A Study of the Mechanisms Involved in Corrosion Produced by Micro-organisms within Fuel Systems

By

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ABSTRACT

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Fuel tank sludge yielded bacterial, yeast and fungal isolates; several were partially identified. The growth characteristics of mixed and pure cultures, especially Cladosporium resinae, were examined. The corrosion effect of C. resinae was described for stainless steel, mild steel, cupronickel and aluminium alloys in modified sea water or Turner's solution with n-undecane. Growth was assessed by dry weight and subsequent pH, acid content, redox and corrosion potentials in the aqueous environment. Corrosion was assessed by weight loss, pit depths and microscopic appearance. Corrosion rates of the culture filtrates were also determined electrochemically. A laboratory mixed culture and particularly a sludge sample were more corrosive than C. resinae, though the organisms, the metal and the environment all interacted with each other and collectively affected the result. In addition the relative corrosivity was dependant on the exposure time.

Corrosion was more severe below adherent growth; pits, channels, blisters or intergranular corrosion being dependant on the metal under test. In the presence of C. resinae weight loss was generally found to be more severe in Turner's solution than in modified sea water, the losses being up to nine times greater than in the control. Using the linear polarisation technique corrosion rates were continuously monitored in and below the interfacial growth of C. resinae and this showed that both a modified environment and particularly adherent growth enhanced corrosion.

Several organic acids produced by C. resinae were corrosive to the four metal alloys, this seeming to depend on the anion rather than the subsequent pH. C. resinae sequestrated several metal ions, whose presence showed variable inhibition of germination, growth and adherence, and modified acid production and enzyme activity of the fungus.

C. resinae exerted a corrosive effect which depended on the metal, the environment, adherence and exposure time. The action of differential aeration cells, localised concentration of corrosive products and selective removal of metal ions probably played a role in corrosion by C. resinae.

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Dedicated to Mum and Dad

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CHAPTER 1

INTRODUCTION

1.1 Historical Development and Current Awareness of Microbial Corrosion

The phenomenon of microbial corrosion was reported in 1891 by Garrett[62] who postulated that increased corrosion of a lead covered cable could be attributed to the metabolites of bacterial activity, in this case ammonia, nitrites and nitrates. By 1910, Gaines[61] produced evidence which clearly indicated that iron and sulphur bacteria were responsible in part for the corrosion of buried ferrous metals. In many instances the corrosion was also associated with the presence of oxygen. The importance of sulphate reducing bacteria to corrosion was established in 1934 by von Wolzogen Kuhr and van der Vlugt[213] who reported on the corrosion of ferrous metal buried in an anaerobic clay soil. They proposed that the organisms played a direct role in the corrosion mechanism, utilising cathodic hydrogen and thus stimulating the anodic dissolution of iron. A variety of both anaerobic and aerobic microorganisms continued to be implicated in corrosion; in 1953, Uhlig[210], reported on the role of organisms, such as the slime forming bacteria, fungi, algae, protozoa and diatoms in corrosion and commented that their primary contribution to this process consisted of the formation and maintenance of differential aeration and concentration cells.

Corrosion of airframe structures became cause for major concern in the early 1960's; the first significant problem was that of sulphate reducing bacteria in gasoline fuel systems[179]. The sulphides produced caused a number of pump failures in Valetta and Hastings aircraft[120] A dramatic increase in microbiological fuel problems occurred in the aircraft industry with the introduction of jet aircraft and the concomitant change from aviation gasoline, used in piston engined aircraft, to turbine fuels.

Most modern aircrafts use integral fuel tanks, i.e. built in sealed compartments. Conditions for growth, e.g. temperatures around 30 - 35^{o}C, free water, kerosene, oxygen and trace elements, generally allow growth of a variety of fungi, yeasts and bacteria[179] with the fungus, Cladosporium resinae, almost always predominant[56, 79].

The effects of such contamination include blockage of fuel

pipes, gauges and filters by a thick sludge. The main danger is the extremely corrosive conditions that exist underneath fungal mats which are attached to the aluminium alloy tank[179]. Penetration of protective coatings and wing planks can be quite rapid and there have been several cases where complete perforation has occurred; the first indication of a problem being a leakage of fuel from the wing.

Ten out of 72 samples from jet aircraft fuel systems were found to be contaminated[55] and yielded 43 isolates; 6 Gram positive cocci, 22 Gram positive bacilli, 9 Gram negative bacilli and six fungi (Table 1.1). Edmond and Cooney[55] found that only five of the 43 isolates grew well in a mineral medium with JP-4 fuel as the sole carbon source. Three of these were Pseudomonas aeruginosa and two were Cladosporium isolates. Although Bacillus species represented 37% of the isolates in the study none of them were capable of utilising hydrocarbon and Edmond and Cooney dismissed them as being transient contaminants or mere survivors in the fuel system. It is more likely that by utilising the by products of hydrocarbon breakdown, e.g., alcohols, aldehydes, acids, hydroperoxides, phenols, carbonyls, ketones and esters[221] they represent part of the ecosystem and contribute to fuel system malfunctions. Various aspects of control have been reviewed by Park[154] and Karrick[107]. The problem is now generally considered to be under control but continuing care is necessary for this to be maintained.

The recent widespread introduction of gas turbine powered warships has resulted in extensive evidence of microbial growth. Many aspects of the growth have been tackled but very little effort has been made to evaluate the potential corrosion of metals used in naval construction. The problems of ships' fuel system contamination is discussed later.

There have been several reviews[14,96,101,132,137,138,157,178,204] published on microbial corrosion and it is significant that in the majority of case histories reported the role of sulphate reducing bacteria is mentioned. In 1971, Hill[83] reviewed the extensive problem of corrosion of machinery components and finished articles. The mechanisms were attributed to aggressive metabolites such as organic acids. Microbial corrosion is an interdisciplinary subject which

requires an understanding of the many areas involved,

Table 1.1

Identification of Isolates from Jet Fuel Systems

Identification	No. of isolates
Staphylococcus aureus	1
S. epidermidis	1
Sarcina flava	4
Bacillus spp.	16
Unidentified bacilli	4
Pseudomonas aeruginosa	3
Brevibacterium spp.	2
Streptomyces spp.	2
Flavobacterium spp.	1
Herellea spp.	3
Penicillium spp.	2
Rhodotorula spp.	2
Cladosporium spp.	2

for example, microbiology, biochemistry, chemistry and metallurgy. The literature often lacks a complete discussion regarding the data presented, weaknesses reflecting a researcher's lack of expertise in specific areas. In order to predict and control corrosion there must be more collaboration between qualified experts from various disciplines, for example the construction engineer should take into account potential biological problems as well as chemical and physical considerations. In order to aid this interdisciplinary approach, UMIST[110] have recently developed 'ranking tables' to emphasise microbial corrosion aspects of various environments.

In addition, the techniques for dealing with many of the organisms involved have been adapted primarily from clinical microbiology and are generally inadequate for dealing with the

organisms involved in microbial corrosion; that is, their isolation, growth, identification or evaluation of their role in the corrosion process. Hill[89,90] has suggested a new approach for the investigation of microbial spoilage and corrosion with the aim of developing a series of relevant tests giving each spoilage organism a 'numerical profile' which will convey significant characteristics as well as identity.

