settled on all of these test surfaces (Edyvean & Terry, 1983), but that diatom settlement was slightly lower on cathodically protected bare steel than on painted unprotected steel. The cathodically protected bare steel was covered by a rough, calcareous scale which may have been detrimental to diatom settlement (Edyvean & Terry, 1981).

A sensitive radio-respirometric technique was used by Maxwell and Hamilton (1985) to study effects of cathodic protection on the activity of intact microbial biofilms. Steel coupons (50 D) were either freely corroding or cathodically

protected (at -950 mv) were immersed in a harbour for 147-167 days. Subsequently the activity of aerobic, anaerobic and SRB populations were found to be greater on freely corroding, than on protected coupons. Although an active population of SRB developed on the surface of cathodically protected coupons, sulphide was only detected on freely corroding coupons. High pH generated by cathodic protection may deter the settlement of microorganisms. Maxwell & Hamilton concluded that the lower activity of the biofilm on protected steel was not due to an inhibitory effect of the cathodic protection itself. The build-up of bulky corrosion product on unprotected steel provided a large surface area and more favorable conditions for biofilm formation and development of anaerobic zones.

A more recent study (Hamilton, Moosavi and Pirrie, 1988) attempted to establish a direct correlation between the extent of corrosion and one or more indices of biological activity for SRB. Coupons (both freely corroding and cathodically protected at -1.05 V S.C.E.) were immersed offshore at various depths for 1-2 years. Subsequently, biological activity of SRB was measured in terms of amount of sulphide produced, viable numbers and rate of sulphate reduction determined with a radio-respirometric assay. Hamilton et al (1988) found that SRB were active on cathodically protected and freely corroding steel coupons, but meaningful differences between the levels of SRB biological

activity on these different substrata were not demonstrated.

In another comprehensive study (Edyvean, 1984) microbial fouling occurred rapidly on unprotected and protected steel (at -950 mV), although the species diversity and fouling density was initially greater on protected steel. Moss (1981) noticed that cathodically protected steel was colonised by bacteria and microalgae much more rapidly in the initial stages of exposure than freely corroding steel. After 50 days immersion (Edyvean, 1984) microfouling of bacteria, fungi, diatoms and blue-green algae was very similar on protected and unprotected steel. Diatoms and other microalgae became enmeshed in the corrosion product on unprotected steel. The copious amounts of mucilage they produced helped consolidate the corrosion product onto the steel surface. Microorganisms readily settled on the cathodically protected steel. Many were partially or completely covered by precipitating calcareous scale. The mucilage produced by the microfouling was incorporated into the scale.

Guezennec and Therene (1988) investigated the influence of cathodic protection on the growth of SRB and steel corrosion in marine sediments. Samples were cathodically protected at between -800 mV Ag.AgCl and -1100 mV Ag.AgCl. Protection at -900 mV Ag.AgCl was not sufficient to stop SRB-enhanced corrosion completely. Lipid analysis of SRB indicated their proliferation

in the sand immediately surrounding the cathodically protected steel. Indeed the significant amounts of cathodic hydrogen produced at the lowest potentials (1000 and 1100 mv Ag.AgCl) promoted the growth of hydrogenase positive SRB in the sediment immediately surrounding the metal, allowing the subsequent growth of other SRB species.

Investigations have shown that interactions between cathodic protection and microfouling organisms are controversial and not well defined. On the one hand cathodic hydrogen may encourage the proliferation of SRB which are able to utilise it as an energy source; on the other hand low pH at the metal/electrolyte interface may affect the settlement of bacteria and discourage biofilm growth. Corrosion product on freely corroding steel may provide a larger surface area for bacterial attachment but its continual dissolution in the anodic reaction may influence biofilm density. The relative importance of these factors during their complex interaction in the different test situations studied is reflected in the disparity between results.

3.0 MATERIALS AND METHODS

3.1 FLOW LOOP DESIGN

A flow loop containing artificial seawater was designed to simulate open sea conditions (Funge-Smith, Robinson & Seal, 1988A). The flow loop consisted of a PVC reservoir (100 l capacity) filled with artificial seawater. The flow loop is shown in FIGURE 3.1.A. and in a photograph, FIGURE 3.1.B. A magnetic pump (Arbo type R5/50) circulated water through the piping at a rate of 10 l min^{-1} (current velocity of 0.6 m s^{-1}). The flow loop itself was built of PVC pipe (George Fischer) with an internal diameter of 19 mm. In order to study microbial corrosion in flowing water conditions a corrosion probe of annular steel coupons was designed to be incorporated into the part of the flow loop constructed of PVC piping. A parallel study of microbial corrosion under slower water flow conditions was to be conducted using steel coupons immersed in the water reservoir. The artificial seawater supported a mixed population of marine bacteria cultured in two fermenters.

FIGURE 3.1.A

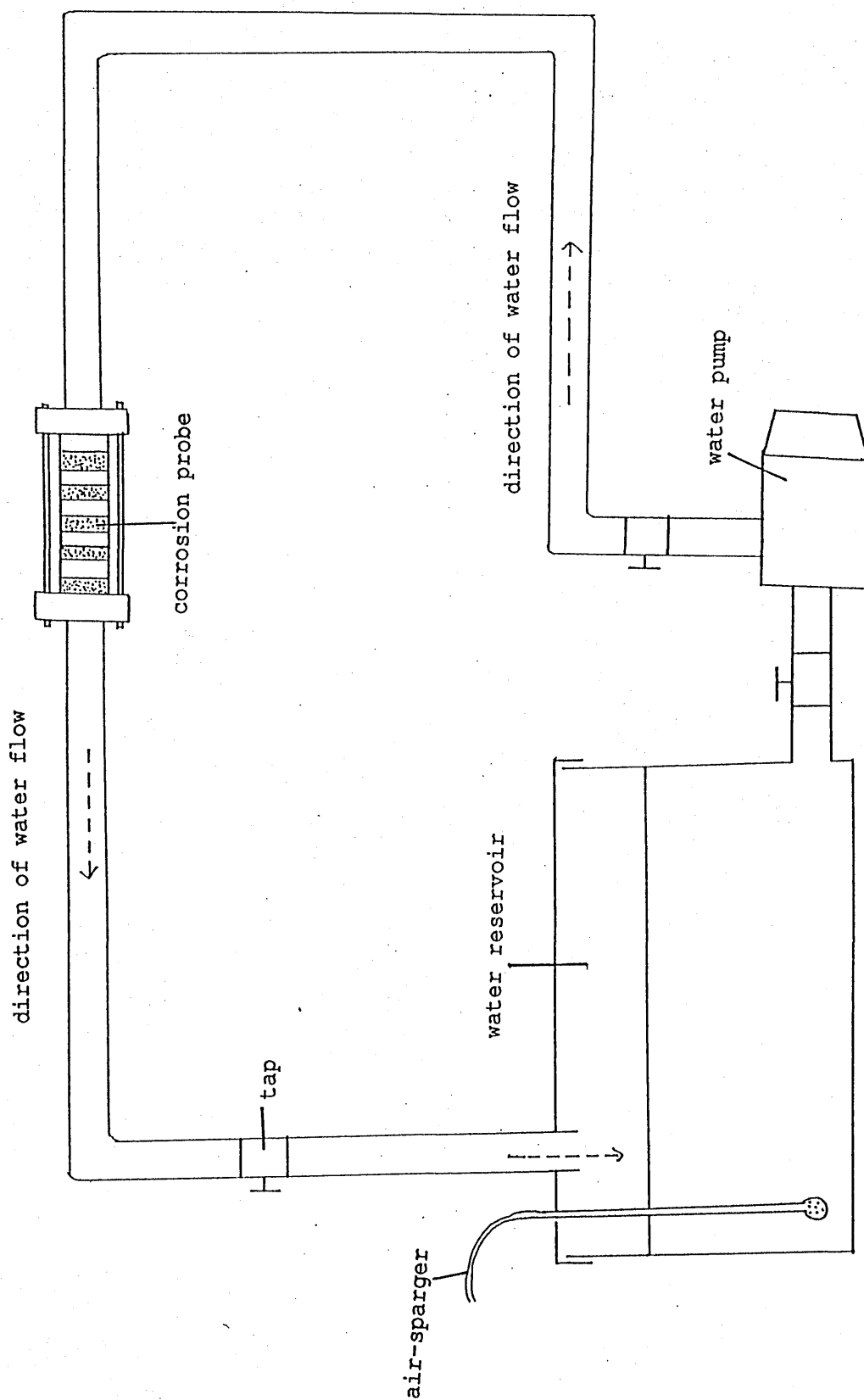
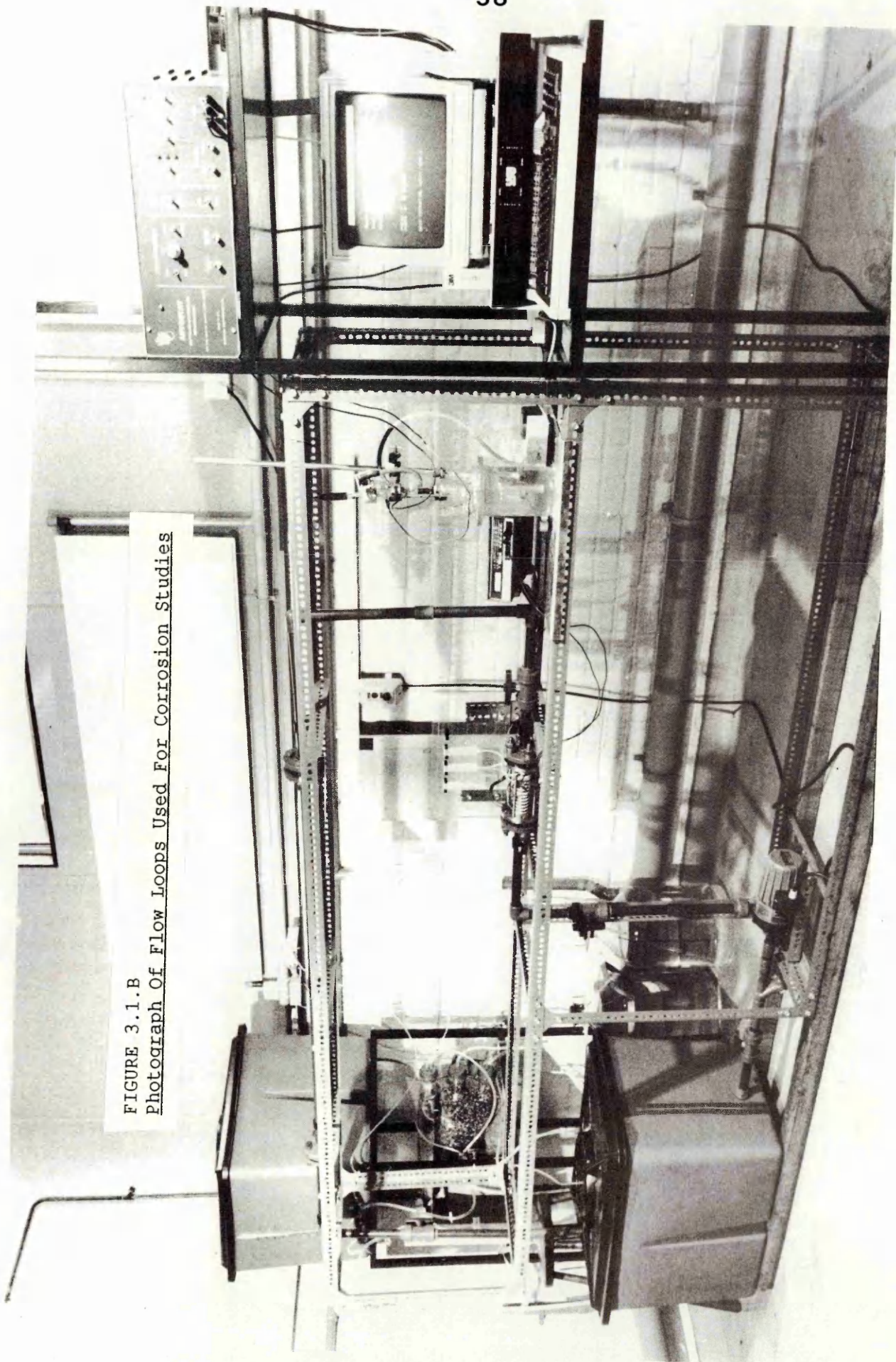
Diagram Of Flow Loop Used For Corrosion Studies

FIGURE 3.1.1.B
Photograph Of Flow Loops Used For Corrosion Studies



3.2 ENVIRONMENTAL CONDITIONS WITHIN THE FLOW LOOP

3.2.1 Seawater

An artificial seawater (Instant Ocean) was used to provide a medium in which the constituent ions were present in constant quantities. The salt was dissolved at a concentration of 3.5 % in tap water and stored before use to allow dissolved chlorine to disappear. The brand of artificial seawater used did not have any additional sources of carbohydrates.

3.2.2 Temperature Of Seawater

The temperature of the seawater was measured periodically with a thermometer.

3.2.3 Concentration of Dissolved Oxygen In The Seawater

The seawater was continually sparged with air, and it was intended that the water would be saturated with oxygen. The concentration of dissolved oxygen in the seawater was determined using the Winkler titration method (Golterman, Clymo & Ohnstad, 1978).

3.2.4 Flow Rate Of Circulating Seawater

The magnetic pump circulated the water through the piping at a speed of 0.6 m s^{-1} . The Reynolds number (of 8225) was calculated for the conditions in the flow loop. It predicted the flow regime in the loop would be turbulent. A turbulent flow, and not laminar flow was thought to be desirable as it would simulate conditions on steel surfaces in the open sea sea.

3.2.5 The pH Balance Of The Seawater

The pH in seawater usually remains fairly stable due to the carbonate/bicarbonate buffer system. The pH of artificial seawater was periodically measured with a pH electrode (Cranwell CR/95).

3.2.6 Carbohydrate Concentration In The Seawater

Twice weekly 0.3 g of glucose was added to the water in the flow loop. As the flow loop contained about 100 l of water this resulted in an initial glucose concentration of 3.0 mg l^{-1} . The actual carbohydrate level will be influenced by its rate of utilisation by heterotrophic microorganisms, and by the photosynthesis of any microalgae in the flow loop. The carbohydrate concentration was measured using a colorimetric

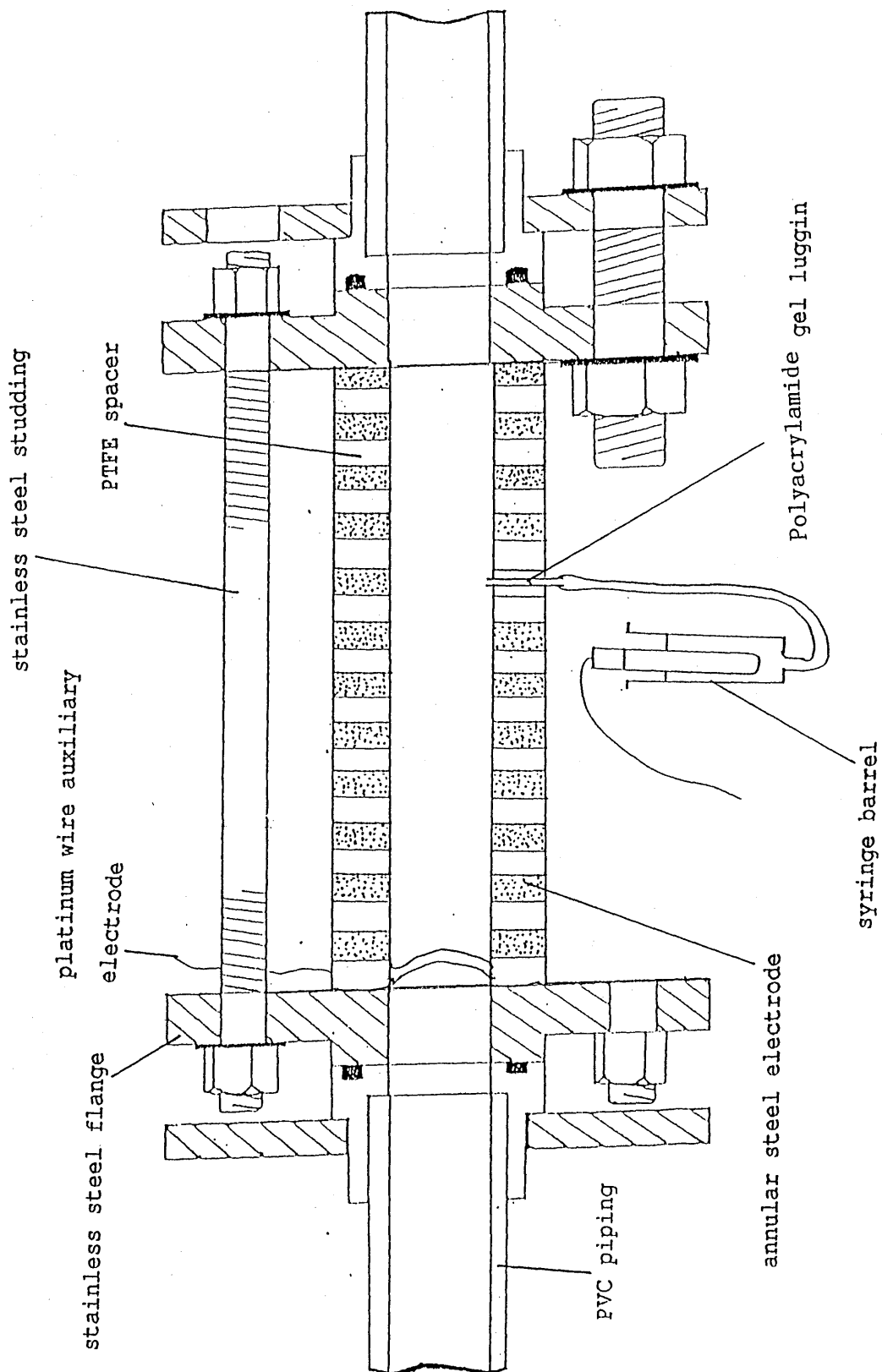
method based on the measurement of a coloured precipitate formed on the reaction of sulphuric acid and phenol with carbohydrates (Golterman et al, 1978). The absorbance of the coloured precipitate was measured at 490 nm using a spectrophotometer (Cecil CE594). This method is useful for measuring total carbohydrate concentrations as the same equivalent colour is measured with different sugars. The water sample was filtered through a sintered glass filter to remove larger contaminants and bacteria. A calibration curve using glucose solutions was prepared for this assay (refer to Appendix A).

3.3 CORROSION PROBE DESIGN

The corrosion probe (refer to FIGURE 3.3) was designed so that corrosion of steel and the development of a biofilm could be easily monitored (Funge-Smith et al, 1988A). The probe consisted of annular steel electrodes machined from carbon-manganese steel plate (BS 4360 Grade 50 D), with a surface area exposed to the seawater of 2.98 cm^2 each. These electrodes were separated by annular PTFE spacers. A standard calomel reference electrode was used to measure the potentials of the steel electrodes and it made contact through a polyacrylamide salt bridge saturated with potassium chloride solution (refer to Appendix B). Some annular electrodes had been cut into four equal quarters. It was intended that these should be reset inside a PTFE ring using

FIGURE 3.3

Diagram Of Corrosion Probe Incorporated Into The Flow Loop



epoxy resin and incorporated into the corrosion probe. The quartered rings were to be used for observation under the scanning electron microscope. Unfortunately these rings were impractical as they would not fit flush in the corrosion probe resulting in leaks and so were not used.

The corrosion probe was designed to investigate the influence of the development of a biofilm on steel corrosion. In addition the effects of cathodic protection on biofilm development were also studied. The corrosion probe was assembled from the annular steel coupons and PTFE spacers in four main sections. The first two sections contained six sets of triplicate, preweighed coupons. These freely corroding coupons were removed periodically from the loop and weight losses determined. Corrosion rates calculated from weight loss data were compared with those gained from electrochemical measurements. The third section consisted of coupons cathodically protected at -850 mV S.C.E. via a potentiostat (Thompson). The steel coupons were treated to remove any surface grease which may have affected bacterial settlement as the probes were assembled (refer to Appendix C). Electrochemical corrosion measurements of coupons in the last section of the probe were taken using an 'Autostat', a modified potentiostat interfaced with a microcomputer (BBC Master Series).

3.4 CORROSION STUDIES USING STEEL COUPONS IMMERSSED IN THE RESERVOIR OF THE FLOW LOOP

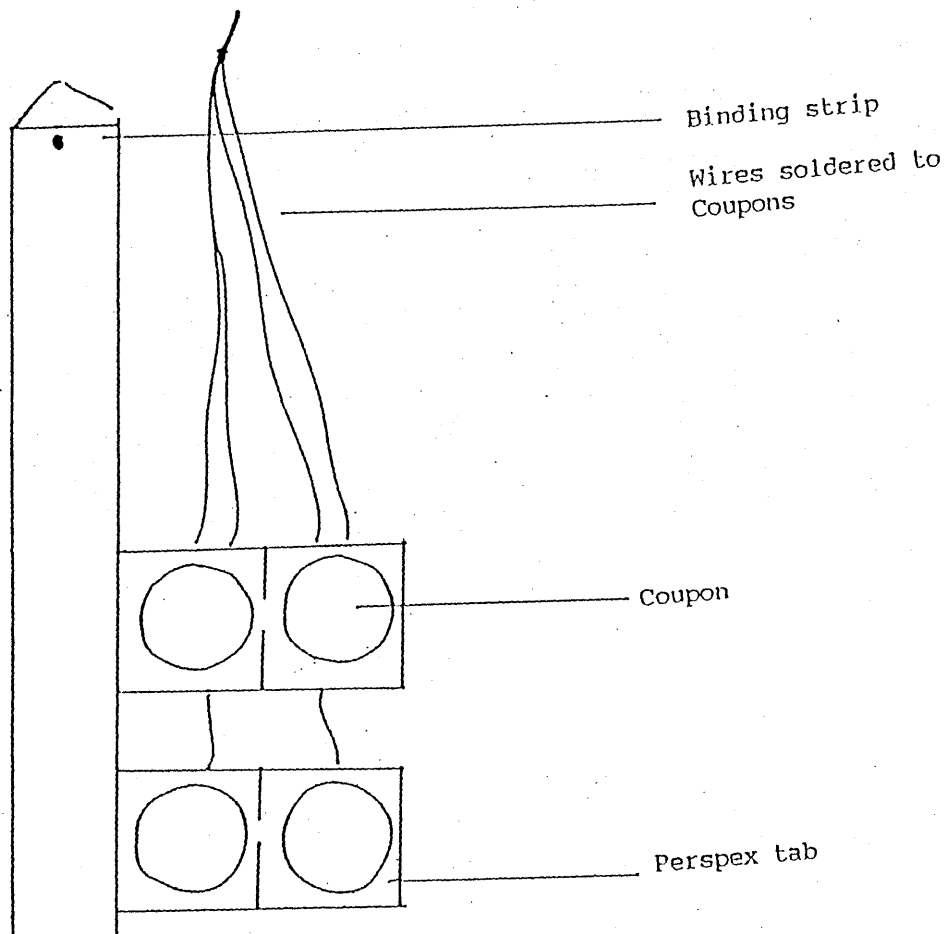
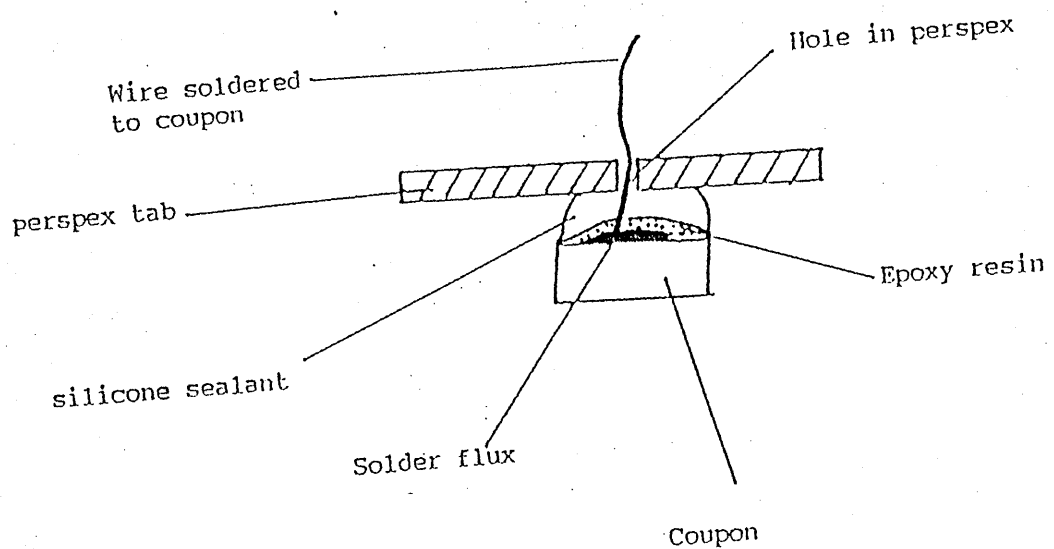
An investigation into the influence of different levels of cathodic protection on the development of biofilms was conducted using steel coupons immersed for 270 days. It was hoped that this study would overcome some of the problems associated with the corrosion probe: firstly, sufficient numbers of coupons could not be built into a probe for all of the tests required, if more than about ten coupons were built into a probe they came out of alignment and the probe leaked; secondly, the large size and shape of the annular coupons meant they could not be used for some of the microbial enumeration techniques. Cylindrical steel coupons were machined out of the same piece of steel that was used for the annular steel electrodes. The dimensions of the steel coupons were 12 mm diameter and 4 mm thick. This gave a surface area exposed to the seawater of 2.64 cm^2 . The cylindrical coupons were immersed in the reservoir and different levels of cathodic protection applied via potentiostats. The different levels of cathodic protection investigated were -850 mV S.C.E (standard level of cathodic protection) and -950 mV S.C.E (overprotection used to counteract the effects of iro. sulphide produced by SRB metabolism). The development of biofilms on cathodically protected coupons was compared with that on freely

corroding ones.

The cathodically protected coupons were mounted in the reservoir by means of a length of electrical wire soldered to the reverse face of each coupon (refer to FIGURE 3.4). The soldered area was covered with epoxy resin to stop galvanic corrosion between the lead/tin flux and the steel coupon. The wired coupons were mounted on small perspex tabs using silicone sealant (Silastic). The perspex tabs were held in plastic report binding strips which were suspended in the reservoir. All the coupons were degreased before immersion (refer to Appendix C).

Freely corroding coupons were also immersed in the reservoir. Some of these coupons were preweighed and used to determine weight loss due to corrosion. Again electrochemical corrosion measurements were taken using the 'Autostat'.

FIGURE 3.4

Method Of Mounting Steel Coupons In The Reservoir Of The Flow LoopSection Through Mounting

Periodically coupons were removed and the following tests conducted: the glucose turnover activity by heterotrophic bacteria; and the sulphate reduction activity by SRB in the biofilm were quantified using radioassays. These results were compared with viable spread plate counts made of anaerobic and aerobic heterotrophic bacteria in the biofilm; SRB were also enumerated using dilution series of liquid medium.

The following table summarises the corrosion studies and biological tests done using the corrosion probe and coupons in the reservoir of the flow loop:

Test done	Corrosion probe	Coupons in reservoir
Corrosion tests		
LPR scans	yes	yes
Polarisation scans	yes	yes
Weight loss coupons	yes	yes
Biological tests		
Radio-respirometric assays	no	yes
Viable counts	no	yes
SEM examination	no	yes

3.5 BACTERIAL FERMENTERS PRODUCING INOCULUM FOR THE FLOW LOOP

3.5.1 Aerated Gravel-Packed Fermenter

In order to provide a supply of marine bacteria to inoculate the water circulating in the flow loop a gravel-packed fermenter was constructed (Funge-Smith, 1988A). This fermenter was designed to provide a habitat across which oxygen and carbohydrate gradients extended, encouraging the growth of a mixed population of marine bacteria. Briefly, the fermenter consisted of a large, glass vessel filled with gravel to provide a large surface area for biofilm formation (FIGURE 3.5.1). The upper-most gravel was continually sparged with air which, it was intended, would set up an oxygen concentration gradient through the gravel.

Twice weekly one litre of liquid was removed from the middle of the fermenter and a further litre drained from the bottom. This inoculum was added to the reservoir of the flow loop. The removed inoculum was replaced with two litres of seawater with 0.3 g of glucose dissolved in it which was added to the top of the gravel.



FIGURE 3.5.1
Photograph Of Aerated Gravel-Packed Fermenter

3.5.2 Monitoring Bacterial Concentrations produced By The Aerated Gravel-Packed Fermenter

In order to check that the bacterial output of the aerated fermenter was fairly constant, viable counts of heterotrophic aerobic and anaerobic bacteria and also of SRB were conducted at monthly intervals.

Enumeration of Viable Heterotrophic Bacteria

Inoculum samples from the lower and middle layers of the gravel-packed fermenter were collected in sterile universal bottles. Serial dilutions were made from 10^{-1} to 10^{-8} in duplicate using Postgate B media as a dilutant (refer to Postgate, 1984, p 30-33). Aliquots of 0.1 ml from each dilution were spread onto duplicate plates of marine agar (Difco). Duplicate plates of each dilution were incubated aerobically at 30 °C for 7 days and under anaerobic conditions at 30 °C in anaerobic jars (BBL Gas Pak Systems) for 14 days.

Enumeration of Viable SRB

Universal bottles containing serial dilutions of inoculum in Postgate B media were filled to the top with more media to exclude oxygen. They were incubated at 30 °C for 14 days.

Positive growth of SRB was shown by a blackening of the media (due to the presence of FeS) and for the production of H₂ gas by SRB. Growth of SRB in different dilutions was converted into a count as follows:

Dilutions with positive growth	Concentration of SRB (Viable cells per ml)
10 ⁻¹ only	1 - 9
10 ⁻¹ - 10 ⁻²	10 - 99
10 ⁻¹ - 10 ⁻³	100 - 999
10 ⁻¹ - 10 ⁻⁴	1,000 - 9,999 etc.

3.5.3 Stagnant Fermenter

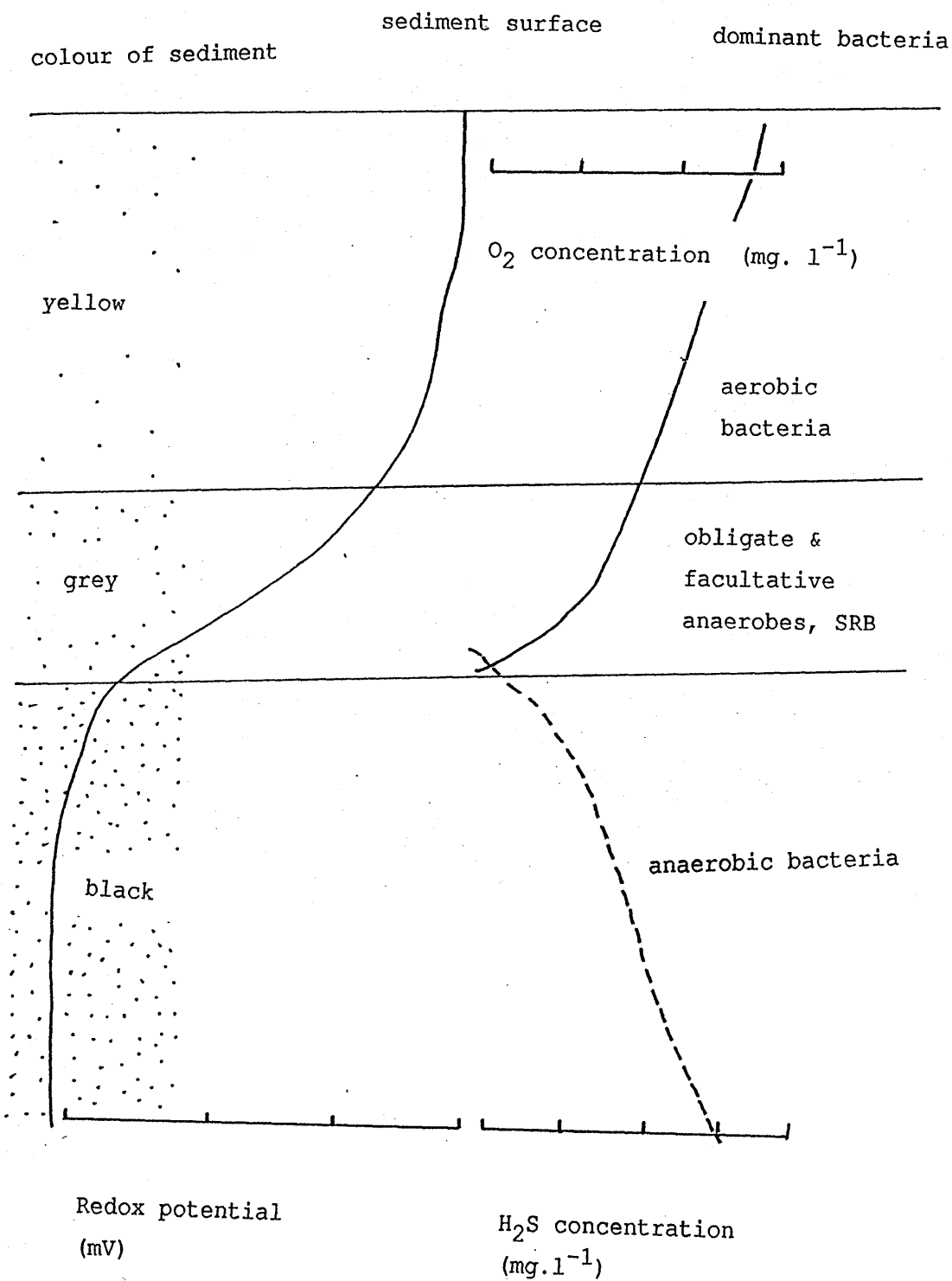
It was found that the aerated fermenter provided a mixed population of bacterial types with which to inoculate the flow loop but counts of viable SRB were low. In order to increase the concentration of SRB another fermenter was set up providing a largely anoxic environment.

Five litres of mud were gathered from Solva Harbour near St. Davids, Pembroke. The mud had a high organic content and was collected from the redox-discontinuity layer about 20 cm below the surface (refer to FIGURE 3.5.3). This is a zone of rapid transition where the redox potential drops rapidly between areas of aerobic and anaerobic decomposition (Fenchel & Riedl, 1970).

FIGURE 3.5.3

Changes In Physical And Chemical Properties Of Sediments Across
The Redox Discontinuity Layer

(After Fenchel & Reidl, 1970)



It is marked by a layer of grey sand due to the deposition of iron sulphide. Redox-discontinuity layers are typically rich in SRB, obligate and facultative anaerobes.

The sediment was mixed with an equal volume of horticultural perlite and put into a fermenter consisting of a 10 l plastic water carrier. The perlite was mixed with the sediment to provide a larger surface area for a bacterial biofilm to colonise and to ensure that the fermenter drained freely.

Twice weekly 0.5 l of liquid was drained from the stagnant fermenter and added to the reservoir of the flow loop. This liquid was replaced with an equal volume of nutrient solution twice weekly. Each litre of nutrient solution was based on artificial seawater plus the following additions:

- 0.1 g ascorbic acid
- 0.1 g thioglycollic acid
- 6.0 g sodium lactate
- 3.0 g glucose
- 2.0 g yeast extract

The pH of the final solution was adjusted to 7.5 before use. The ascorbic and thioglycollic acids are reducing agents to promote the growth of anaerobes requiring a very low redox potential.

3.5.4 Monitoring Bacterial Concentrations Produced By The Stagnant Fermenter

In order to check that the bacterial output from the stagnant fermenter was fairly constant, monthly counts of heterotrophic aerobic and anaerobic bacteria and also of SRB were performed using the techniques previously described.

3.5.5 Enumeration Of Viable Planktonic Bacteria In The Reservoir Of The Flow Loop

Counts of viable heterotrophic aerobic and anaerobic bacteria and also of SRB were performed on a sample of water removed from the reservoir of the flow loop.

3.6 THE DETERMINATION OF CORROSION RATES OF STEEL COUPONS

The corrosion rates of steel coupons in the corrosion probe and immersed in the reservoir were monitored at regular intervals using both electrochemical techniques and weight loss methods.

3.6.1 Electrochemical Corrosion Measurements

Two electrochemical corrosion measurement techniques employed were Linear Polarisation Resistance (LPR) Scans and Full Polarisation Scans.

Method Of taking LPR Scans

LPR scans of steel coupons were taken using the 'Autostat' and BBC Master computer. Electrode potentials were measured with reference to a calomel reference electrode. In the case of the corrosion probe this made electrical contact with the steel coupons with a polyacrylamide salt luggin, or it was dipped directly into the reservoir in the case of the coupons immersed in the reservoir. LPR scans were initially conducted daily and then less frequently at twice weekly intervals. The scans were run on the same five duplicate coupons over the entire duration of each investigation. Each LPR scan lasted 4 minutes and the potential was controlled at a scanning rate of 10 mV min^{-1} . During an LPR scan the corrosion potential was determined, then the potential was raised gradually to 10 mV above this value, then controlled to 10 mV below the corrosion potential and finally raised back to the original corrosion potential. Graphs of electrode potential against current density were plotted and the corrosion current calculated using the

Stern-Geary equation.

The LPR technique of measuring the corrosion rates relies on the linear relationship between the potential and current density in the region of the corrosion potential (E_{corr}). A small voltage (E) was applied to the steel working electrode, altering its electrode potential away from the corrosion potential. This caused a small current (I) to flow from the platinum auxiliary electrode to the steel working electrode. The slope of the linear part of the plot (FIGURE 3.6.1.A) of potential versus current density is termed the polarisation resistance (R_p).

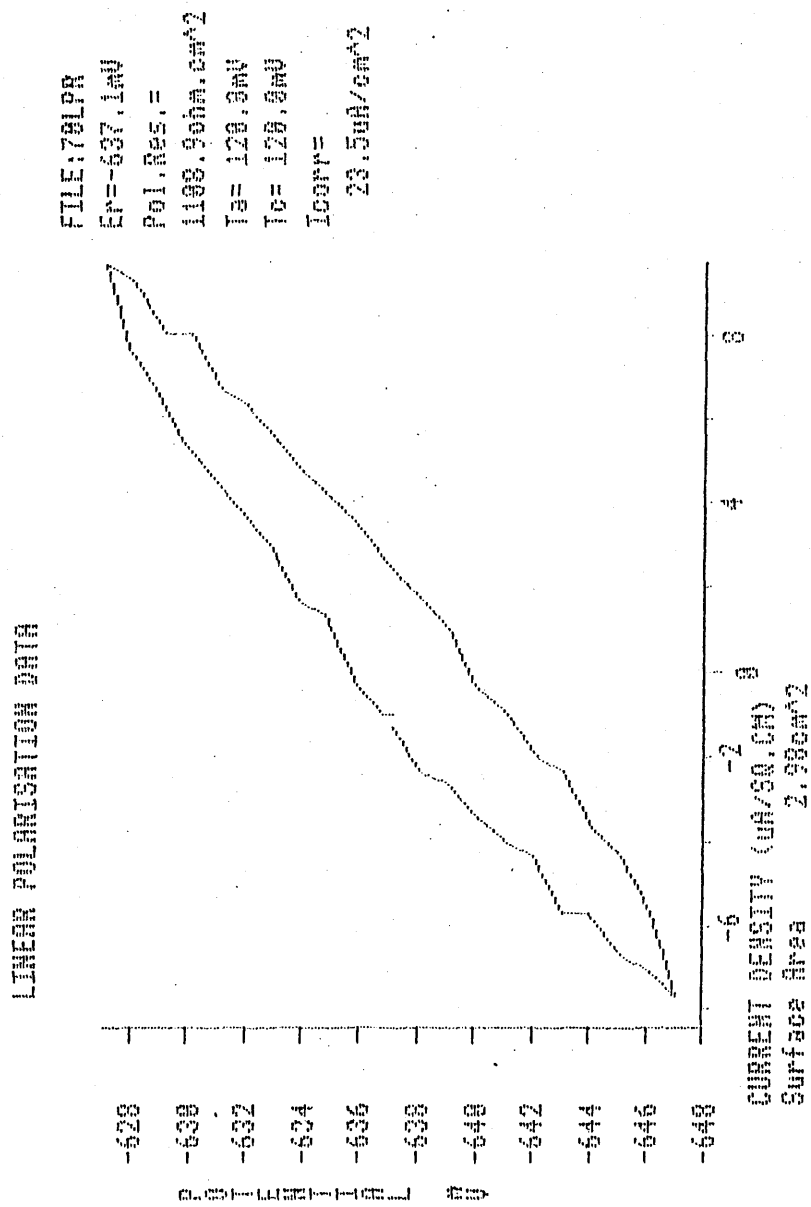
$$R_p = \frac{\text{(Change in I)}}{\text{(Change in E)}}$$

The Stern-Geary equation describes the linear relationship between the potential and the current density, it can be used to calculate the corrosion current (I_{corr}).

$$I_{\text{corr}} = \frac{b_a \times b_c}{(b_a + b_c) \times 2.3 \times R_p}$$

Where R_p is the polarisation resistance determined at potentials close to E_{corr} and b_a and b_c are Tafel constants. This equation shows that the corrosion rate is inversely proportional to R_p or directly proportional to the reciprocal slope of the potential versus the current density curve at potentials close to E_{corr} .