Despite all the microbial corrosion work which has been carried out in the past and the protection techniques which have been developed, microbial corrosion incidents continue to occur. In 1977, a survey[214] showed that with regard to the incidence of corrosion British Industry could be divided into two main groups. Firstly, the gas, oil, water supply and iron and steel industries form a group which, having in the past suffered considerable trouble from microbial corrosion, have set up research laboratories to study and advise on corrosion problems and now routinely protect all underground pipelines and submerged metal structures. Chemical and food manufacturing firms also pay due regard to microbial corrosion. Likewise the aircraft industry is very corrosion conscious and having suffered from microbial fuel contamination problems in the past has introduced maintenance procedures which have almost eliminated their microbial corrosion problems. Secondly, in the engineering, building and construction, shipping and parts of the manufacturing industry there is still cause for concern. A continuing occurrence of what ought to have been recognised as typical microbial corrosion situations was reported. They involved underground pipelines, fuel storage tanks, ships and marine installations and circulating or enclosed oil and water systems. In nearly all cases where microbial corrosion was reported the structures were unprotected or the protection was unsatisfactory either because of poor application, poor quality, or damage caused during installation.

It is difficult to assess the economic significance of microbial corrosion. Corrosion failures are often due to several mechanisms, including microorganisms, so that to attribute costs to microbial corrosion alone is not always feasible. The cost of protective coatings and cathodic protection may only be about 1% of

the contract cost of certain pipelines but this is a worthwhile expenditure as the cost of replacing gas and water distribution mains has been given as £300 - £400 million per year[214]. However, until industries accept the reality of microbiological factors in corrosion, problems such as high and unnecessary replacement costs will persist.

1.2 The Electrochemical Nature of Corrosion

Most metals exist in nature in the combined state. Their ores or natural compounds must be subjected to pyro-metallurgical and chemical refining processes involving an input of energy to bring them into the metallic state. It follows that the metallic state represents a condition of high energy content. It is to be expected that the natural tendency of metals is to combine with other substances and to revert to a lower energy state with a corresponding release of energy. This decrease in free energy is the driving force of corrosion reactions[37].

The corrosion of metals in contact with aqueous solutions is now firmly established as an electrochemical process, detailed accounts of which are well documented in the literature[27,58,118,186]. The present discussion has been limited to that required to interpret the microbiological involvement in the processes.

The initial reaction of a metal immersed in an aqueous system is dissolution of that metal as metallic cations releasing an excess of electrons, for example:

$$Fe \longrightarrow Fe^{2+} + 2e$$

If no mechanism were available to remove the electrons, the system might be expected to come to equilibrium, the positively charged ions being attracted to the negatively charged metal. In nature this does not happen very often as various mechanisms operate to continually remove the electrons. In near-neutral solutions in the presence of oxygen, electrons are consumed at the cathode by the reduction of oxygen to hydroxyl ions:

$$O_2 + 2H_2O + 4e \longrightarrow 4OH^-$$

The overall reaction is the formation and subsequent precipitation of insoluble products formed by the reaction of ferrous ions (liberated

from the anode) with hydroxyl ions, and frequently oxygen in solution, for example[27]:

$$Fe^{2+} + 2OH^- \longrightarrow Fe(OH)_2$$

When the upper limit of solubility of ferrous hydroxide is reached, a white product will start to precipitate from solution which, in oxygenated conditions, will be rapidly oxidised to form ferric hydroxide,

$$4Fe(OH)_2 + O_2 + 2H_2O \longrightarrow 4Fe(OH)_3$$

This is unstable and subsequently loses water to form hydrated ferric oxide, FeOOH, or Fe_2O_3 (red rust),

$$Fe(OH)_3 \longrightarrow FeOOH + H_2O$$

Such insoluble products may be loose and bulky or become firmly adherent to the metal surface to which they are protective to a greater or lesser extent.

In the absence of oxygen the usual cathodic reactions are the reduction of hydrogen ions or water;

$2H^+ + 2e \longrightarrow H_2$ <u>or</u> $2H_2O + 2e \longrightarrow H_2 + 2OH^-$

Where the hydrogen ion concentration is great enough, i.e. at low pH values, its reduction occurs readily (both in the presence and absence of oxygen). Hydrogen evolution from the surface of the metal will occur. In neutral solutions, in the absence of oxygen, the hydrogen remains on the surface of the metal and the rate of reaction becomes so low that it is ineffective in removing electrons and the system reaches equilibrium; this is known as polarisation. If the hydrogen was removed (depolarisation) corrosion would be expected to occur again. This mechanism forms the basis of the cathodic depolarisation theory discussed later.

The overall process is determined by factors such as the heterogeneity of the surface, random failure of a tenuous pre-existing oxide film, microgeometry of the metal surface, variation in oxygen concentrations in the immediate environment (causing differential aeration cells) or variation in concentrations of aggressive anions (causing concentration cells). These phenomena usually encourage localised pitting corrosion, which is also exacerbated by the action of certain microorganisms.

1.3 Forms of Corrosion

The form, appearance and distribution of the attack, in particular the degree of localisation and the topography of the affected area are all important characteristics of different types of corrosion[27]. Identification of the form of corrosion is helpful in understanding its cause and in suggesting ways of decreasing attack. It is not uncommon for several forms of corrosion to occur at the same time, although one form usually predominates[36].

General corrosion is attack to an approximately uniform depth of the whole of the surface under consideration, but it may vary from the very uniform attack often caused by acids to the more uneven attack such as may result from atmospheric exposure[30]. The attack may be rapid or slow and may leave the metal clean or coated with corrosion products[36]. This form of corrosion is the easiest to deal with since due account of it can be made at the design stage of a component.

Semi-local corrosion (even or uneven) is somewhat similar in character to general attack, but is confined to areas of restricted size which are nevertheless large compared with the depths of the attack. While the attack remains of this type there is not a great danger of perforation of the metal by corrosion.

Localised corrosion can take the form of either pits on an otherwise smooth surface of metal or increased corrosive attack at crevices. Outwardly both forms of corrosion are similar, but the causes are not necessarily the same[212]. The term pitting is used when the area of attack is so localised that the width is of the same order or less than the depth of attack[69]. In such cases the possibility of the metal failing by perforation is high and hence the depth of attack is of major importance. The danger of this type of attack may be further defined by subdivision into wide, medium and narrow pits[30].

Pitting of passive metals usually requires the presence of an aggressive ion like Cl^- or Br^- which can displace the oxygen of the passive film at favoured sites. Should such ions make contact with the metal surface at any point, the potential of the metal becomes much more active, forming an anode, and the rate of metal dissolution

is greatly increased over the rate when oxygen of the passive film is intact[212].

Localised attack may occur in a crevice due to the limited access of electrolyte to the area within the crevice. This results in the formation of a concentration cell due to differences in salt, hydrogen ion, or more commonly, oxygen concentration. Both pitting and crevice corrosion affect mostly passive metals, e.g. aluminium and it's alloys, the stainless steels and the nickel base alloys. In non-passive metals, e.g. carbon steels, copper and zinc the tendency for similar localised corrosion to take place is less pronounced[212].

Intergranular or intercrystalline attack is selective corrosion of the grain boundaries or closely adjacent material without appreciable attack on the grains or crystals themselves[24]. The grain boundary region can be anodic and attack very severe owing to the large cathode to anode area. The attack causes a loss of strength and ductility out of all proportion to the amount of metal destroyed, and hence is a serious form of corrosion. Intergranular attack occurs in many different alloys.

Other forms of corrosion are less commonly the result of microbial interaction. For example, blistering, exfoliation, dezincification, graphitisation, impingement, cavitation and deposition attack. Descriptions of these are reviewed in the literature[27,186].

1.4 Corrosion Testing - General Principles

The reliable way to determine the corrosion rate of a metal is to expose the metal to the environment under service conditions and to assess the corrosion by means available in that particular case[202]. In nearly all instances this is slow and costly making it necessary to simulate and accelerate the corrosion process.

Irrespective of the test method or its purpose the underlying principles are the same. In designing any corrosion test, there are three major considerations which must be given preliminary study, namely, the environment, the test metal, and the means of appraising the corrosion damage. These are discussed at length by

La Que[118] and are mentioned briefly below. The environmental factors to be considered are 1) composition of the environment; 2) condition of immersion; 3) volume of test solution; 4) length of exposure; 5) temperature; 6) aeration; and 7) velocity. There are several factors concerning the test metal which must be considered including surface condition, identification and number of replicate specimens, and the means of supporting the specimens. For many metals consideration of the effects of internal and external stresses and the specific heat treatment of the specimens being used are also important.

The extent and progress of corrosion can be evaluated in a number of ways. A visual examination, possibly using optical devices is usually made. Observations should be described in detail to include colour and any unusual features such as pits, blisters, exfoliation or intergranular corrosion. Corrosion damage may be assessed by weight loss or gain, depth of pitting, change in the mechanical strength, change in electrical properties, consumption of oxygen and evolution of hydrogen[118]. The choice depends primarily on the type of test or the particular type of corrosion being evaluated. Generally several of these methods are used together in evaluating the degree of attack.

1.4.1 Electrochemical Corrosion Testing

When a metal specimen is immersed in a corrosive medium, both reduction and oxidation processes occur on its surface. Typically the specimen is oxidised to corrosion products at anodic sites and the medium is reduced at cathodic sites. Because of the electrochemical nature of most corrosion processes, electrochemical techniques can be used to study them. Measurements of current-potential relationships under carefully controlled conditions can yield information on corrosion rates, coatings and films, passivity, pitting tendencies and effects of inhibitors and oxidisers on specimens.

In electrochemical corrosion testing two direct approaches are apparent: control the current (i.e. corrosion rate) and measure the resulting potential, or control the potential (i.e. oxidising

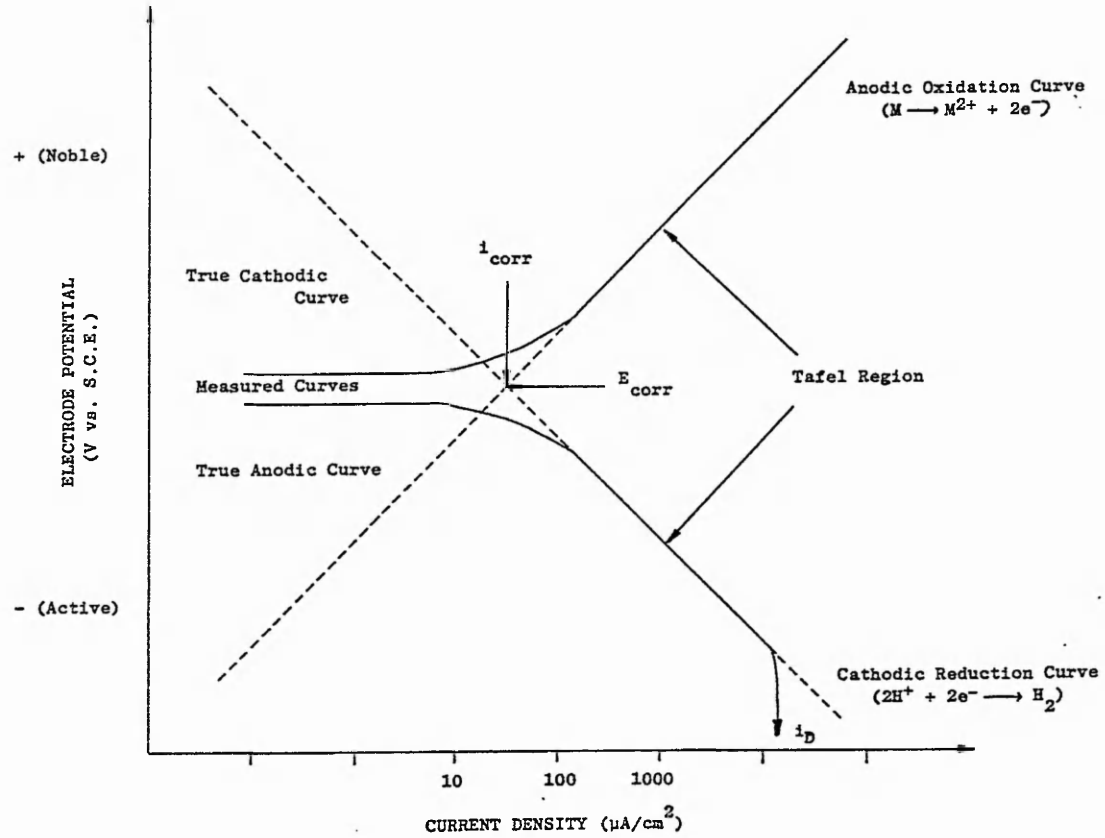
power) and measure the resulting current. In each case the potential of an electrode in a conducting media is changed by the flow of current in the electrolytic cell. This change in potential from a reversible or steady state value as a result of current flow is known as polarisation[41].

When a specimen is in contact with a corrosive liquid and the specimen is not connected to any instrumentation, as it would be "in service", the specimen assumes a potential (relative to a reference electrode) termed the corrosion potential, E_{corr}. A specimen at E_{corr} has both anodic and cathodic currents present on its surface. However these currents are exactly equal in magnitude so there is no net current to be measured. E_{corr} can be defined as the potential at which the rate of oxidation is exactly equal to the rate of reduction[161]. If the specimen is polarised slightly more positive (noble) than E_{corr} then the anodic current predominates at the expense of the cathodic current. Figure 1.1 is a schematic diagram illustrating experimental and true polarisation curves. As the specimen potential is driven further positive the cathodic current component becomes negligible with respect to the anodic component. If the specimen is polarised in the negative (active) direction then the cathodic current predominates and the anodic component becomes negligible. The mathematical relationship which exists between polarisation and anodic or cathodic current can be found in the literature[60,194].

There are three fundamental types of anodic and cathodic polarisation namely, activation, concentration and resistance polarisation[41]. Under activation polarisation the reaction sequence at the metal-electrolyte interface controls the electrochemical process. One example is the corrosion that occurs in media containing a high concentration of active species, such as relatively concentrated acids. Under activation control, anodic and cathodic data for potential versus the logarithm of the applied current density give linear behaviour when the amount of polarisation, which is called over voltage, is more than about 50 mV from the open circuit corrosion potential. An explanation for the deviation of the observed curve (solid line) from the theoretical (dotted line) at potentials within

Figure 1.1

Polarisation Curves - Measured and True



KEY

i_{corr} - Corrosion current density

E_{corr} - Open circuit corrosion potential

i_D - Limiting diffusion current density

50 mV may be found in the literature[41]. When the reaction rates are controlled by the diffusion of species in the bulk electrolyte to the metal-electrolyte interface, concentration polarisation is observed. This behaviour usually occurs when the concentration of reducible species is small, for example, corrosion in aerated salt solutions. In such cases there is a precipitous change in potential at the limiting diffusion current density as illustrated in Figure 1.1. Resistance polarisation can result from an IR potential drop during electrochemical measurements in low conductivity solutions.