FIGURE 3.6.1.A

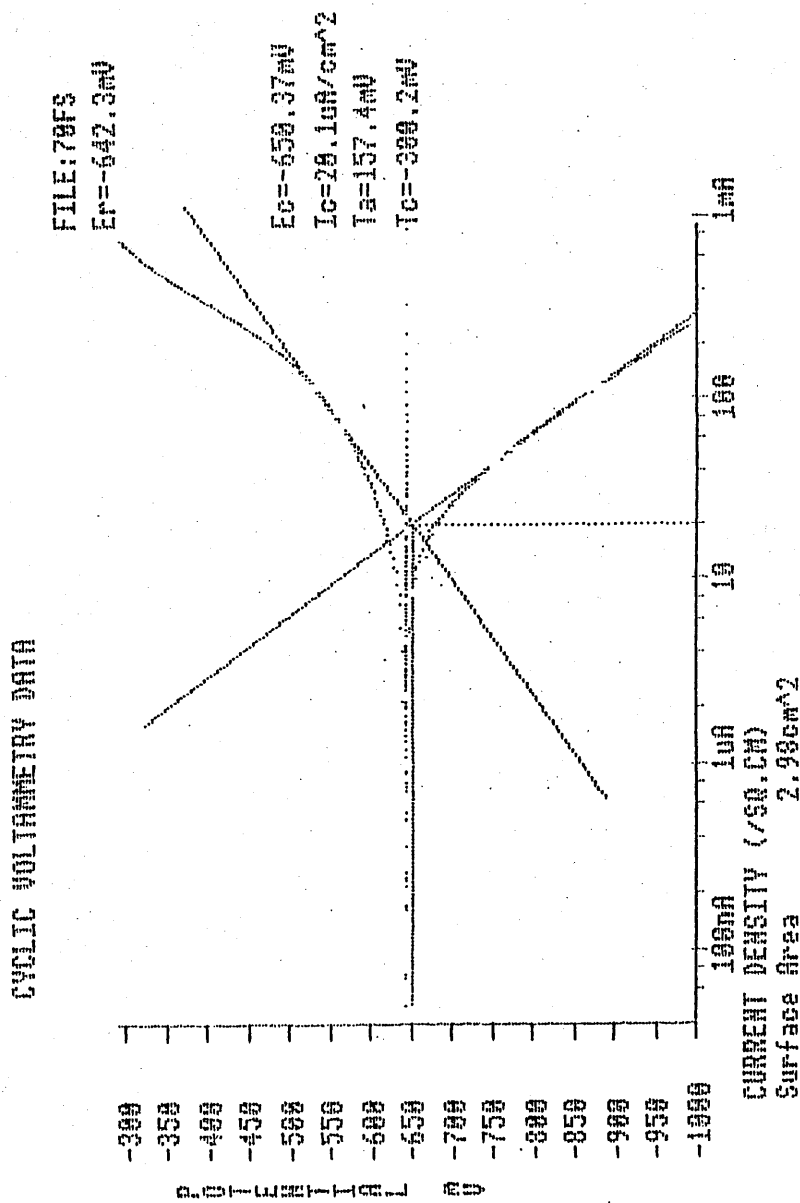
LPR Scan

In order to calculate the corrosion current using the Stearn-Geary equation the Tafel constants must be known. The Tafel constants were assumed both to be 120 mV/decade by the 'Autostat' programme. This assumption may result in inaccuracies as the exact values of b_a and b_c may vary substantially with environmental conditions over the course of a corrosion test (Shreir, 1976, p 20.37).

Method Of Taking Full Polarisation Scans

Full polarisation scans were conducted periodically on coupons in situ in the reservoir or in the corrosion probe. Firstly, the steel working electrode was polarised to 300 mV below its corrosion potential, followed by sweeping to 300 mV above its corrosion potential at a rate of 10 mV min^{-1} . Coupons were only used once for full polarisation scans and were subsequently discarded due to disruption of the biofilm when cathodic hydrogen is evolved. When polarisation data was plotted on logarithmic axes it could be used to determine the corrosion current (I_{corr}) through Tafel extrapolation. The Autostat computer programme was used to fit lines on the linear Tafel regions of anodic and cathodic polarisation curves giving values for the Tafel constants (this is shown in FIGURE 3.6.1.B). It should be noted that in this study the cathodic curve was a straight line whereas the anodic region was curved.

FIGURE 3.6.1.B

Full Polarisation Scan Of Steel Coupon

Tafel extrapolation has been used to determine actual values for the Tafel constants (I_a and I_c), from which the corrosion current (I_c) was determined.

Extrapolation was done by first extending the cathodic curve back to the corrosion potential. The point at which the anodic and cathodic Tafel extrapolations intersect in the region of the corrosion potential corresponds to the corrosion current. The Tafel Constants determined from the full polarisation scans were used to replace the estimated values of 120 mV/decade in the LPR scans, giving revised corrosion rates.

3.6.2 Determination Of Steel Corrosion Rates Using Weight loss Coupons

Periodically, triplicate preweighed coupons were removed from the corrosion probe or from the reservoir and any loose corrosion product removed. The coupons were then held at a cathodic potential and electrolytically cleaned in a solution of tri-ammonium citrate to remove adhering corrosion product (refer to Appendix D). Mean weight losses were calculated.

3.7 TECHNIQUES USED TO STUDY BIOFILM DEVELOPMENT ON THE COUPONS IMMERSED IN THE RESERVOIR

Periodically coupons were removed from the reservoir and the biofilm examined using the scanning electron microscope; viable counts of biofilm bacteria and radio-respirometric assays to measure sulphate reduction and glucose turnover rates were made.

3.7.1 Preparation of Coupons For Observation With Scanning Electron Microscope

Duplicate coupons were fixed by freeze-drying or in osmium tetroxide followed by critical point drying before viewing with the SEM.

Fixation By Freeze-Drying

Firstly, coupons were immersed in liquid nitrogen in a polystyrene cup until it stopped bubbling. Sections of corrosion product were removed from freely corroding coupons with a sharp blow from a craft knife cooled in liquid nitrogen. Coupons and sections of corrosion product were freeze-dried at -50 °C (Edwards Pirani 10 freeze drier) for at least 24 hours. After freeze-drying, coupons were mounted on SEM stubs and coated with

carbon or gold.

Fixation In Osmium Tetroxide

Coupons were put into separate glass vials, immersed in 2% aqueous osmium tetroxide solution and fixed at 4 °C overnight in the dark. After fixation the vials were allowed to reach room temperature before the fixed material was washed with distilled water for 15 minutes with two changes. Whilst changing solutions the fixed material was left in the glass vial, the top two thirds of the solution removed and then topped up with the next solution. The fixed material was not exposed to the atmosphere which would result in artefacts caused by surface tension effects.

After washing, the fixed material was dehydrated through a graded ethanol series from 10 % to 90 % with 15 minutes in each 10 % step. The 90 % ethanol was replaced with 95 % ethanol for 15 minutes. The fixed material was then immersed in absolute ethanol for 15 minutes with 2 changes. The stock of absolute ethanol was kept over a molecular sieve to remove traces of water.

The ethanol was replaced with water-free acetone in a graded series: 2:1, 1:1, 1:2 (ethanol:acetone) and finally into pure

acetone. A stock of water-free acetone was kept over a molecular sieve. The fixed material was critical point dried (CPD 750) before mounting on SEM stubs and coating in carbon or gold.

Use of SEM

Prepared stubs were examined using an ABT 50 or a Cambridge 250 Stereoscan scanning electron microscope. Stubs were viewed using a low accelerating voltage (usually 17 KV) to avoid surface damage, and a short working distance and small spot size to provide better resolution.

3.7.2 Taking Viable Counts of Aerobic And Anaerobic Heterotrophs And SRB

Duplicate coupons were removed from the perspex mounts with sterile forceps and placed in sterile universal bottles containing 10 cm³ of Postgate B media each. The universals were put into an ultrasound bath for 5 minutes, then vigorously vortex mixed and the treatment repeated. This treatment seemed to remove visible scale from the coupon leaving it bright and shiny. Serial dilutions in duplicate were made in Postgate B media to determine viable numbers of SRB. Aliquots (0.1 cm³) of each dilution were spread onto duplicate plates of marine agar (Difco). These plates were incubated at 30 °C under aerobic and

anaerobic conditions to enumerate heterotrophic bacteria.

Viable SRB were not detected on the coupons probably due to the prolonged sonication. The method of removing the biofilm from the coupons was altered. Coupons were put into Postgates B media containing a non-ionic surfactant (0.1 % Tween 80) to help disperse the bacteria. The universals were vortex mixed then sonicated for only 20 seconds and vortex mixed again.

3.7.3 Radio-respirometric Assay To Measure Sulphate Reduction Activity And Glucose Turnover

The relative amounts of sulphate reduction activity by SRB and glucose turnover by aerobic heterotrophic bacteria were determined by a radio-tracer technique (refer to Maxwell & Hamilton, 1984). The radioassays were performed using $\text{Na}_2^{35}\text{SO}_4$ and universally labelled ^{14}C glucose (Amersham). Radioassays were first performed using duplicate coupons from each treatment, this number was subsequently increased to five due to variable results.

Method Of Sulphate Reduction Assay

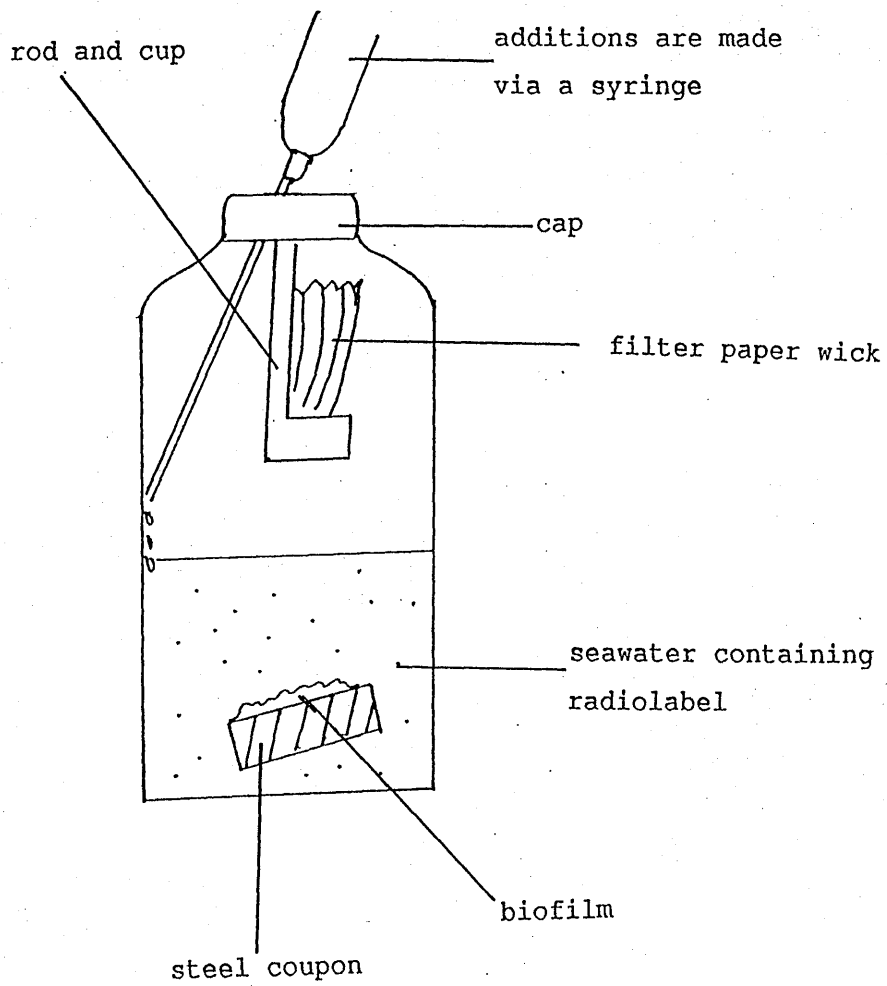
Coupons were removed from the reservoir and put into half-ounce glass bottles. A stock solution of $\text{Na}_2^{35}\text{SO}_4$ in artificial

seawater was prepared to give a final concentration of 10 uCi per 3 cm³. Three cm³ of this stock solution was pipetted into each half-ounce bottle containing a coupon. Lids were crimped onto the vials and they were incubated at room temperature in a shaking water bath for 7-8 hours. During the incubation period an air headspace was used as this most closely resembled the aerobic conditions in the reservoir of the flow loop.

After incubation the lid from each bottle was removed and a filter paper wick and holder inserted into the headspace (refer to FIGURE 3.7.3). The filter paper wicks (Whatman No. 1, 4 x 8 cm) were folded into an accordian and placed into the cup of a plastic holder on a rod. Lids were replaced and the aerobic headspace replaced with one of oxygen free nitrogen. Then 1 cm³ of anaerobic zinc acetate (1 M) added to each bottle. This addition reacts with labelled hydrogen sulphide to form non-volatile, labelled zinc sulphide. The seawater was then acidified with 2 cm³ of anaerobic hydrochloric acid (6 M). This lowered the pH of the seawater below one and renders labelled sulphide volatile as labelled hydrogen sulphide. To trap the labelled hydrogen sulphide on the wick 0.8 cm³ of anaerobic zinc acetate was added to each wick. Trapping was performed overnight in a shaking water bath.

FIGURE 3.7.3

Incubation System Used During Radio-Respirometric Assay To Measure Glucose Turnover Or Sulphate Reduction Activity By A Biofilm



After, trapping the wicks were carefully removed and placed in mini-vials containing 4 cm³ of liquid scintillant (Beckman Ready Value). The cup of each holder was rinsed with 0.5 cm³ of distilled water which was added to the mini-vial. To determine the actual activity of the stock seawater 0.5 cm³ was added to a mini-vial containing 4 cm³ of scintillant in triplicate. The radioactivity associated with the wicks and stock seawater was quantified with a liquid scintillation counter (Beckman LS7000) after 24 hours. This time allowed labelled sulphide to equilibrate with the scintillant.

Quantification of sulphate reduction activity was calculated by determining the ratio of labelled sulphate to unlabelled sulphate in the seawater. The sample counts were then corrected for counting efficiency and partitioning of sulphur isotopes using a formula derived by Jorgenson and Fenchel (1974):

Rate of sulphate reduction =

(n mol. cm⁻³. hr⁻¹)

$$\frac{\text{DPM evolved} \times 1.06 \times [\text{sulphate}]}{\text{DPM added} \times \text{Incubation time (hr)}} \times \frac{1}{\text{Surface area of coupon (cm}^2\text{)}}$$

DPM = disintegrations per minute is equal to CPM (counts per minute) corrected for the counting efficiency determined from the quench correction curve (refer to Appendix E).

$$\text{DPM added} = \text{DPM of } 0.5 \text{ cm}^3 \text{ seawater stock} \times 6 =$$

$$\text{DPM of } 3.0 \text{ cm}^3 \text{ used in each assay}$$

[sulphate] = Seawater contains 4.09 g l^{-1} of sodium sulphate
Molecular weight of sodium sulphate is 142

$$\frac{4.09}{142} = 0.0288 \text{ mol l}^{-1}$$

Therefore 3 cm^3 of seawater stock contains:

$$\frac{3}{1000} \times 0.0288 = 8.64 \times 10^{-5} \text{ moles of sulphate.}$$

$$\text{Surface Area of Coupon} = 2.64 \text{ cm}^2$$

Method Of Glucose Turnover Radioassay

The method of radioassay to determine glucose turnover was similar to that used to measure sulphate reduction. The quantity of universally labelled glucose used per radioassay was 1 uCi. Incubation and trapping occurred under aerobic conditions as $^{14}\text{CO}_2$ is not readily oxidised. Potassium hydroxide (1 M) was used to trap $^{14}\text{CO}_2$ on the wick.

Seawater contains a very small concentration of glucose which is difficult to quantify. The amount of ^{14}C glucose added during the radioassay may alter the size of the glucose pool available for use by heterotrophic bacteria and significantly increase their activity. The amount of labelled glucose added was

calculated to give a final concentration of 5 ppm glucose carbon in the seawater.

Unlike the glucose concentration, seawater contains a high concentration of sulphate, so the addition of the small quantity of labelled sulphate is assumed to have a negligible effect on the overall sulphate concentration. Indeed, since the large sulphate concentration does not limit the rate of sulphate reduction by SRB, the exact amount of labelled sulphate does not need to be known.

By taking these factors into account the glucose turnover is expressed as the relative turnover of the added radiolabel.

3.8 STERILE FLOW LOOP: A CONTROL FOR CORROSION MEASUREMENTS

A flow loop was assembled (Funge-Smith, Robinson & Seal ,1988B) to provide a controlled environment to measure corrosion rates of steel coupons with similar levels of aeration as in the other flow loop but without the influence of marine microorganisms.

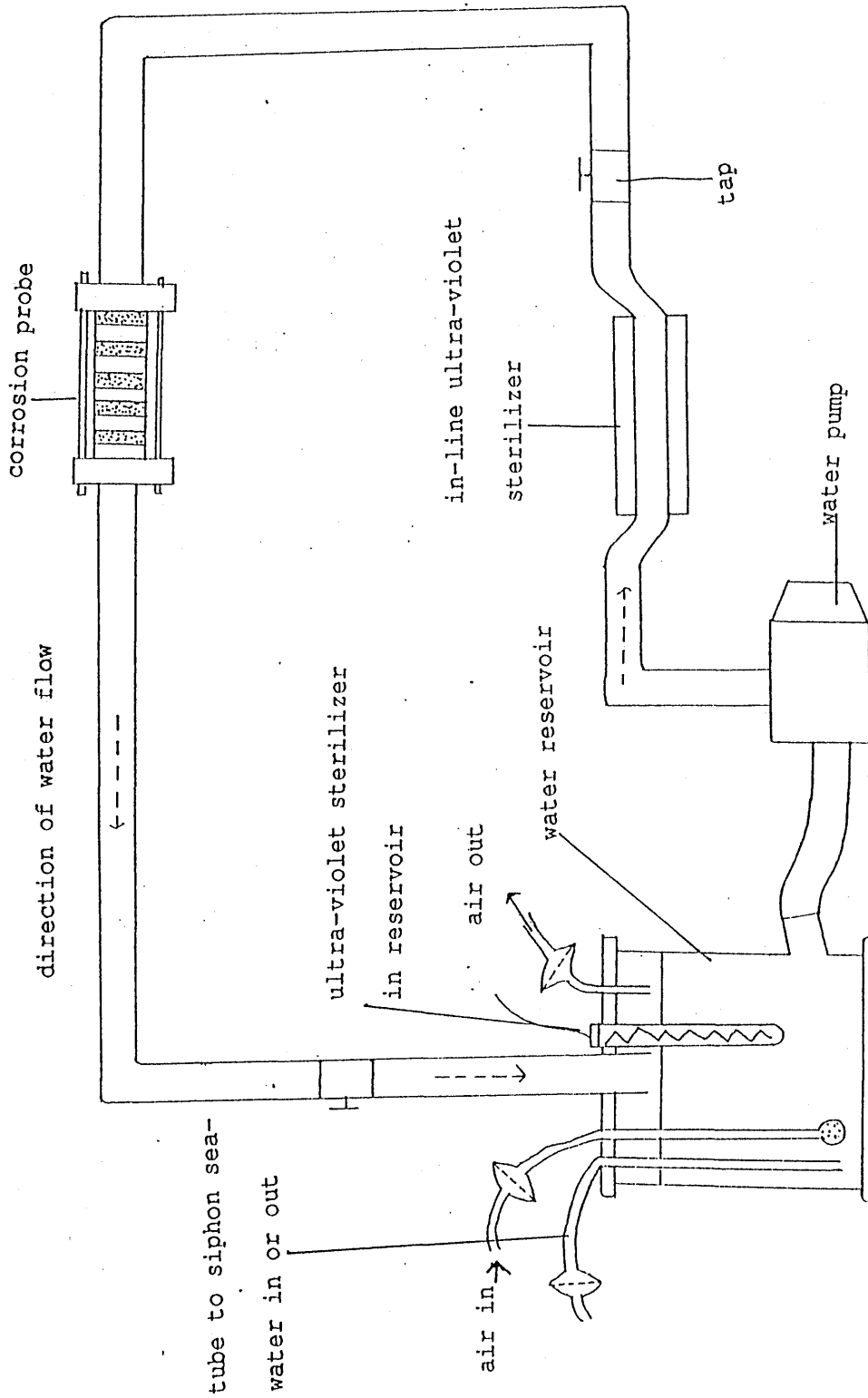
3.8.1 Sterile Flow Loop Design

The sterile flow loop (refer to FIGURE 3.8.1) was similar in design to the one already described. The reservoir of the sterile loop had a much smaller volume (only 5 l) than that of the first loop. It was considered, perhaps wrongly, that a smaller volume of water would be easier to keep sterile. The small volume of water in the flow loop (8 l total) resulted in problems later on. The sterile flow loop was to be filled with artificial seawater which was sparged with oxygen.

Maintaining Sterility Within The Flow Loop

Sterility within the sterile flow loop was to be maintained by two ultra-violet light sterilisers. One was placed in the reservoir itself, the other was in-line with water pumped through

FIGURE 3.8.1

Diagram Of Sterile Flow Loop

it. Ultra-violet light alone could not be expected to maintain the sterility of the flow loop. It may be fairly effective in killing planktonic bacteria but it cannot penetrate around corners and inactivate sessile bacteria. It was suggested that water could be filtered periodically to remove bacteria and organic detritus. This idea was impractical as an in-line filter with a small enough pore size (0.2 μm) would have provided too big a resistance for the pump to operate. Filtering water would not have any effect on sessile bacteria adhering to the walls of the flow loop.

To discourage the growth of heterotrophic microorganisms a carbohydrate source was not added to the artificial seawater.

Another method of maintaining sterility that was to be used in conjunction with the use of UV was the periodic use of a biocide. It was appreciated that the biocide should have a negligible effect on the corrosion behavior of the steel.

Running Of The Sterile Flow Loop

The flow loop was dismantled, the pipes and reservoir soaked in a solution of detergent (Decon 90), scrubbed out and rinsed. Bacterial filters were fitted to all the air inlet and outlet pipes to stop air-borne contamination. A corrosion probe of

annular steel electrodes was built and incorporated into the loop. The corrosion probe consisted of coupons on which LPR and Full Scans could be taken, and preweighed coupons. Before seawater could be put into the flow loop the surfaces were sterilised with a biocide, Emulcid (Thor Chemicals). This biocide was rigorously tested for its influences on steel corrosion (refer to Appendix F). Emulcid (1 % solution) was found to inhibit steel corrosion markedly but the effect was not residual, thus once the Emulcid solution had been rinsed off the steel it exhibited normal corrosion behavior. Eight litres of Emulcid solution (1 % in distilled water) were circulated in the flow loop for 4 hours. This volume of biocide was sufficient to fill the reservoir of the flow loop, excluding air pockets in which potential sources of contamination could survive. The loop was drained and 8 litres of sterile distilled water (autoclaved at 15 psi for 20 minutes) were circulated for an hour. This rinsing procedure was repeated.

Corrosion rates measured with LPR scans were found to be very high (about 10 times greater than expected) after the flow loop had been running for 24 hours. The temperature in the sterile flow loop reached 33.6 °C, compared with 22 °C in the other flow loop. The two ultra-violet sterilizers probably combined with effects of friction of the small volume of circulating water caused this unacceptable temperature rise.

These problems could probably have been overcome by fitting a heat-exchanger into the flow loop. Unfortunately, due to financial constraints of the project this idea could not be implemented and the sterile flow loop was abandoned.

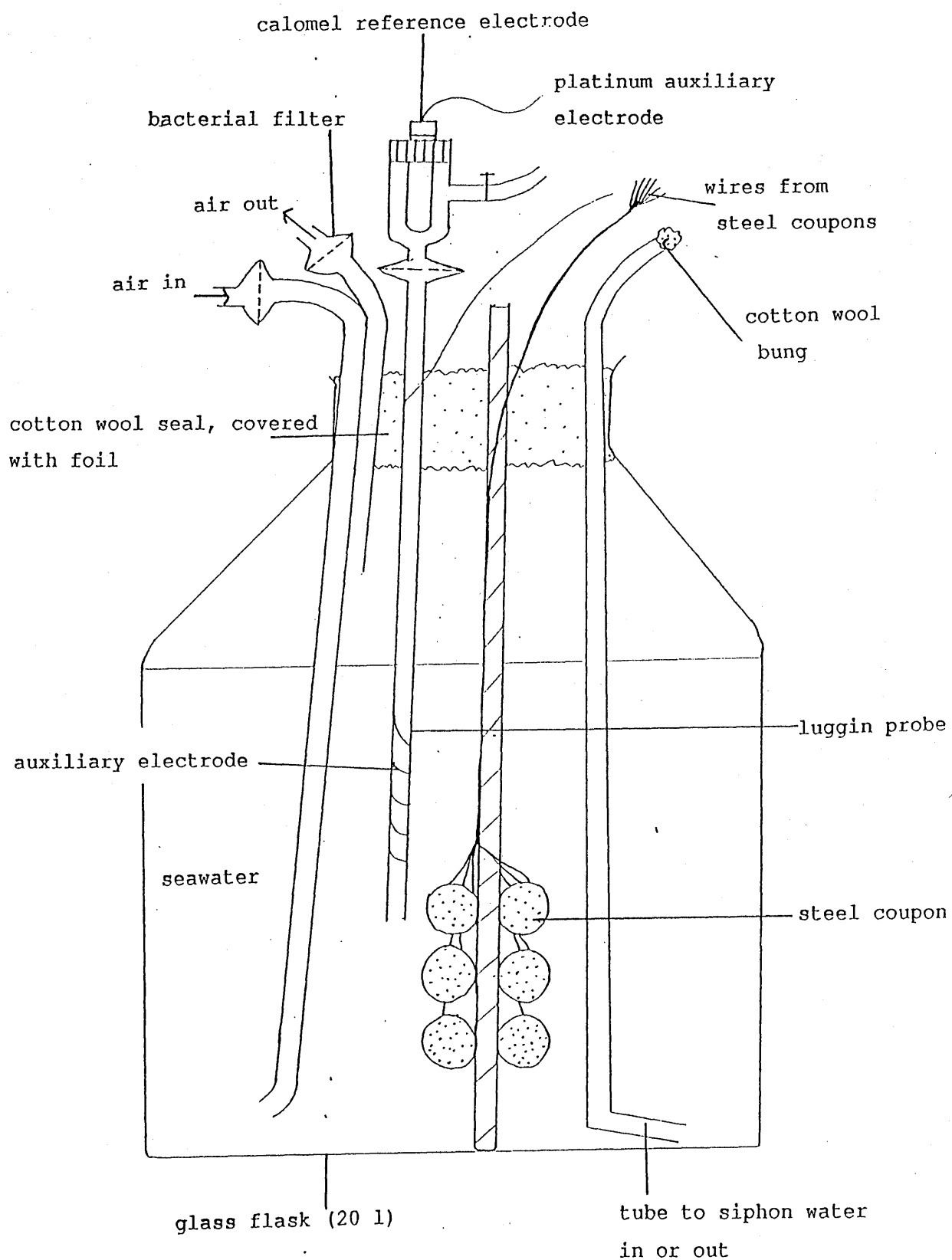
3.8.2. Alternative Sterile Control

An alternative sterile control was designed in which steel corrosion could be investigated using electrochemical and weight loss techniques. The equipment was based on 20 l and 5 l glass flasks filled with seawater which could be easily sterilised.

Electrochemical measurements were to be taken on coupons immersed in a 20 l glass flask containing seawater (refer to FIGURE 3.8.2). Cylindrical steel coupons with wires soldered to the reverse were attached to a length of glass rod with silicone sealant and insulating tape. The steel coupons stuck to the glass rod with the electrical wires were immersed in isopropanol for 24 hours to degrease and sterilise them. Steel coupons were not sterilised by autoclaving as they corroded.

A glass flask (20 l capacity) was filled with 15 l of artificial seawater. Into the neck of the flasks were inserted: a glass tube going to the bottom of the flask to syphon water in or out; a similar glass tube through which air would be pumped;

Diagram Of Alternative Sterile Control Used To Take Electrochemical Corrosion Measurements



a glass tube to be used as a luggin probe; a length of platinum wire to be used as an auxiliary electrode was wound around the glass luggin; a short length of glass tube as an air outlet. All of the glass tubes were fitted with bacterial filters to stop air-borne contamination. A short length of plastic tube was stuck to the side of the neck of the flask with silicone sealant and a bung was put into the top of this. The neck of the flask was sealed with cotton wool, foil wrapped over it and sealed with autoclave tape. The flask and glass tubes was autoclaved at 15 psi for half an hour.

In a sterile flow cabinet the bung was removed from the plastic tube in the neck of the 20 l flask and the steel coupons mounted on the glass rod quickly inserted. The sterile bung was replaced. The flask was set up with a continuous air sparge through the water.

Electrochemical measurements were taken by using the Autostat. The glass tube luggin probe had a bacterial filter inserted in the top end. A glass holder above the bacterial filter contained a calomel reference electrode. A pump was used to draw water from the flask up the glass luggin probe through the bacterial filter to make contact with the calomel electrode. The bacterial filter was a disposable type usually employed for filter sterilising liquids out of syringes (Acrodisc of 0.2 μ m

pore size, Gelman Sciences U.K.) so it was replaced each time corrosion measurements were taken. The sterility of the flask was checked after the investigation with triplicate spread plates of marine agar incubated under aerobic conditions.

A similar arrangement was used to determine corrosion rates with weight loss coupons. Five replicate preweighed coupons were stuck to glass rods and sterilised in isopropanol. They were inserted into three sterile glass flasks (5 l capacity) filled with seawater. Air was sparged continuously into the water. All of the weight loss coupons were removed from each flask at intervals, electrolytically cleaned and weight losses determined. The sterility of each flask was checked by triplicate spread plates of marine agar incubated under aerobic conditions.

3.9 INVESTIGATION INTO STEEL CORROSION IN REAL MARINE ENVIRONMENT

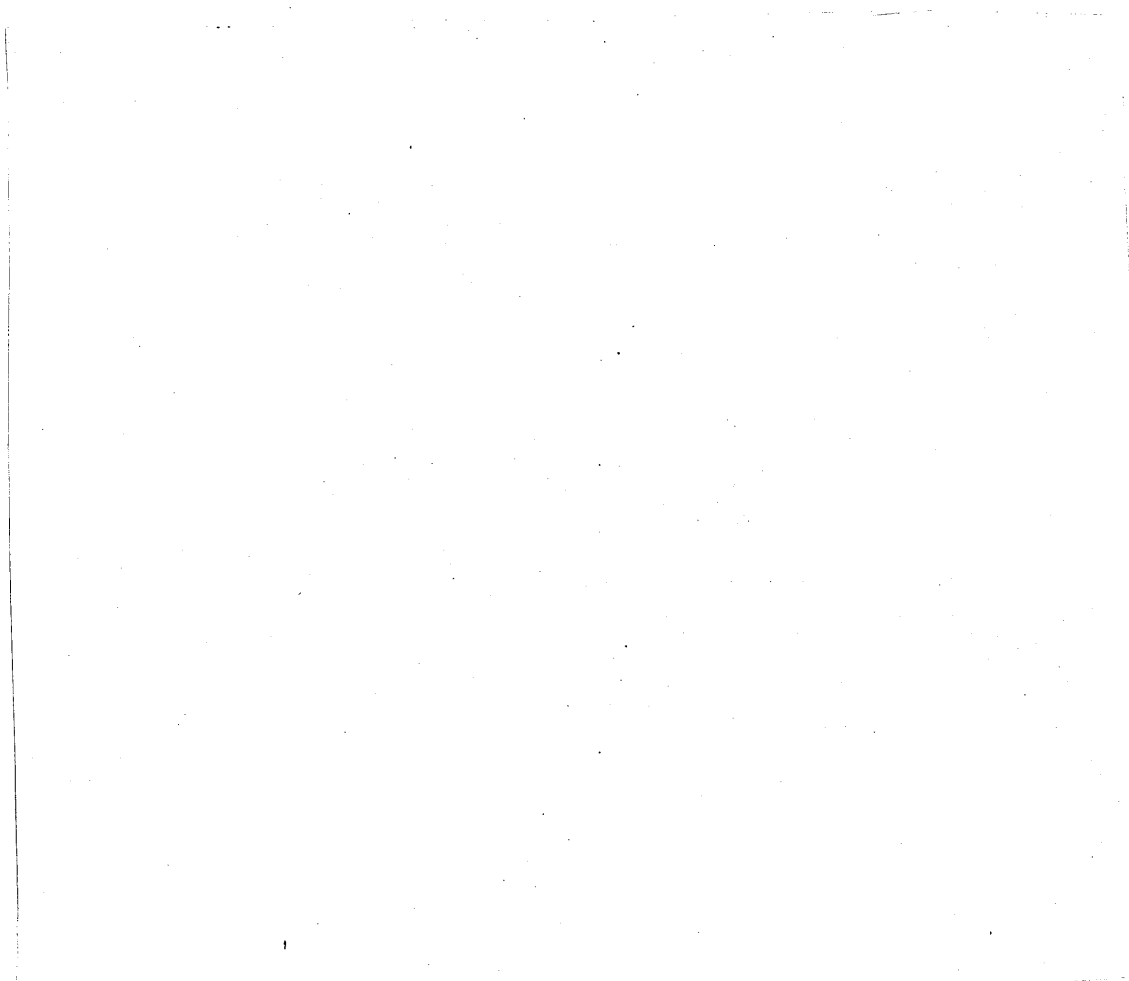
It was important to compare corrosion data gained at Cranfield using the flow loops which simulated marine environment with genuine marine conditions. In order to do this a corrosion probe and cylindrical coupons have been sited at the Shell Research Station on the Ferry Pier, Holyhead. At this research station water is pumped from 20 m below the sea surface into Portacabins on the Pier in which the corrosion monitoring equipment was placed.

3.9.1. Construction Of A Corrosion Probe

A corrosion probe was built of annular steel electrodes and mounted on the inside wall of a Portacabin, seawater was pumped through it at a rate of 30 l per minute (flow velocity of 1.8 m s^{-1}). The probe consisted of 12 steel electrodes, 6 of which were cathodically protected at -850 mV S.C.E. by a potentiostat (Thompson). The remaining coupons were freely corroding and their corrosion potentials were continuously recorded. The rest potentials were measured against a robust calomel reference electrode (Kent Instruments) mounted horizontally adjacent to the probe. This reference electrode was used to overcome problems of gel filled salt bridges drying out or being expelled under high

water pressure. The platinum wire auxilliary electrode was wound round the inside of a section of pipe next to the reference electrode and cemented to it with epoxy resin. The corrosion probe is shown in the photograph, FIGURE 3.9.1.

The annular steel working electrodes were wired to different channels of a data logger. This recorded the potentials at which coupons were cathodically protected, the voltage used to protect the coupons over a 25 Ohm resistor and the individual corrosion potentials of the freely corroding coupons. Corrosion measurements were taken successfully for 34 days before the water flow was interrupted by a pump failure.



4.0 RESULTS

4.1 CONDITIONS WITHIN THE FLOW LOOP INOCULATED WITH BACTERIA

The flow loop contained artificial seawater with a salinity of 3.5 ‰. Its temperature fluctuated slightly with the seasons as a heat exchanger was not fitted into the loop. The temperature of the seawater ranged from a maximum of 24 °C in the summer to a minimum of 17 °C in the winter and was usually about 19 °C or 20 °C. Using the Winkler titration the concentration of dissolved oxygen was 3.25 mg l⁻¹. The saturation concentration of dissolved oxygen varies with air pressure, water temperature and salinity. The seawater in the flow loop was approximately one third saturated with dissolved oxygen. The pH of the seawater was in the range of pH 8.5-9.0. The total carbohydrate concentration determined using a colorimetric assay was 16 mg l⁻¹. The count of viable bacteria showed that 1.4 x 10⁴ cfu per cm³ of heterotrophs incubated under aerobic conditions and 6.0 x 10³ cfu per cm³ of heterotrophs incubated under anaerobic conditions were present. Positive results for SRB using Postgates B media were not gained.

4.2 MONITORING BACTERIAL CONCENTRATIONS PRODUCED BY THE AERATED GRAVEL-PACKED AND STAGNANT FERMENTERS

Viabie aerobic and anaerobic bacteria and viable SRB were enumerated at approximately monthly intervals from both fermenters. The results of these viable counts are shown graphically in FIGURE 4.2.A and FIGURE 4.2.B.

Levels of heterotrophic bacteria from the aerated gravel-packed fermenter incubated under aerobic and anaerobic conditions remained fairly constant in the upper and lower layers of the fermenter. Numbers of heterotrophic bacteria were not significantly different between these regions. Numbers of viable SRB remained at a steady state in the lower layer of the fermenter. Numbers of viable SRB in the upper layer increased, this may be correlated with a slight increase in the numbers of viable heterotrophs incubated under anerobic conditions in this region. It was anticipated that aerobic bacteria would become dominant in the upper gravel layer. This region was adjacent to the air-sparging and to the source of enriched seawater. Higher viable counts of SRB were gained from this layer than from the lower gravel layer.

lower gravel layer was intended to provide an anoxic environment in which anaerobic heterotrophs and SRB would

FIGURE 4.2.A
Graph Showing Results Of Viable Counts Of Heterotrophic Bacteria And Of
SRB From The Aerated Gravel-Packed Fermenter

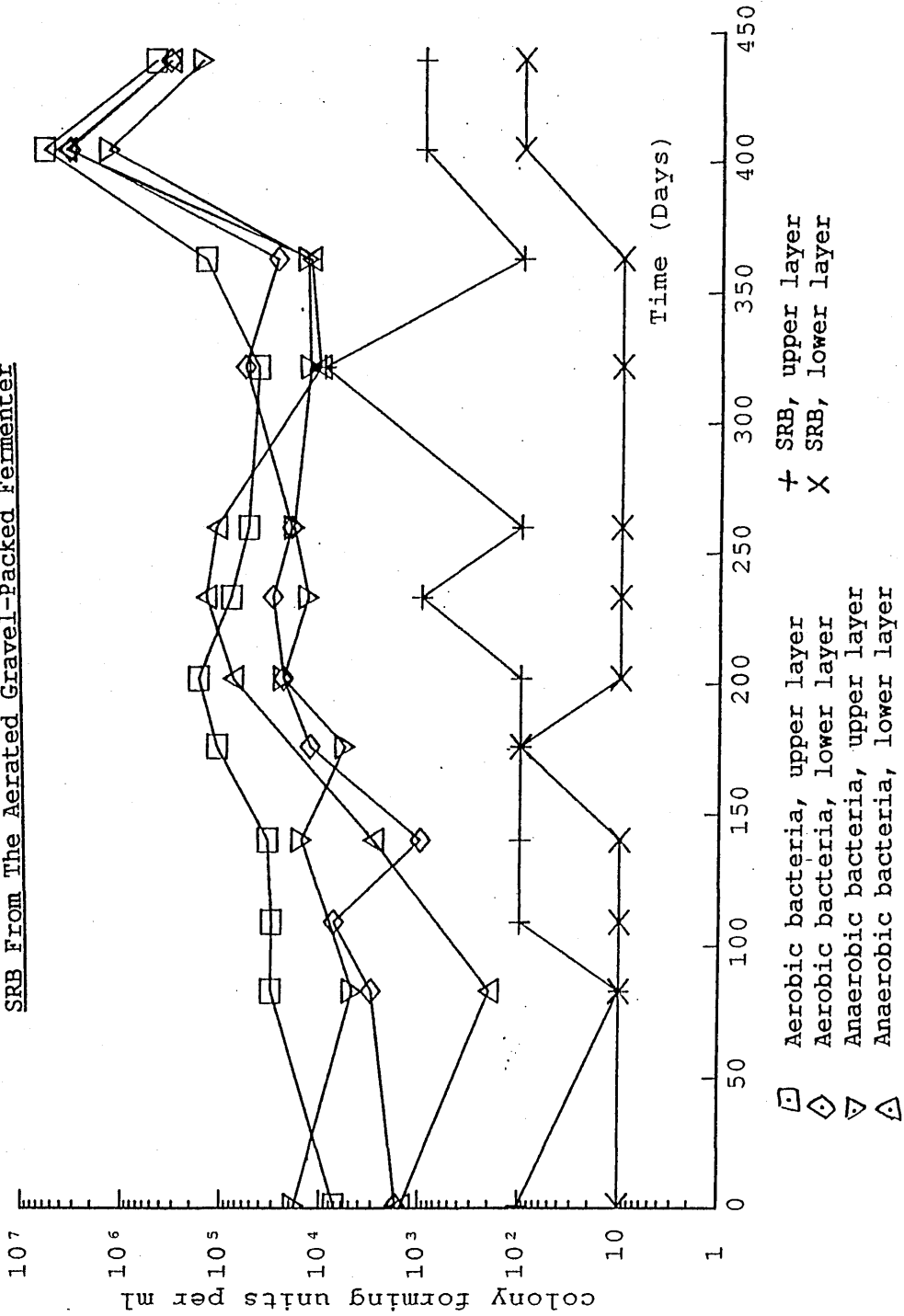
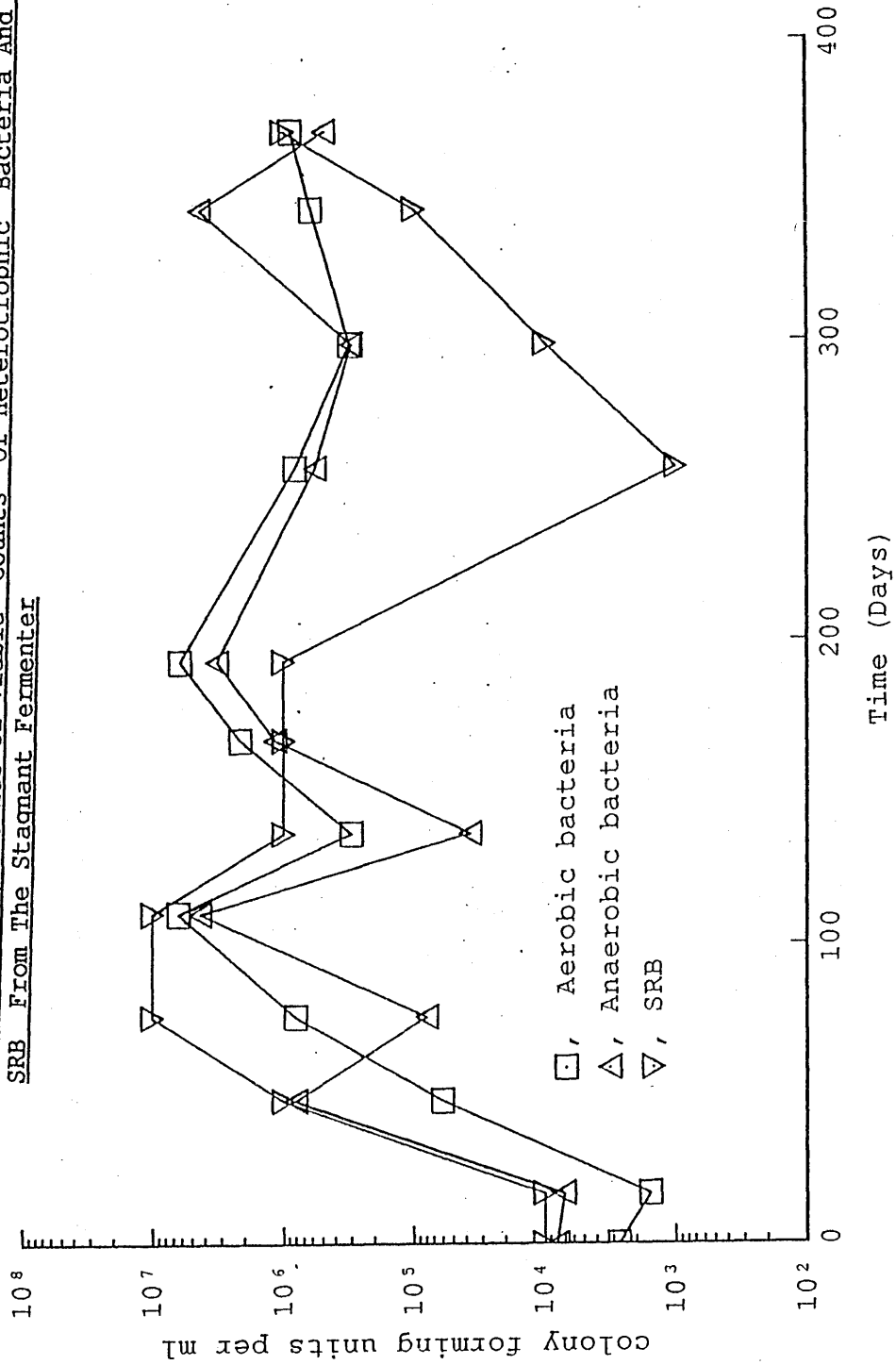


FIGURE 4.2.B

Graph Showing Results Of Viable Counts Of Heterotrophic Bacteria And Of SRB From The Stagnant Fermenter



flourish. The gravel-packed fermenter was to be compared to a typical marine sediment in which the end-products of fermentation in the upper aerobic zone diffuse into the lower zone and support the growth of anaerobes. This did not occur although the upper layer of the fermenter (which is closest to the carbon source) supported a denser community of aerobes and anaerobes. Although the fermenter was designed to simulate conditions in a marine sediment it differs from this environment due to the large particle size of the gravel. The development of a lower anoxic zone is dependant on the diffusion rate of oxygen downwards into the lower layer being less than its rate of use by respiring aerobes in the upper zone. An anoxic zone develops in sediments which are relatively impermeable to oxygen-carrying water due to the small grain size.

The aerated gravel-packed fermenter succeeded in providing a habitat supporting a balanced range of marine microorganisms to inoculate the flow loop, although counts of SRB were low. No particular group of bacteria became dominant in this fermenter to the exclusion of other groups.

Bacterial concentrations from the stagnant fermenter increased initially during the 60 days after it was set up. The fermenter subsequently produced approximately 10^6 cfu per ml of heterotrophic bacteria incubated under aerobic and anaerobic

conditions. Similar concentrations of SRB were produced. Concentrations of viable bacteria produced by this fermenter remained stable and increased the proportion of SRB added to the flow loop.

4.3 EXAMINATION OF COUPONS IMMERSSED IN THE RESERVOIR OF THE FLOW LOOP USING SCANNING ELECTRON MICROSCOPE

Coupons were prepared for examination with SEM after 45, 85, 128, 180 and 218 days immersion in the reservoir of the flow loop. Some typical electron micrographs of freely corroding and of cathodically protected coupons are presented (FIGURES 4.3.A - 4.3.J). All of the electron micrographs of coupons removed after 180 days immersion (in a study set up by S. Funge-Smith) were taken using too high an acceleration voltage (about 30 kV). This resulted in a loss of surface detail and gave images of too high contrast.

Scanning electron microscopy revealed the surface of coupons to be covered in an extensive network of bacterial mucilage. This 'glycocalyx' was present on all coupons. Its abundance did not seem to be affected by the application of different levels of cathodic protection, or by the period of immersion. Fixation by freeze-drying preserved the bacterial mucilage, showing that it extended over almost the entire surface of the coupons.

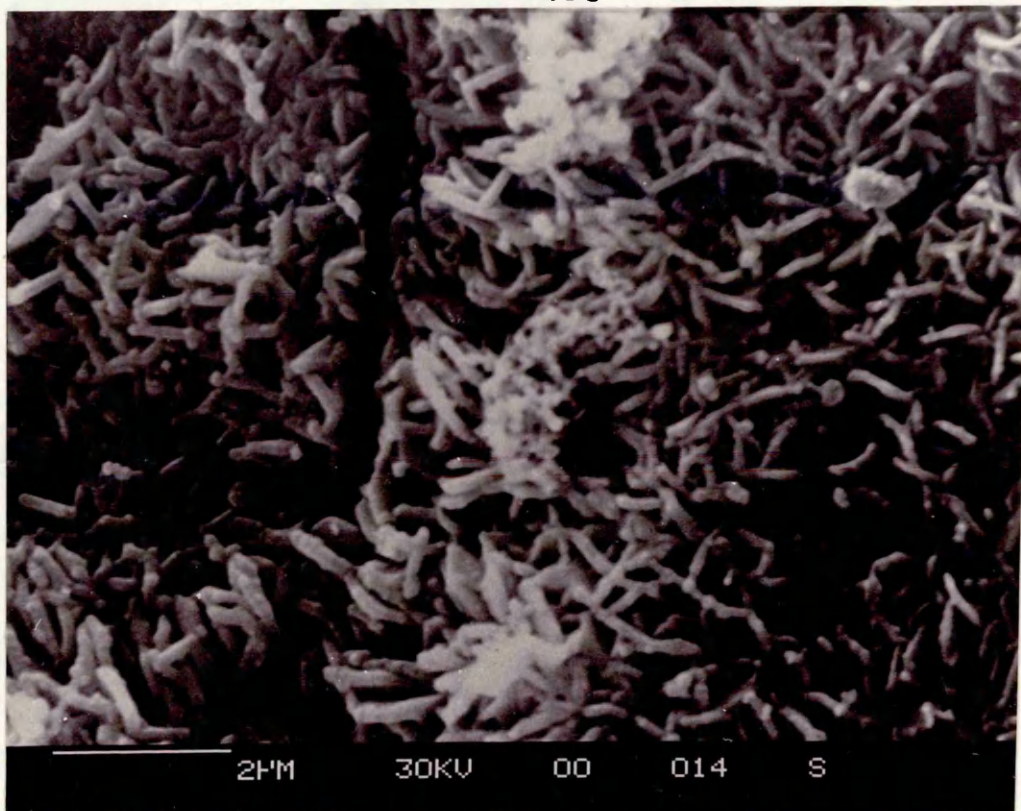


FIGURE 4.3.A

6 months immersion, freely corroding, critical point dried & gold coated.

Mass of small rod bacteria on a section of corrosion product.

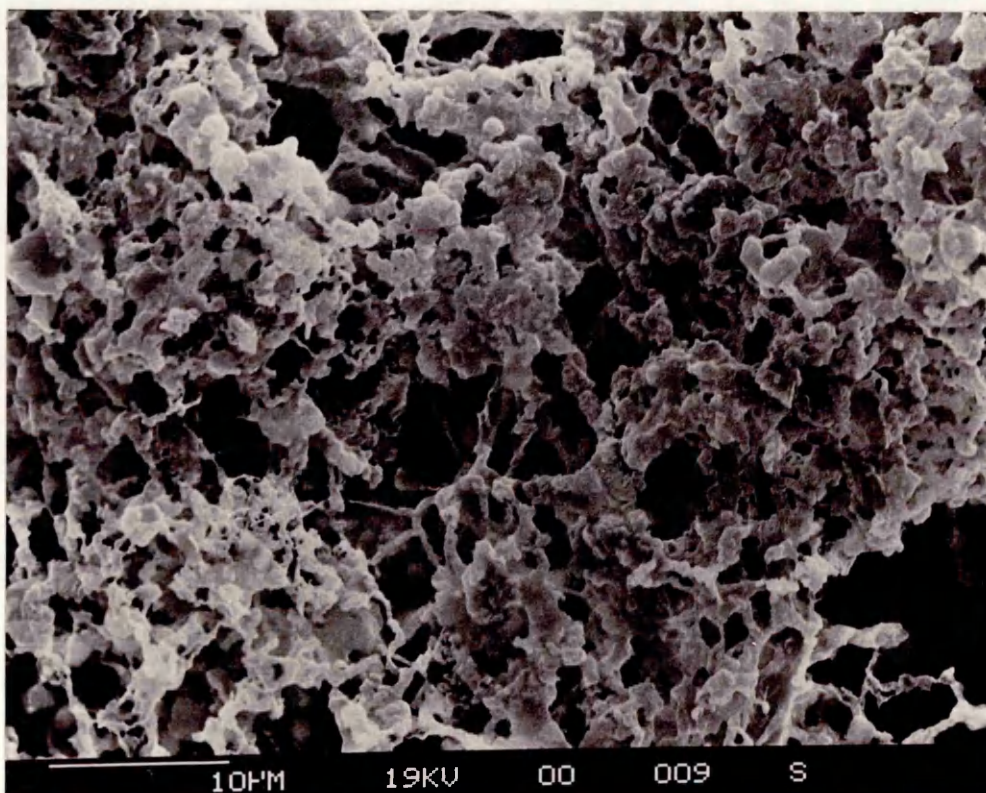


FIGURE 4.3.B

6 months, cathodically protected at -850 mV S.C.E., freeze-dried & gold coated.

Network of mucilage that covers bacterial cells on the surface of calcareous scale.

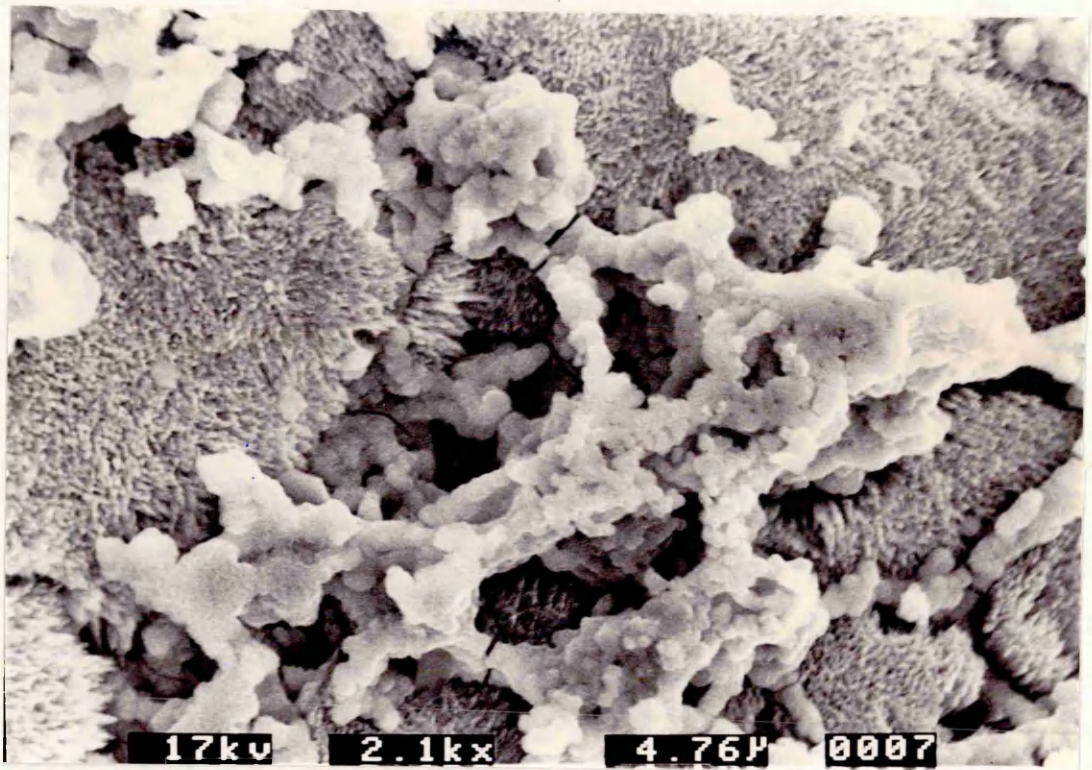


FIGURE 4.3.C

45 days immersion, freely corroding, freeze-dried, carbon coated.

Bacterial cells on corrosion product enrobed in a sheath of extracellular mucilage.

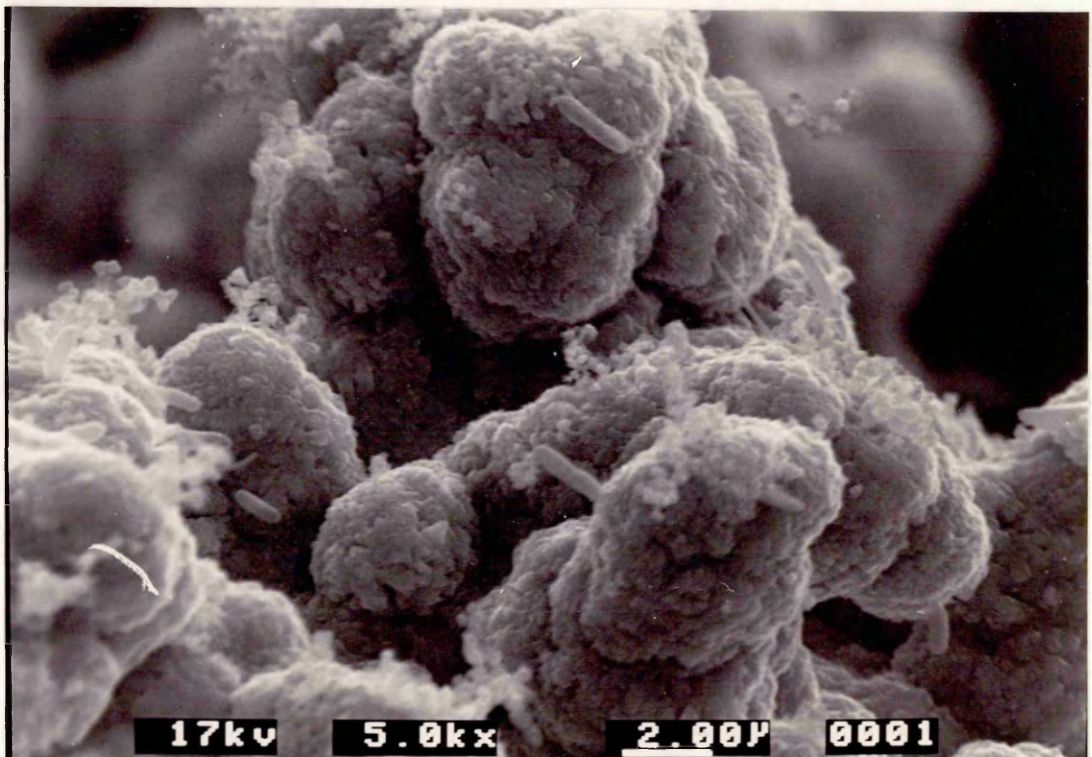


FIGURE 4.3.D

128 days immersion, freely corroding, critical point dried, carbon coated.

Rod bacteria adhering to corrosion product within a section about 150 µm from the surface.

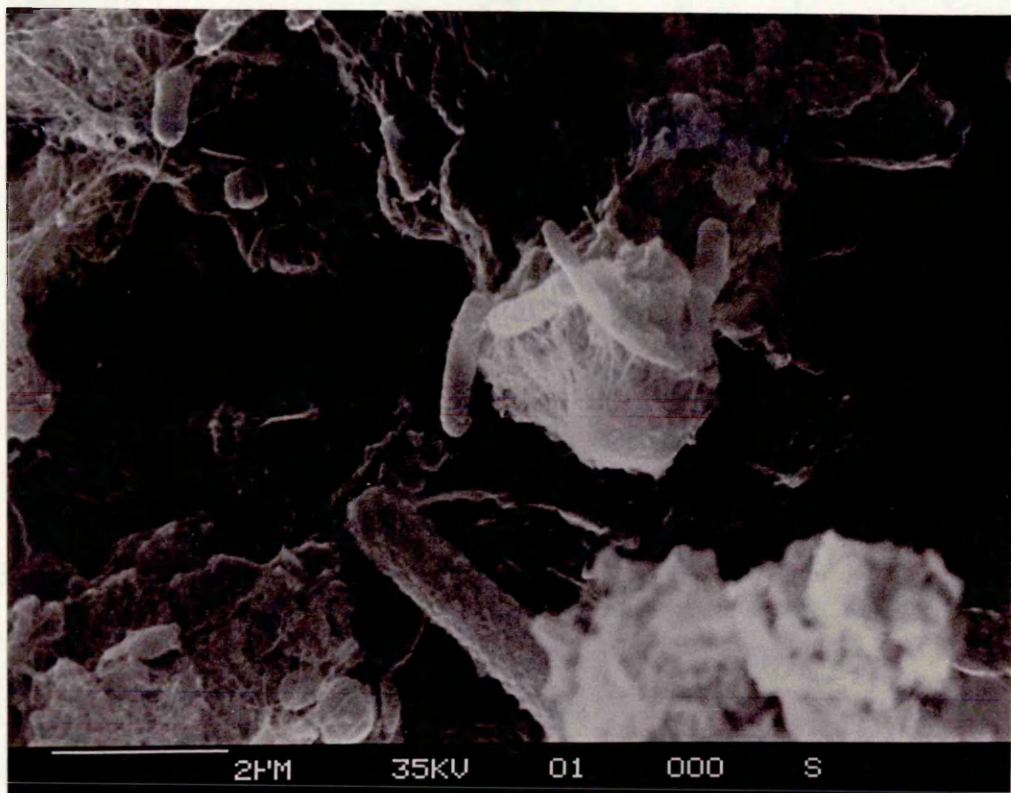


FIGURE 4.3.E

6 months immersion, freely corroding, critical point dried & gold coated.

Rod and cocci bacteria on sectioned corrosion product about 50 µm from the outer surface. Some fibrillar-like material is present on the right-hand side of the picture. This is a typical feature of bacterial mucilage following dehydration during fixation.

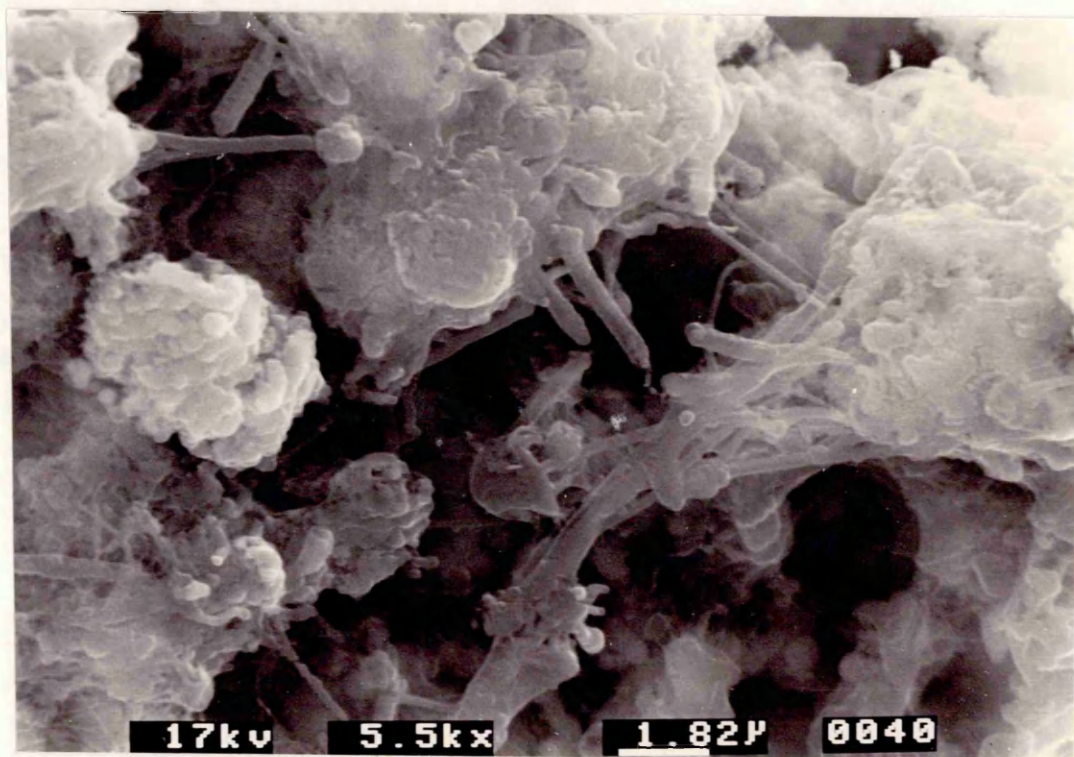


FIGURE 4.3.F

85 days immersion, freely corroding, critical point dried, carbon coated.

Rod bacteria emeshed in dehydrated mucilage. The porous nature of the corrosion product is clearly visible.

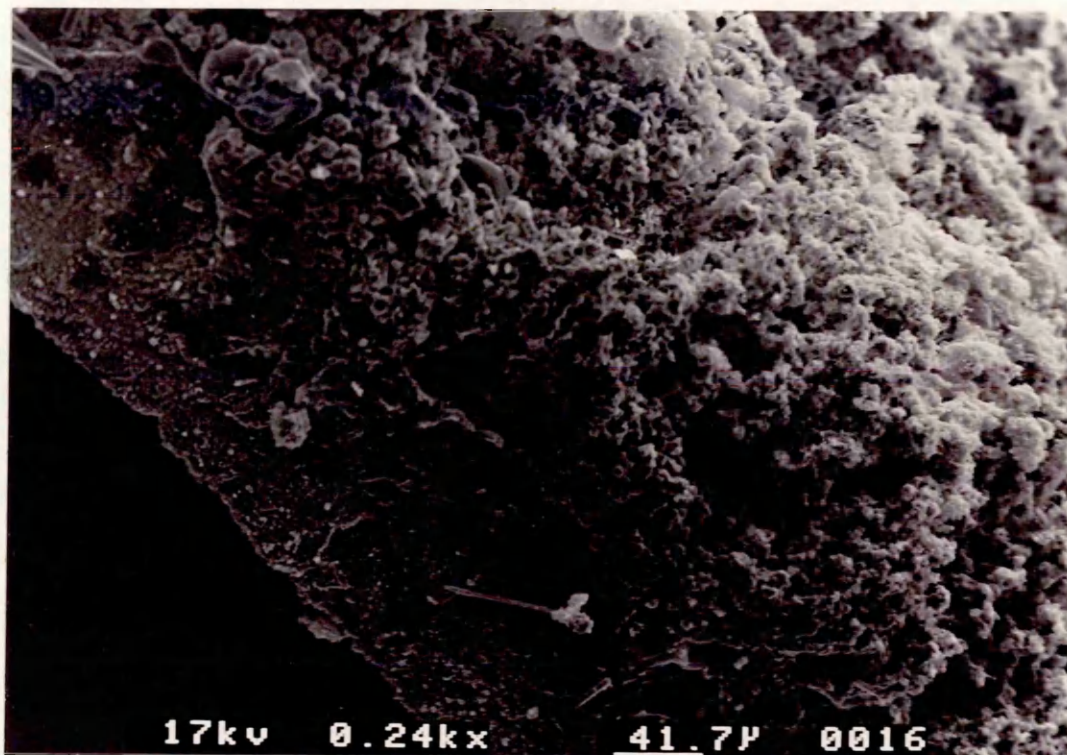


FIGURE 4.3.G

85 days immersion, freely corroding, critical point dried, carbon coated.

Section through corrosion product showing the porous, open structure of the outermost layer and the dense corrosion product adjacent to the metal surface.

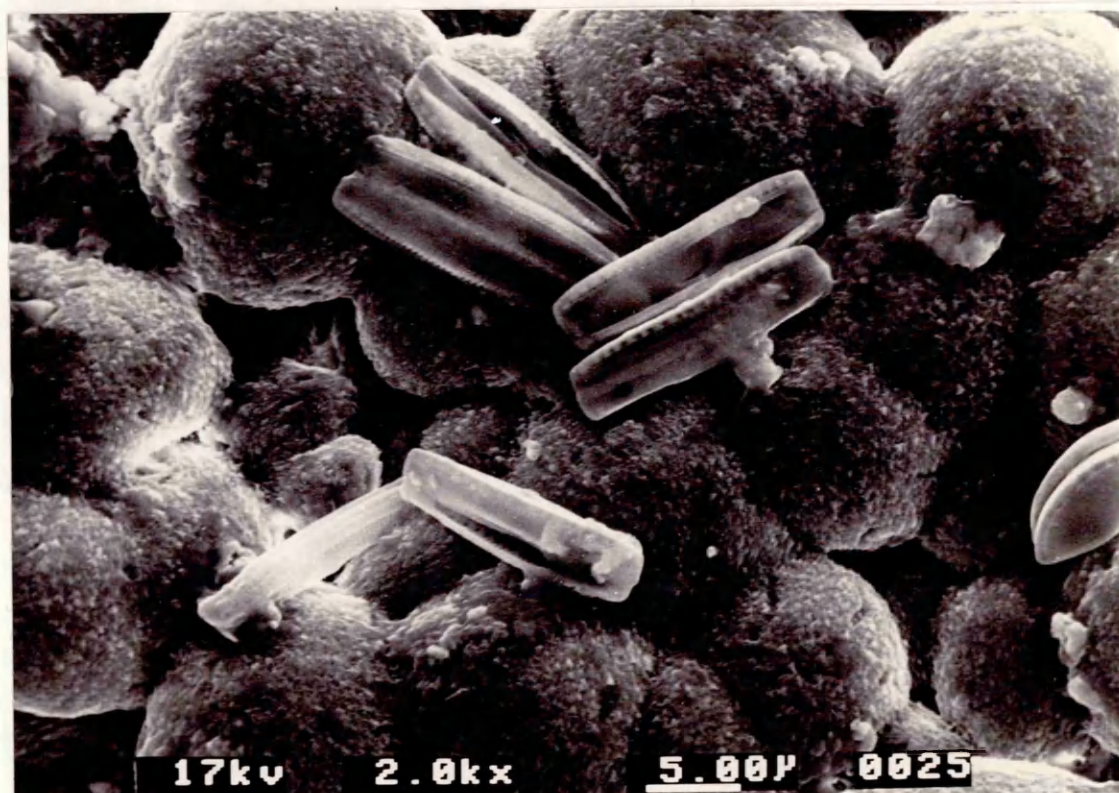


FIGURE 4.3.H

45 days immersion, cathodically protected at -950 mV S.C.E., freeze-dried, carbon coated.

Diatoms on the surface of calcareous scale. Each cell is enclosed by 2 silica valves, forming a frustule. Some of the diatom use mucilage to adhere to the substratum.

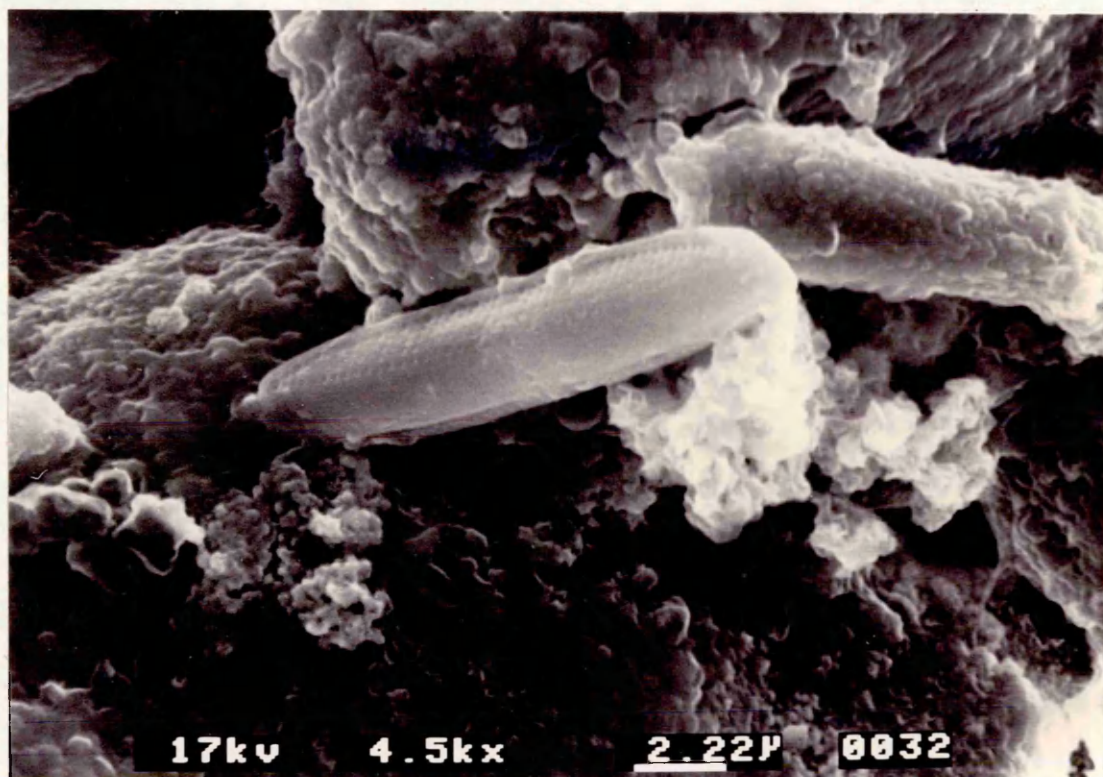


FIGURE 4.3.I

45 days immersion, freely corroding, freeze-dried, carbon coated.

Two diatoms on the surface of calcareous scale. Their extracellular mucilage has been preserved by the freeze-drying.

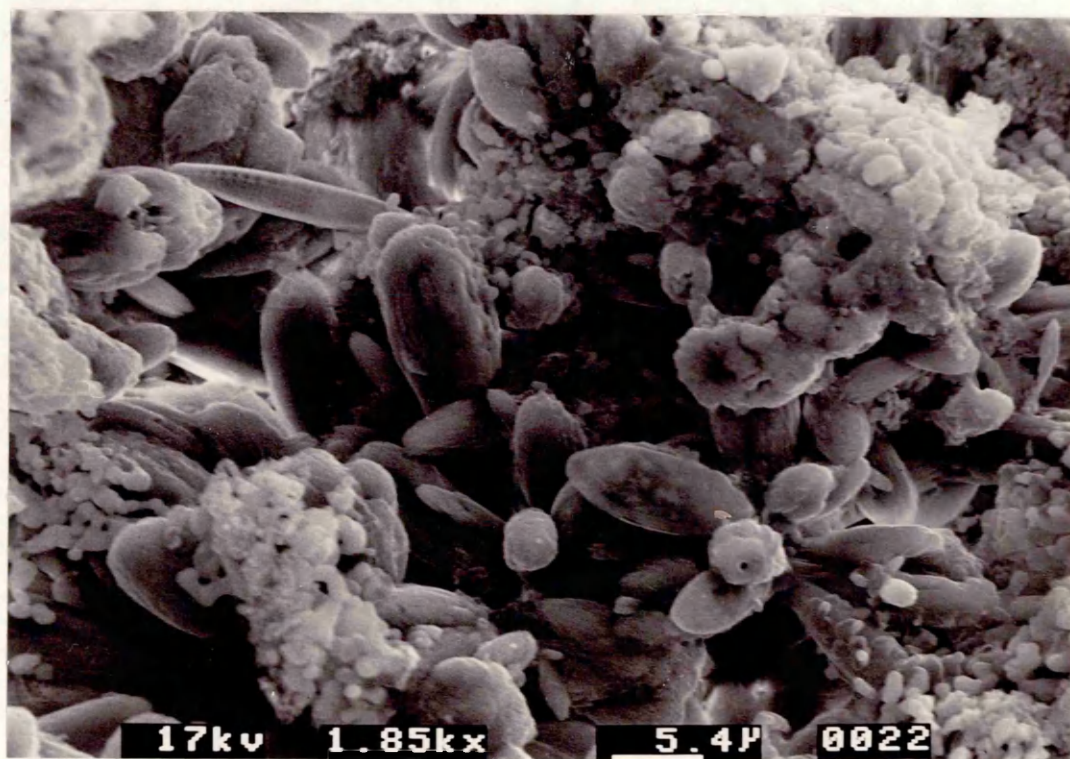


FIGURE 4.3.J

128 days immersion, freely corroding, freeze-dried, carbon coated.

Diatoms covering the surface of corrosion product. In some areas in which diatom growth was particularly dense they were arranged in concentric circles, giving a 'rosette-like' appearance.

Critical point drying (CPD) dehydrated the mucilage into fibrillar-like material revealing the bacteria beneath. Most of the surface bacteria were rod shaped, generally up to about 3 μm long. Coccoid bacteria were observed less frequently but were often quite abundant locally.

Diatoms were also observed on some coupons regardless of the length of immersion or application of cathodic protection. Diatoms were present as individuals or as occasional groups. Often the cells were arranged in concentric circles, resulting in a rosette-like appearance. Fixation by CPD removed the sheath of mucilage in which the diatoms were also enveloped and revealed the minute pores in the sculptured frustule. The diatoms were oval shaped or elongated, rather like a razor shell, and were 6 to 12 μm long. The oval diatoms may have been Cocconeis sp., a non-filamentous and non colonial diatom.

This study showed that the surface of corrosion product on freely corroding coupons consisted of rounded protrusions that were generally covered in minute, pointed crystals, or occasionally flattened, angular ones. EDAX analysis showed that the surface corrosion product was rich in iron but also contained calcium, chlorine, phosphorus, silicon and magnesium. Examination of sections of corrosion product removed after 45 days onwards showed that it was composed of two distinct layers. The

outermost, thicker layer had a porous, sponge-like appearance. The thinner layer adjacent to the steel surface was very dense, with infrequent pores and seemed to be composed of elongated crystals. Bacteria were sometimes found within the porous outer layer of corrosion product but they were not very numerous and were difficult to locate. The corrosion product could also be distinguished as two distinct layers without the use of the electron microscope. An outer, thicker layer of reddish brown corrosion product was fairly loose and could be easily removed from the coupon. Adjacent to the metal surface was a thin, dense black layer which adhered very firmly to the metal surface and was sometimes difficult to dislodge, even with a scalpel.

The surface of coupons protected at -850 mV and -950 mV S.C.E. were covered in a scale, the surface of which was composed of many rounded lobes. EDAX analysis revealed calcium to be the dominant element in the scale with some magnesium also present.

4.4 VIABLE COUNTS OF BACTERIA FROM COUPONS IMMERSSED IN THE RESERVOIR OF THE FLOW LOOP

Results of viable counts of bacteria on freely corroding coupons and on ones cathodically protected at -850 and -950 mV S.C.E. have been plotted as histograms (FIGURES 4.4.A - 4.4.F). Heterotrophs were enumerated using spread plates incubated under aerobic and anaerobic conditions, SRB were enumerated in liquid culture. Viable counts were done after 28, 44, 85, 117, 168, 211 and 270 days. Viable counts done until day 117 used only one or two replicates of each treatment, subsequently three were used. Prolonged periods of sonication were used to disrupt the biofilm in counts until day 168. This treatment killed the SRB and may also have depressed numbers of viable heterotrophs. Bacteria enumerated on coupons cathodically protected at -950 mV S.C.E. after 28 days immersion were not sonicated for a long period of time. These factors make the results gained difficult to interpret.