Since the electrical data, either current or potential, are amenable to precise measurement, controlled polarisation has become an important technique in corrosion testing. By measuring the impressed current density as a function of potential, the corrosion resistance of a specimen can be estimated over a wide range of oxidising conditions. Instruments are available for corrosion testing both in the laboratory and in the field.

The 'linear polarisation', also referred to as 'polarisation resistance', technique is particularly advantageous in that corrosion rates can be measured rapidly (within ten minutes), the specimen isn't destroyed, low rates can be detected and it allows continuous monitoring. Also correlation between corrosion rates measured by linear polarisation and convlentional weight loss is often good[161].

1.5 Theories of Microbial Corrosion

In nature, most of the microbially caused corrosion probably occurs as a result of more than one mechanism, either simultaneously or progressively, and by combinations of microorganisms. It is difficult to establish what the predominating mechanism might be, especially in non-laboratory environments where there are continuously changing physical, chemical and biological parameters. A further complication is the fact that corrosion may take place in the absence of microorganisms. The question becomes one of establishing how much of the corrosion process, if any, is due to the activities of microorganisms.

It is now considered that microbial involvement in the

corrosion process does not involve any new mechanism[204]. Corrosion in aqueous environments is caused by electrochemical reactions and microorganisms are thought to enhance these reactions by one or more of the following mechanisms: a) cathodic depolarisation b) production of corrosive metabolic products c) production of differential aeration and concentration cells on the metal surface d) disruption of protective films (natural and applied) e) breakdown of corrosion inhibitors.

There have been several reviews to this effect[38,86,100,138,158] though Tiller[203,204] preferred to discuss the mechanisms in terms of aerobic or anaerobic conditions, and Pope et al.[157] in terms of specific metals.

1.5.1 Cathodic Depolarisation

According to conventional electrochemical theory the corrosion rate of iron in neutral deaerated water should be negligible due to polarisation of the cathode. However, it has been demonstrated that in the presence of anaerobic sulphate reducing bacteria the corrosion rates of ferrous materials can be extremely high. For example, the corrosion rate of mild steel in a sterile culture medium and in a laboratory culture of Desulfovibrio desulfuricans was 2.6 and 206 milli inches per decimetre^2 per day (m.d.d.) respectively[203]. Such bacteria may utilise cathodic hydrogen in the dissimilatory reduction of sulphate thus depolarising the cathode. The classical theory suggests the following mechanism:-

Anode Reaction	$4Fe \longrightarrow 4Fe^{2+} + 8e$
Dissociation of water	$8H_2O \longrightarrow 8H^+ + 8OH^-$
Cathode Reaction	$8H^+ + 8e \longrightarrow 8H$
Cathode Depolarisation	$SO_4^{2-} + 8H \longrightarrow S^{2-} + 4H_2O$
Corrosion Product	(i) $Fe^{2+} + S^{2-} \longrightarrow FeS$
	(ii) $3Fe^{2+} + 6OH^- \longrightarrow 3Fe(OH)_2$
Overall Reaction	$4Fe + SO_4^{2-} + 4H_2O \longrightarrow FeS + 3Fe(OH)_2 + 2OH^-$

If this mechanism is correct, the ratio of corroded iron to iron sulphide would be 4:1. In practice wide deviations occur ranging from 0.9:1 to 50:1[203].

Support for the classical theory has been based on the understanding that certain strains of the sulphate reducing bacteria e.g. Desulfovibrio desulfuricans and D. vulgaris contain the enzyme, hydrogenase, and so utilise the cathode hydrogen for the reduction of a suitable substrate. Hydrogenase negative strains, for example, Desulfotomaculum orientis have been shown to be completely inactive in this mechanism. However, evidence collected by various workers (reviewed by Tiller[203]) shows that other factors must be involved. Such evidence includes the rate of corrosion being independent of the hydrogenase activity in a number of different strains of sulphate reducing bacteria[18] and the hydrogenase negative organism showing itself to be quite as aggressive as the Desulfovibrio species. Over the years much published work has suported the classical theory[19,94] but later work suggested an alternative mechanism involving sulphide, and iron sulphide films and the presence of free sulphur may be important[16,109]. It was found[139] that certain strains of Desulfovibrio spp. are able to grow by fumarate dismutation in sulphate free medium. Actively growing cultures of hydrogenase positive strains caused some depolarisation of mild steel but corrosion rates were very low compared with those in cultures reducing sulphate. In addition chemically prepared iron sulphide itself was found to depolarise cathodic steel in bacteria free systems. It is now known that iron sulphides (of which there are several)[166] are cathodic to iron, though they do not have a lasting effect in a sterile system. The role of the bacteria could therefore be a) to 'regenerate' (or depolarise) iron sulphide, enabling it to remain cathodic b) to produce more iron sulphide by their growth reaction, or even c) to bring fresh iron sulphide surfaces constantly into contact with the steel by their movement[138].

The corrosiveness of iron sulphides depends on composition, pH and temperature[204]. Although the cathodic

characteristics of iron sulphides have been known for some time it is now thought that elemental sulphur may be a more important corrodent. Under aerated conditions elemental sulphur may be formed and can act in the same manner as dissolved oxygen in promoting the corrosion of steel[176]. The sulphur appears to promote corrosion by a concentration cell mechanism, that is an anodic area develops underneath a porous material which shields the steel from dissolved sulphur; the corresponding cathodic area being the adjacent region where dissolved sulphur is readily available as a cathodic reactant.

Ferrous metals (mainly mild steel and cast iron) have received the most attention in the literature with regard to this type of corrosion, because of their widespread use in underground pipes. Also at risk are piers and sheet piles, etc. in the lower reaches of rivers, and frequently the hulls of berthed ships which are in contact with estuarine mud. Corrosion of aluminium and its alloys in aircraft fuel tanks has often been due to the action of sulphate reducing bacteria[13,75,99].

It is apparent that although extensive research has been carried out to clarify the mechanisms by which sulphate reducing bacteria function in the corrosion process the mechanism remains uncertain.

1.5.2 Formation of Corrosive Products

1.5.2.1 Organic Acids

Many obligate and facultative anaerobes have a fermentative metabolism resulting in production of organic acids. In addition several fungi excrete considerable amounts of organic acids during normal oxidative metabolism. The acids are aggressive to many metals and to metal alloys, such as aluminium bronze where they may specifically attack one phase of the alloy[86].

The medium of an anaerobic culture of E. coli in which corrosion of carbon steel was enhanced was found to contain acetic, α-ketoglutaric, succinic and lactic acids[7]. It was suggested that these metabolites were responsible for accelerating corrosion by dissolution of the iron oxide formed on the metal surface. Such disruption of the natural protective layer is discussed later.

A severe case of corrosion in the diffusion batteries of a sugar beet factory has been reported[4] due to the anaerobic production of organic acids by Lactobacillus delbruckii. Cellulose-decomposing bacteria were reported to have oxidised the bitumen-bound hessian coating of lead lined cables[34]. Etching caused mainly by acetic and butyric acids occurred which initially was mistakenly attributed to phenols in the bitumen, and named 'phenol corrosion'.