FIGURE 4.4.A

Histogram Showing Viable Counts Of Heterotrophs Incubated Under Aerobic Conditions From Freely Corroding Coupons Immersed In The Reservoir Of The Flow Loop

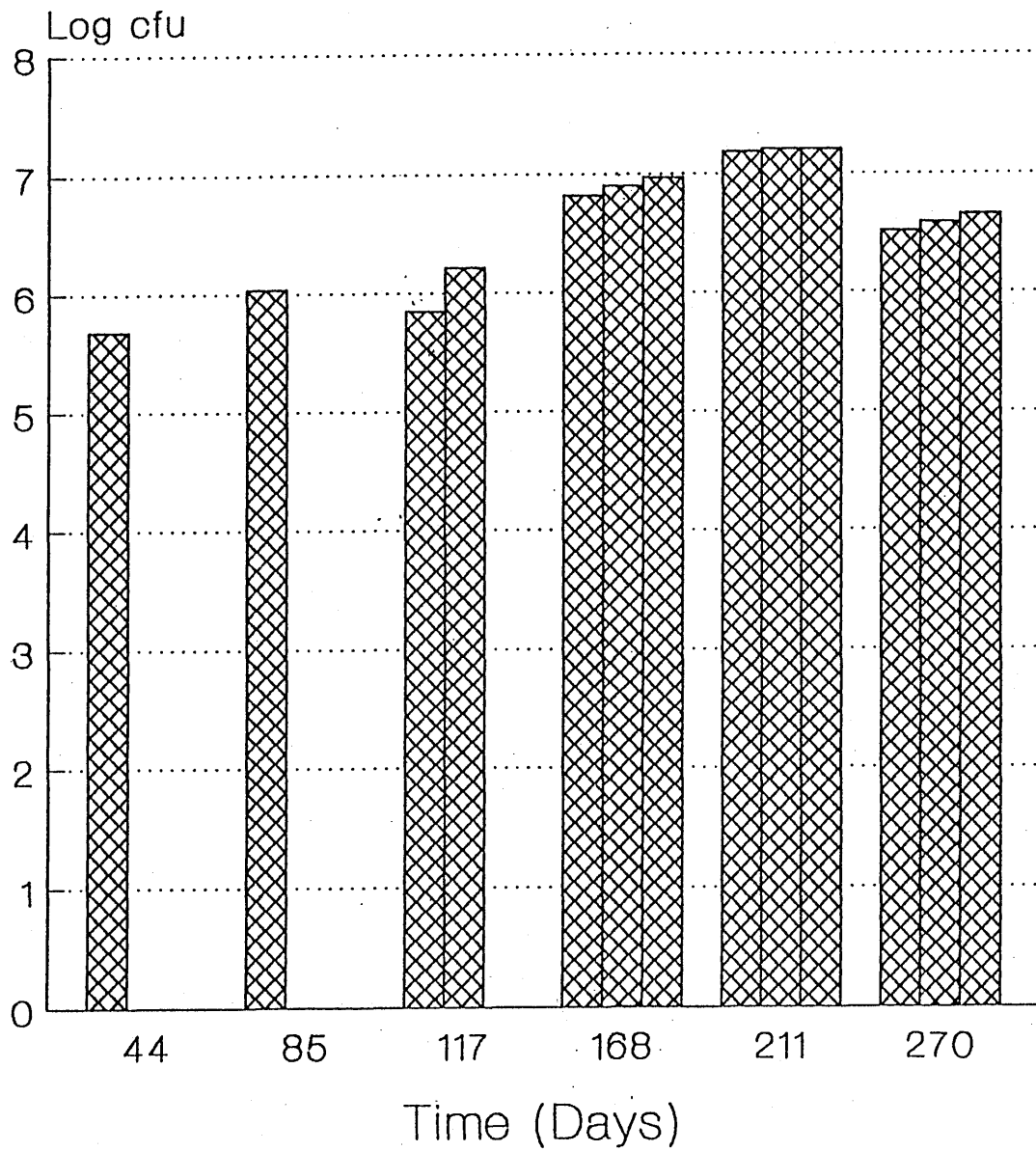


FIGURE 4.4.B

Histogram Showing Viable Counts Of Heterotrophs Incubated Under Anaerobic Conditions From Freely Corroding Coupons Immersed In The Reservoir Of The Flow Loop

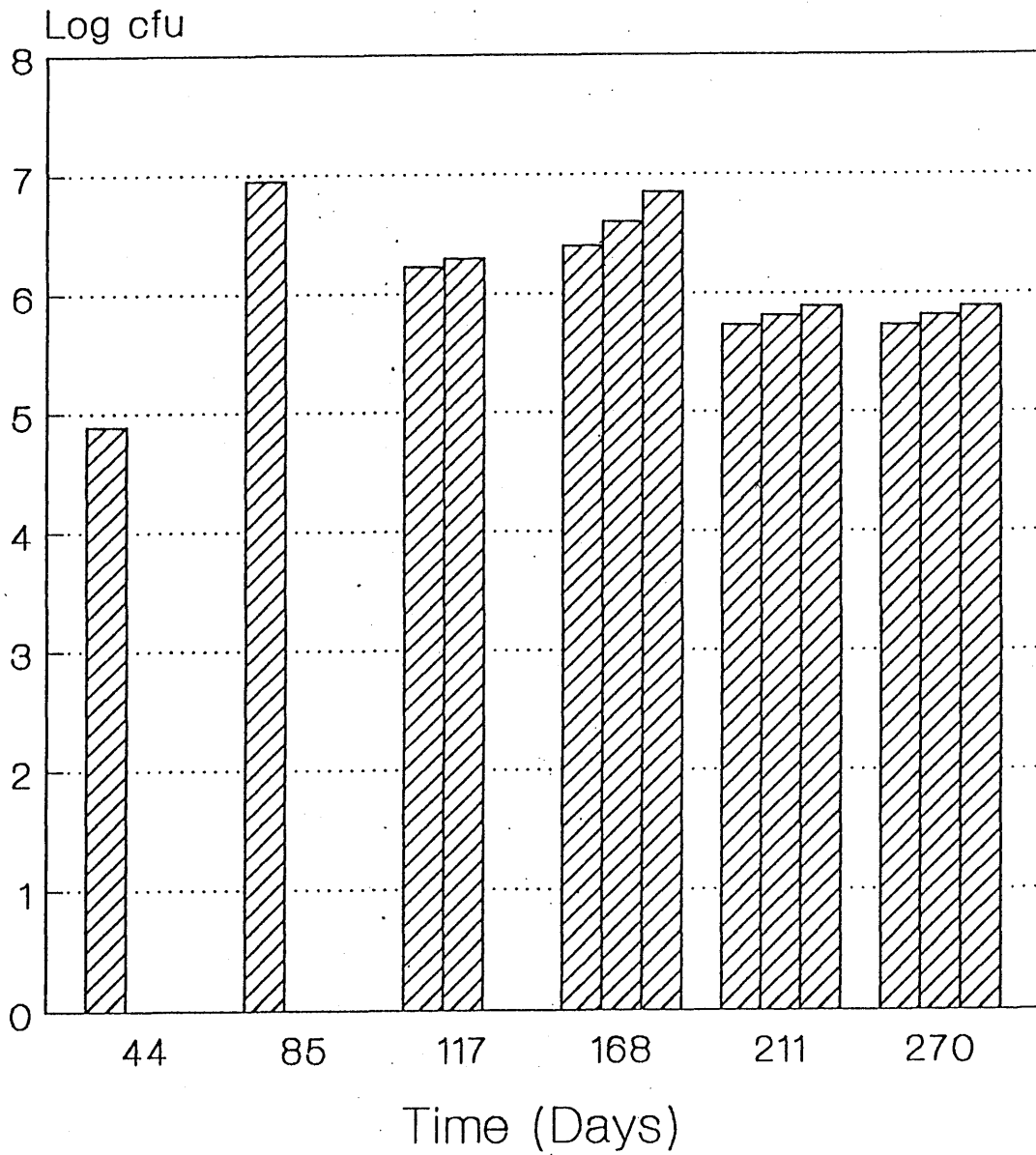


FIGURE 4.4.C

Histogram Showing Viable Counts Of Heterotrophs Incubated Under Aerobic Conditions From Coupons Cathodically Protected At -850 mV S.C.E. Immersed In The Reservoir Of The Flow Loop

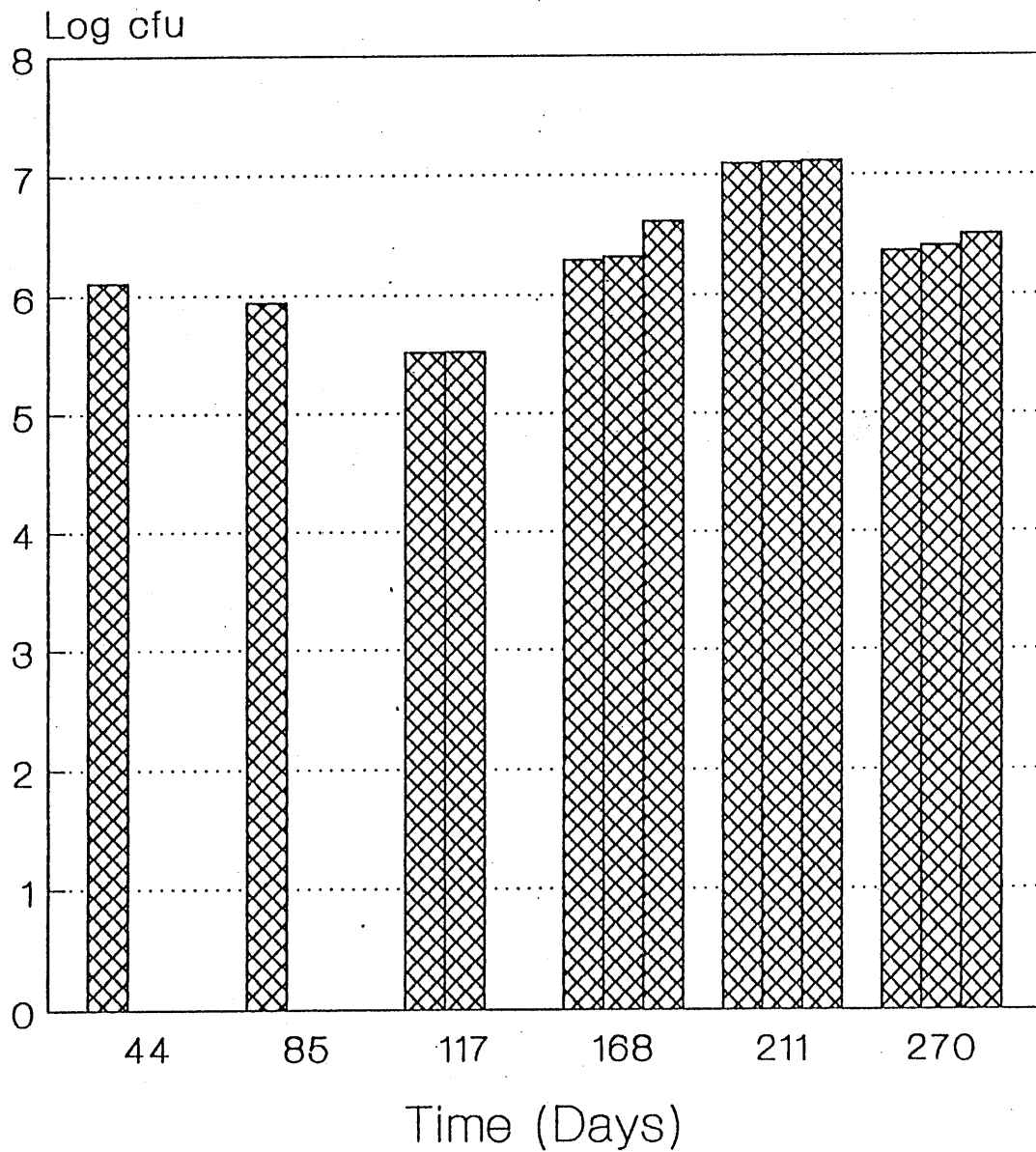


FIGURE 4.4.D

Histogram Showing Viable Counts Of Heterotrophs Incubated Under Anaerobic Conditions From Coupons Cathodically Protected At -850 mV S.C.E. Immersed In The Reservoir Of The Flow Loop

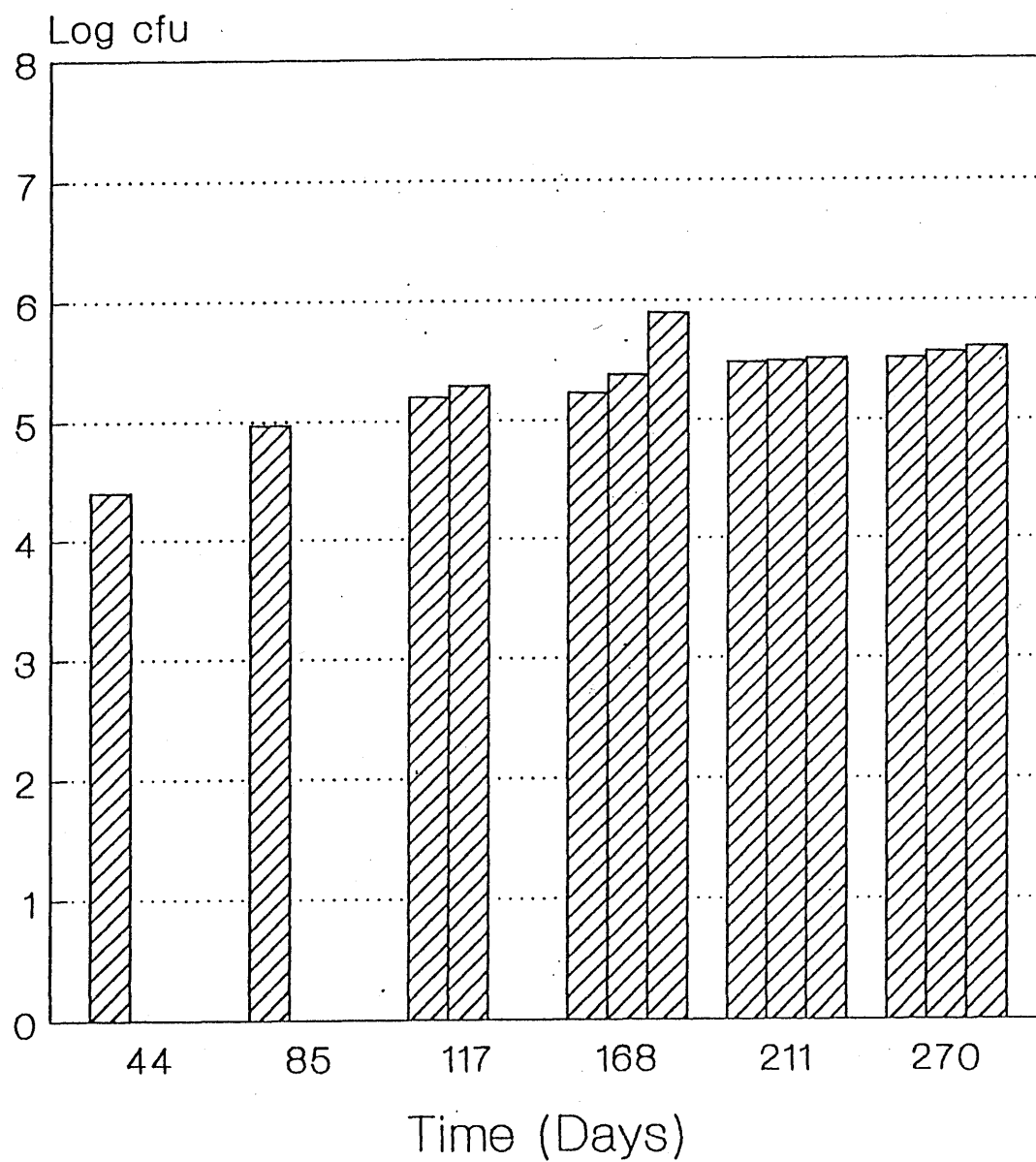


FIGURE 4.4.E

Histogram Showing Viable Counts Of Heterotrophs Incubated Under Aerobic Conditions From Coupons Cathodically Protected At -950 mV S.C.E. Immersed In The Reservoir Of The Flow Loop

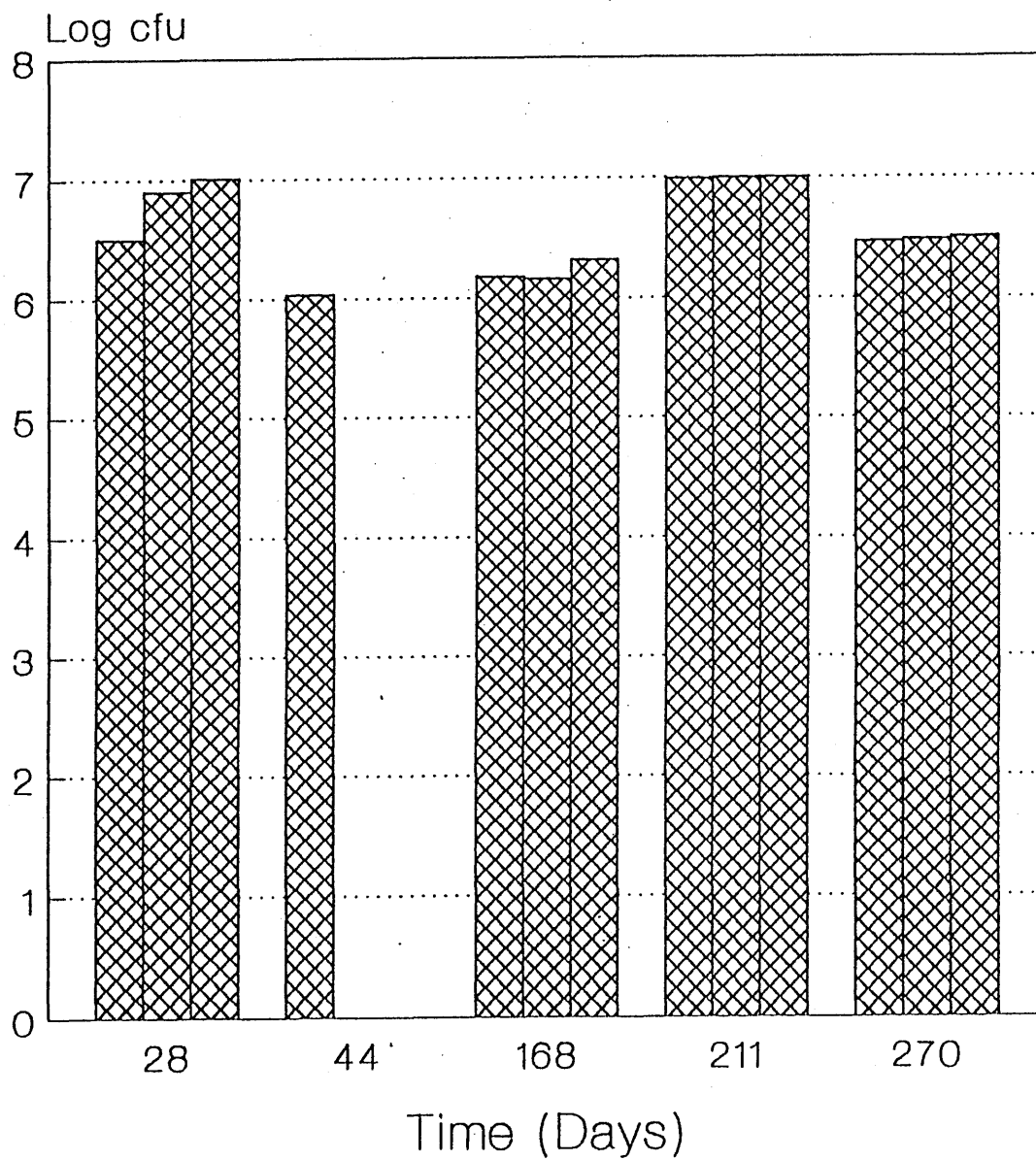
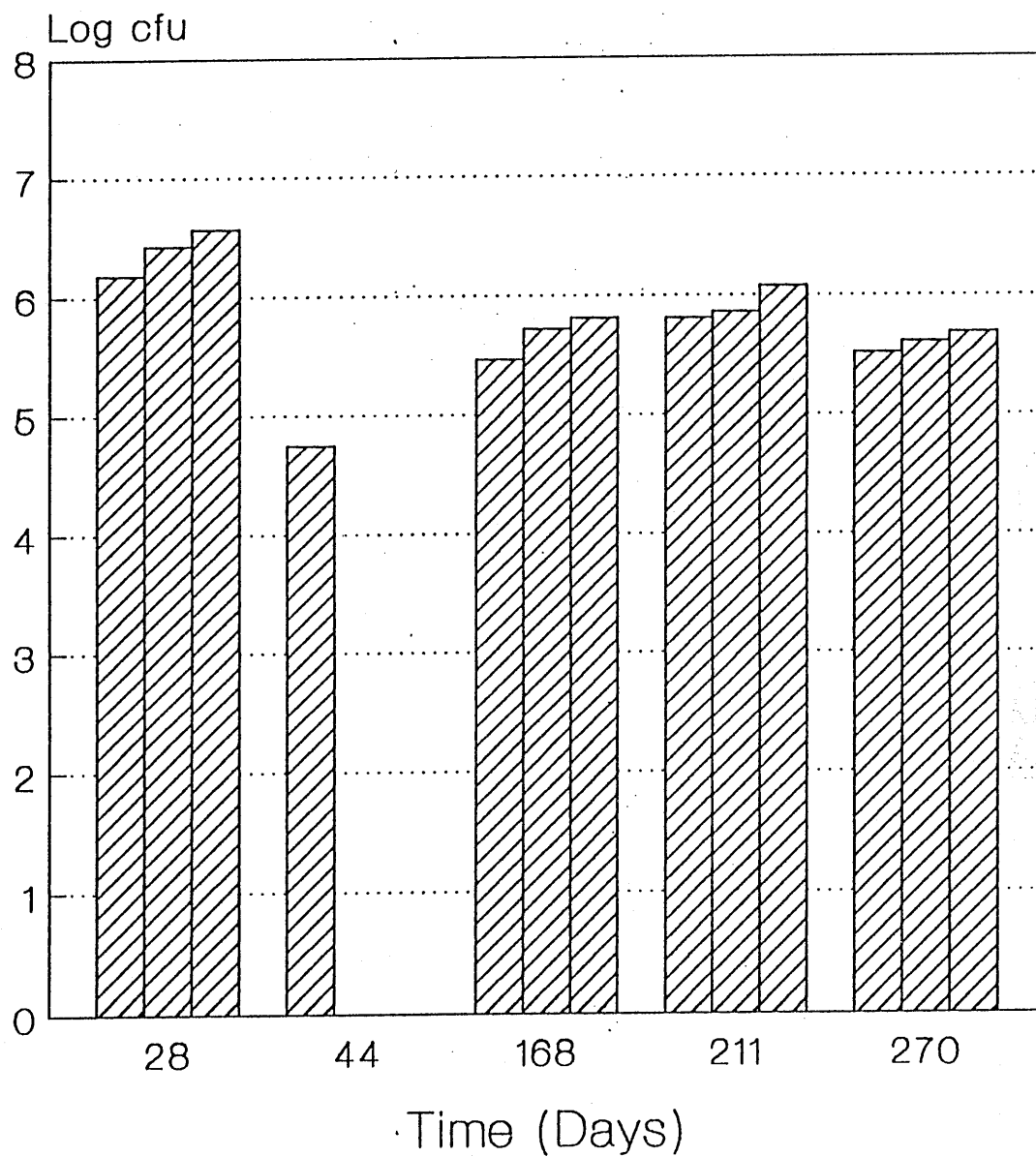


FIGURE 4.4.F

Histogram Showing Viable Counts Of Heterotrophs Incubated Under Anaerobic Conditions From Coupons Cathodically Protected At -950 mV S.C.E. Immersed In The Reservoir Of The Flow Loop

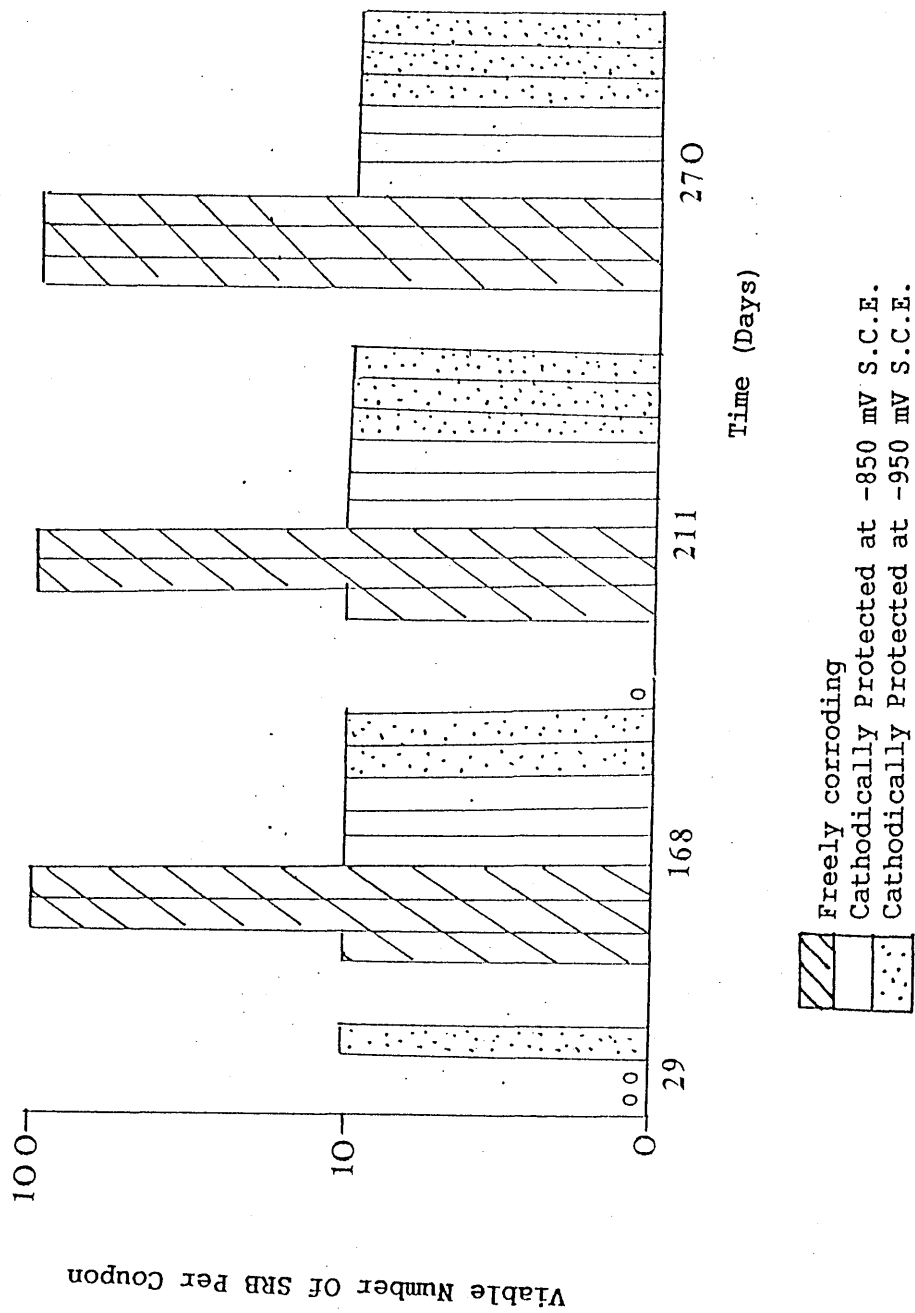


Counts of viable heterotrophs when enumerated either under aerobic or anaerobic conditions were quite similar, generally differing from each other by less than an order of magnitude. Trends in numbers of heterotrophs during the course of the 270 day study were not observed. The condition of the steel coupon, whether it was corroding or cathodically protected did not seem to affect numbers of viable bacteria on it.

Viable counts for SRB after 211 days (FIGURE 4.4.G) which did not use prolonged sonication have shown trends. Numbers of SRB were greater on freely corroding coupons than on cathodically protected ones. The degree of cathodic protection employed did not affect numbers of viable SRB.

FIGURE 4.4.G

Histogram Showing Viable Counts Of SRB From Coupons
Immersed In The Reservoir Of The Flow Loop



4.5 RADIO-RESPIROMETRIC ASSAYS TO MEASURE GLUCOSE TURNOVER AND SULPHATE REDUCTION IN THE BIOFILM OF COUPONS IMMERSSED IN THE RESERVOIR OF THE FLOW LOOP

Results of radio-respirometric assays to measure glucose turnover and sulphate reduction activity have been plotted as histograms (FIGURES 4.5.A, 4.5.B). Early results were rather patchy as there were problems in getting the radioassay running smoothly. Initially only two replicates of each treatment were used. This number was later increased to five to try to overcome the large variability in results and problems with results spoiled by contaminated wicks.

FIGURE 4.5.A

Histogram Showing Rates Of Glucose Turnover By Biofilms
On Coupons Immersed In The Reservoir Of The Flow Loop

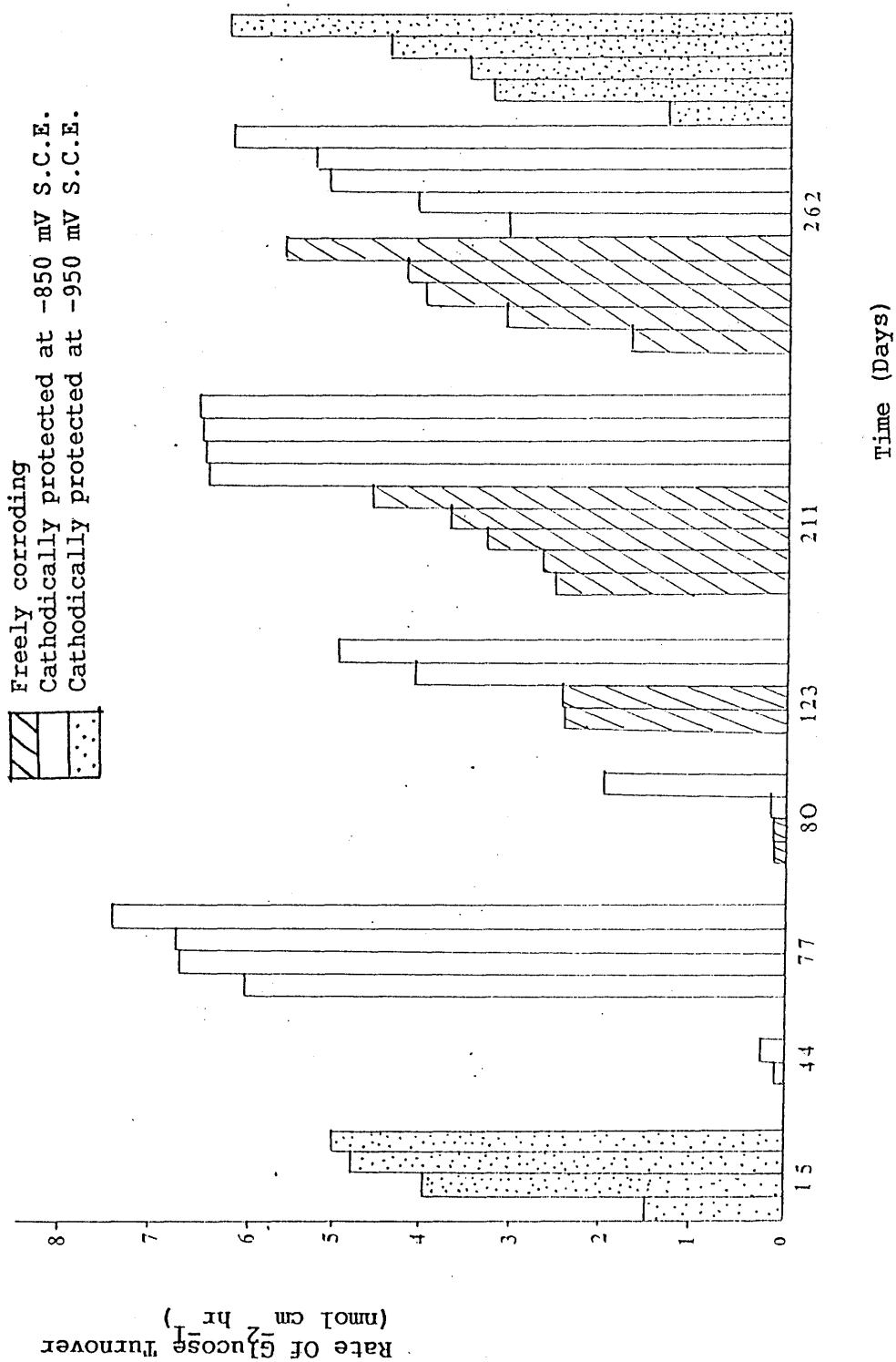
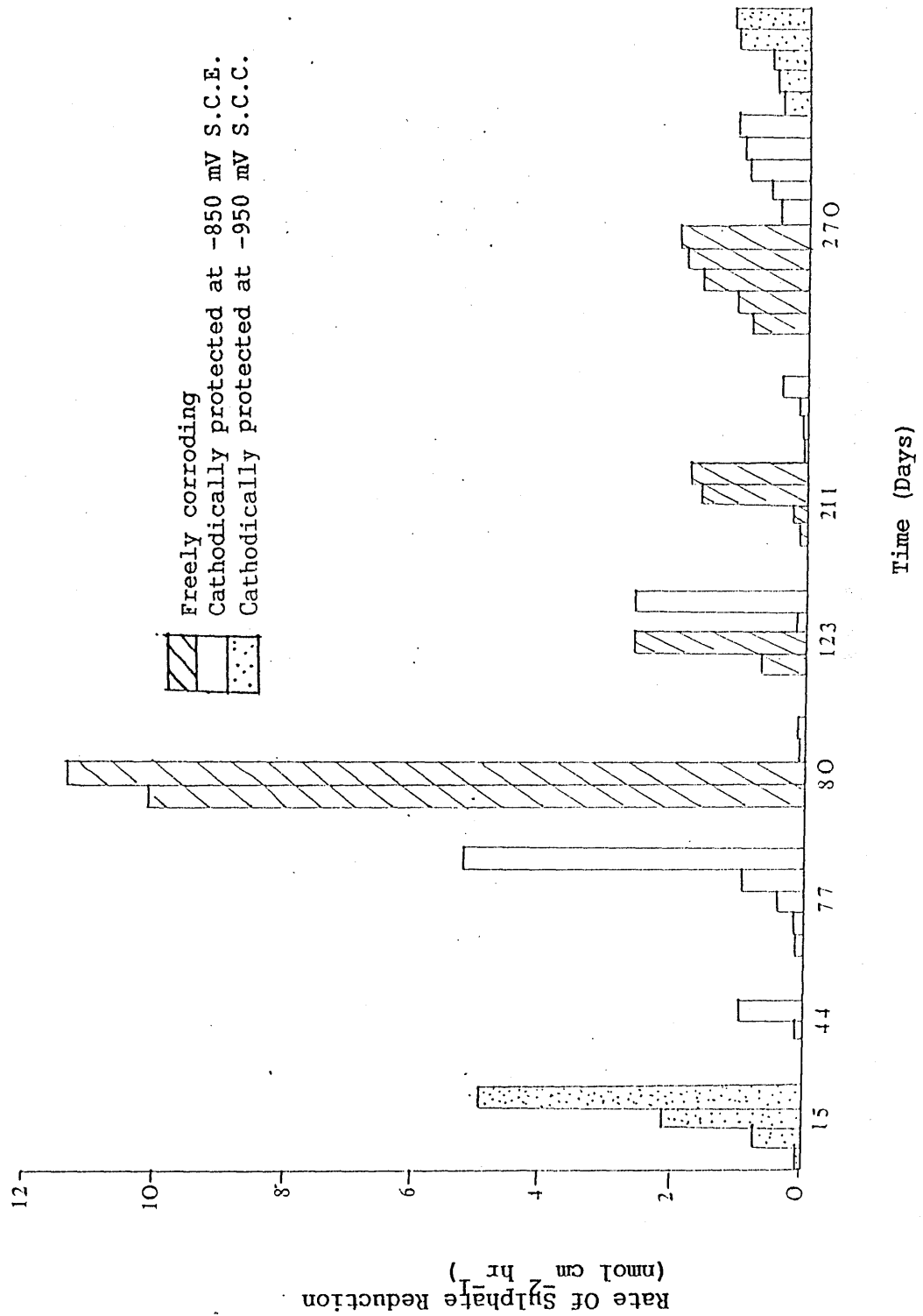


FIGURE 4.5.B

Histogram Showing Rates Of Sulphate Reduction By Biofilms
On Coupons Immersed In The Reservoir Of The Flow Loop



Early radio-respirometric assay results indicate that rates of glucose turnover were generally higher on cathodically protected coupons than on freely corroding ones. The radioassay of day 262 does not show any significant differences between glucose turnover rates on freely corroding or cathodically protected coupons. The level of cathodic protection used did not appear (results day 262) to alter rates of glucose turnover measured. The limited results gained do not show if rates of glucose turnover increase or decrease during the study.

The radio-respirometric assay to measure sulphate reduction initially gave very variable results. Some early results (days 80 and 123) and later ones (day 262) indicate that sulphate reduction rates may have been higher on freely corroding coupons than on cathodically protected ones. The rates of sulphate reduction seemed to be unaffected by the level of cathodic protection applied (results day 270). Trends in rates of sulphate reduction with time were not established due to the limited results.

4.6 CORROSION MEASUREMENTS USING THE CORROSION PROBE IN THE FLOW LOOP

4.6.1 LPR Measurements

LPR scans were taken twice a week on the same five coupons for 265 days after the probe was constructed. Graphs of mean corrosion current (FIGURE 4.6.1.A), mean rest potential (FIGURE 4.6.1.B) and mean polarisation resistance (FIGURE 4.6.1.C) were plotted, each value is the mean $\pm$ S.D. of five determinations.

During the first 20 days the mean corrosion current fell rapidly from 99 uA cm^{-2} (1.15 mm yr^{-1}) to 25 uA cm^{-2} (0.29 mm yr^{-1}). The mean corrosion current stabilised at $20\text{--}30 \text{ uA cm}^{-2}$ ($0.23\text{--}0.35 \text{ mm yr}^{-1}$) until about day 120 when it increased steadily. This increase, if significant, may have been due to SRB activity as the biofilm matured.

FIGURE 4.6.1.A

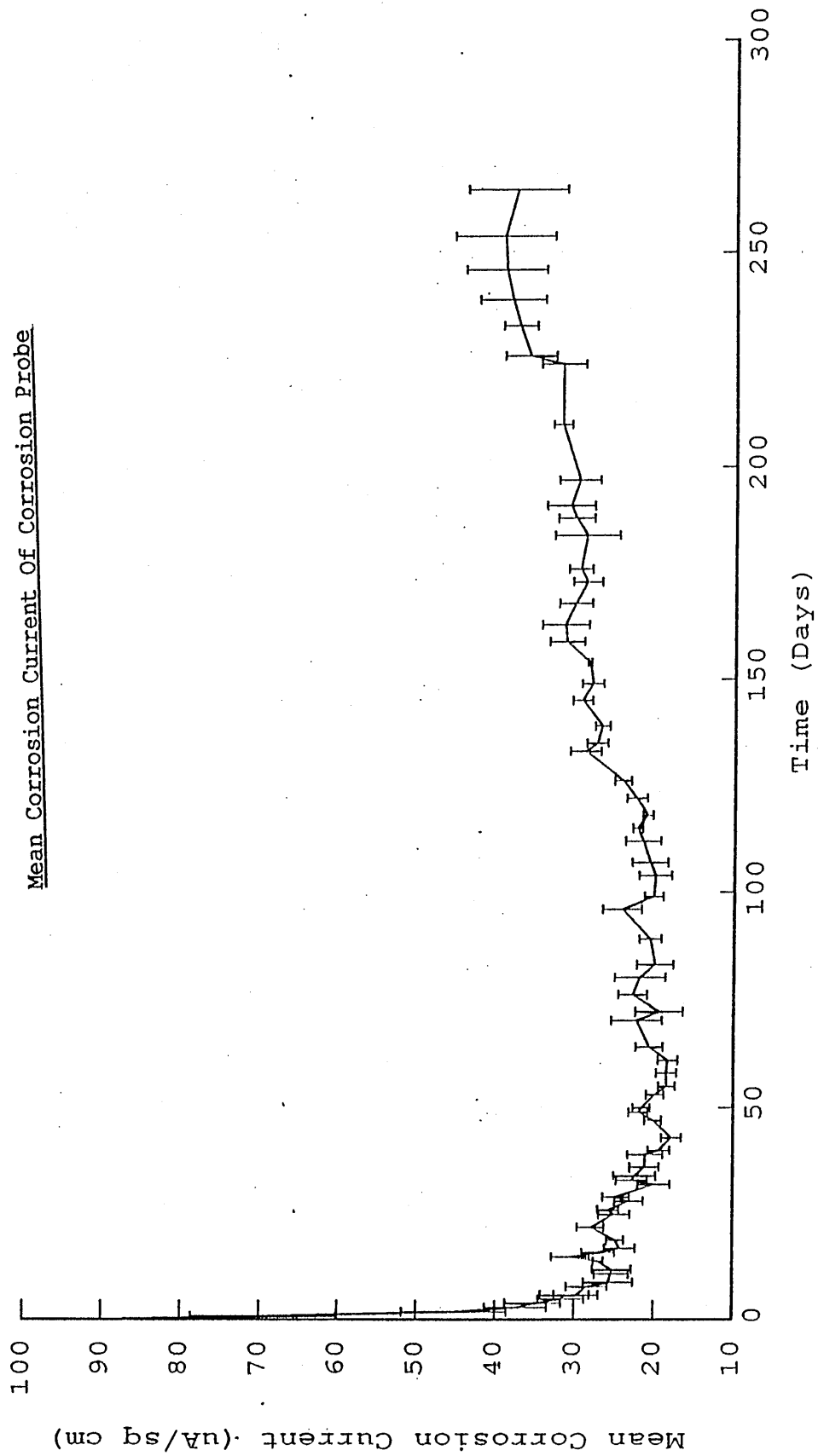
Mean Corrosion Current Of Corrosion Probe

FIGURE 4.6.1.B

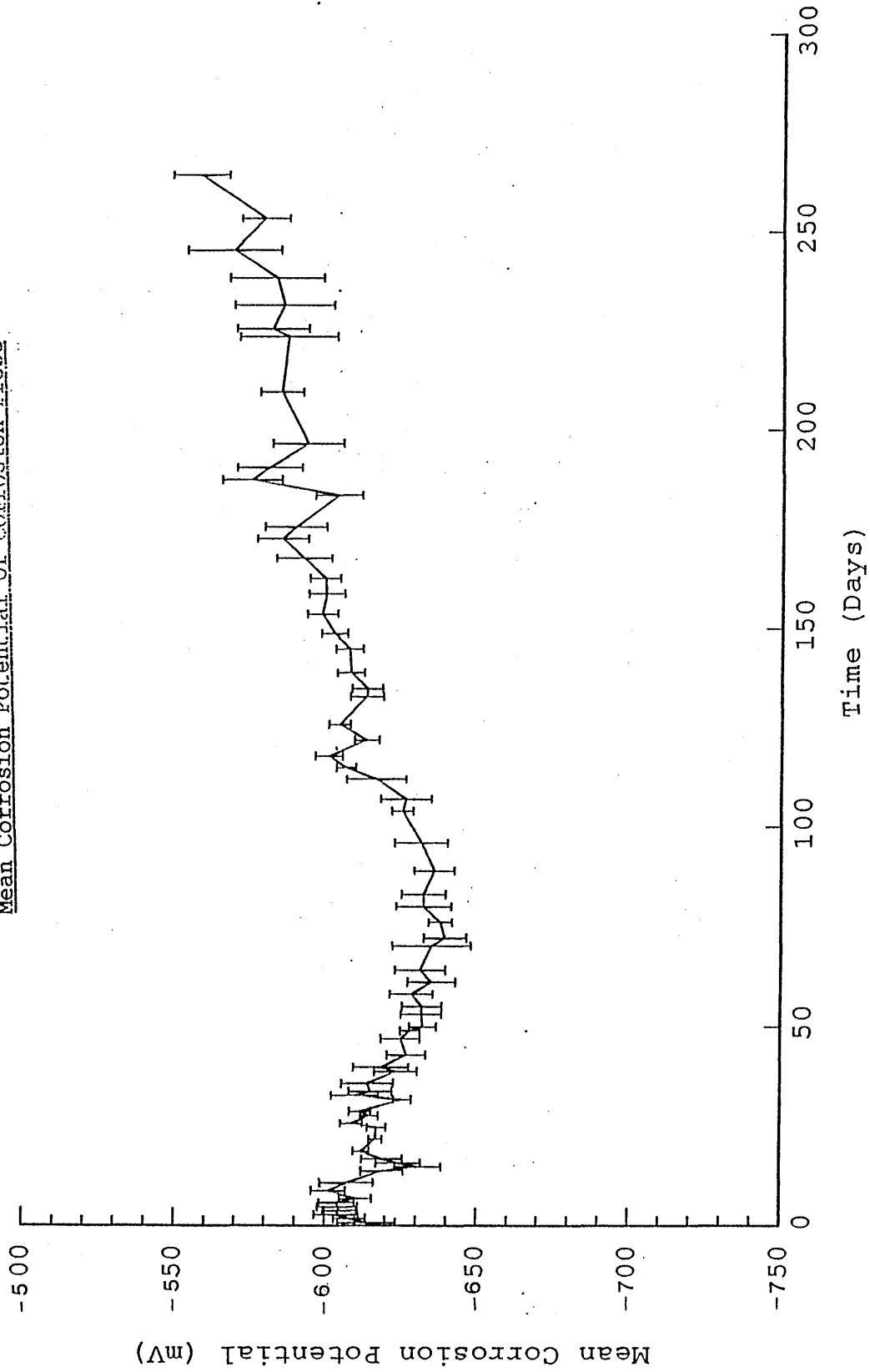
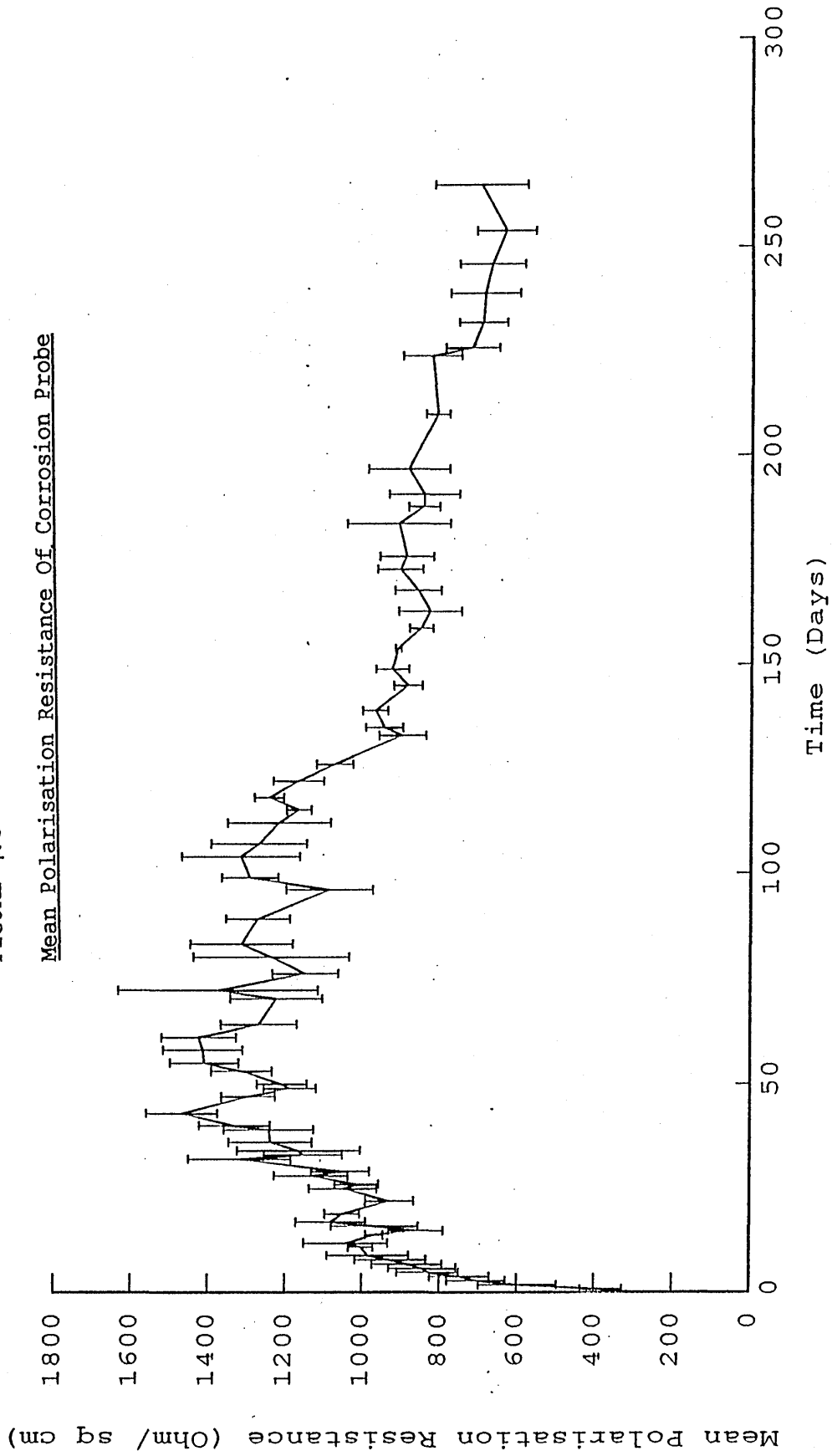
Mean Corrosion Potential Of Corrosion Probe

FIGURE 4.6.1.C



The mean corrosion potential increased during the first 20 days as the corrosion current rapidly declined. From days 20 to about 120 it slowly fell, then stabilised at about -640 mV S.C.E.. A shift in the mean corrosion potential (as occurred during the first 20 days) resulted from polarisation of the anodic or cathodic reactions. From day 120 onwards the corrosion potential did not stabilise but increased steadily. This may have correlated with the increase in the mean corrosion current.

The polarisation resistance is inversely related to the corrosion current. It increased during the first 50 days as the corrosion current fell. It then remained fairly stable until about day 120 when it steadily declined as the corrosion current increased.

4.6.2 Weight Loss Data

After 15, 33, 90, 227 and 264 days triplicate weight loss coupons were removed from the corrosion probe and mean weight losses determined. These are tabulated below.

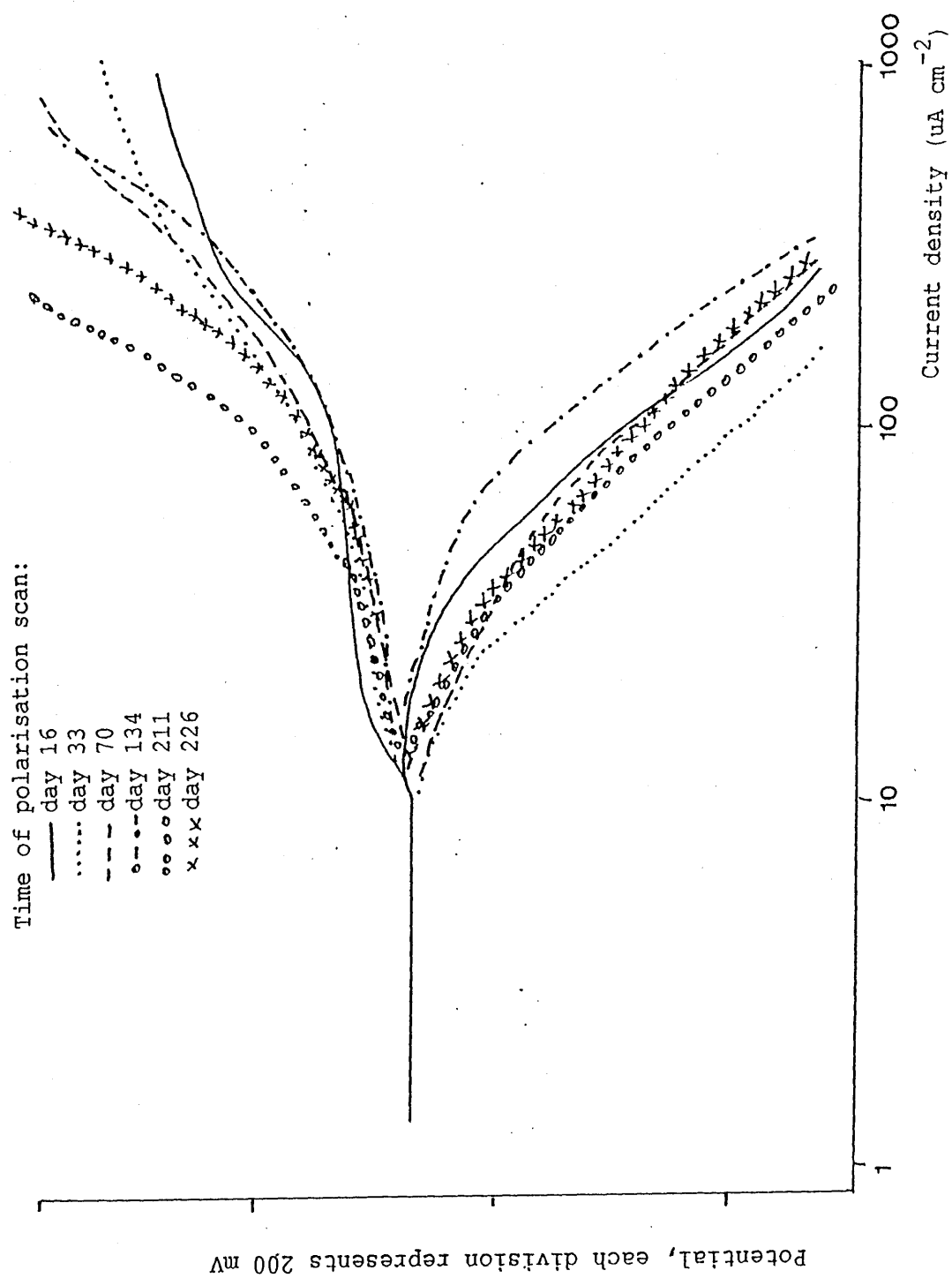
Table recording mean weight loss data from the corrosion probe:

Day coupons were removed	Mean weight loss (mg)
15	47.9 $\pm$ 4.80
33	73.5 $\pm$ 1.41
90	114.2 $\pm$ 0.99
227	289.8 $\pm$ 38.72
264	308.2 $\pm$ 49.92

4.6.3 Full Polarisation Scans Of The Corrosion Probe

Full polarisation scans were taken on different coupons after 16, 33, 70, 134, 211 and 226 days. The results of all these polarisation scans were plotted on the same axes (FIGURE 4.6.3) after the different corrosion potentials were superimposed onto the same point. These results (if significant as no replicate

FIGURE 4.6.3

Full Polarisation Scans Of Corrosion Probe

scans were done) show that the cathodic curves did not differ markedly. The anodic curves were progressively polarised.

4.7 CORROSION MEASUREMENTS USING COUPONS IMMERSSED IN THE RESERVOIR OF THE FLOW LOOP

4.7.1 LPR Measurements

LPR measurements were taken twice a week on the same five coupons for 270 days subsequent to their immersion. Graphs of mean corrosion current (FIGURE 4.7.1.A), mean rest potential (FIGURE 4.7.1.B) and of mean polarisation resistance (FIGURE 4.7.1.C) were plotted, each value is the mean $\pm$ S.D. of five determinations.

During the first ten days the mean corrosion current declined very rapidly from 44 uA cm^{-2} (0.51 mm yr^{-1}) to 22 uA cm^{-2} (0.26 mm^{-1}). It continued to steadily decline until about day 40 when it stabilised at about $15\text{-}20 \text{ uA cm}^{-2}$ ($0.17\text{-}0.23 \text{ mm yr}^{-1}$) until about day 100. From about day 100-180 the range of corrosion rates between the five coupons widened, some corroded twice as fast as others. After this period corrosion rates decreased slightly to the level observed during days 40-100. Corrosion rates of coupons in the reservoir were generally lower than those of coupons in the probe.

FIGURE 4.7.1.A

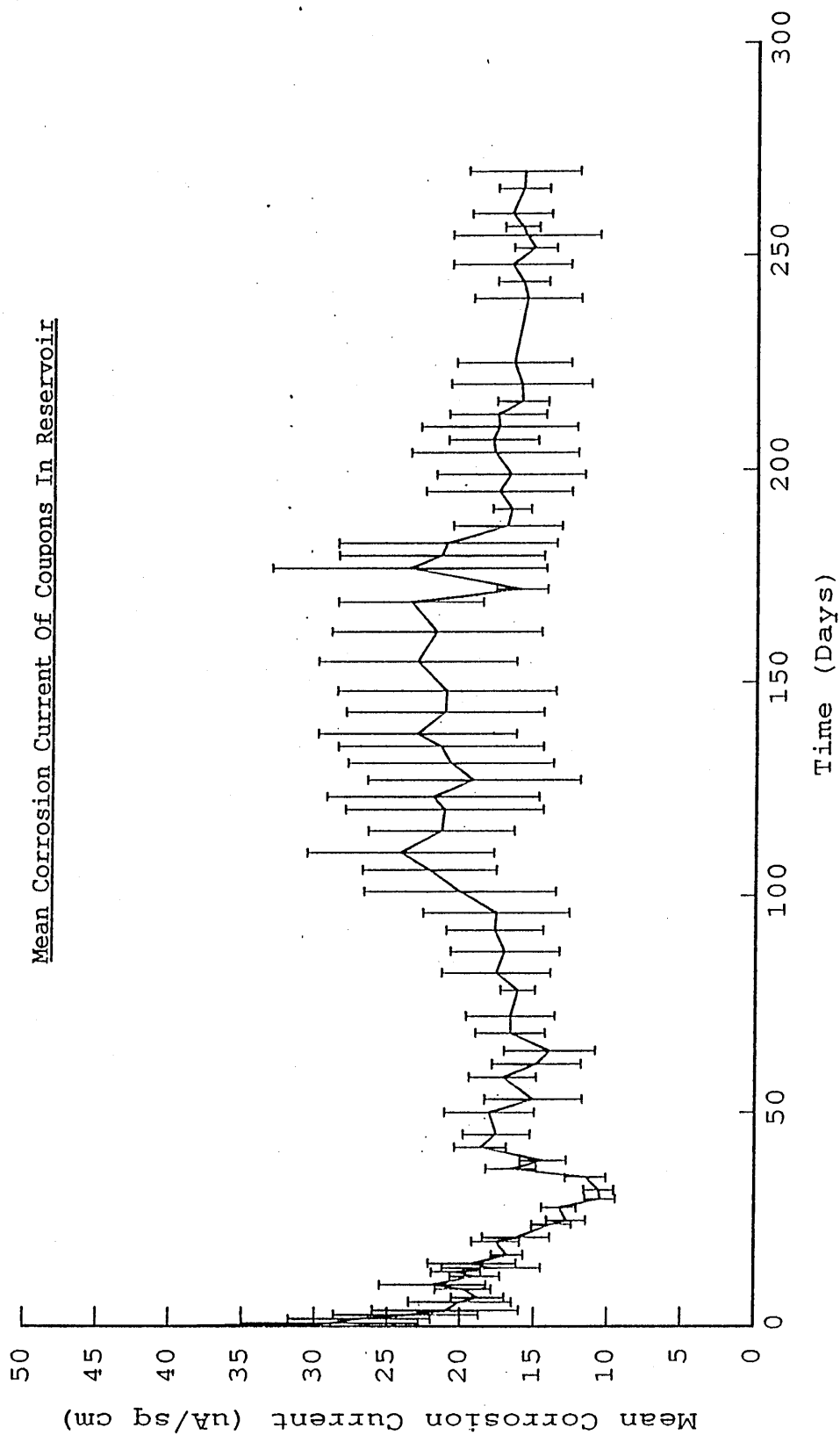
Mean Corrosion Current Of Coupons In Reservoir

FIGURE 4.7.1.B

Mean Corrosion Potential Of Coupons In Reservoir

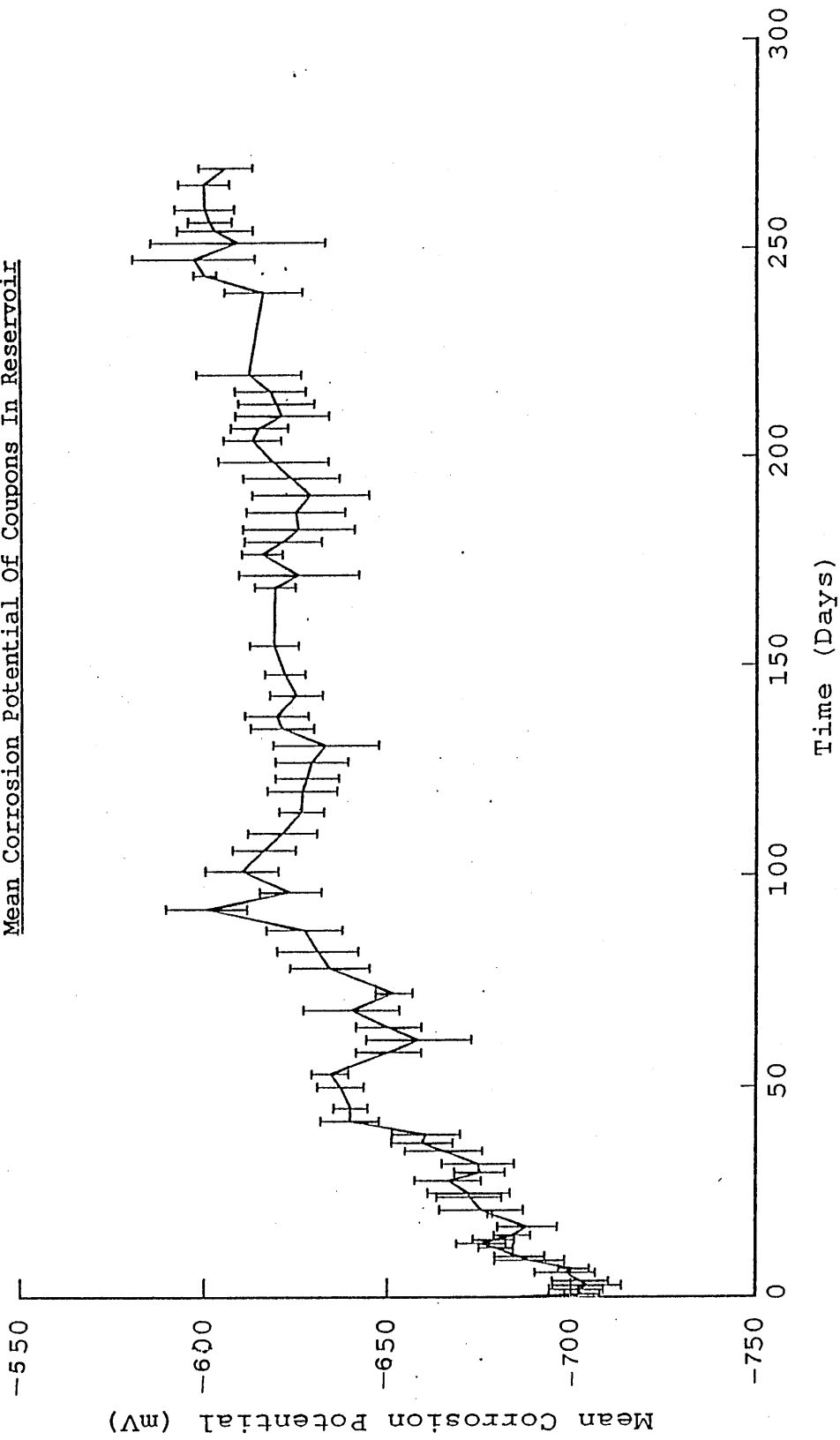
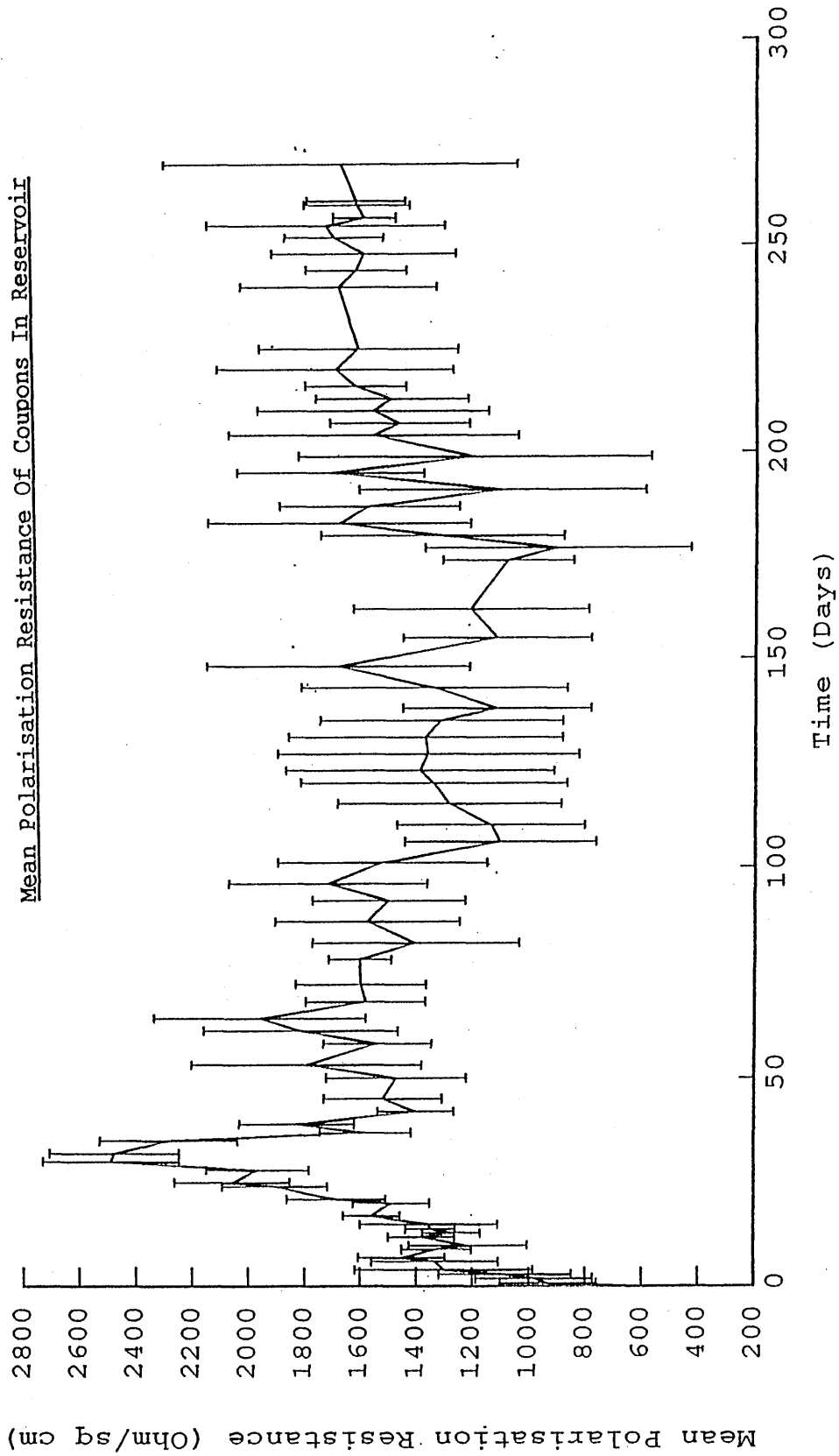


FIGURE 4.7.1.C



The mean corrosion potential of the coupons in the reservoir was about -700 mV S.C.E. at day zero and increased to about -620 mV S.C.E. after 100 days. Fluctuations in the mean rest potential corresponded to changes in the mean corrosion current. During the first 40 days the mean corrosion current rapidly dropped whilst the mean corrosion potential increased. Either the anodic polarisation or cathodic depolarisation was much greater than the other, causing a shift in the corrosion potential as the corrosion current fell. From about days 100-180 the mean corrosion potential was fairly stable, corresponding with the elevated but fairly stable corrosion current. After this the mean corrosion potential rose slightly.

Trends in the mean polarisation resistance showed an inverse relationship to changes in the corrosion current. Variations in polarisation resistance values of the five coupons were very wide, especially in the latter part of the study after about 100 days.

4.7.2 Weight Loss Data

After 15, 30, 80, 138, 170 and 270 days triplicate weight loss coupons were removed from the corrosion probe and the mean weight losses determined.

Table recording mean weight loss data from the coupons in the reservoir:

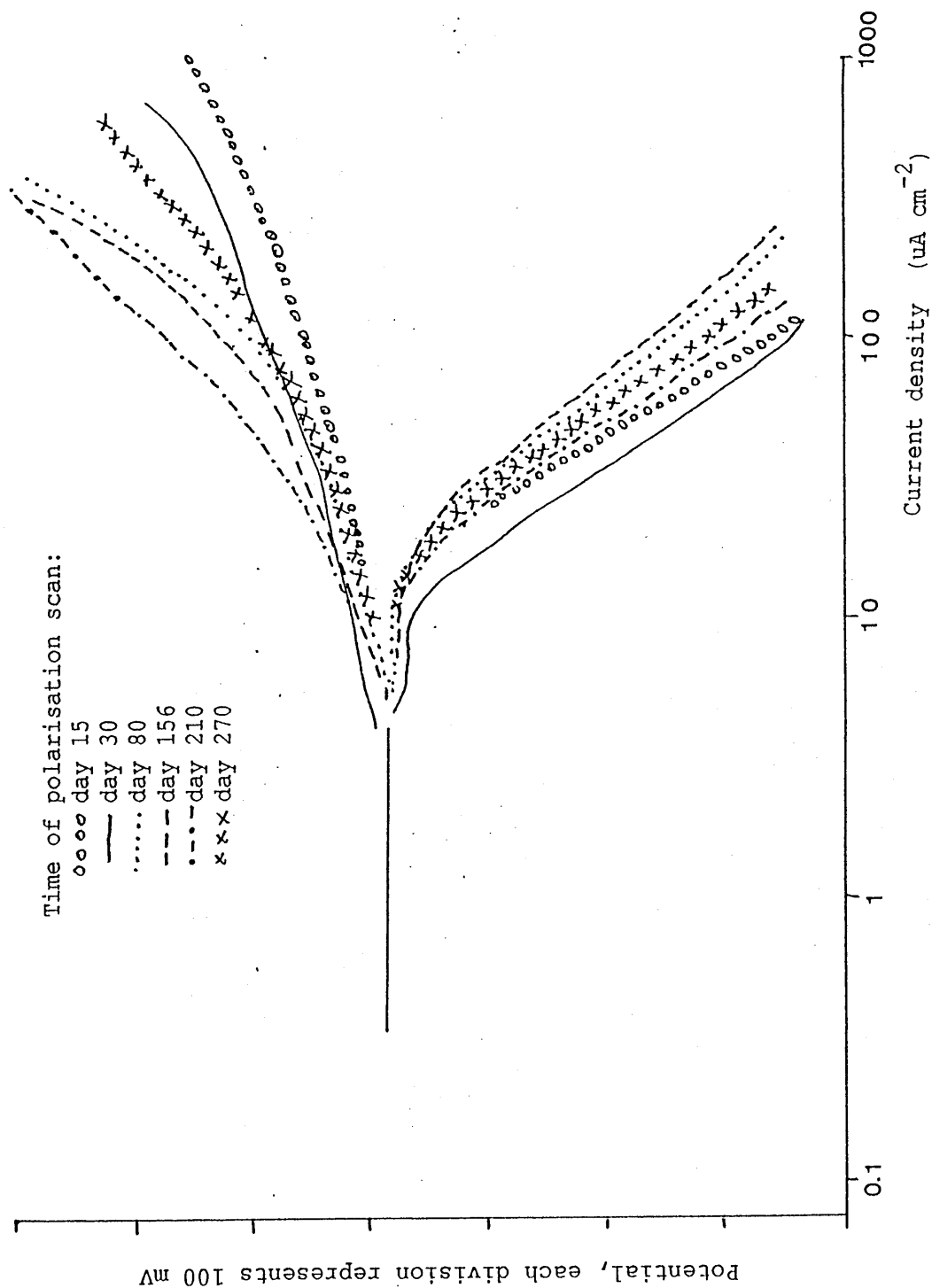
Day coupons were removed	Mean weight loss (mg)
15	24.7 ± 0.83
30	36.0 ± 1.30
80	76.5 ± 6.25
138	107.3 ± 2.05
170	135.8 ± 10.02
270	232.5 ± 10.36

4.7.3 Full Polarisation Scans Of Coupons In The Reservoir

Full polarisation scans were taken of different coupons after 15, 30, 80, 156, 210 and 270 days. The results of all of these polarisation scans were plotted on the same axes (FIGURE 4.7.3), the different corrosion potentials were superimposed onto the same point. The cathodic curves do not appear to have been depolarised whereas the anodic curves were progressively polarised.

FIGURE 4.7.3

Full Polarisation Scans Of Coupons Immersed In The Reservoir Of
The Flow Loop



4.8 CORROSION STUDIES USING THE PROBE AT HOLYHEAD PIER

Corrosion measurements were taken successfully for 34 days when, unfortunately the pump supplying the probe broke down. Results of readings of the corrosion potential taken at 12 hourly intervals have been plotted versus time (FIGURE 4.8.A). Each value is the mean $\pm$ S.D. of five determinations taken on different coupons. FIGURE 4.8.B records the current through a 25 Ohm resistor used to cathodically protect six coupons at -850 mV S.C.E.. Again, readings taken at 12 hourly intervals were plotted.

Initially, the mean corrosion potential was approximately -800 mV S.C.E. and it decreased rapidly during the first two days. A similar trend was evident with the coupons in the reservoir of the flow loop. The mean corrosion potential subsequently rose during the next 20 days to about -640 mV S.C.E., where it remained fairly stable. This increase was similar to trends in the mean corrosion potential of coupons in the reservoir of the flow loop.

FIGURE 4.8.A

Graph of Mean Corrosion potential Of Holyhead Corrosion Probe

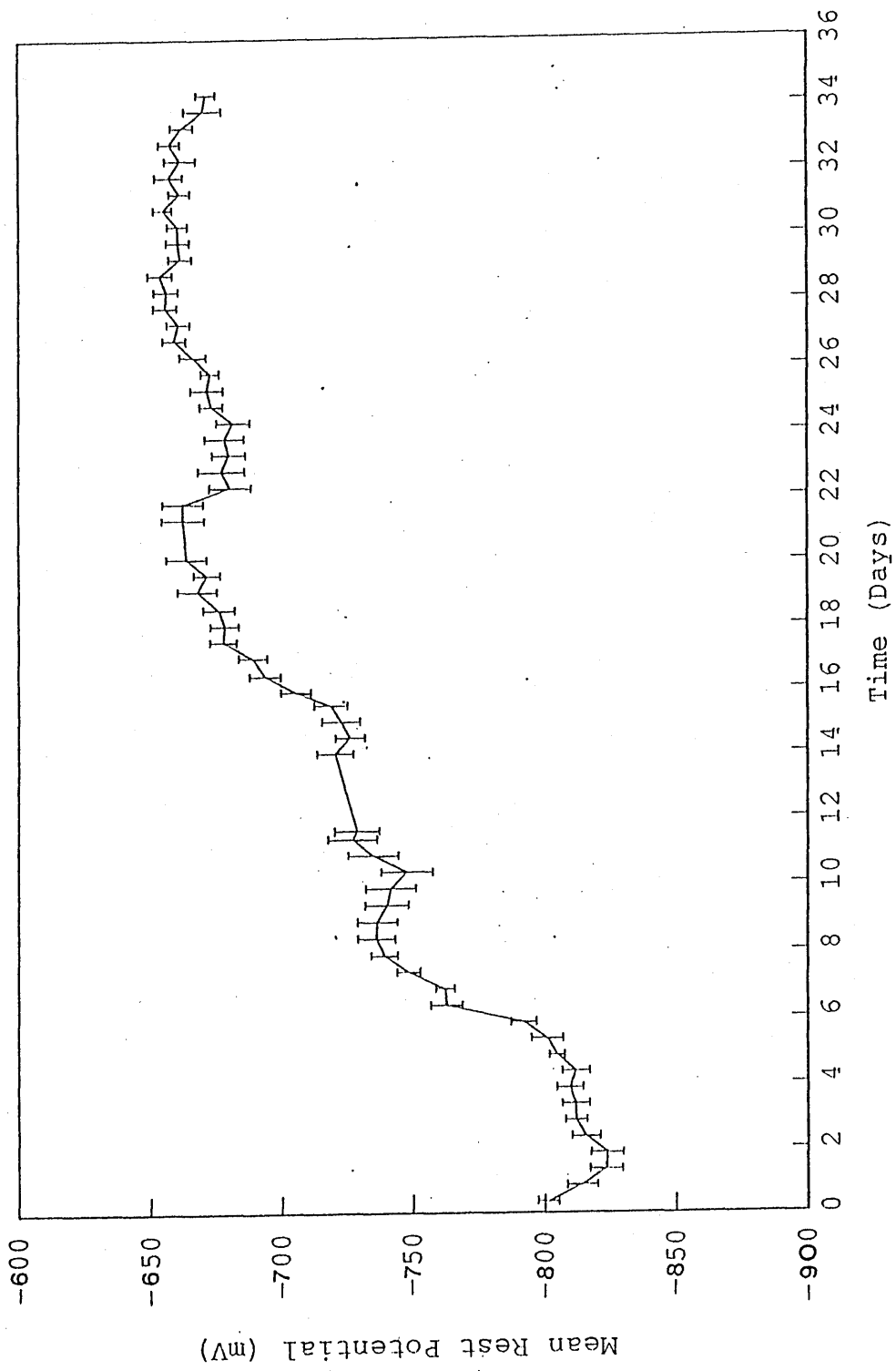
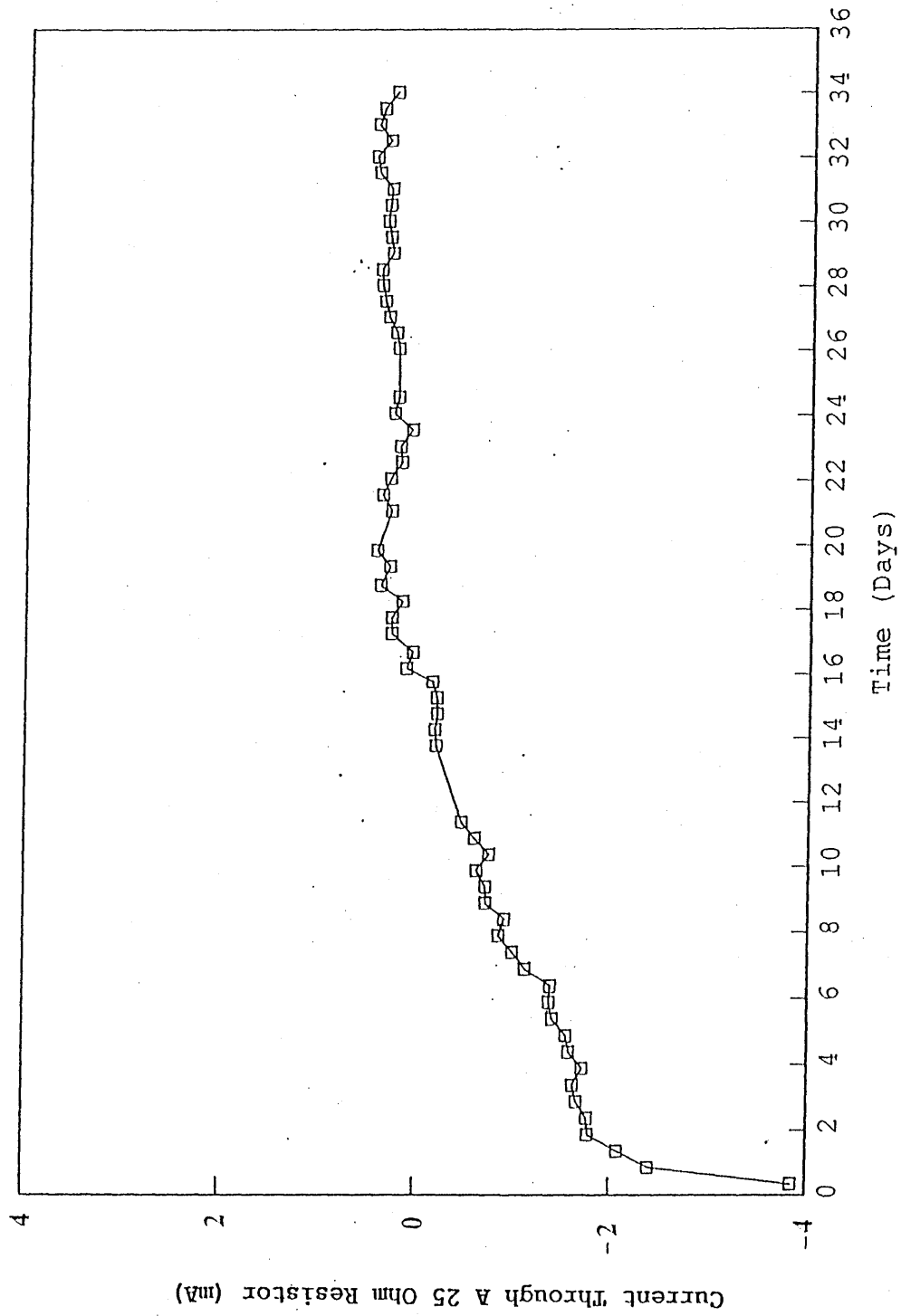


FIGURE 4.8.B

Graph Of Current Used For Cathodic Protection Of Holyhead
Corrosion Probe



With the cathodically protected coupons a constant measuring resistor of 25 Ohms was used so the voltage was proportional to the current (as $V = I.R$). For example after 0.37 days the current used to protect the 6 coupons at -850 mV S.C.E. was :

$$I = \frac{V \text{ (mV)}}{R \text{ (Ohm)}}$$

$$I = \frac{116.2}{25}$$

$I = 4.64 \text{ mA}$ for 6 coupons or 0.26 mA cm^{-2} .

The plot of current versus time reveals that the current needed to protect the coupons during the first day was very high, this value rapidly fell during the next two days. Subsequently the current needed declined slowly until about 20 days when it was fairly stable.

4.9 CORROSION MEASUREMENTS FROM THE STERILE CONTROL

4.9.1 LPR Measurements

LPR scans were taken regularly on the same five coupons for 110 days after the sterile control was set up. Graphs of mean corrosion current (FIGURE 4.9.1.A), mean corrosion potential (FIGURE 4.9.1.B) and of mean polarisation resistance (FIGURE 4.9.1.C) were plotted, each value is the mean $\pm$ S.D. of five determinations.