Cladosporium resinae has frequently been associated with the corrosion of aluminium fuel tanks, particularly in aircraft[28,44,45,120,136,149,179]. The culture broths of this organism grown in glucose or n-alkanes as the carbon source contained carboxylic acids (mono C10; di-C7, C10, C11, C12) and components of the tricarboxylic acid cycle such as citric, iso-citric, α-ketoglutaric, succinic and malic acids[124,136,188]. Various organic acids (citric, fumaric, α-ketoglutaric, maleic, malic, pyruvic and succinic) form metallic salts of copper, tin and zinc in sterile conditions[25] though the presence of oxygen is necessary for the formation of these salts. Thus, electrochemical oxidation of metals may be followed by acid removal of the oxide film formed[178].

An examination of the interactions between enzymes, organic acids and 21 metals found the organic acids, as represented by glycolytic, tricarboxylic, amino and fatty acids, to be more active as corrosive agents than were the enzymes tested[193].

The effect of six concentrations of fumaric, itaconic, malic and aspartic acids, methionine and glycine on copper segments was examined[135]. Corrosion was assessed as weight of

copper salts formed. It was found that the various acids produced differing amounts of soluble/insoluble salts. No corrosion was noted at 1 and 10 ppm and only negligible amounts were observed at 100 ppm. Concentrations of 1,000 ppm were necessary to produce organic acid-metal corrosion. As concentrations were increased above 1,000 ppm acid-metal corrosion increased.

The acids, by supplying an excess of hydrogen ions, provide an excellent electron sink. In the presence of organic acids, active metals, which have an oxidation potential higher than hydrogen, corrode by the following mechanism[18]:-

$$M^{o} \longrightarrow M^{2+} + 2e$$

The reduction taking place would be:

$$2\ \underset{\text{Acid}}{(Org^{-}H^{+})} + 2e \longrightarrow H_2 + 2Org^{-}$$

which is followed by precipitation of an organic salt of the metal:

$$M^{2+} + 2Org^{-} \longrightarrow \underset{\text{Salt}}{(M(Org)_2)}$$

With metals having a lower oxidation potential than hydrogen the same product is achieved by neutralisation of the organic acid by cathodically produced alkali:

$$M^{o} \longrightarrow M^{2+} + 2e$$

$$H_2O + \frac{1}{2} O_2 + 2e \longrightarrow 2OH^{-}$$

Then;

$$2\ \underset{\text{Acid}}{(Org^{-}\ H^{+})} + 2OH^{-} \longrightarrow 2H_2O + 2\ Org^{-}$$

$$M^{2+} + 2\ Org^{-} \longrightarrow \underset{\text{Salt}}{(M(Org)_2)}$$

In summary, two mechanisms for corrosion by organic acids have been proposed. Firstly, the disruption of the oxide film and secondly the provision of an electron sink at the cathode and a

subsequent anion to utilise the metal cations at the anode. A third mechanism involving the production of concentration cells where growth comes into close contact with the surface has been proposed[138]. This is described later. The occurrence of one or more of these mechanisms may, depending on the specific situation, be active at any one time.

1.5.2.2 Mineral Acids

Most metals are rapidly attacked by oxidising acids such as nitric and concentrated sulphuric. Copper base materials are particularly susceptible whereas stainless steels can be fairly resistant[186]. Ammonia can be oxidised by the bacterium Nitrosomonas to nitrous acid and this can in turn be oxidised to nitric acid.

A case has been reported[158] of serious pitting of stored and buried lead cables. In this instance a paper lining within the lead cover had been degraded to carbon dioxide by bacteria and the main corrosion product was carbonate.

Corrosion which occurs in an environment containing a substantial amount of sulphuric acid can usually be attributed to the activity of autotrophic bacteria of the genus Thiobacillus. The following series of interlinked reactions lead to the formation of free sulphuric acid[162]:

$$2H_2S + 2O_2 \longrightarrow H_2S_2O_3 + H_2O$$

$$5Na_2S_2O_3 + 4O_2 + H_2O \longrightarrow 5Na_2SO_4 + H_2SO_4 + 4S$$

$$4S + 6O_2 + 4H_2O \longrightarrow 4H_2SO_4$$

The organisms are capable of oxidising elemental sulphur and sulphur compounds (sulphite, thiosulphate, tetrathionate, etc.) and can create and survive in solutions of 10 - 12% sulphuric acid[158]. A pH as low as 0.6 has been reported in cultures of T. thiooxidans[14].

The most important single problem caused by these organisms is the corrosion of concrete sewers. Evolution of hydrogen sulphide from the sewage leads to the deposition of

inorganic sulphur compounds on the roof of the pipe, and these are oxidised to sulphuric acid by the bacteria. Iron pipes carrying polluted effluents, concrete manhole covers, cooling towers, iron gas mains, statues, buildings and stonework have been corroded for similar reasons[158]. Actual reported situations of metallic corrosion by sulphuric acid produced by these organisms are relatively rare though it seems likely that many cases must exist where corrosion of this kind has taken place[38]. However, it has long been recognised that inadequate protection of ferrous pipes laid in aggressive soils allows serious corrosion[14]. On the basis of either weight loss data in laboratory cultures of Thiobacilli or polarisation curves it has been indicated that oxidation of reduced sulphur compounds by these organisms can greatly increase the corrosion rates by lowering pH values and removing protective films from the surface[95].

An associated organism T. ferrooxidans is particularly interesting since it is used in the recovery of metals from low grade ores. It is important in the present context because of its ability to produce sulphuric acid from the mineral pyrite (FeS_2). It was estimated that a million tons of sulphuric acid was contributed annually to the drainage area of the Ohio river due to the oxidation of pyritic deposits in the mines of Western Pennsylvania by this organism[121]. Such acidic mine waters, common in bituminous coal mines and gold mines can cause serious corrosion of pumping machinery[158].

The probable reactions[123] are:

$$2FeS_2 + 7O_2 + 2H_2O \longrightarrow 2FeSO_4 + 2H_2SO_4$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \longrightarrow 2Fe_2(SO_4)_3 + 2H_2O$$

$$FeS_2 + Fe_2(SO_4)_3 \longrightarrow 3FeSO_4 + 2S$$

$$2S + 3O_2 + 2H_2O \longrightarrow 2H_2SO_4$$

1.5.2.3 Other Products

Many organisms are able to produce ammonia from nitrites or nitrates (in oxygen depleted conditions) or from a variety of nitrogen containing organic substances such as proteins, amines,

and amino acids. The ammonia is aggressive towards copper containing alloys[38,86]. It has been shown to cause stress corrosion cracking of brass[211].

Amino acids have been demonstrated to accelerate the corrosion of copper[178]. It was found that 23 hours after spore germination by Puccinia coronata, 20 amino acids or amino acid derivatives were located in the distilled water surrounding the spores. Cystine produced by bacterial action on proteinaceous matter in sea water has been shown to increase the rate of impingement attack on 70:30 brass[169]. The attack in this case seemed to be due to the formation of a discontinuous film on the metal surface resulting in intensified attack at certain points.

Mannitol produced in sea water by the action of bacteria on sea-weeds was found to accelerate corrosion of 70:30 brass[168]. It was suggested that hydrogen acceptors capable of causing depolarisation of cathodes were formed during mannitol fermentation in heavily polluted waters.

By chromatographic purification it was found that aggressive substances of high molecular weight (greater than 5,000) were formed by cultures of Pseudomonas aeruginosa[13]; this may be indicative of enzymic corrosion.