FIGURE 4.9.1.A

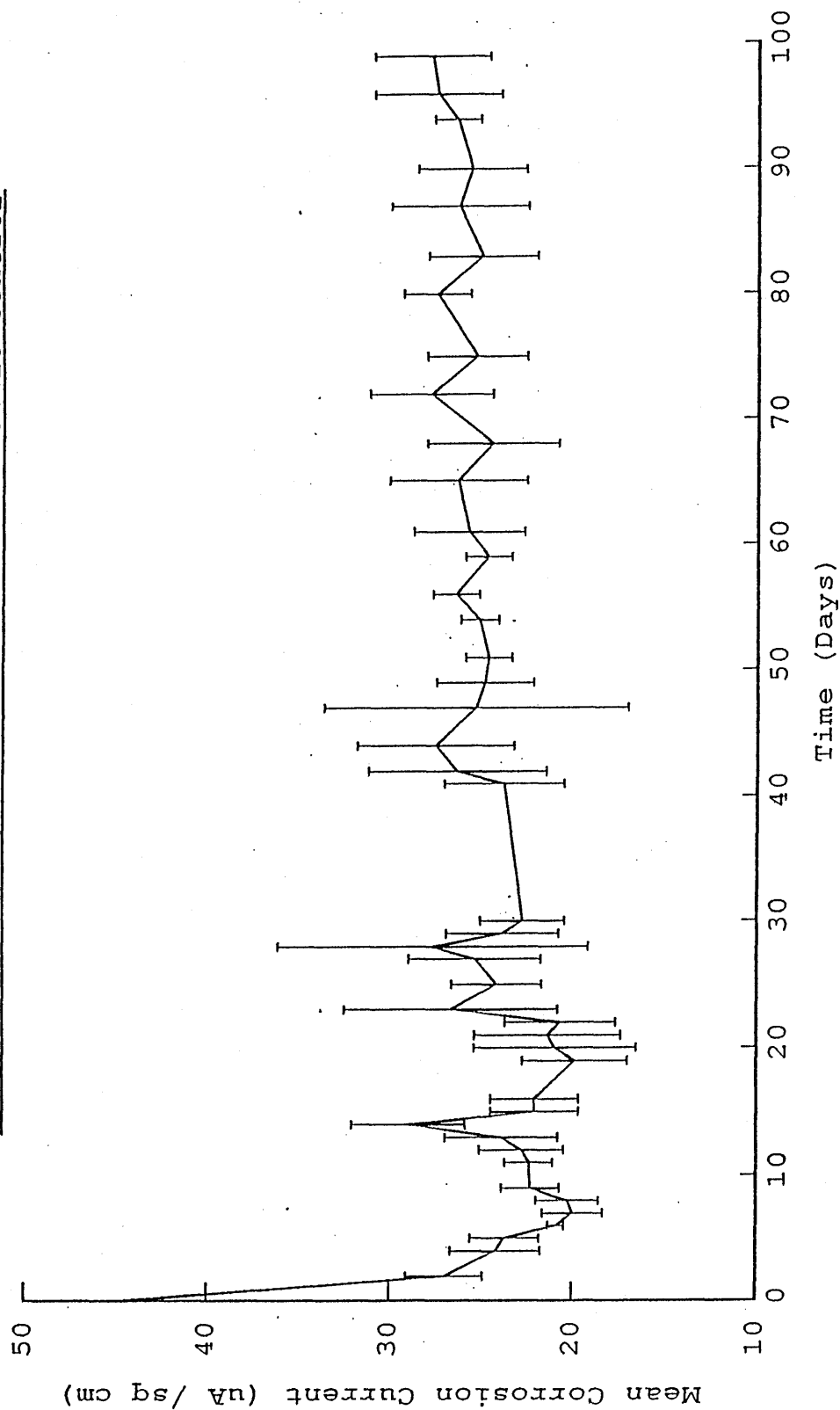
Graph Of Mean Corrosion Current Of Sterile Control

FIGURE 4.9.1.B
Mean Corrosion Potential Of Sterile Control

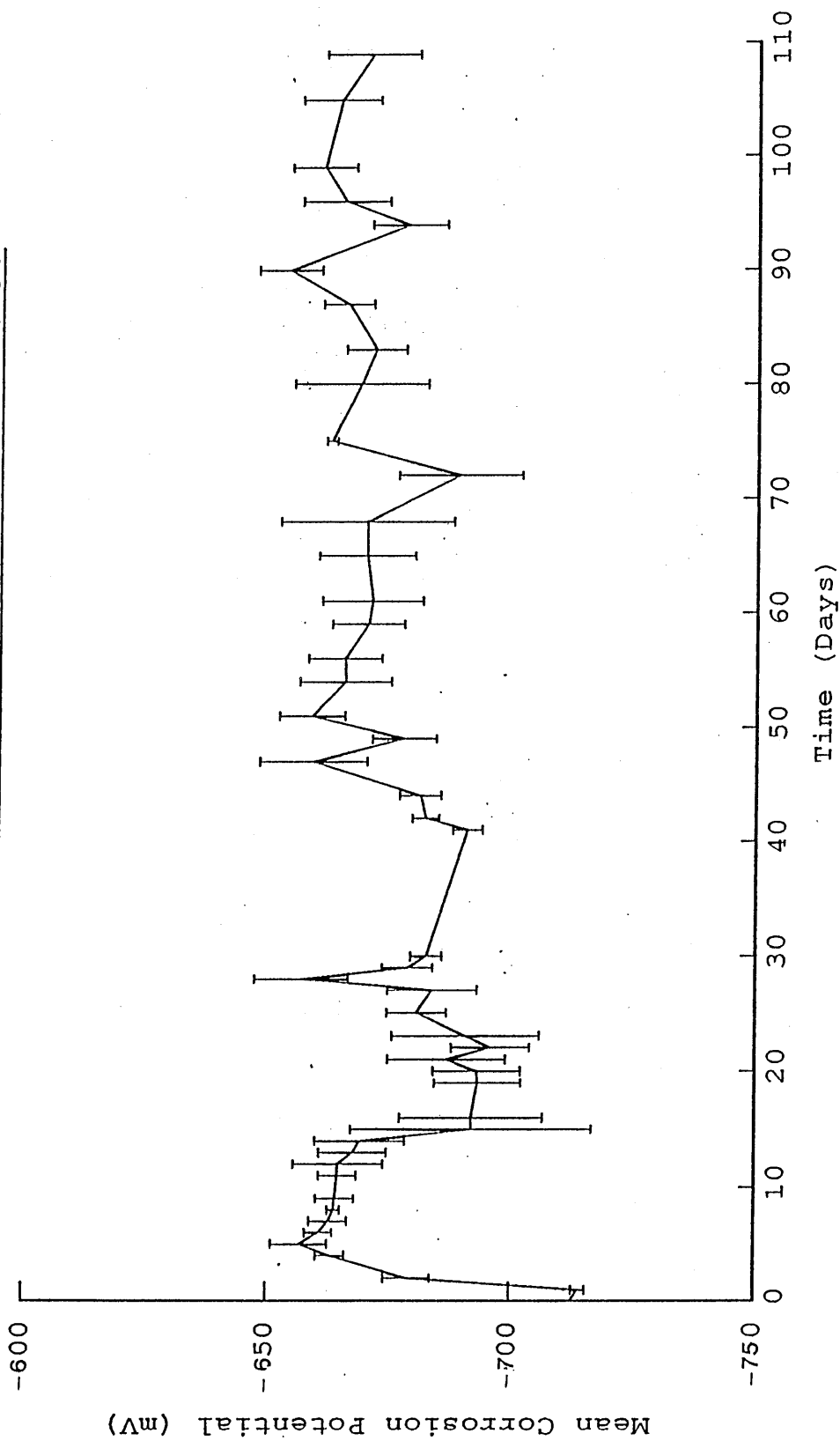
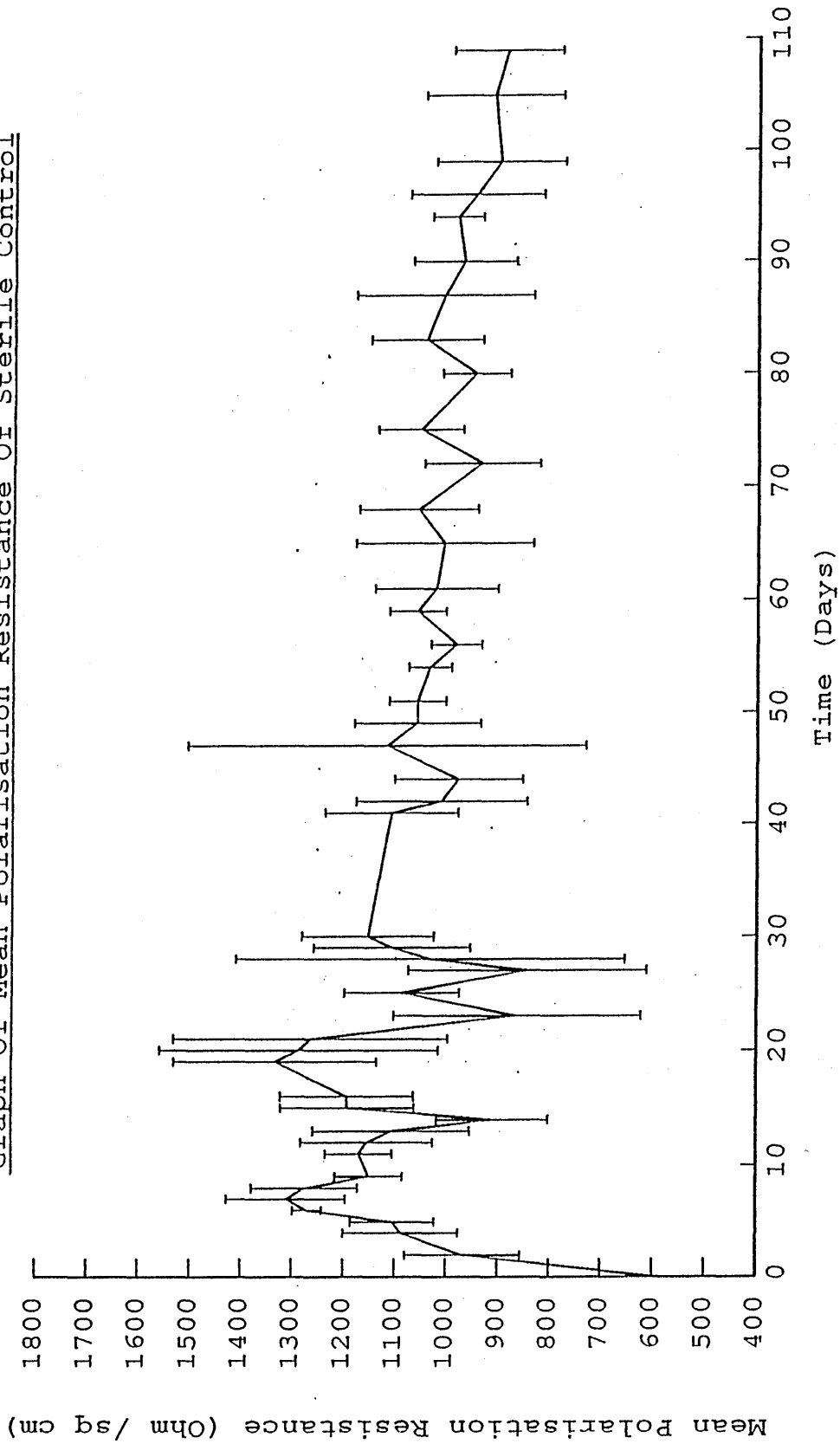


FIGURE 4.9.1.C

Graph Of Mean Polarisation Resistance Of Sterile Control

The mean corrosion current rapidly fell during the first eight days from 46 uA cm^{-2} (0.53 mm yr^{-1}) to 20 uA cm^{-2} (0.23 mm yr^{-1}). After a period in which the value fluctuated it increased slightly from about day 40 onwards.

The mean corrosion potential increased very rapidly during the first eight days as the corrosion current fell. After this it fluctuated but generally stayed between -660 mV S.C.E. and -690 mV S.C.E. .

The mean polarisation resistance had an inverse relationship to the mean corrosion current.

4.9.2 Weight Loss data

After 15, 30 and 110 days immersion five replicate weight loss coupons were removed from the five litre flasks and mean weight losses determined.

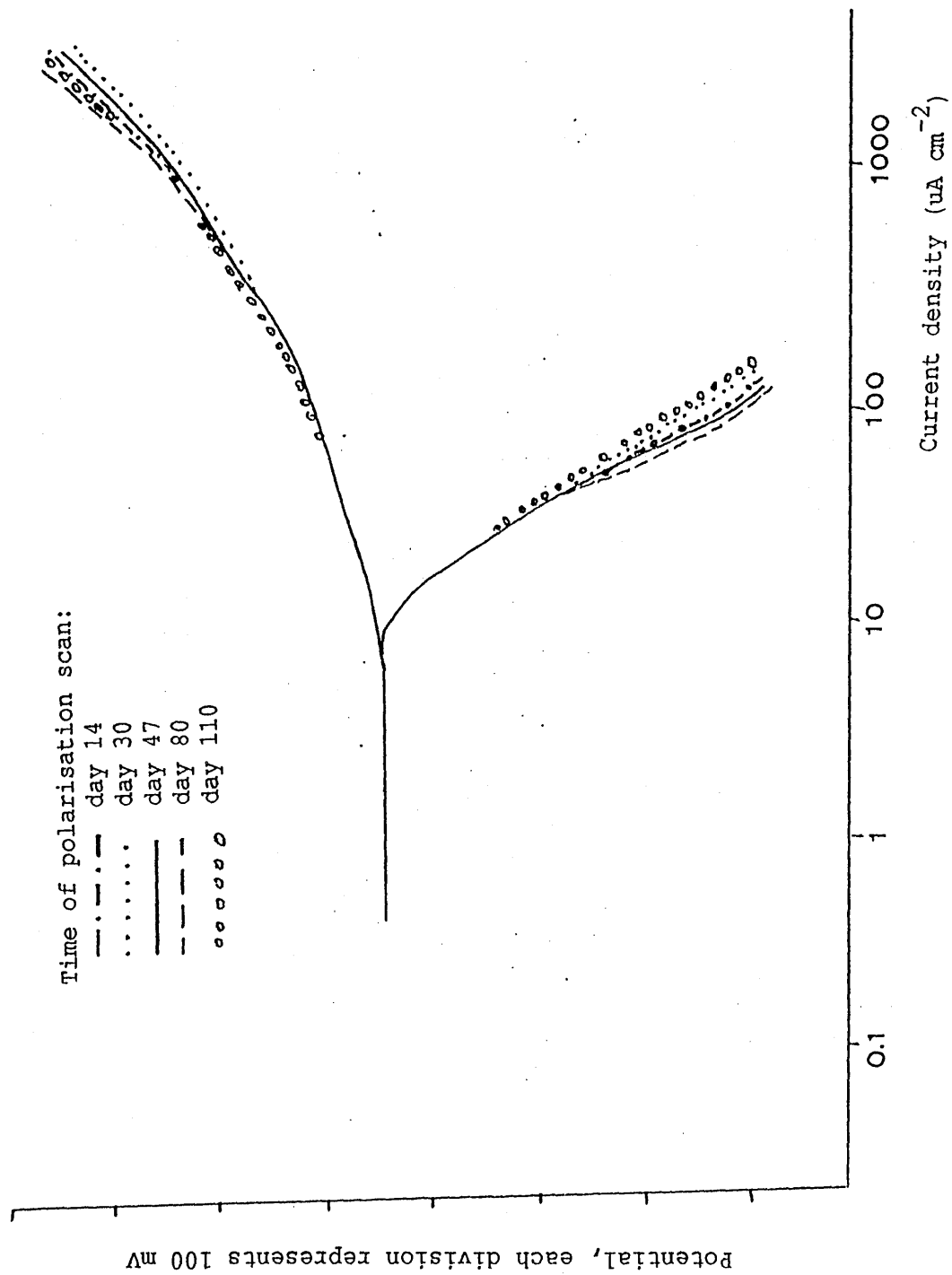
Table recording mean weight loss data from the sterile control:

Day coupons were removed	Mean weight loss (mg)
15	23.8 ± 1.73
30	29.8 ± 2.52
110	93.9 ± 6.90

4.9.3 Full Polarisation Scans Of The Sterile Control

Full polarisation scans were taken on different coupons after 14, 30, 47, 80, and 110 days. The results of all of these polarisation scans were plotted on the same axes (FIGURE 4.9.3) with the different corrosion potentials superimposed on the same point. The anodic curves were not polarised nor were the cathodic curves depolarised.

FIGURE 4.9.3

Full Polarisation Scans Of Sterile Control

5.0 DISCUSSION

5.1 EXAMINATION OF COUPONS IMMERSSED IN THE RESERVOIR OF THE FLOW LOOP WITH SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy revealed that the biofilm on freely corroding and cathodically protected coupons was dominated by rod bacteria with a lesser proportion of cocci and a few stalked rods. Demsey (1981) studied the attachment of marine bacteria to a range of substrata over up to 4 weeks. Different bacteria that were observed included rods, cocci and stalked bacteria as well as prosthecate forms. These bacteria were attached with cell structures such as flagella, pilli and stalks. Only stalks were observed in the flow loop study but bacteria were attached with strands of extracellular mucilage. Specimens that were prepared by freeze drying revealed that some cells were attached by an enveloping sheet of mucilage. Dempsey (1981) also showed that rod bacteria were attached with mucilage sheets and strands.

In several scanning electronmicroscopy investigations the importance of a dense, complex matrix of extracellular mucilage in the structure of the mature marine biofilm has been demonstrated (Jackson & Jones, 1988; Surinach, Hoffman &

Bernhardt, 1984; Edyvean, 1984; Characklis & Cooksey, 1983; Dempsey, 1981). This study was no exception, a complex network of mucilage extended over the surface of all of the coupons. The stringy, so called fibrillar nature of the mucilage was an artefact of fixation by critical point drying (Richards & Turner, 1983) in which it became dehydrated. The extracellular mucilage has a range of functions in addition to its adhesive role (Dempsey, 1981; Hamilton, 1987) such as protection against predators and antifoulants. Another interesting postulation is that mucilage binds extracellular enzymes, so increasing the surface area available for digestion and absorption (Dempsey, 1981). It was considered that the mucilage acted in this way rather like a placenta for the bacteria (Dempsey, 1981). Some studies have followed the development of a mature biofilm on substrata (Jackson & Jones, 1988; Dempsey, 1981). The latter investigation revealed that initially single cells attached to the substratum and divided to form small colonies. These increased in size forming a confluent biofilm over the surface; eventually the film thickened into a dense community of bacteria and mucilage. A dense biofilm covered in mucilage developed on a glass coverslip immersed in seawater for 4 weeks at 4 °C (Dempsey, 1981). Unfortunately, these early and probably most interesting stages in biofilm development were missed in this study. Coupons were not removed from the reservoir until 45 days immersion. By this time mature, dense biofilms had developed on

all the coupons regardless of whether they were cathodically protected or not.

Some of the coupons (both freely corroding and cathodically protected ones) were colonised by diatoms. These non-colonial diatoms were present as individual cells but were occasionally very dense locally. Jackson & Jones (1988) noted that during the development of bacterial films diatoms began to colonise the surface, firstly as single cells and later developing into dense sheets of cells on the surface.

Coupons immersed in the flow loop were covered in a layer of brown corrosion product. The corrosion product was easily distinguished as two layers: an outer, thicker brown layer which was easily removed with a scalpel; and a thin, dense, black layer which adhered strongly to the metal surface and was often difficult to dislodge. EDAX analysis revealed the presence of a large iron peak with calcium, chlorine, silicon, magnesium and phosphorus. These latter elements are present in fouling organisms such as in the silica-rich frustule of diatoms. Examination of sections with the electron microscope showed that two layers could be distinguished as an outer, porous layer and a thinner, dense layer adjacent to the metal surface. Edyvean (1987) was able to separate rust on steel immersed for 100 days into 3 layers: a loose, red rust layer on the outside; a firmer

dark brown layer; and then closest to the steel surface a dense, firmly attached black layer. EDAX analysis and x-ray diffraction showed this black layer to be composed almost entirely of magnetite. The firm, brown layer was composed of goethite and lepidocrocite with lesser amounts of magnetite, iron sulphide and sodium chloride. The loose, red rust layer was composed of lepidocrocite and wustite, together with sodium chloride. Chlorine, silicon and calcium EDAX peaks were also present indicating the presence of fouling organisms (Edyvean, 1987). The long term study by Southwell et al (1974) showed that corrosion product on steel continuously immersed in seawater could be distinguished as two layers: an outer layer of corrosion product; and a soft, black layer rich in sulphides and with active SRB next to the metal surface. Covering these layers of corrosion product was a layer of fouling organisms (Southwell et al). Hamilton, Moosavi & Pirrie (1988) also found that corrosion product on steel immersed offshore for 1-2 years had clearly defined layering. In this study the corrosion product on coupons immersed in the reservoir, especially in the latter stages of the investigation sometimes formed rounded protuberances up to 5 mm long.

EDAX analysis revealed that the dominant cation in the outer surface of the calcareous scale on cathodically protected coupons was calcium with some magnesium. In another study

(Edyvean, 1987) the scale furthest from the metal surface was made up entirely of calcium compounds, whilst magnesium was the dominant element of the scale closest to the metal surface. Unfortunately in this investigation a method of sectioning the scale was not found.

5.2 VIABLE COUNTS OF BACTERIA IMMERSSED IN THE RESERVOIR OF THE FLOW LOOP

Counts of viable heterotrophs from the same treatment when incubated under aerobic or anaerobic conditions were quite similar. Incubating spread plates under anaerobic conditions does not distinguish between obligate anaerobes and facultative anaerobes. Obligate anaerobes are bacteria inhibited or killed on exposure to oxygen. Facultative anaerobes are bacteria which grow either in the presence or absence of oxygen provided the nutrient conditions are favourable. This group includes bacteria which have an anaerobic type of metabolism but which are not sensitive to oxygen, as well as bacteria which can shift their metabolism from aerobic to anaerobic, depending on the environmental conditions. Likewise, incubating plates under aerobic conditions does not distinguish between obligate aerobes and facultative aerobes. Thus facultative bacteria which can grow in the presence or in the absence of oxygen will be counted under both conditions. If the biofilms contained a high

proportion of facultative bacteria viable counts incubated under aerobic and anaerobic conditions will inevitably have been similar.

Enumeration of viable heterotrophs did not reveal any marked increases or decreases over the course of the 270 day study. This correlates with the SEM study in which trends in the nature and density of biofilms with time were not observed. Early data gained from viable counts was not very reliable due to prolonged sonication of the inoculum, and as insufficient replicates were done. More reliability could be placed on the later results (days 168 onwards) as increased numbers of replicates were used. During this latter stage of the study the biofilms on freely corroding and on cathodically protected coupons had probably matured to a stable state without large fluctuations in numbers of heterotrophs.

In addition, numbers of viable heterotrophs were not influenced by the deployment of cathodic protection. It might be expected that greater numbers of heterotrophs would be enumerated on the freely corroding coupons than on the cathodically protected ones due to the larger surface area of the corrosion product compared to the calcareous scale. This difference would probably have differed by less than an order of magnitude.

Interestingly greater numbers of SRB were detected on freely corroding coupons than on cathodically protected ones. SRB are as active on cathodically protected as on freely corroding steel (Sanders & Hamilton, 1986). Cathodic protection promotes the production of cathodic hydrogen which many SRB are able to oxidise in addition to other electron donors (Pankhania, 1988). It is possible that this additional energy source (cathodic hydrogen) on cathodically protected steel might favour the growth of SRB. Pankhania (1986) polarised steel to -100 mV and then to -1400 mV and found that the growth of a pure culture of SRB was stimulated greatly by the over potential. It was concluded that their growth was limited by the amount of hydrogen available from the steel electrode. In this study it is possible that other factors such as high pH, and oxygen concentration gradients were operating, deterring the growth of SRB on cathodically protected coupons. The diagram (FIGURE 5.2.A) of a pH profile in the electrolyte adjacent to the metal surface shows that the pH at the metal/electrolyte interface is determined by the rate of hydroxyl ion production and OH^- removal resulting from either diffusion or convection, or both (Hartt, Culberson & Smith, 1984). It is possible that calcareous scale will act as a physical barrier to the diffusion of hydroxyl ions from the metal surface maintaining a high pH through much of the scale (refer to FIGURE 5.2.B). This may render the scale adjacent to the metal surface unfavorable for the growth of SRB due to high

FIGURE 5.2.A

Schematic illustration Of The pH Profile In The Electrolyte
Adjacent To A Cathodically polarised Metal Surface
(After Hartt, Culberson & Smith, 1984)

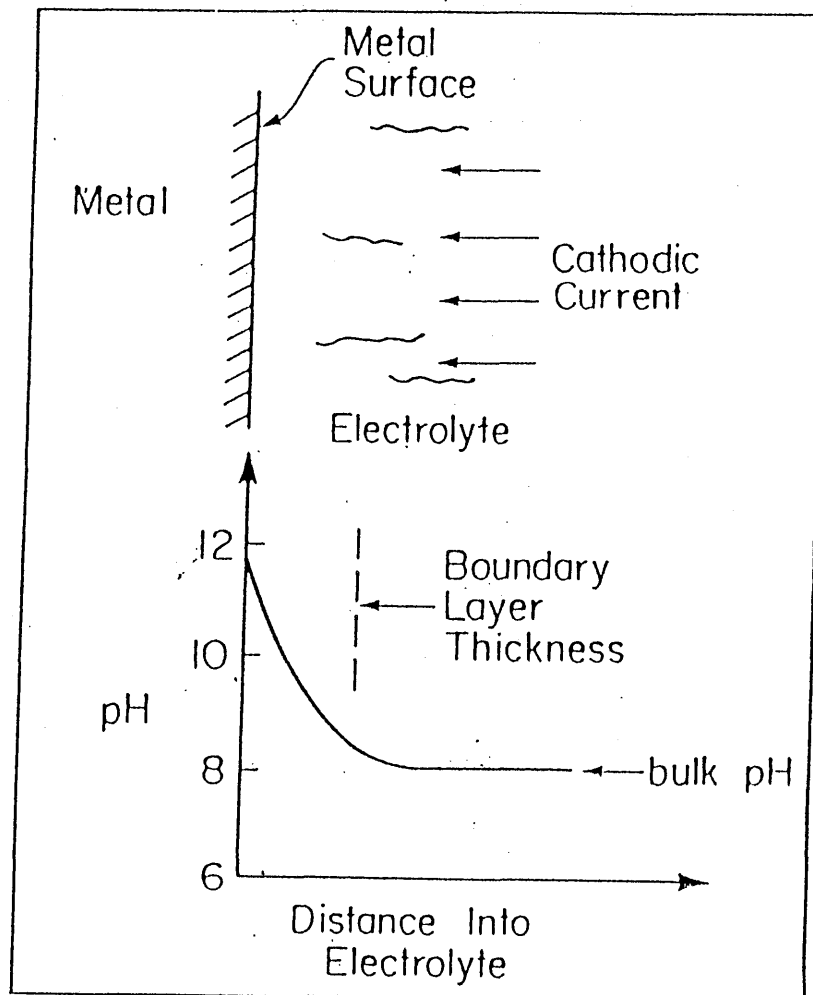
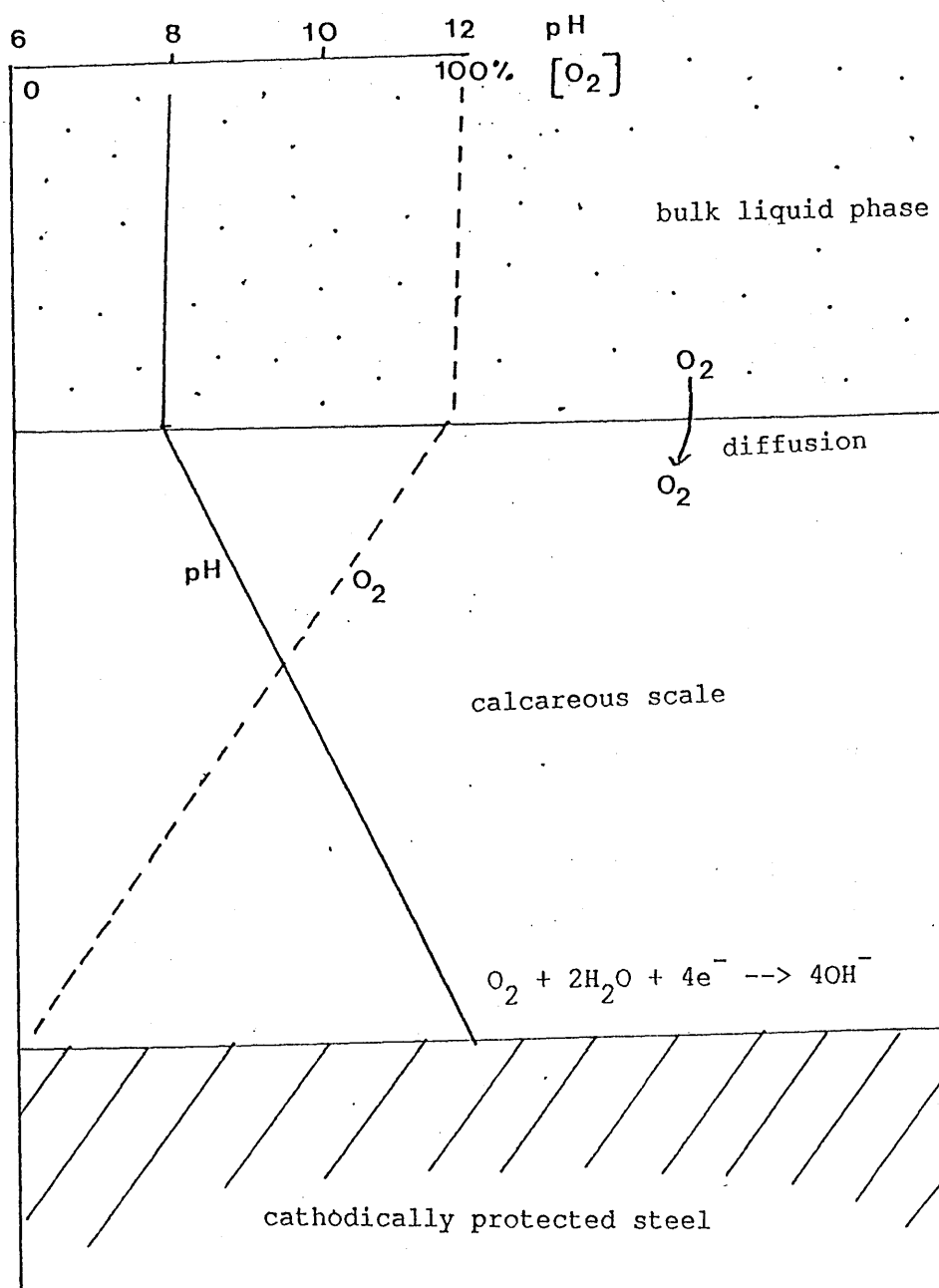


FIGURE 5.2.B

Diagram Showing pH and Oxygen Diffusion Gradients Through A Section Of Scale On Cathodically Protected Steel



pH. If an oxygen concentration gradient extends through the scale the anoxic zone adjacent to the metal surface may be favourable for the growth of SRB in terms of the lack of oxygen but their growth may be inhibited by the high pH there.

In contrast to these results Hamilton, Moosavi & Pirrie (1988), did not find that numbers of SRB cultured from freely corroding test coupons were higher than numbers cultured from cathodically protected ones (at -1.05 V S.C.E.). Nor was any correlation gained between the numbers of viable SRB and the amount of corrosion that had occurred. Hamilton et al concluded that viable counts of SRB cultured from test coupons were of no value whatsoever in gaining a measure of what corrosion had already occurred or would occur in the future.

The use of viable count procedures in microbial ecology has been discredited, for various reasons many years ago (refer to Fletcher, Gray & Jones, 1987, p4-5). As already discussed, the validity of enumerating 'aerobic' and 'anaerobic' heterotrophs separately is very suspect due to the unknown proportion of facultative heterotrophs. Viable counts involve disruption of the biofilm which has unknown effects on viability of bacteria within the complex consortia of it.

5.3 RADIO-RESPIROMETRIC ASSAYS TO MEASURE GLUCOSE TURNOVER AND SULPHATE REDUCTION

The radio-respirometric technique is a sensitive method by which rates of glucose turnover and sulphate reduction can be determined in intact biofilms still adhering to the metal surface. This complex technique was hampered initially by difficulties in establishing its smooth running, and the use of insufficient replicates. Another problem was the lack of controls that could be performed. In another study (Gilbert, Attwood, David, Morgan & Herbert, 1987) biofouled coupons were autoclaved and the rate of sulphate reduction of the dead but intact biofilm was determined to be $1.75 \text{ nM cm}^{-2} \text{ hr}^{-2}$. Most of the rates of sulphate reduction in this study were below this value which casts doubt on whether rates were due to SRB activity. Gilbert et al (1987) immersed the steel studs in an anaerobic flow loop and a thick sulphide film was present on the steel surface. In contrast the flow loop in this study was aerated so a thinner sulphide layer may have formed and so lower rates of sulphate reduction by an autoclaved biofilm would be expected. Even so, most of the rates of sulphate reduction were very low. It is impossible to tell if they were due to SRB or a spontaneous chemical reduction of sulphate. Definite trends in rates of glucose turnover or sulphate reduction were not shown by the results obtained.

Useful results gained in the comprehensive study by Maxwell and Hamilton (1985) revealed that the glucose turnover was significantly greater on freely corroding steel than on protected steel. This was attributed to the build-up of corrosion product giving a far greater surface area for bacterial attachment and metabolism. Rates of glucose turnover were determined under aerobic and anaerobic conditions. The rate of glucose turnover under anaerobic conditions on protected steel was very low, suggesting that significant zones of anaerobiosis had not developed in the biofilm. Maxwell & Hamilton (1985) also found lower activity of anaerobic heterotrophs and of SRB on cathodically protected coupons. In this study lower numbers of viable SRB were also detected on cathodically protected coupons than on corroding ones but this did not correlate with significantly lower numbers of anaerobic heterotrophs on these coupons.

In a more recent investigation (Hamilton, Moosavi & Pirrie, 1988) an attempt was made to obtain a direct correlation between the corrosion and one or more indices of biological activity. Biological activity of SRB was determined by viable counts, amount of sulphide produced and the sulphate reduction activity. Steel coupons were freely corroding or cathodically protected (-1.05 V S.C.E.) and immersed at various depths for 1-2 years.

This study was not able to draw any close parallels between the extent of corrosion and any of the three indices used to measure the biological activity of SRB. In general high values of sulphate reduction of in situ biofilms were associated with high corrosion rates but there were some exceptions. When cathodic protection was employed any correlations between remaining corrosion rates and sulphate reduction activity disappeared. Cathodic protection did not show any definite trend in promoting or inhibiting SRB activity as measured by the three biological indices. Hamilton et al (1988) were seriously concerned about the accuracy and validity of the ^{35}S -sulphate reduction assay when carried out in a single test-tube: Firstly sulphate has been shown to be limiting in the assay; secondly seawater contains a sulphate concentration of 29 mM and it is not known how much 'cold' sulphate is trapped within each biofilm. It was concluded that the importance of SRB in offshore microbial corrosion was related to the chemical and physical nature of the sulphides produced by them at the metal/ biofilm interface.

5.4 CORROSION STUDIES FROM COUPONS AND PROBE IN THE FLOW LOOP AND THE HOLYHEAD CORROSION PROBE

5.4.1 Changes In Corrosion Current Of Coupons And Probe In The Flow Loop And Holyhead Probe

The mean corrosion currents of the coupons in the reservoir and of the corrosion probe were initially high, decreasing very rapidly in the first 10 to 20 days (refer to FIGURES 4.7.1.A and 4.6.1.A). Corrosion of steel in seawater is generally under cathodic control, limited by the rate of oxygen diffusion to the cathodic sites. Southwell et al (1974) in a long term investigation (16 years) found that steel panels corroded very rapidly at first, but then quickly developed a fouling cover which provided appreciable protection to the metal. The mean corrosion rate of the coupons in the reservoir remained relatively stable (excluding period between days 100 to 180) after the initial drop. The mean corrosion current of the probe showed a slightly different trend in the latter stages, after a period of stability it increased slowly. The initial, rapid decrease in corrosion rates (Southwell et al, 1974) of steel subjected to intermittent immersion in the intertidal zone was followed by a linear relationship, reached within a year. In this period the corrosion rate remained at a steady-state high

rate and continued virtually unchanged through the 16 years of exposure. Southwell et al (1974) attributed this latter period of a increased corrosion to the development of anaerobic zones on the metal surface with the proliferation of SRB. Their investigation showed that SRB became active on the metal surface within a year of the exposure of the steel in the sea. Similar trends were recorded by Humble (1948); during a 10 month study weight losses revealed that steel corroded very rapidly initially, then the corrosion rate stabilised after about 150 days.

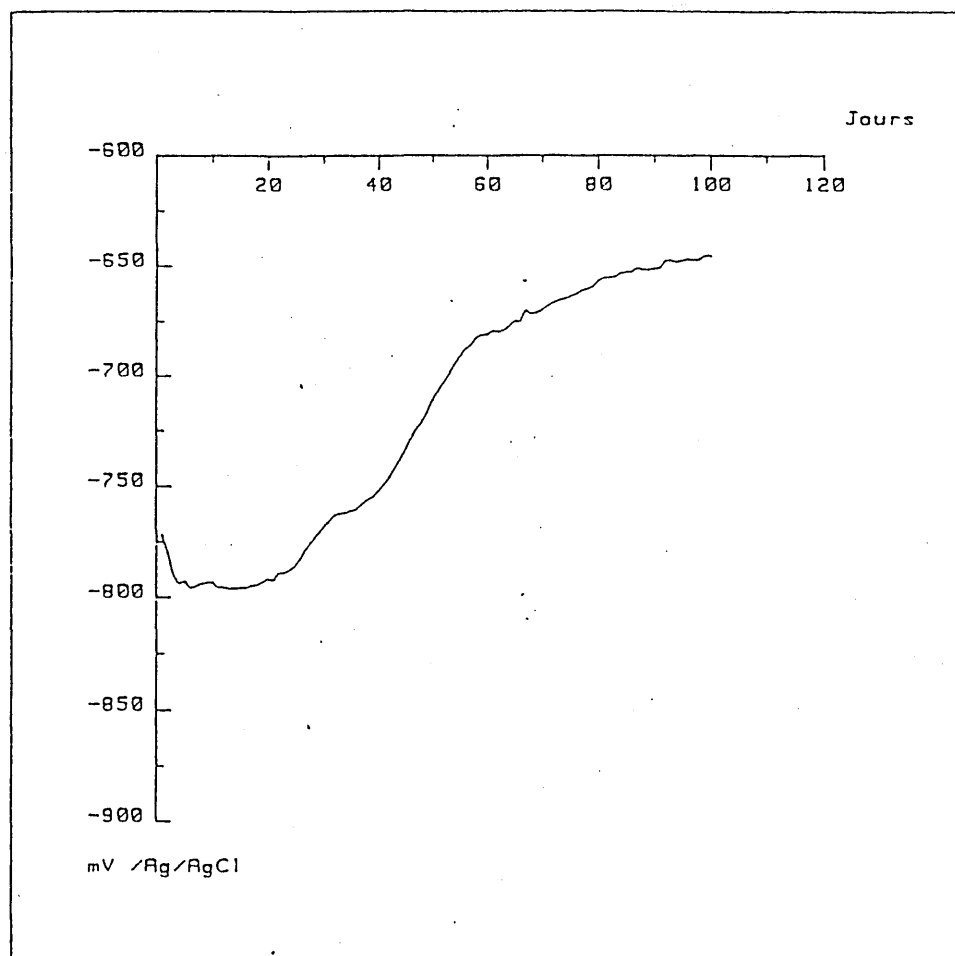
There is some difficulty in comparing the study by Southwell et al (1974) with this one as it extended over such a prolonged time period. The first panels in the study by Southwell et al were not removed until a year of exposure (from which the initial high weight losses were calculated); this entire investigation lasted less than a year. Southwell himself pointed out that corrosion rates reported in literature are usually obtained from short-term tests averaged over the entire period of exposure, but that marine corrosion of carbon steel normally proceeds very rapidly initially and then levels off to a linear relationship. Although this study has been short term (when compared to the investigation by Southwell et al) it has attempted to measure corrosion rates at frequent intervals throughout the test and avoid this pitfall.

5.4.2 Changes In Corrosion Potential Of The Probe And Coupons In Flow Loop And Holyhead Probe

The mean corrosion potentials of coupons in the reservoir (FIGURE 4.7.1.B) and of the flow loop and Holyhead probe (FIGURE 4.8.A) gradually increased with time to more noble values. The mean corrosion potentials of the Holyhead probe and of the coupons in the reservoir increased rapidly at first, then the potential shift became slower and it finally stabilised. Investigations of stainless steel corrosion (Varjonen, Harkkarainen, Nurmiaho-Lassila & Salkinoja-Salonen, 1988) and of steel corrosion in marine sediments (Guezennec & Therene, 1988) also revealed that increases in corrosion potential were initially very rapid (refer to FIGURE 5.4.2). The plot of potential versus time of the Holyhead probe and of steel in marine sediments (Guezennec & Therene, 1988) are very similar. In the latter study a stable potential was observed between 0 and 30 days (0 and 6 days Holyhead probe); it subsequently increased due to the activity of SRB, and the formation of a passivation layer on the surface. It was concluded that the formation of an iron sulphide film due to the activity of SRB resulted in the ennoblement of the metal. Care must be taken when comparing stainless steel to carbon steel but it is interesting to note that size of the range of corrosion potential

FIGURE 5.4.2.

Corrosion Potential Of Mild Steel Immersed In Marine Sediments
(After Guezennec & Therene, 1988)



from the initial value to its later, stable value was much greater for the corrosion of stainless steel in natural seawater (about 300 mV) than in artificial seawater (about 70 mV), (Varjonen et al, 1988). Parallels occurred with this study as the potential range was about 180 mV for the Holyhead corrosion probe and less than 100 mV for the coupons in the reservoir.

5.4.3 Weight Loss Data From Probe And Coupons In The Flow Loop

Weight loss coupons were employed in this corrosion study so as not to be totally reliant on electrochemical corrosion measurements and their associated problems. Purdy, Rosser & Robertson, (1984) investigating corrosion problems and control of sessile bacteria concluded that LPR probe instantaneous corrosion rate measurements generally had poor correlation with weight loss coupons. The raw weight losses recorded in the results section can be manipulated to gain corrosion rates in mg per day per coupon during time intervals between the removal of weight loss coupons.

Table recording mean rates of weight loss from the corrosion probe:

Period of time (Days)	Rate of weight loss ₁ per coupon (mg day ⁻¹)
0 - 15	$\frac{47.9}{15} = 3.2$
16 - 33	$\frac{(73.5 - 47.9)}{(33 - 16)} = 1.5$
34 - 90	$\frac{(114.2 - 73.5)}{(90 - 34)} = 0.73$
91 - 227	$\frac{(289.8 - 114.2)}{(227 - 91)} = 1.29$
228 - 264	$\frac{(308.2 - 289.8)}{(264 - 228)} = 0.52$

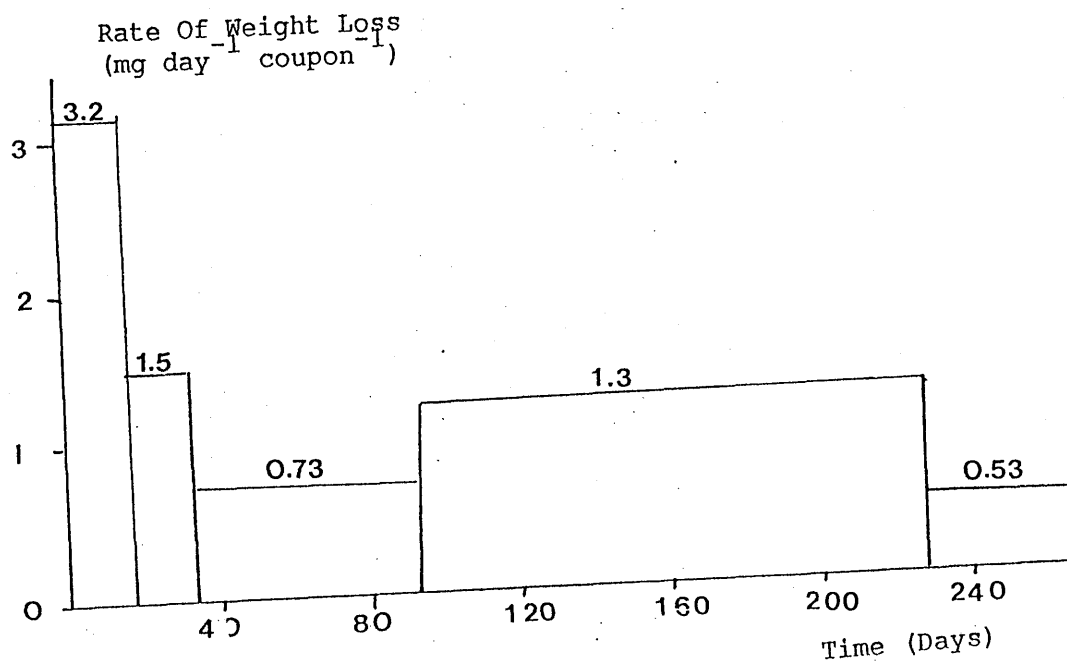
Table recording mean rates of weight loss from coupons in the reservoir:

Period of time (Days)	Rate of weight loss ₁ per coupon (mg day ⁻¹)
0 - 15	$\frac{24.7}{15} = 1.7$
16 - 30	$\frac{(36.0 - 24.7)}{(30 - 16)} = 1.2$
31 - 80	$\frac{(76.5 - 36.0)}{(80 - 31)} = 0.83$
81 - 138	$\frac{(107.3 - 76.5)}{(138 - 81)} = 0.54$
139 - 170	$\frac{(135.8 - 107.3)}{(170 - 139)} = 0.92$
171 - 270	$\frac{(232.5 - 135.8)}{(270 - 171)} = 0.98$

These mean rates of weight loss have been plotted as histograms (FIGURE 5.4.3). The histograms show that the corrosion rates initially decrease. The corrosion rate of the coupons in the reservoir increases slightly from day 138 onwards. The corrosion rate of the corrosion probe also starts to increase during the latter part of the experiment excluding the data from days 228 - 264.

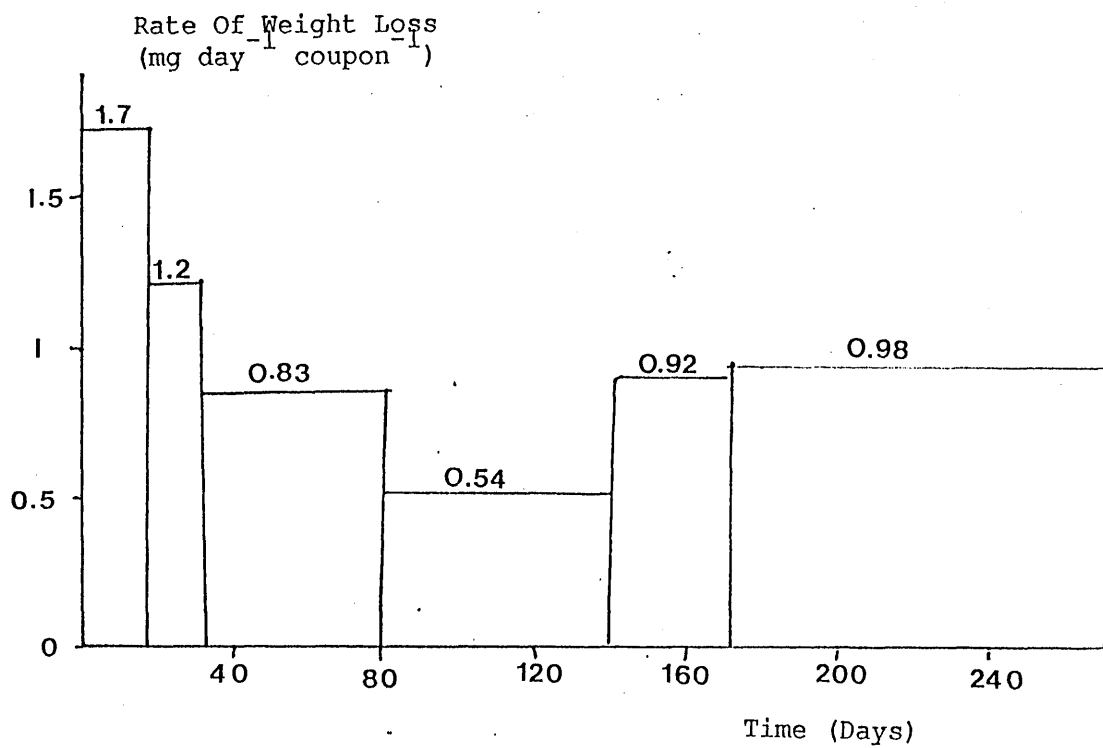
FIGURE 5.4.3

Histogram Of Rates Of Weight Loss From Corrosion Probe



FIGURE

Histogram Of Rates Of Weight Loss From Coupons Immersed In The Reservoir



5.4.4 Comparison of Corrosion Rates From Weight Loss Data Of The Probe And Coupons In The Reservoir With LPR Scans

It was necessary to try to correlate LPR results with rates of weight loss due to some inherent inaccuracies in the former technique. The LPR technique basically measures the electrical resistance of the corroding steel surface. The amount of corrosion current is determined by the size of the electrical resistance. Any factor contributing to the overall electrical resistance such as biofilm development may depress the measured corrosion current, yet corrosion may be actively occurring under the biofilm. Additionally the assumed Tafel constants ($120 \text{ mV decade}^{-1}$) used by the autostat may not have been accurate if polarisation of the anodic or cathodic reactions occurred. An Acceleration factor was used to correct the results from the LPR scans. A revised corrosion current was calculated from the acceleration factors taking the corrected Tafels constants into account.

A condensed form of the Stern-Geary equation is:

$$I_{\text{corr}} = \frac{B}{R_p}$$

Where B equals a constant.

If $b_a = b_c = 120 \text{ mV/decade}$, then $I_{\text{corr}} = \frac{26}{R_p}$

Corrected value of B (mV) =

$$\frac{\text{Actual mean weight loss (from coupons)}}{\text{Predicted mean weight loss (from LPR scans)}} \times 26$$

Acceleration factor = $\frac{\text{Corrected value of B}}{26}$

The weight loss can be predicted from the LPR scans by determining the area beneath the curve of the plot of mean corrosion current versus time, this figure is the total charge passed in a period of time. If the total charge passed is known in a period of time the associated metal loss can be calculated using Faraday's law.

Faraday's law states that to produce one mole of singly charged ions one Faraday or 96500 Coulombs is needed.

2 x 96500 Coulombs is needed to produce 56g of Fe^{2+}

For example the total charge passed for the probe from days 0 - 15:

If 1 uA of current flows for 1 second then 1 uC of charge is passed.

To convert the charge passed into weight loss:

$$\frac{1 \text{ uC cm}^{-2}}{\text{charged passed}} = \frac{56}{193000} \times \frac{1}{1000,000} = 2.9 \times 10^{-10} \text{ g of Fe/cm}^{-2}$$

In one day metal loss =

$$2.9 \times 10^{-10} \times (60 \times 60 \times 24) = 2.5056 \times 10^{-5} \text{ g/cm}^{-2}/\text{day}$$

For a current of 37.23 uA cm⁻² metal loss =

$$37.23 \times 2.5056 \times 10^{-5} = 9.33 \times 10^{-4} \text{ g/cm}^{-2}/\text{day}$$

As each coupon had a surface area of 2.98 cm²

Weight loss predicted from LPR scans = 2.78 mg/coupon/day

Table recording acceleration factors from the corrosion probe determined from day 0 until the the removal of the weight loss coupons:

Period of time (Days)	Acceleration factor
0 - 15	1.15
0 - 33	0.982
0 - 90	0.704
0 - 227	0.696
0 - 265	0.967

Table recording acceleration factors from the coupons in the reservoir determined from day 0 until the removal of the weight loss coupons:

Period of time (Days)	Acceleration factor
0 - 15	1.12
0 - 30	0.976
0 - 80	0.837
0 - 138	0.637
0 - 170	0.610
0 - 270	0.694

These acceleration factors were applied to the mean corrosion currents and revised corrosion currents were plotted versus time (FIGURE 5.4.4.A and FIGURE 5.4.4.B). The corrosion current of the probe rose markedly in the latter part of the investigation, the corrected corrosion current did not increase so much during this time. The corrected corrosion current from the coupons in the reservoir was similar to the original one but the latter corrosion rates were depressed. This method of calculating acceleration factors was unsatisfactory in one respect. As an example the corrosion data from the probe from day 228 - 265 was corrected with an acceleration factor of

FIGURE 5.4.4.A

Mean Corrosion Current From Corrosion Probe Adjusted With Acceleration factors Determined From Weight Losses

AC; acceleration factor calculated from day 0 to day of removal of weight loss coupons.

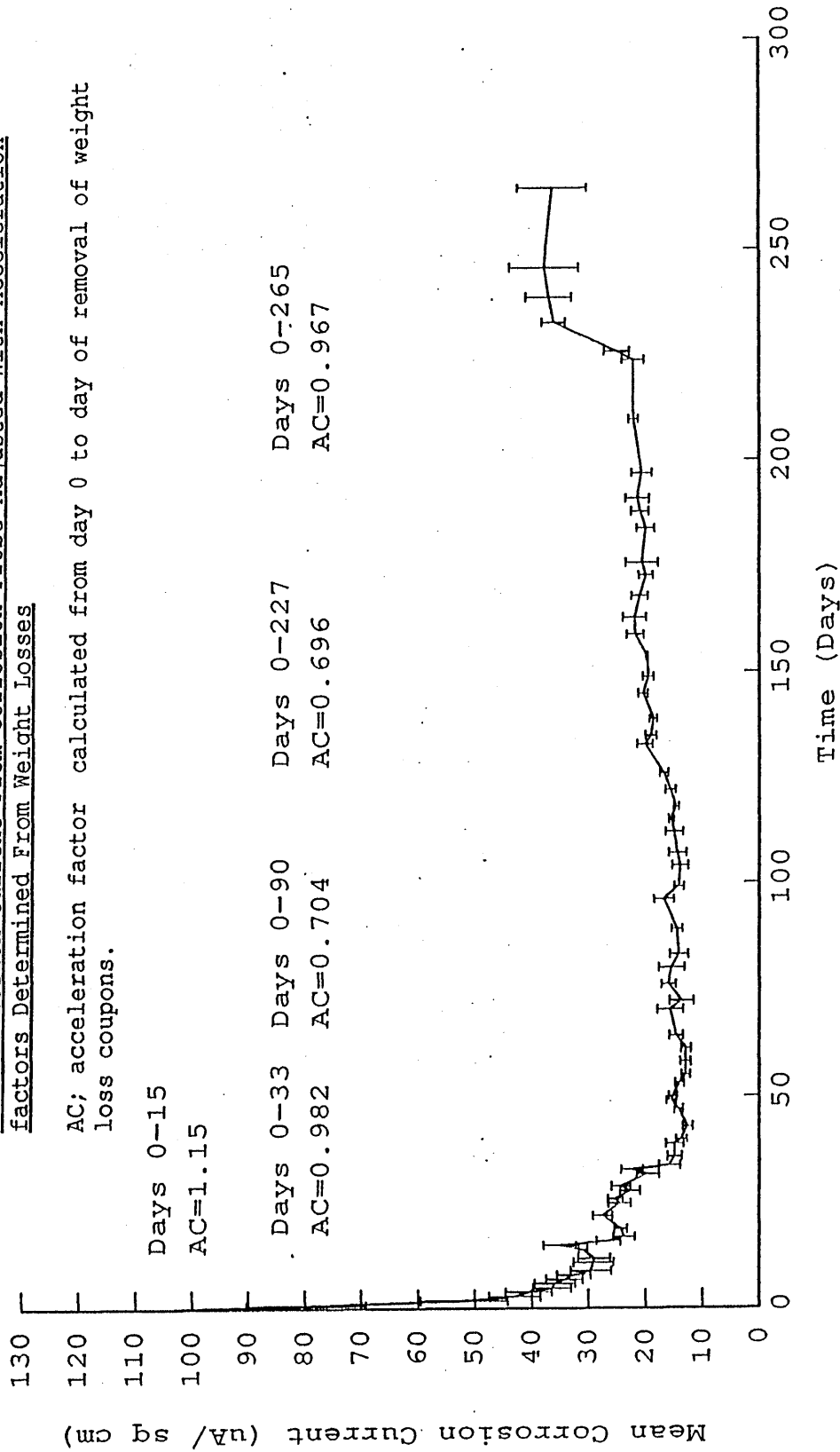
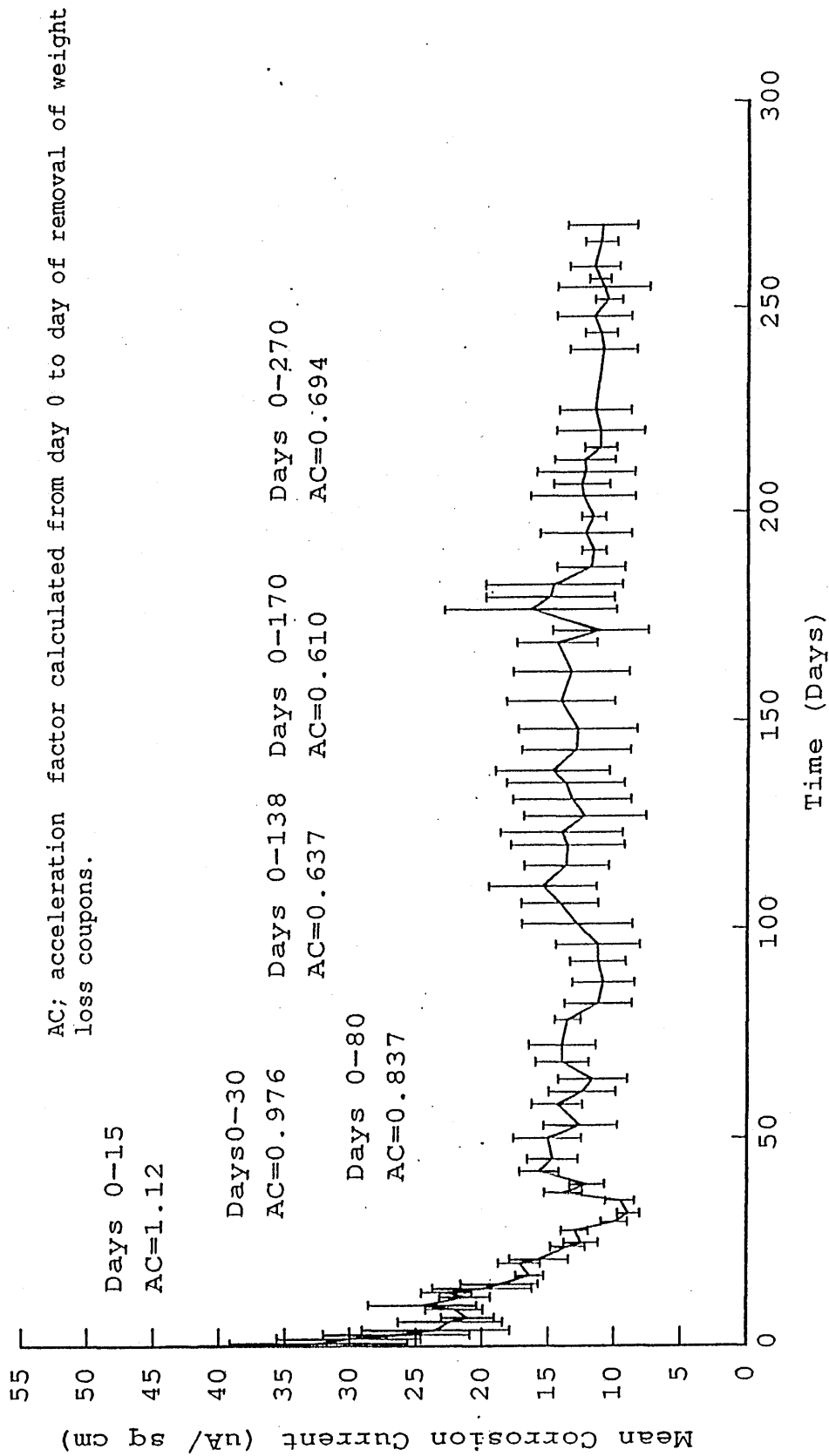


FIGURE 5.4.4.B

Mean Corrosion Current From Coupons In Reservoir Adjusted With Acceleration Factors Determined From Weight Losses



0.987 which had been derived from an actual weight loss and predicted weight loss occurring from 0 - 265 days. This latter acceleration factor was a product of, and dependant on all the previous corrosion trends before day 228.

This problem was overcome by manipulating the corrosion data in the same way as the histograms previously plotted of mean corrosion rates for time intervals between the removal of weight loss coupons. Acceleration factors were derived from corrosion rates calculated for time intervals and compared with corrosion rates predicted from LPR scans for the same time interval.

Table recording acceleration factors from the corrosion probe determined at time intervals between the removal of weight loss coupons:

Period of Time (Days)	Acceleration factor
0 - 15	1.15
16 - 33	0.806
34 - 90	0.468
91 - 227	0.686
228 - 264	0.172

Table recording acceleration factors from the coupons in the reservoir determined at time intervals in between the removal of weight loss coupons:

Period of time (Days)	Acceleration factor
0 - 15	1.12
0 - 30	1.17
0 - 80	0.738
0 - 138	0.396
0 - 170	0.517
0 - 270	0.864

Revised corrosion currents were calculated from these acceleration factors and plotted versus time (FIGURE 5.4.4.C and FIGURE 5.4.4.D). These graphs have a step-like appearance as acceleration factors were calculated for set time intervals, defined by days on which weight loss coupons were removed. In reality the acceleration factors would have been continually changing during these time intervals as changes in anodic polarisation and cathodic depolarisation occurred. These subtle changes cannot be measured by the relatively long periods between the removal of weight loss coupons, in this respect the plots must not be interpreted too literally.

FIGURE 5.4.4.C

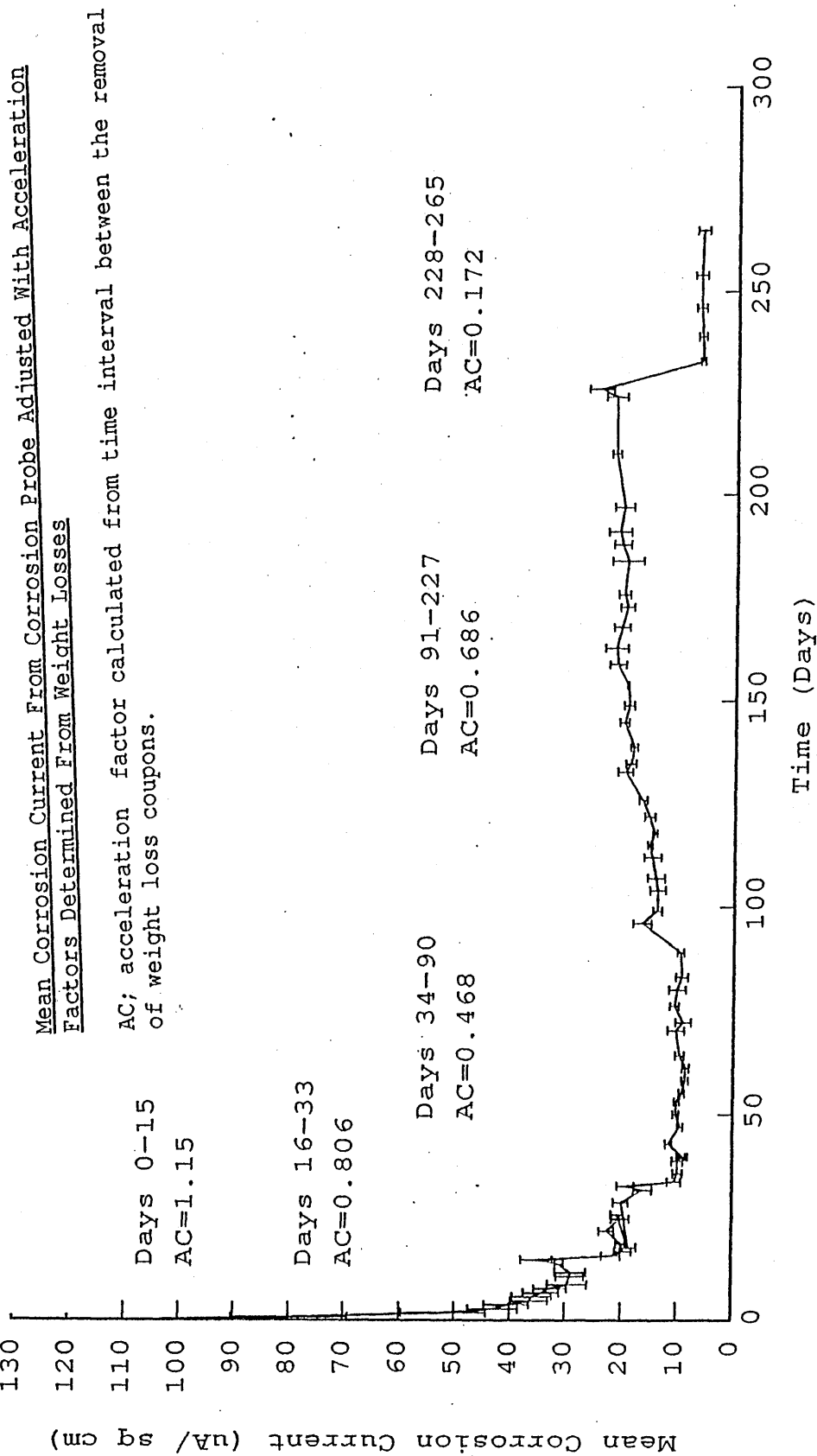
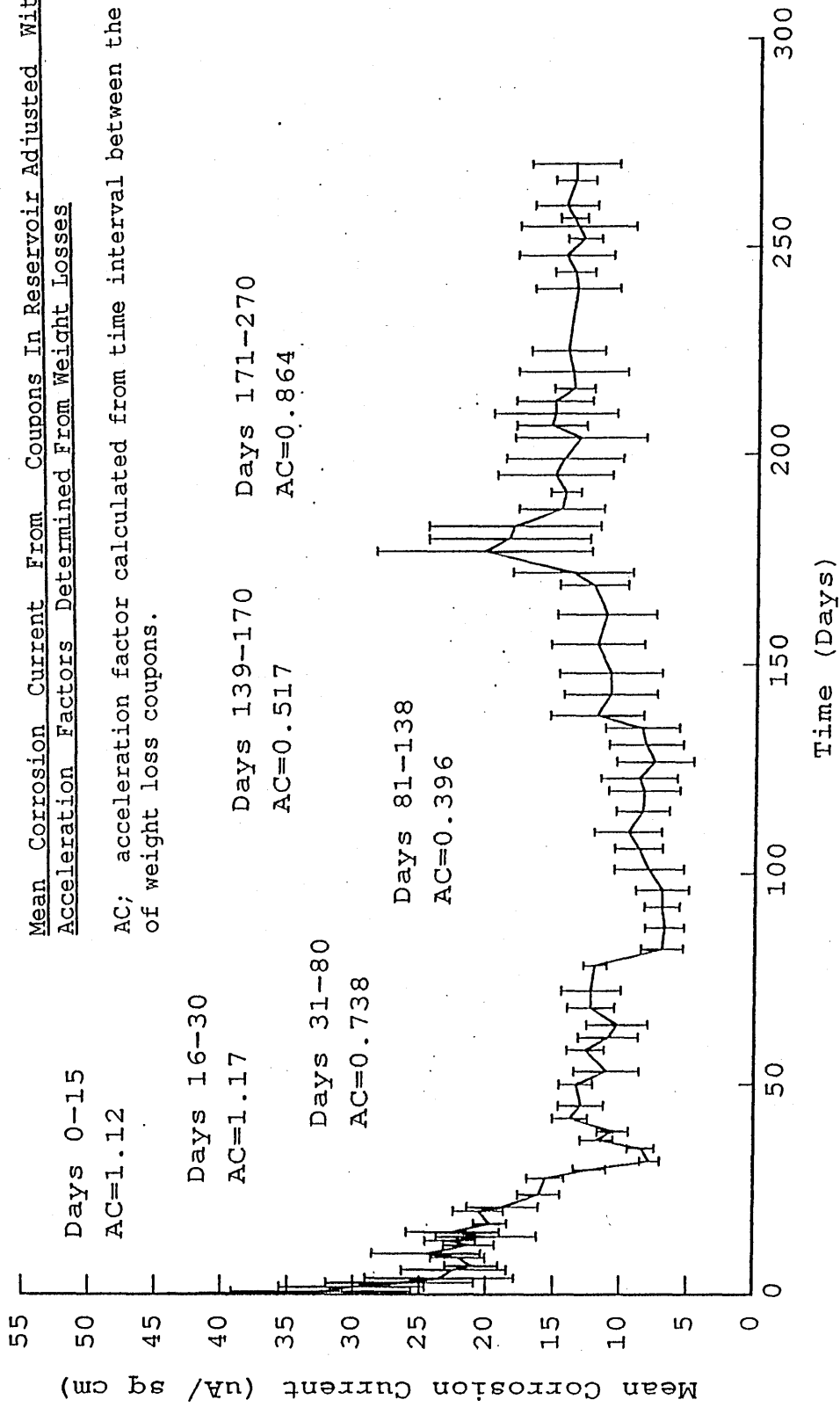


FIGURE 5.4.4.D

Mean Corrosion Current From Coupons In Reservoir Adjusted With Acceleration Factors Determined From Weight Losses

AC; acceleration factor calculated from time interval between the removal of weight loss coupons.



The corrected corrosion current of the corrosion probe still shows a slight increase from day 120 to 227, but the size of increase is reduced. The small size of the acceleration factor from day 228 to 265 resulted in a much reduced corrosion current. The corrected corrosion current of the coupons in the reservoir increased in the latter part of the investigation from about day 130 onwards. These corrosion currents determined from LPR results, when adjusted with weight loss data, show similar trends to the histograms of weight losses. These, results from the coupons in the reservoir especially, indicate that a rise in the corrosion rate did occur in the latter part of the investigation. This rise may perhaps have been due to the proliferation of SRB in the biofilm as proposed by Southwell et al (1974). It cannot be conclusively attributed to the action of SRB as biological tests did not show an absence of viable SRB before this rise nor was significant cathodic depolarisation detected.

5.4.5 Full Polarisation Scans Of The Probe And Of Coupons In The Flow Loop

Full polarisation scans taken on different coupons indicate that in both cases the cathodic curves did not alter markedly but the anodic curves were progressively polarised. The autostat programme was used to fit lines to the anodic and cathodic curves of the polarisation curves giving specific values to the Tafel constants. It should be noted that a linear Tafel region was not easily distinguished on the bowed anodic curves and the positioning of the line fitted onto it was rather subjective. Acceleration factors were calculated by using the Stearn-Geary relationship and comparing these Tafel constants to the assumed Tafel constants used of $120 \text{ mV decade}^{-1}$.

For example day 16, $b_a = 91.8$ and $b_c = 353.9$

$$\frac{(b_a \times b_c)}{(b_a + b_c)} \text{ using the above Tafel constants} = 73$$

If the assumed values of $120 \text{ mV decade}^{-1}$ are substituted into the Stearn-Geary relationship the answer = 60

$$\text{Acceleration factor} = \frac{73}{60} = 1.22$$

Table of acceleration factors calculated from Tafel constants derived from full polarisation scans on the corrosion probe:

Day	b_a	b_c	Acceleration factor
16	91.8	353.9	1.22
33	135.9	329.3	1.60
70	157.4	300.2	1.72
134	185.7	385.3	2.08
211	170.1	299.7	1.81
226	186.7	317.8	1.97

Table of acceleration factors calculated from Tafel constants derived from full polarisation scans of the coupons in the reservoir:

Day	b_a	b_c	Acceleration factor
15	77.3	395.1	1.08
30	85.4	366.8	1.16
80	105.4	305.0	1.31
156	143.8	307.7	1.78
210	149.4	307.1	1.67
270	102.9	333.6	1.31

Changes in these acceleration factors with time differ from those derived from weight loss data. Acceleration factors derived from weight loss data may represent changes in Tafel constants more accurately, due to the increased number of replicates and because one method of electrochemical corrosion measurements (polarisation scans) is not being used to calibrate another one (LPR scans).

Metabolic activities of biofilms can increase corrosion rates by influencing the anodic or cathodic processes or both of these reactions. Examination of the graphs of the full polarisation scans from the probe and coupons in the reservoir shows that the cathodic curves were not systematically depolarised and the anodic curves were progressively polarised. The actual values for b_a determined from the polarisation curves correlate with this as they increase as the Tafel region gets steeper, similarly there is not a discernable trend in the actual values for b_c .

It would be useful at this stage to consider the effects of cathodic depolarisation and anodic polarisation on the corrosion current (I_{corr}) and the corrosion potential (E_{Corr}). FIGURE 5.4.5 depicts three different scenarios in which the change in anodic reaction rate is greater than the change in the cathodic one, or vice versa, or where the changes in the two reaction rates are equal.

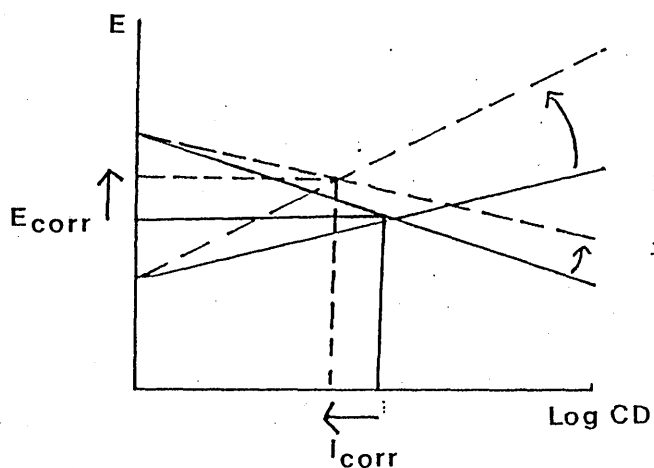
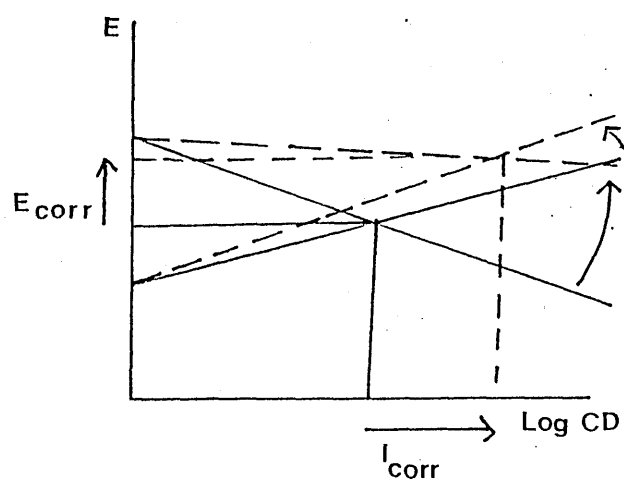
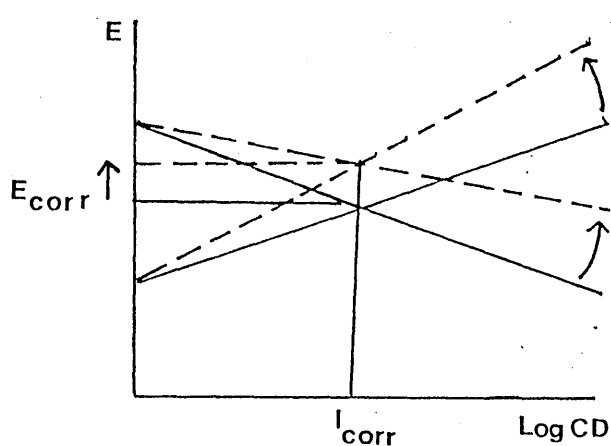
These events are summarised below:

	Effect on I_{corr}	Effect on E_{corr}
Scenario 1 Change $b_a \gg$ change b_c	decreases	increases
Scenario 2 change $b_c \gg$ Change b_a	increases	increases
Scenario 3 change $b_c =$ change b_a	unchanged	increases

From the plots of I_{corr} and E_{corr} versus time of the probe and coupons in the reservoir it can be seen that generally during the first few weeks the corrosion rate decreases whilst the corrosion potential increases. This indicates that the change in b_a was greater than the change in b_c (scenario 1).

FIGURE 5.4.5.

Diagram Illustrating Effects Of Cathodic Depolarisation And Anodic Polarisation On The Corrosion Current And Corrosion Potential Of Steel



Although viable counts and radio-respirometric assays have shown that SRB were active on the freely corroding coupons in the reservoir, cathodic depolarisation was not observed but anodic polarisation was. Many investigations have shown depolarisation of the cathode by SRB (Booth, Elford & Wakerley, 1968; Booth & Tiller, 1960, 1968; Costello, 1974; Pankhania, Moosavi & Hamilton, 1986). Anodic polarisation by microfouling is less well documented. Polarisation studies of mild steel in cultures of SRB (Booth & Tiller, 1960) revealed significant anodic polarisation. Also various cultures of marine algae have been shown to polarise the anode (Edyvean & Terry, 1983). Anodic polarisation by Enteromorpha in aerobic conditions has been attributed to the formation of protective films such as ferric (Fe(III)) compounds by the oxidation of the initial ferrous Fe(II) corrosion product by any oxygen left. Anodic polarisation occurred in anoxic conditions under a decomposing mat of Enteromorpha due to the SRB associated with it (Edyvean & Terry 1983). Sulphide films are associated with SRB and sulphide minerals have been shown to effect both anodic polarisation and cathodic depolarisation (Mara & Williams, 1972).

5.4.6 Cathodically Protected Coupons In The Reservoir And Holyhead Probe

The current density needed to cathodically protect the coupons (at -850 mV S.C.E.) in the Holyhead probe was very high initially, it subsequently declined until a stable value was attained after about 20 days. Wolfson & Hartt (1981) obtained similar results for carbon steel protected at different cathodic potentials ranging from -780 mV to -1033 mV S.C.E.. This decrease in the current density can be correlated with the deposition of a calcareous scale. When steel is cathodically protected in aerated seawater concentration polarisation occurs, so that the current density required decreases with exposure time. The deposition of calcareous scale compounds the oxygen concentration even more (Hart *et al*, 1984). In marine sediments cathodic hydrogen generated at the lower potentials (-1000 and -1100 mV Ag/Ag Cl) especially promoted the growth of hydrogenase positive SRB in the sediment adjacent to protected steel, allowing the subsequent growth of other SRB species (Guezennec & Therene, 1988). Lipid analysis of the biofilm showed that SRB were active on cathodically protected steel causing the deposition of an iron sulphide film. Indeed the activity of SRB rendered a cathodic potential of -900 mV Ag/Ag Cl insufficient to protect the steel and some corrosion occurred (Guezennec & Therene, 1988). It is not known if SRB were active in the

biofilm on the corrosion probe at Holyhead.

5.5 CORROSION STUDIES FROM THE STERILE CONTROL

5.5.1 Changes In Corrosion Measurements From Sterile Control

The mean corrosion current of the sterile control (FIGURE 4.9.1.A) followed similar trends to the corrosion rates of the coupons (FIGURE 4.7.1.A) and probe (FIGURE 4.6.1.A) in the flow loop. An initially high corrosion rate declined very rapidly during the first eight days and then increased very slowly in the latter part of the experiment. The mean corrosion potential (FIGURE 4.9.1.B) increased slightly during the course of the experiment, but the range of increase (about 60 mV) was smaller than the range of increase in the corrosion probe at Holyhead (about 180 mV), and the range of increase in the coupons in the reservoir (about 100 mV). The potential of freely corroding stainless steel in artificial or sterile seawater is generally 200 to 300 mV lower than that in natural seawater (Johnsen & Bardel, 1986).

5.5.2 Weight Loss Data From Sterile Control

The mean weight losses from the sterile control can be used to calculate corrosion rates in mg per day per coupon during time

intervals between the removal of weight loss coupons.

Table recording mean rates of weight loss from the sterile control:

Period of time (Days)	Rate of weight loss per coupon (mg day ⁻¹)
0 - 15	$\frac{23.8}{15} = 1.59$
16 - 30	$\frac{(29.8 - 23.8)}{(30 - 16)} = 0.40$
31 - 110	$\frac{(93.9 - 29.8)}{(110 - 31)} = 0.81$

Rates of weight loss were again high at first, then decreased rapidly. The same trend was observed with the coupons and corrosion probe in the flow loop. In the sterile control a biofilm would not have formed but the accumulation of corrosion product was sufficient to slow down the corrosion rate, probably by limiting the diffusion of oxygen to the metal surface. From these results it is difficult to assess if a biofilm did accelerate steel corrosion in the laboratory-based rig.

5.5.3 Comparison Of Corrosion Rates From Weight Loss Data From The Sterile Control With LPR Scans

Acceleration factors were calculated from the weight loss data gained in comparison with weight losses predicted from LPR scans using Faradays law. Acceleration factors were determined from day 0 until the removal of the weight loss coupons.

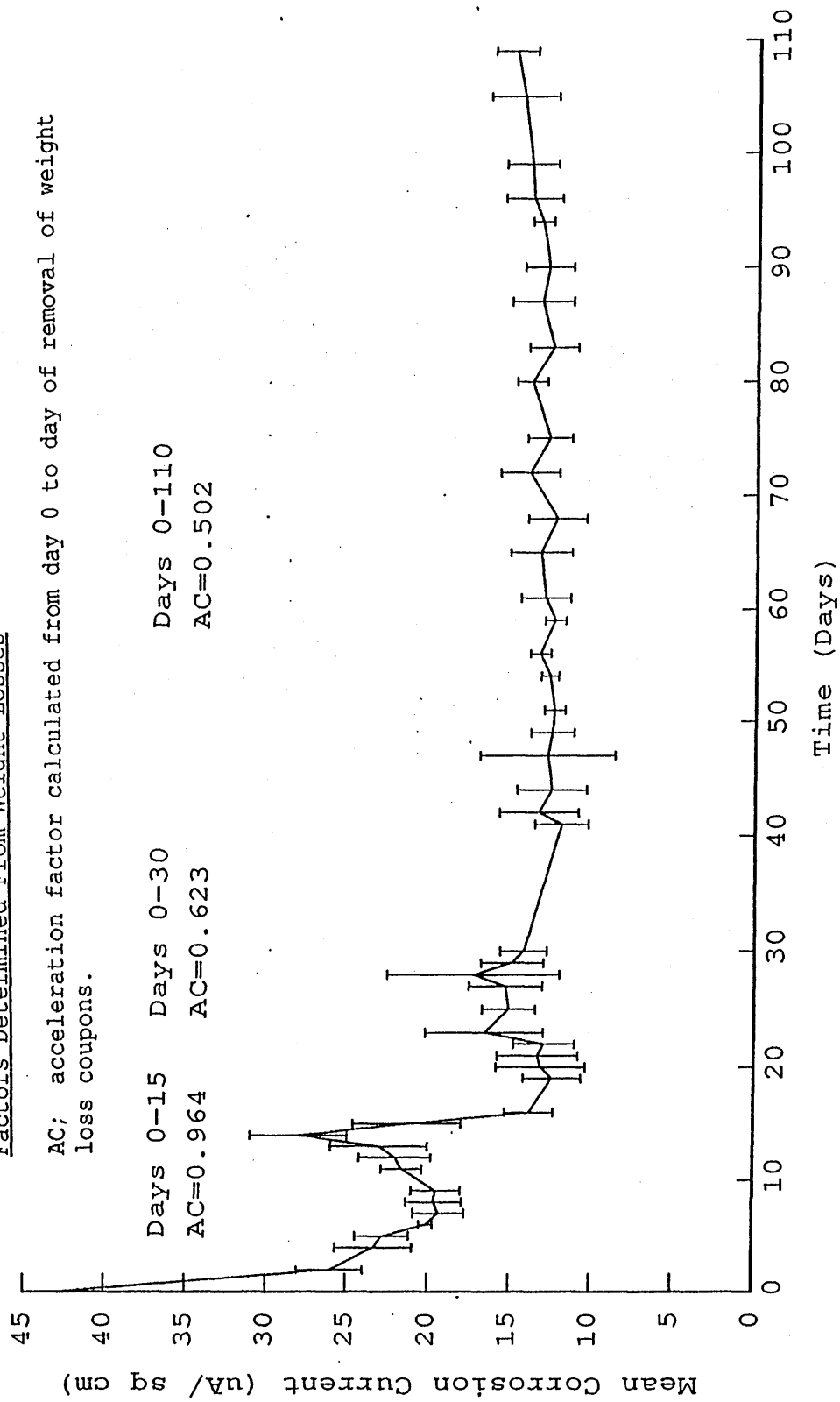
Table recording acceleration factors for the sterile control determined from day 0 until the removal of the weight loss coupons:

Period of time (Days)	Acceleration factor
0 - 15	0.964
0 - 30	0.623
0 - 110	0.502

These acceleration factors were applied to the mean corrosion currents and revised corrosion currents were plotted versus time (FIGURE 5.5.3.A). The revised corrosion current declined rapidly in the initial stages before stabilising.