Many microbial growth products (e.g. proteins) reduce interfacial tension between oil and water and promote the formation of stable mixtures which aggravate corrosion[39,86,116].

Sulphides produced by anaerobic bacteria e.g. Desulfovibrio desulfuricans in fuel tanks, attack not only the aluminium or steel but also dissolve in the fuel and attack silver (for example, in parts of some aircraft fuel pumps) and copper and its alloys[86].

Elemental sulphur has been reported to cause very rapid corrosion of iron and steel[100,176]. Conditions favourable to elemental sulphur formation in the soil are due to sulphate reduction during periods of poor aeration and oxidation of sulphide during periods of greater aeration.

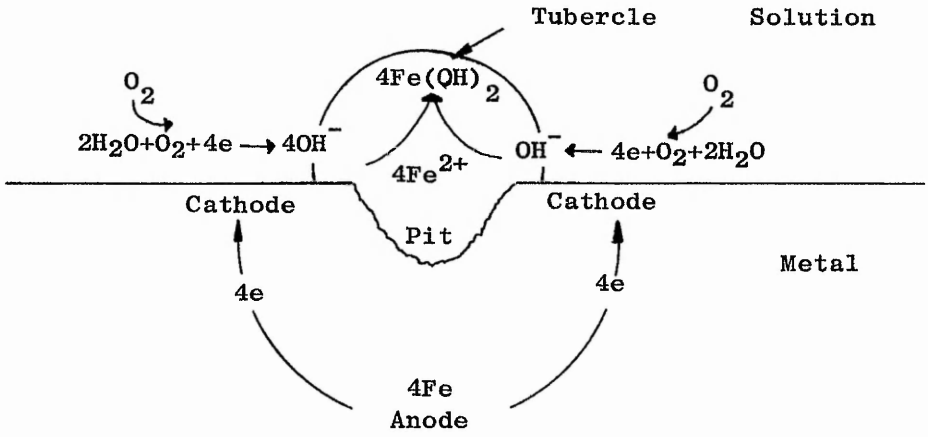
1.5.3 Formation of Concentration Cells

In addition to microbial corrosion due to the production of aggressive chemical substances, it is known that the mere presence of microbes on a metal surface may cause corrosion. Therefore, all types of microbes that can grow in a coherent colony or clump are potentially corrosive[31,52,200] including those algae, protozoa, diatoms and fungi as well as bacteria which produce highly hydrated gelatinous slimes allowing adherence to metal surfaces. Such microbial fouling may well precede macrofouling[175] by barnacles, mussels and seaweed especially in the marine environment. Typical examples of the microflora involved include the filamentous algae Chlorophyta and Cyanophyta, bacteria of the species Pseudomonas aeruginosa as well as species of Flavobacterium, Aerobacter, Gallionella and Sphaerotilus[203]. Fungal genera often identified include Trichoderma, Monilia, Penicillium and Cladosporium. The corrosive effects of microalgae have recently been reported[200].

The development of a microbial mass adhering tightly to a metal produces a non-uniform oxygen distribution on the metal surface. More oxygen would be found at the edge of the mass than under it. Utilisation of oxygen by microorganisms would be expected to increase this difference in oxygen concentration. The poorly aerated surface becomes the anode of the differential aeration cell while the better aerated regions away from the deposits, provide the balancing cathodic reaction. Figure 1.2 illustrates this mechanism. The metal goes into solution at the anode and reacts with the hydroxyl ions formed at the cathode by the combination of water, oxygen and free electrons. As a result of the very large cathode/anode surface area ratio corrosion will be severe[86]. A classic example of this type of problem is the fouling of industrial cooling water systems and heat exchangers[203] where contamination of the water by airborne aerobic bacteria and spores is common. Another example of this type of problem is the external fouling of steel structures in industrialised river estuaries and other environments.

Figure 1.2

Pitting by Differential Aeration



The corrosion pits of aluminium tanks containing jet fuel are believed to be due to such a mechanism[100] caused by mats of Cladosporium resinae and pseudomonads adhering to the surface. Sulphate reducing bacteria may also be present in the anaerobic region beneath the mats.

Another form of concentration cell is via tubercle formation which is encouraged by the presence of the iron oxidising bacteria Gallionella, Sphaerotilus and Leptothrix[203]. These organisms obtain their energy for carbon dioxide fixation by the oxidation of ferrous ions to ferric ions, with the consequent accumulation of ferric hydroxide on, for example, the internal surfaces of a ferrous water distribution pipeline. Eventually these act as shields and prevent the access of oxygen to the surface of the material at their base and in consequence differential aeration cells are set up. The problem is often aggravated by the development and growth of sulphate reducing bacteria at the base of the tubercle where conditions of anaerobiosis prevail. Several workers[2,176] consider that the outer edge of the tubercle has a higher population of sulphate reducers than the interior. This is considered to be due to the inhibitive properties of the sulphide. Hence under these conditions it is suggested that sulphate reduction takes place at or near the surface of the tubercle and the sulphide produced diffuses back into the nodule to react with ferrous ions to precipitate more iron sulphide.

The presence of a microbial mass on the surface of a metal may also change the electrolyte beneath and around the mass and cause corrosion by a chemical concentration cell effect. The possible changes due to the wide variety of metabolic activities by microorganisms singly and in mixed populations would appear to be infinite.

It would appear that for corrosion to occur by the formation of concentration cells there has to be intimate contact between the microorganism and the metal[52]. This may be considered as the first stage in the microbial corrosion process. A better understanding of the factors which affect adhesion may enable this

stage to be controlled or even eliminated. Such factors, recently reviewed,[52] include mechanism of attachment, effects of the chemical and physical nature of the surface, cell growth conditions, environmental composition, temperature and fluid velocity.

1.5.4 Destruction of Natural and other Protective Films

The high resistance to corrosion exhibited by a number of metals and alloys of commercial importance, such as stainless steels, aluminium and certain copper alloys, is dependent upon the formation of a tenacious, passive oxide layer on the surface of the metal. A supply of oxygen must therefore be available at all times to maintain the oxide film. However, the dissolved oxygen in contaminated waters is likely to be at a very low level and under such conditions the protective film would not be maintained thus corrosion, probably by the creation of differential cells, may begin[86].

Exposed metals are generally protected by the application of an organic, inorganic or metallic coating. In some cases the coating provides a source of nutrient for microorganisms and may undergo rapid deterioration rendering the underlying metal liable to corrosion in a relatively short period of time. The situation can be further aggravated if the metabolic product is itself a corrosive substance. The correct choice of coating is therefore of paramount importance for situations in which a component must remain in service for very long periods with the minimum of inspection.

1.5.5 Breakdown of Corrosion Inhibitors

While a number of corrosion inhibitors are also capable of inhibiting microbial growth, several commonly used inhibitors notably nitrites, phosphates and benzoates, contain elements essential to the metabolism of organisms. Such inhibitors may therefore be removed from the system allowing metallic corrosion

to proceed; for example, nitrites used to inhibit the corrosion of iron have been found to be utilised by Nitrobacter agilis in cooling systems and rendered ineffective[131]. In laboratory experiments, it was demonstrated[13] that hydrocarbon utilising bacteria, reduced nitrate, an inhibitor of aluminium corrosion, to nitrite, which is ineffective as a corrosion inhibitor for aluminium. Again corrosion will be further aggravated if a corrosive metabolic product is formed.

1.5.6 Selective Removal of Metallic Ions

It has been hypothesised[77] that "the corrosion of (aluminium) alloys by microorganisms, particularly aerobic bacteria, results from the removal of certain metallic ions (major or minor) from the basic structure of the alloy by extracellular enzyme activity". This resulted from the observation that greater amounts of corrosion products were evident in environments containing lesser quantities of nutrients, suggesting that the microorganisms were obtaining their metal requirements from the alloys. For example, the accumulation of zinc and magnesium from aluminium alloys was suspected[78]. It was found that an Aspergillus[201] both dissolved and accumulated copper and that various bacteria[5] accumulated aluminium from a solid medium. Preliminary work by Engel & Swatek[57] showed that several fuel contaminants concentrated Ca, Fe, Mg and Al but not Zn from various metal alloys. They investigated the uptake of Al, Mg, Fe, Mo, and Cu by two Pseudomonas spp. and a Micrococcus sp. and found Al to be the most highly concentrated by the cells.

Thus corrosion may be enhanced either by dissolution and uptake or by sequestration of the corroding material thus providing a continuously depolarised surface. The concentration of metals by bacteria may either be inside the cell as a metabolic function or on the outside of the cell as a non-specific adsorption or a metabolically controlled reaction.

1.6 Hydrocarbon Utilisation

Developing awareness of the detrimental role of hydrocarbon degrading microorganisms has been responsible for increasing attention being given to such problems as the combating of microbial destruction of oil in deposits, corrosion of metals, plugging of petroleum reservoirs, degradation of drilling mud additives, deterioration of aviation turbine fuel, spoilage of cutting oil emulsions and degradation of asphalts[127].

The first species reported to grow on hydrocarbons was Botrytis cinerea in 1895[141]. It is now known that thousands of microorganisms are able to utilise various hydrocarbons. Lonsane et al.[127] produced a codification of bacteria, yeast, actinomyetes and fungal hydrocarbon utilising organisms on the basis of type and nature of the substrate.

Hydrocarbons within the range C_4 to C_{20} and particularly the soluble short chain components are taken up by many microorganisms. Growth is usually at the oil-water interface, it's rate being limited by the available interfacial area[97]. The hydrocarbons may be taken up as vapour[220], solution or dispersed droplet phase[68]. Uptake of substrate is affected by relative concentrations of alkane and cell protein, pH, temperature and mixing efficiency[68]. Several hydrocarbon utilising yeasts and C. resinae release surfactants into the medium which emulsify hydrocarbons and increase their adhesive properties to the cell[68,188]. This increased adherence of substrate to cell results in increased growth[216]. The n-alkane partitions from the medium directly to the cell surface and may transiently become part of the phospholipid component of the cell membrane before being released into the cytosol[100].

Microorganisms generally grow at the oil-water interface, their rate of growth being limited by the available interfacial area[97].

Several mechanisms for the initial catabolism of n-alkanes by microorganisms have been proposed[40,59,65,114]. The most common route involves terminal oxidation to the primary

alcohol, followed by dehydrogenation to the aldehyde and then to the corresponding chain length fatty acid[80]. There is evidence to support this pathway in many filamentous fungi[114] and Walker and Cooney[215] reported that cell free extracts of C. resinae converted labelled hexadecane to the primary alcohol, aldehyde and acid.

The subsequent β-oxidation of fatty acids yields acetyl CoA which enters the tricarboxylic acid cycle[182]. McKenzie et al.[136] demonstrated that C. resinae produces citric, isocitric, cis - aconitic, α -ketoglutaric and oxaloacetic acids from hydrocarbon oxidation.

The available evidence indicates that n-alkane grown microorganisms utilise the TCA and glyoxylate cycles and do not possess any novel enzymes or pathways.

1.7 Cladosporium resinae - Growth and Nutrition

It is generally believed that C. resinae plays a major role in most cases of fuel system fouling[56,79,86,149,208].

Cladosporium resinae was first isolated (as Hormodendron resinae Lindau) from the resin of Pinus excelsa near Hamburg[125]. The perfect state of C. resinae (Amorphotheca resinae) has been observed in soil[183] and in mineral oil[185] but the fungus is usually isolated from soils, air and fuel in its asexual state[184]. Many forms of C. resinae have been isolated and 14 have been placed with the American Type Culture Collection[106]. De Vries[48,49] described the fungus as having olive green colonies covered with a dense powdery pale greyish brown layer of conidia, and regularly septate brownish hyphae which remained sterile or produced conidia of mean width 5.6 x 2.64 μm.

Although ubiquitous in nature, C. resinae appears to have a low competitive ability in general habitats, but is capable of exploiting certain specialised ecological niches, either by consuming substrates unavailable to other organisms, or by surviving in extreme environments[149]. For example, it was found that a variety of pesticides at concentrations of 20,000 ppm did not inhibit growth of C. resinae on either glucose or

hexadecane[33]. Pesticides can be concentrated in surface oil slicks[181] and this may account for the enrichment of C. resinae in oil polluted waters[1].

Culture conditions, for example, nitrogen concentration, inoculum level, type of carbon source[151] and humidity,[184] markedly affect the morphology of C. resinae. Thus care must be taken when measuring the response of C. resinae to environmental variables. There is little correlation between spore production and growth of C. resinae[149] which invalidates the use of spore counts as an assessment of growth under different conditions.

Parbery[150] found that even in replicates where uniform inoculum and quantities of medium were used, growth of C. resinae was variable. In addition, growth appeared to be related to the surface area of the medium exposed to the atmosphere, smaller areas probably limited the gaseous exchange of oxygen and carbon dioxide. He found that although agitation increased aeration and hence growth in a glucose medium, growth decreased when media containing fuel were shaken. He concluded that shaking cultures of C. resinae was deleterious to growth.

C. resinae has been found to grow over the pH range of 3.0 to 9.6[32] with an optimum pH of 5.8[208]. By the production of organic acids[136,188,207] C. resinae has been found to reduce the medium pH to less than 2.0 during growth[149]. C. resinae has been found to grow on kerosene over the range of 0 to 50°C[150] with an optimum of 25 to 30°C. Turner[207] found an optimum temperature of 30°C for C. resinae growing on malt-yeast-peptone-glucose agar.

Hendey[79] tested the ability of C. resinae to grow in different concentrations of sea water, with kerosene as carbon source. He concluded that although concentrations greater than 30% slightly retarded growth, 100% sea water had relatively little effect on development and growth. Other workers[140,191] have reported that C. resinae does not grow in undiluted sea water and Dieso. The sea water in ships' fuel systems must therefore be modified in order to allow the prolific growth of C. resinae that is often observed. Such modifications will include those made by

the growth of bacteria and yeasts, which have been shown to appear first in the ecosystem[117], absorption of soluble components from the fuel and the presence of organic and inorganic contaminants, corrosion products and fuel additives.

C. resinae can utilise an extensive range of carbon sources[149] including sugars, n-alkanes, cycloalkanes, aromatics, aliphatic alcohols, aliphatic carboxylic acids and complex hydrocarbons. It has been suggested[149] that C. resinae may be unique in its ability to utilise such a wide range of often complex or toxic organic compounds and that this may explain its success in specialised environments.

Growth of C. resinae on n-alkanes has been reported for chain lengths seven to nineteen[33,148,199]. There is some discrepancy over which chain length produces the highest biomass, both undecane[33] and hexadecane[199] have been reported to do so.