FIGURE 5.5.3.A

Mean Corrosion Current From Sterile Control Adjusted With Acceleration Factors Determined From Weight Losses



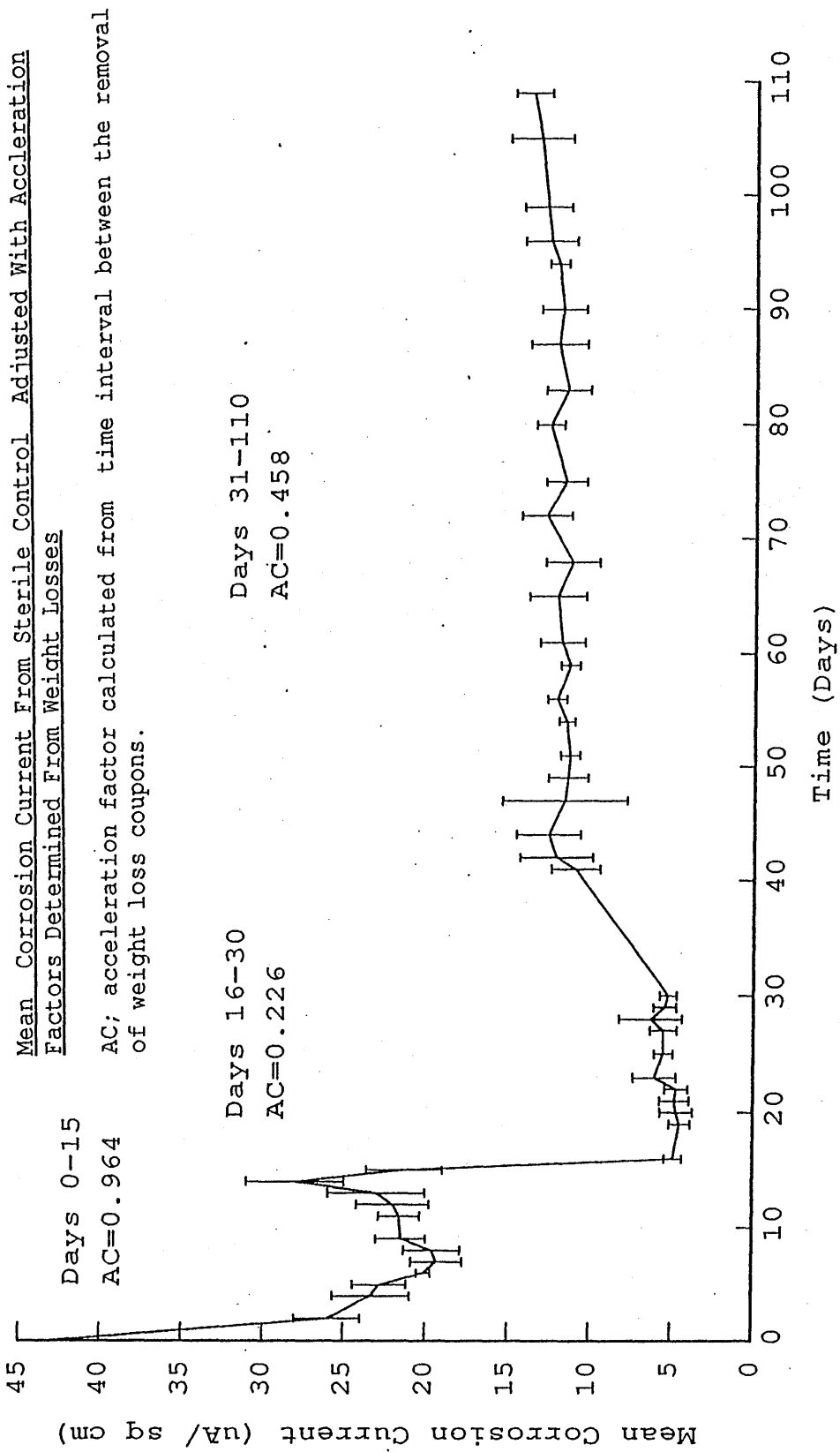
The corrosion data was manipulated to give acceleration factors for time intervals between the removal of weight loss coupons.

Table recording acceleration factors from the sterile control determined at time intervals between the removal of weight loss coupons:

Period of time (Days)	Acceleration factor
0 - 15	0.964
16 - 30	0.226
31 - 110	0.458

Revised corrosion currents were calculated using these acceleration factors and plotted verses time (FIGURE 5.5.3.B). This plot illustrates the difficulties in creating identical conditions in three different glass flasks in which the weight loss coupons were immersed. Although a careful check was made visually on the amount of air sparged into each flask it is possible that oxygen concentrations differed between the flasks causing a wide range of corrosion rates.

FIGURE 5.3.3.B



5.6 Full Polarisation Scans Of The Sterile Control

Full polarisation scans taken on different coupons in the sterile control show that neither anodic polarisation nor cathodic depolarisation have occurred. Cathodic depolarisation was not observed in the flow loop studies but anodic polarisation was. The biofilm on the steel surfaces in the flow loop may be polarising the anode, perhaps due to the formation of a sulphide film. The corrosion product that developed in the presence of marine microorganisms had a dense, black layer (perhaps of FeS) that was difficult to dislodge from the steel surface. Underneath it the steel was deeply pitted with surface layers dissolved away corresponding to the positioning of former tubercles. Visually the corrosion product that developed in the absence of bacteria differed as a black layer was not present and the brown corrosion product was very loose, it could be easily washed off. The surface of the coupons removed from the sterile control were not deeply pitted but appeared smooth to the naked eye.

The 'Autostat' programme was used to fit lines to the anodic and cathodic curves of the polarisation scans, giving specific values to the Tafel constants. Acceleration factors were calculated by using the Stearn-Geary relationship and comparing these Tafel constants to the assumed values of $120 \text{ mV decade}^{-1}$.

Table of acceleration factors calculated from Tafels constants derived from full polarisation scans of the sterile control:

Day	b_a	b_c	Acceleration factors
14	68.0	404.7	0.97
30	85.8	292.9	1.11
47	75.6	365.5	1.04
80	101.0	349.2	1.31
110	103.4	311.1	1.29

Changes in these acceleration factors with time differ from those derived from weight loss data. As already mentioned there are probably flaws in trying to use one electrochemical corrosion measurement to calibrate another.

5.6 EXPERIMENTAL CONSIDERATIONS AND LIMITATIONS OF THE FLOW LOOP

The flow loop was designed to simulate the marine environment and facilitate the investigation of the early stages of biofouling and colonisation of the fouling layer by SRB. In a laboratory flow loop important parameters that are known to affect the speed, nature and extent of biofilm development can be controlled. Some of these major parameters which were controlled include: temperature, ionic composition of the seawater, pH, flow characteristics, oxygen concentration and light supply. The control of these important parameters enabled relatively stable conditions to be maintained, not subject to changes of season as in the real marine environment.

Artificial seawaters such as Instant Ocean may be used to mimic the basic ionic composition of 'average' seawater but they lack the complex organic macromolecules unique to real seawater. Metal surfaces immersed in real seawater are covered in a conditioning film of spontaneously adsorbed macromolecules (Fletcher & Marshall, 1982A; Niehof & Loeb 1974). Surfaces immersed in seawater lacking organic matter do not become conditioned (Niehof & Loeb, 1974). Conditioning of a surface favours the settlement of planktonic bacteria. It is possible

that initial stages of biofilm development may have been influenced by the use of an artificial seawater. The use of aged, filtered seawater would have overcome this, but artificial seawater is much more convenient.

The circulating seawater was sparged with oxygen to simulate marine conditions in which SRB and other obligate anaerobes proliferate in anoxic regions of a biofilm adjacent to the metal surface. Numbers of viable SRB detected and rates of sulphate reduction within the biofilm on coupons immersed in the reservoir were too low. It was considered, in retrospect, that the seawater should have been sparged with nitrogen, and reducing agents such as ascorbic or thioglycollic acid (Postgate, 1984) added to encourage the development of low redox potentials. This would have altered the nature of the study, perhaps representing conditions in water injection systems, but may have amplified any effects of SRB colonisation on rates of steel corrosion.

The growth of sessile bacteria in a biofilm rather than planktonic ones is encouraged by a short residence time of seawater. The residence time used in the flow loop was very long. A total of five litres of inoculum was added each week to a volume in excess of 100 l circulating in the loop. A short residence time could have been achieved by the continual displacement of 'spent' seawater from the reservoir via an

overflow pipe. An investigation into biofilm formation (McCoy, Bryers, Robbins & Costerton, 1981) using a 'tubular recycle reactor' resulted in a mean residence time of 3.33 - 5.3 hours and eleven litres of effluent water was produced per hour.

Inoculum from the two bacterial fermenters was added to the flow loop on batch fed basis twice a week. This may have resulted in cyclic variations in growth rates of bacteria within the flow loop. It would have been better to have added it on a continuous basis using peristaltic pumps.

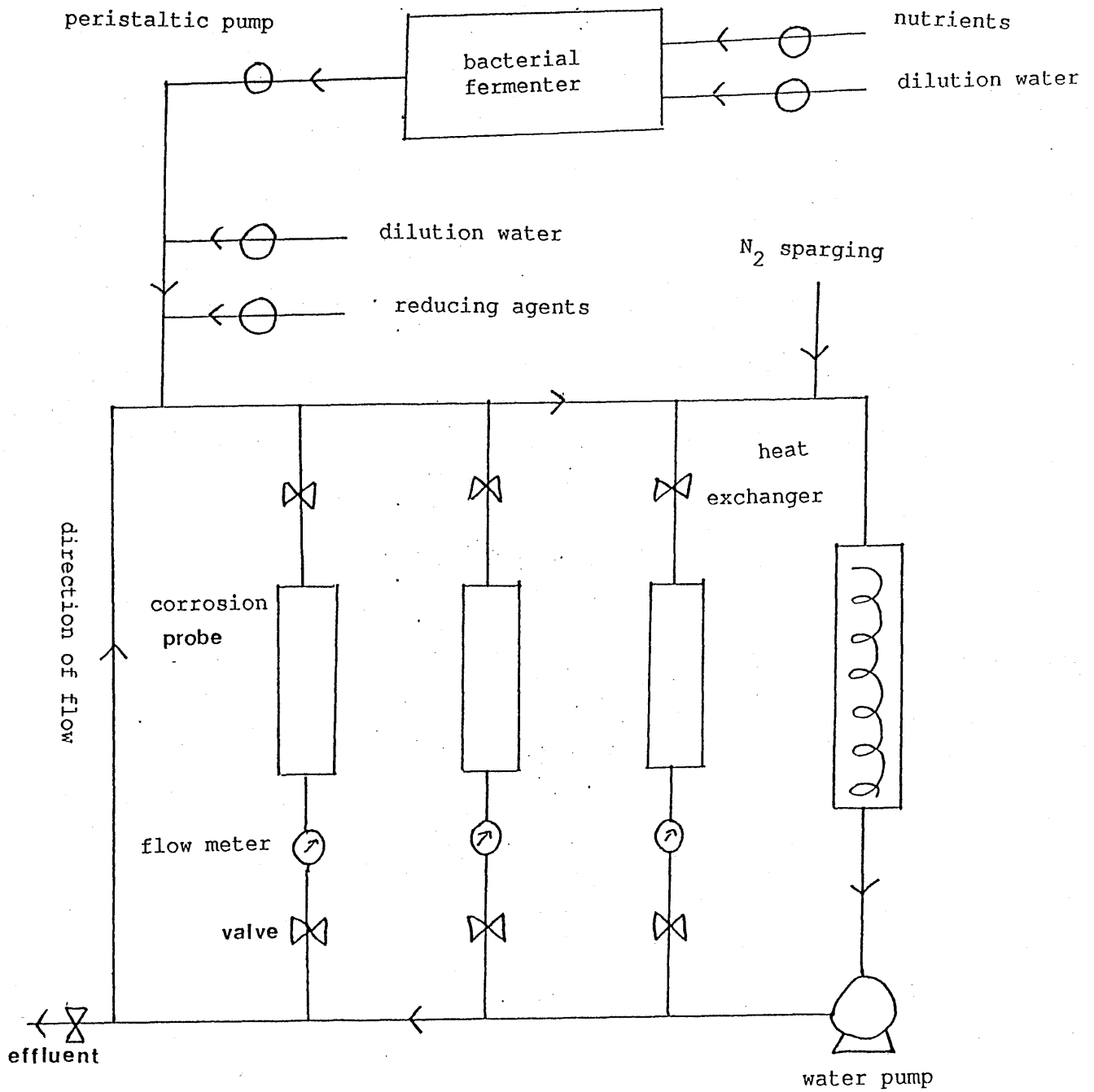
A modified recycling flow loop incorporating these suggestions is shown in FIGURE 5.6. In addition it would have been advantageous to have built in more test sections vertically into the flow loop.

The corrosion probe design was good for taking electrochemical corrosion measurements using the 'Autostat'. Each coupon had a surface area of almost 3 cm^2 which is much more than commonly used in the Robbins Type device. It was envisaged that the corrosion probe could be used for viable counts, radioassays and SEM observation with the aid of split-ring coupons but this ingenious idea was not practical as too many leaks resulted. The design of the probe and flow loop did not take into account the number of replicate coupons necessary to

conduct a biological study and gain meaningful data. It was impossible to build more than about a dozen steel coupons into the corrosion probe as they became misaligned resulting in leaks. The removal of coupons during this study was essential to conduct tests, this labourious procedure took several hours to complete whilst water could not circulate in the loop. By peering down the inside of a corrosion probe it was observed that the steel coupons did not fit flush with the PTFE rings which tended to bulge outwards under pressure. This would have affected in situ hydrodynamics within the probe, perhaps causing variations in biofilm development between coupons. This variability indicates the need for large numbers of replicate samples, which could not be achieved.

A better design of corrosion probe might have consisted of a tube with a series of ports containing easily removable steel coupons. There could be problems of small coupon size and non-flush fitting but these disadvantages are outweighed by their ease of operation and larger replicate numbers possible. It would have been advantageous to investigate the design of commercially available ported tube corrosion monitoring devices.

FIGURE 5.6

Design For Modified Re-circulating Flow Loop

5.7 CONCLUDING POINTS OF DISCUSSION

The combination of corrosion monitoring and a parallel investigation into the biological development of microbial fouling on steel was used to study the effects of microfouling on steel corrosion. Scanning electron microscopy revealed that the abundant biofilm covering all coupons was dominated by rod bacteria with a smaller proportion of cocci cells. Extracellular mucilage produced by microfouling was an integral feature of the biofilm. Heterotrophic bacteria were shown to be abundant in the biofilm by viable counts and viable SRB were present as well. An attempt was made to quantify the metabolic activities in the biofilm in terms of glucose turnover and sulphate reduction by radio-respirometric assays. These indices of biological activity did not show any major differences between the biofilm on cathodically protected coupons and that on freely corroding ones. Trends in biofilm development with time were not observed although this does not mean that they did not occur. Tests for biological activity were not employed early enough in the study (within the first few days of steel immersion) to detect the early stages of steel colonisation by microorganisms. The characterisation of the mature biofilm and the SRB activity within it is important to corrosion monitoring. The relationship between the numbers of viable SRB and amount of

steel corrosion is uncertain. Using the rather crude method of enumerating viable SRB, trends versus time of these anaerobes on coupons in the reservoir were not detected. But it should be appreciated that changes in numbers of viable SRB are not always reflected in changes in the corrosion rate.

Another aim of the investigation was to characterise the effects of different levels of cathodic protection on biofilm development. The structure of corrosion product and of calcareous scale is open, with many pores, so nutrient and oxygen-rich water is probably able to diffuse through it. These factors, combined with the large surface area, provide ideal conditions for biofilm development which was observed with scanning electron microscopy. Calcareous scale differs from the corrosion product which was much thicker, with a dense, pore-less layer adjacent to the metal surface. This may have encouraged the development of an anaerobic layer adjacent to the metal surface and higher numbers of viable SRB on freely corroding coupons than on cathodically protected ones. Two factors complicate discussion of the physical environment within calcareous scale: pH and cathodic hydrogen. The former factor was not measured with pH microelectrodes in this study, and it is not known how far a pH gradient extended through the scale. More cathodic hydrogen would have been produced by cathodically protected steel than freely corroding steel. SRB

are able to utilise cathodic hydrogen as an energy source. In this situation cathodic hydrogen and organic compounds (eg. metabolic products from the oxidation of glucose by heterotrophs) would have been available for use by SRB. It is not known if SRB use cathodic hydrogen in preference to other potential energy sources or if the supply of energy sources was limiting. In consequence it is impossible to tell whether the additional energy source of cathodic hydrogen would stimulate SRB growth or not. The biological tests employed were not able to detect any differences between the mature biofilm on steel protected at -850 mV S.C.E. and that on steel protected at -950 mV S.C.E..

Steel corrosion was monitored throughout the investigation in the laboratory by weight loss coupons and electrochemical methods. Corrosion rates of steel in the flow loop were initially very high, decreased rapidly and then stabilised. This was accompanied by a gradual increase in the corrosion potential. The gradual increase in the corrosion potential of the Holyhead corrosion probe indicates that its corrosion followed similar trends. It was anticipated that a subsequent rise in corrosion rates could be correlated with the proliferation of SRB within the biofilm. LPR results indicated an increase in the corrosion current with the corrosion probe but not with the coupons in the reservoir. Corrosion currents adjusted for weight losses indicate that the increase in corrosion rates, although

diminished in size were not totally an artefact of Tafel constants becoming increasingly inaccurate. The histogram of mean rates of weight loss calculated from the coupons in the reservoir shows a rise in corrosion rate in the latter part of the investigation. This rise cannot be correlated to an increase in heterotrophic activity within the biofilm or a proliferation of SRB measured by any of the biological indices used. It is known that SRB are able to depolarise the cathodic reaction but this was not detected by full polarisation scans. It is probably an over simplification to try to correlate a rise in corrosion rate with a proliferation of SRB without considering other factors such as the amount and state of sulphide-containing compounds on the steel surface.

In the absence of bacteria steel corrosion followed similar trends, decreasing from its initially high rate to a stable value. LPR results reveal that the corrosion rate started to increase in the latter part of the investigation. From these results the development of a biofilm does not seem to have influenced steel corrosion markedly.

These results indicate that although cathodic depolarisation was not detected, anodic polarisation was detected in the presence of a biofilm. Stimulation of the anodic reaction is attributed to iron sulphides deposited under anaerobic conditions in which SRB proliferate. The dense, black layer of corrosion product that developed may have contained iron sulphide and caused polarisation of the anode. It is very difficult to equate this with the lack of cathodic depolarisation which is a well documented feature of SRB in conjunction with iron sulphide.

6.0 CONCLUSIONS

The following conclusions can be drawn from the results gained in this investigation of microbially induced steel corrosion in the marine environment:

1, Scanning electron microscopy indicates that the appearance of the mature biofilm was similar on freely corroding coupons and on cathodically protected ones. Indeed, the appearance of the biofilm was not influenced by the level of cathodic protection employed or the length of exposure.

2, Similar numbers of viable heterotrophic bacteria were found on steel coupons regardless of the application of cathodic protection at different levels, or the period of exposure. Viable counts of SRB show that generally more were present on freely corroding coupons than on cathodically protected ones.

3, Corrosion monitoring using LPR and weight loss coupons reveal that usually corrosion rates were very high initially, rapidly decreased, and then stabilised. A subsequent rise could not be correlated with cathodic depolarisation or the proliferation of SRB.

4, In the absence of bacteria, corrosion rates followed similar trends to those observed when a biofilm was present.

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APPENDICES

APPENDIX A

Calibration Curve For Carbohydrate Assay

The following graph (FIGURE A) is a calibration curve for the quantification of glucose concentrations using the spectrophotometric assay.

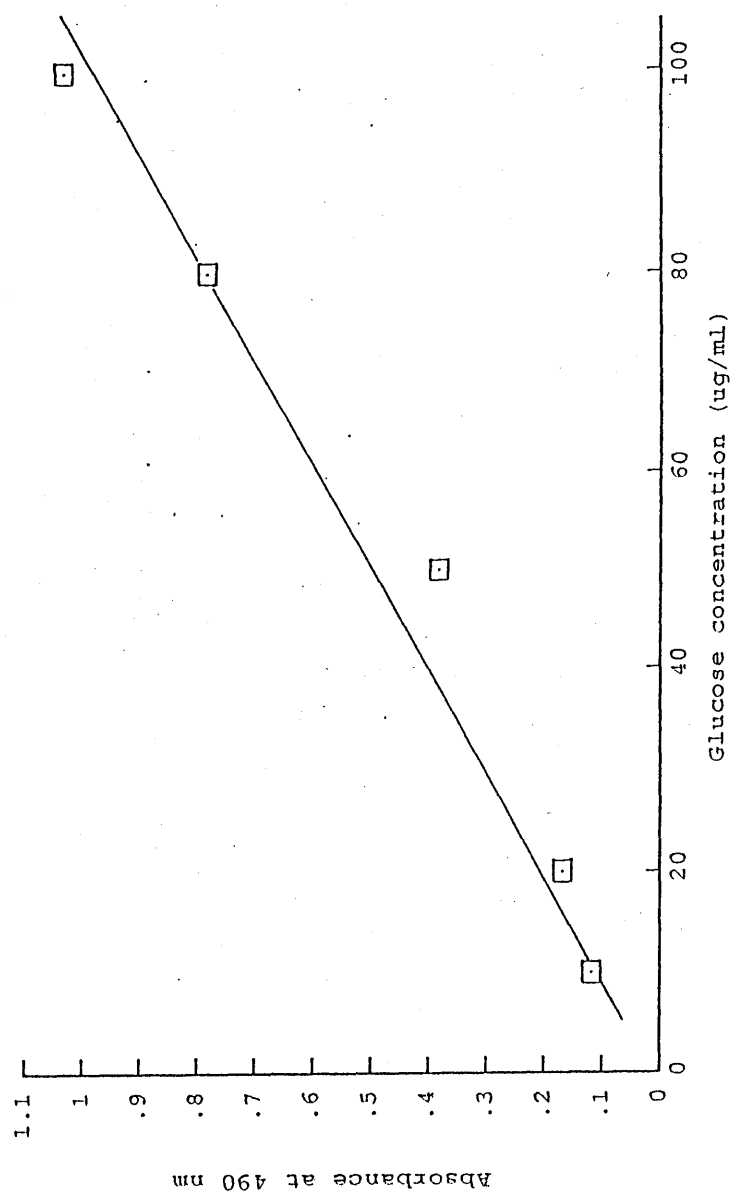
APPENDIX B

Making Polyacrylamide Gel Luggins

Capillary tubes filled with potassium chloride saturated polyacrylamide were used to make contact with the calomel reference electrode and the electrolyte (seawater) in the flow loop. These gel luggins were made in the following manner. Into a beaker containing 50 cm³ of distilled water was added an equal volume of 3.8 M potassium chloride solution. To this was added 15 g of acrylamide, 0.76 g of methylene bisacrylamide and 0.3 g of ammonium persulphate. The mixture was deaerated by sparging

FIGURE A

Calibration Curve For Quantification Of Carbohydrate Concentrations



it with nitrogen gas for 2 minutes. Glass capillary tubes were stood in the mixture and tapped to remove any bubbles. The catalyst, 2 cm³ of tetramethylene diamide, was added to polymerise the mixture.

The calomel reference electrode was placed in a syringe body containing 3.8 M potassium chloride solution. The syringe body was connected to the salt bridge by a length of dialysis tubing.

APPENDIX C

Treatment To Remove Surface Grease From Steel Coupons

Annular steel coupons were immersed in acetone followed by ethanol for one hour, then allowed to air dry. The probe was carefully assembled without touching the inside face of the coupons. Cylindrical steel coupons were mounted then immersed in an aqueous solution of detergent (5 % Decon 90) for 5 hours. Acetone was not used as it degraded the perspex tabs used to mount the coupons. The coupons were then immersed in ethanol for an hour and allowed to air dry.

APPENDIX DElectrolytic Cleaning Of Weight Loss Coupons

Loose corrosion product was removed from the coupon and it was then immersed in an aqueous solution of tri-ammonium citrate (10 % W/V). The coupon was connected to a potentiostat as the working electrode and held at a cathodic potential of -1.083 V against a calomel reference electrode. The circuit was completed with a platinum auxiliary electrode. This electrolytic treatment was continued until all visible corrosion product was removed, usually 1 - 2 hours. The coupon was dried and weighed and treated again for half an hour. This process was repeated until there was no further weight loss. Triplicate control coupons were also electrolytically cleaned to assess the weight loss due to this process. The annular steel electrodes used in the corrosion probe lost an average of 0.0013 g per hour and the cylindrical coupons lost on average 0.0004 g per hour during electrolytic cleaning.

APPENDIX E

Construction of Quench Curves To Quantify Radioassays

During liquid scintillation radioactivity caused excitation of compounds (fluors) which fluoresce (scintillate). The light emitted during scintillation is detected by a photomultiplier in the scintillation counter which converts the photons into an electric pulse, the size of which is proportional to the energy in the original radioactive event. The energy transfer from the radioactive event to the photomultiplier is not 100 % efficient and energy is lost during the transfer stages. One type of energy loss is chemical quenching which occurs if a component of the sample interferes with the energy transfer between different fluors in the scintillation cocktail. The degree of quenching in terms of counting efficiency was determined for each sample as they are not chemically identical. A calibration curve enabled the relative count (ie. CPM) to be converted into an absolute count (ie. DPM).

Construction Of ^{35}S Quench Curve

Into five scintillation vials were put 4 cm^3 aliquots of scintillation cocktail (Beckman Ready Value) and each vial was checked for abnormal levels of background radiation. To each

vial was added 8,700 DPM of standard dioctyl sulphide (Amersham) and the relative count (CPM) of each vial determined using the scintillation counter (Beckman LS7000). The quenching agent, carbontetrachloride was added to each vial at 5 ul increments and the relative count determined until 45 ul had been added in total. Five replicates were performed.

The radioactivity was counted on two different channels. Channel 1 covered the whole of the unquenched B-energy spectrum. Channel 2 was set to cover half of the energy section.

The ratio of these counts:

$$\frac{\text{CPM channel 2}}{\text{CPM channel 1}} = \text{Sample channel ratio (SCR)}$$

The counting efficiency after each addition of quenching agent was determined. The counting efficiency is the CPM from channel 1 divided by the DPM of known standard (8,7000 DPM) multiplied by 100.

Construction Of ^{14}C Quench Curve

This method was similar to that used for the ^{35}S quench curve but 8,7000 DPM of standard n-hexadecane (Amersham) was added to each vial of scintillant.

Calibration curves were plotted (refer to FIGURES E.1 and E.2) of mean counting efficiencies against mean SCR. The CPM from radioassays can be converted to DPM as each count is given a SCR so the counting efficiency can be determined using the calibration curve.

FIGURE E.1

Quench Curve For S^{35} Using Channel Ratio Method

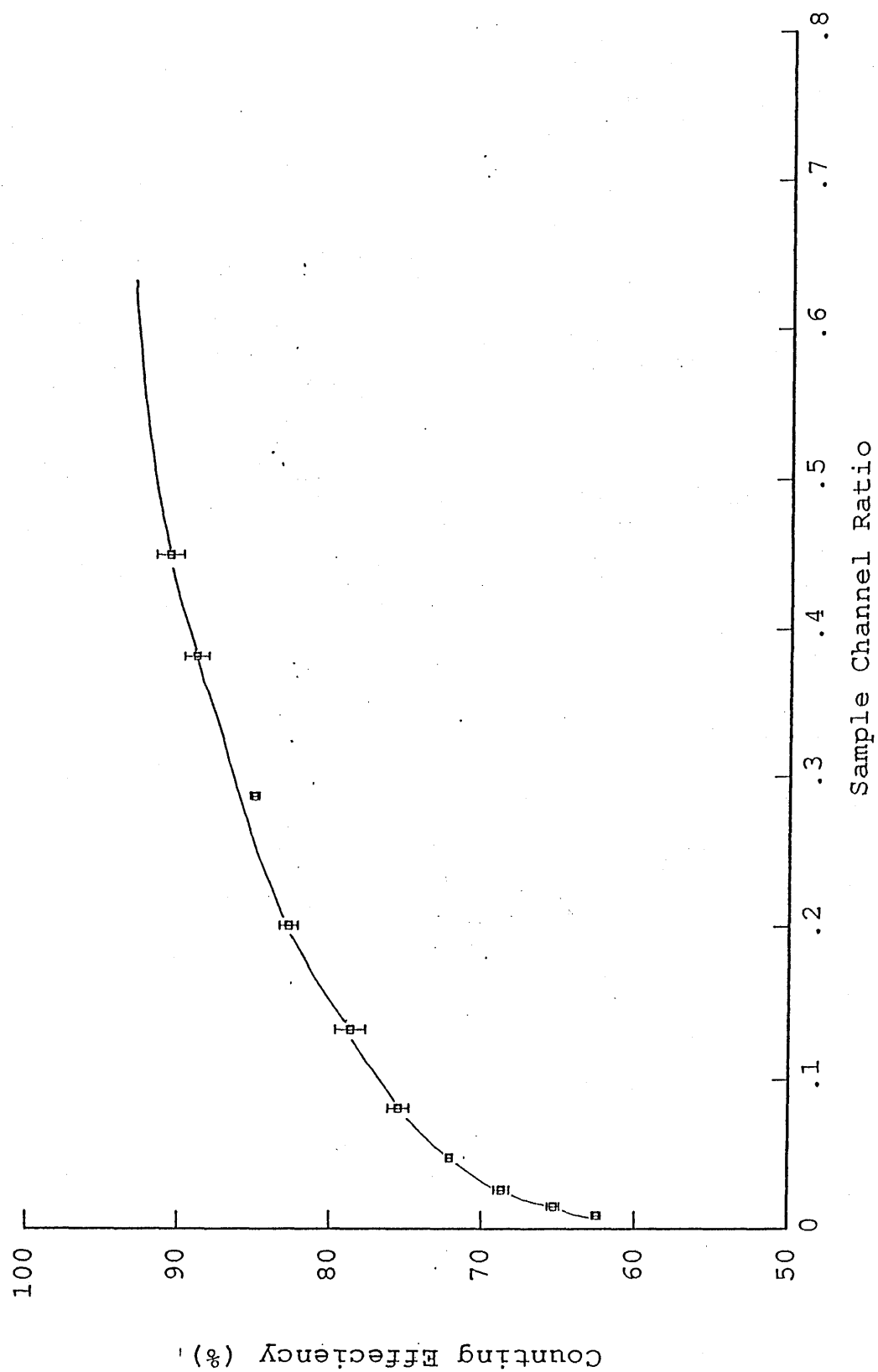
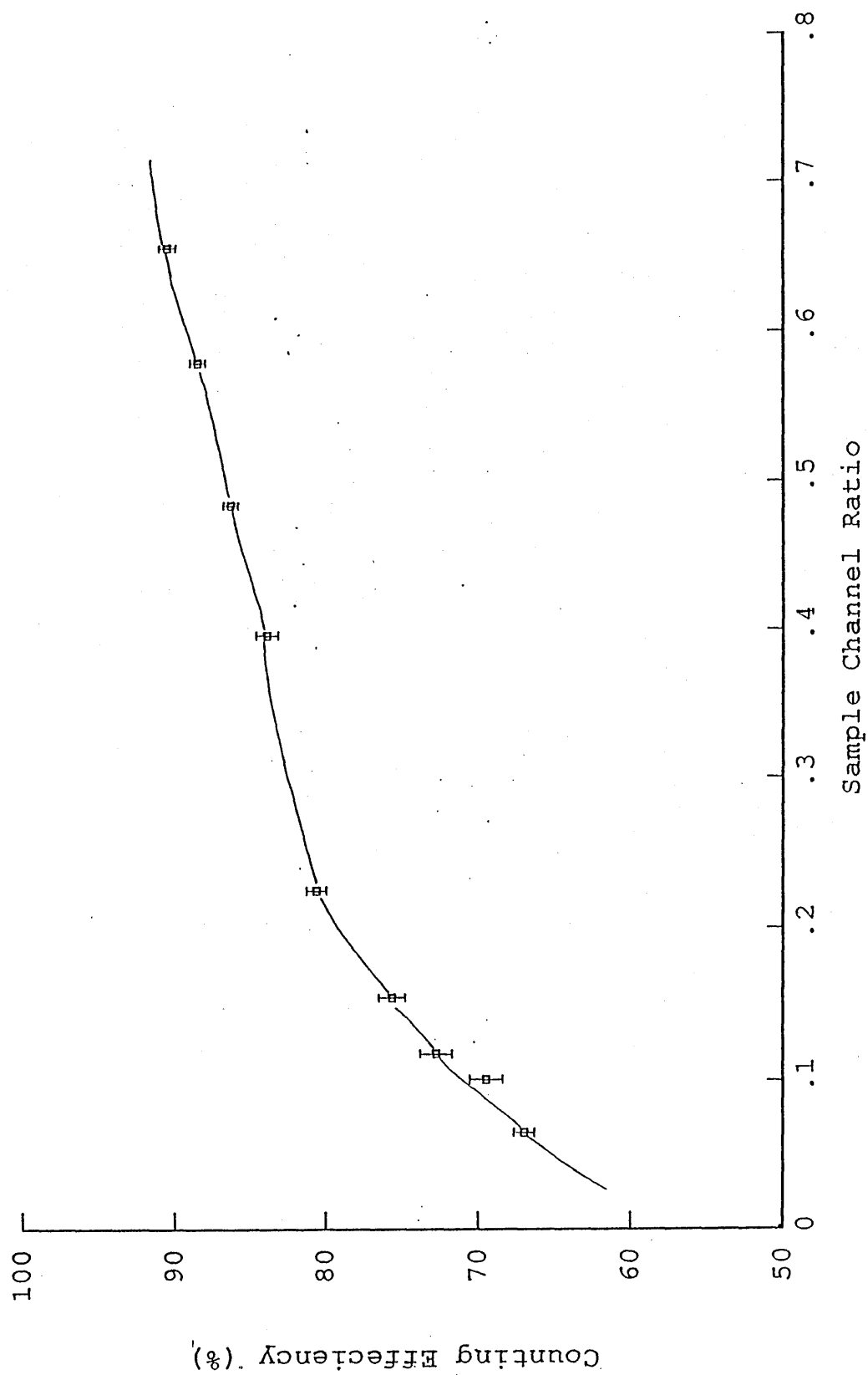


FIGURE E.2

Quench Curve For C^{14} Using Channel Ratio Method



APPENDIX F

INVESTIGATION INTO THE EFFECTS OF EMULCID ON THE CORROSION OF STEEL

The sterile flow loop was to be sterilised with a solution of Emulcid before filling with sterile seawater and then if necessary resterilised with Emulcid. Emulcid is a low toxicity biocide against bacteria and fungal spoilage in the wet state. It is used as a sterilant in metal storage tanks and holding tanks. It is also employed in metal working fluids.

Methods

Weight Loss Study

Ten cylindrical steel (50 D) coupons (12 mm diameter by 4 mm depth) were polished and degreased, and their weights determined. Five of the coupons were placed individually in universal bottles, each containing 15 cm³ of Emulcid solution (1 % in distilled water). The remaining coupons were placed in distilled water as a control. The coupons were immersed for 62 days and then electrolytically cleaned in tri-ammonium citrate before weight losses were determined.

Electrochemical Corrosion Studies

Coupons of 50 D steel were polished and degreased before use. Reproducible results were obtained more easily by initially cathodically polarising each coupon by holding it at -800 mV below the rest potential to clean the surface. Coupons were immersed in an aerated Emulcid solution (1 % in distilled water) plus 0.2 % sodium chloride. The salt was added to provide a great enough concentration of ions to conduct a current. The resistance of distilled water alone was too high to conduct sufficient current.

LPR scans (each lasting 4 minutes at a scanning rate of 10 mV min^{-1}) were performed on each coupon 10, 15, 20 and 25 minutes after cathodic cleaning was completed. Five control coupons were also tested in aerated 0.2 % salt solution.

Results

Weight Loss Study

During the investigation the coupons immersed in distilled water corroded discolouring it. In contrast the coupons immersed in Emulcid solution did not show any visible signs of corrosion. Indeed they were still bright and shiny after 62 days, the

solution was not discoloured.

The following table records the mean weight loss of steel coupons immersed in Emulcid solution and in distilled water:

	COUPONS IMMERSSED IN:	
	DISTILLED WATER	EMULCID SOLUTION
MEAN WEIGHT LOSS		
(g cm ⁻¹ yr ⁻¹)	0.0662 ± 0.0004	4.46 ± 4.91 x 10 ⁻⁴
(mm yr ⁻¹)	0.0838 ± 0.0005	5.65 ± 6.29 x 10 ⁻⁴

Each value is the mean ± of 5 determinations.

Electrochemical Corrosion Study

The following graph (FIGURE F.1) shows the results of LPR scans done on steel coupons immersed in Emulcid solution plus 0.2 % sodium chloride and in 0.2 % sodium chloride.

Discussion

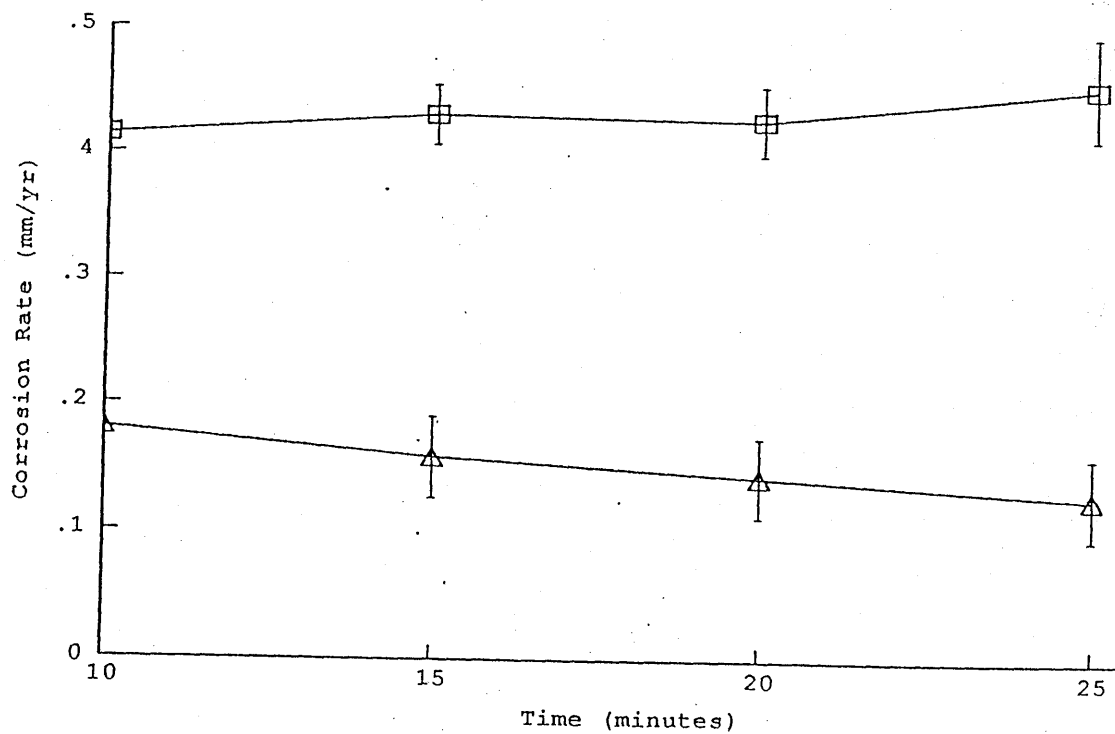
The weight loss study shows that the corrosion rate of steel in distilled water is over a hundred times greater than that in Emulcid solution. Inhibition of steel corrosion in the long term by Emulcid would appear to render it unsuitable for use in the

FIGURE F.1

Graph Showing Effect Of Emulcid On Rate Of Steel Corrosion

Each point is mean $\pm$ S.D. of 5 determinations.

□ , control; △ , 1% Emulcid plus 0.2% NaCl.



sterile flow loop. The Emulcid solution was only to be used occasionally to sterilise the flow loop and would only be circulating for 4 hours. Using electrochemical techniques the rate of steel corrosion in 0.2 % salt solution was significantly higher (approximately twice as much) than that in Emulcid plus salt solution. Emulcid inhibited the corrosion of steel in the short term and in the long term. The LPR scans showed that the corrosion rate of steel steadily decreased in Emulcid solution during the 25 minute interval after cathodic cleaning. This may be due to the build-up of an organic film of Emulcid on the metal surface.

Electrochemical Study Into the Residual Effects Of Emulcid On Steel Corrosion

A further investigation was conducted to determine if the sterilisation of the flow loop with Emulcid solution would influence the corrosion rate of the steel corrosion probe once the sterilant had been replaced with seawater.

Methods

Twelve cylindrical 50 D steel coupons were polished and degreased. Six were placed in a beaker containing aerated Emulcid solution (1 % in distilled water) for 4 hours. This

solution was replaced with distilled water in which the coupons were left for an hour. This treatment was repeated to simulate the conditions in the flow loop when Emulcid was used to sterilise it. The Emulcid pretreated coupons and six control ones were put into aerated salt solution (3.5 % in distilled water) and left to corrode for 14 days.

The coupons were removed and electrochemical methods used to determine their corrosion rates. Each coupon was placed in a cell containing aerated salt solution and left for 15 minutes for the rest potential to stabilise. Three LPR scans (each lasting 4 minutes at a scanning rate of 10 mV min^{-1}) were done at 5 minute intervals. The Emulcid pretreated coupons were treated with Emulcid solution as before. All of the coupons were again immersed in aerated salt solution for a further 14 days. The coupons were removed and the electrochemical measurements repeated.

Results

The following graph (FIGURE F.2) shows rates of corrosion of steel coupons pretreated with Emulcid solution and of untreated coupons tested in 3.5 % salt solution.

Discussion

It has been shown that although Emulcid Solution inhibits steel corrosion the effect is not residual. This investigation to simulate the sterilisation of the flow loop showed there was no significant difference between the mean rates of steel corrosion of untreated control coupons and those pretreated in Emulcid. Emulcid is a suitable biocide for use in the flow loop.

FIGURE F.2

Graph Showing Residual Effect Of Emulcid On Rate Of Steel Corrosion

Each point is mean $\pm$ S.D. of 5 determinations.

□ , untreated control coupons; △ , Emulcid pretreated coupons.