Good growth of C. resinae is supported by either peptone ammonium nitrate, ammonium sulphate or sodium nitrate but as sole nitrogen source little or no growth is obtained with urea or sodium nitrite[134]. The nitrogen content of ship fuel systems is not known but it is likely to vary considerably. Any competitiveness of C. resinae under nitrogen limiting conditions would determine its success. Organic nitrogen sources such as peptides, urea, acrylnitrile, pyridines, quinolines and acridines may be available from sewage or the fuel. A study of petroleum degradation in sea water showed that low concentrations of nitrogen and phosphate were limiting factors[8]. An addition of at least 1.49 g l^{-1} of potassium nitrate and 10 mg l^{-1} of di-sodium hydrogen orthophosphate was recommended to enhance biodegradation. Inorganic nitrogen levels in the relatively unpolluted Southampton water were found to be 0.14 to 1.2 mg nitrate nitrogen; 0.05 to 0.43 mg ammonium-nitrogen and 0.007 to 0.018 mg nitrite nitrogen per litre. Concentrations varied with site and depth of sample.

Nine water samples from ships fuel systems showed an average content of 28 µg of phosphorous l^{-1}[208] whereas unpolluted sea water concentrations of phosphorous vary but average 60 µg l^{-1}[20]. Assuming an approximate theoretical yield of 70 g of

organism per g of phosphorous[208] the availability of phosphorous would appear to be a major limiting factor in fuel system contamination.

Potassium, magnesium and sulphur have a presence of 0.38, 1.3 and 0.9 g l^{-1} respectively in sea water and assuming some sea water enters the fuel system growth is unlikely to be limited[208]. Deep sea water contains relatively high concentrations of calcium (0.5 g l^{-1}), boron (4.4 mg l^{-1}) and chlorine (18.8 g l^{-1}) but manganese, iron, cobalt, copper and zinc are generally only present in traces[20] and may limit growth. The medium developed by Turner[207] contained all these trace elements and was found to give better growth yields than either Bushnell and Haas salts solution[26] or Parbery and Thistlewaite's[152] medium.

1.8 Naval Ships' Fuel Systems

1.8.1 General Description

HMS Exmouth, an anti-submarine frigate, was converted from steam to gas turbine propulsion in 1968 and became the first major warship in the Royal Navy to be propelled solely by gas turbine engines. A combination of Tyne and Rolls Royce 'Olympus' engines have now been adopted as propulsion units for all new frigates, cruisers and destroyers. The United States Navy, Russian Navy and the Royal Swedish Navy also have gas turbine powered warships[208].

At present there are four major classes of R.N. ships powered by gas turbine engines; type 21 and 22 frigates; type 42 destroyers and the Invincible class. The advantages over conventional steam powered plant include rapid start up from cold, low manning requirements, precise and rapid response to controls, compactness and relatively low capital and running costs[142].

However, it is only since the introduction of these engines that the problem of microbiological contamination has been recognised. A new fuel, Dieso, was adopted for use which is a

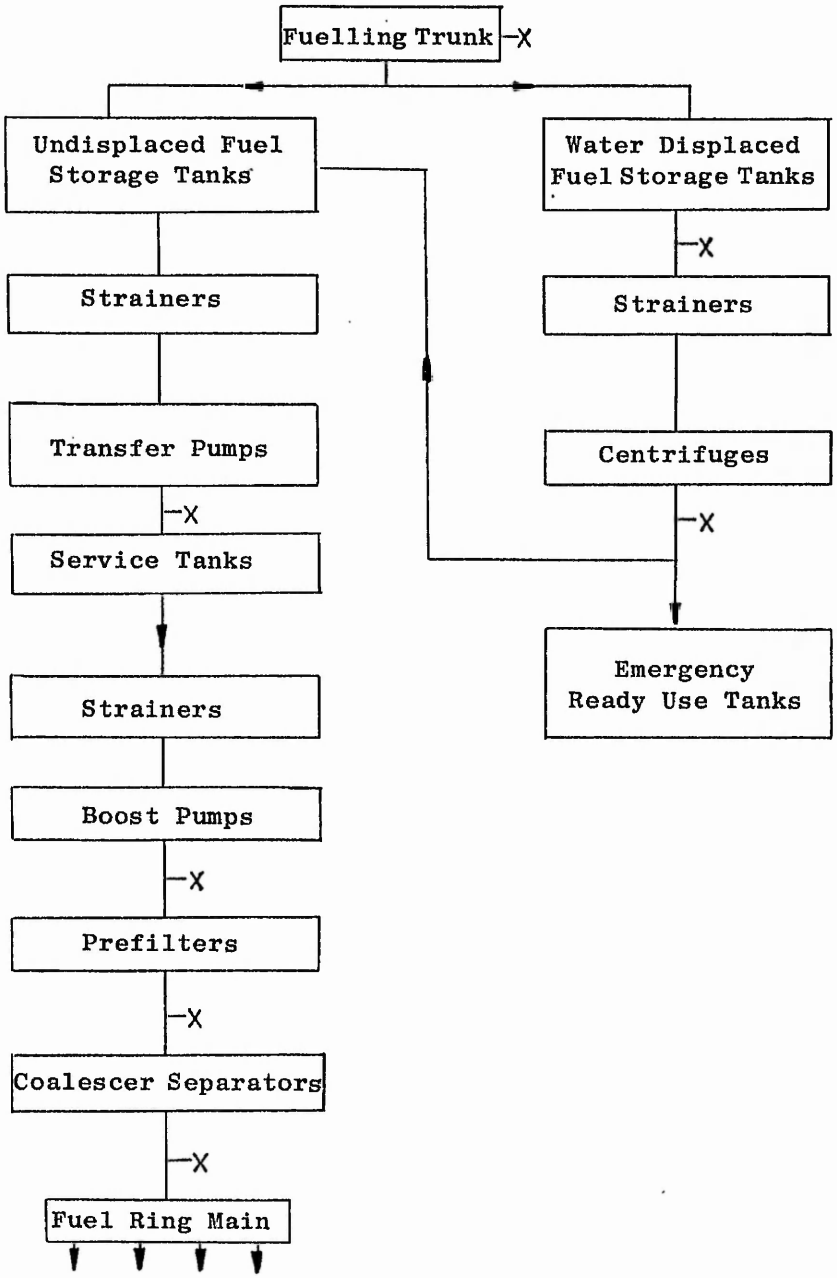
blended light to middle distillate fraction from oil and is supplied to a NATO specification[140]. The fuel for gas turbine engines, unlike steam turbine or Diesel engines must be dry (water content <10 ppm). This is to prevent the ingress of dissolved sodium ions, which, by reaction at high temperatures with the sulphur present in the fuel produce reactive slags, which in turn would attack the surface of the nickel alloy turbine blades[66]. The fuel is therefore stripped of water and solid contamination by a filter and water separator system situated between the service tanks and the engine. Premature blockage of these units by microbiological contamination brought the problem to the fore. Frequent replacement of filters due to high pressure differentials have been reported by a number of ships. In 1975 HMS Sheffield experienced fuel starvation, caused by filter blockage, during manoeuvres[208].

In the Type 21 frigate fuel system Dieso enters the fifteen main storage tanks via the fuelling trunk. These tanks are integral with the ship's hull and contain ledges, baffles and strengthening girders, which reduce bulk fuel movement. Transfer pumps draw fuel from approximately 5 cm above the lowest points in the tanks, through 1.5 mm strainers to the service tanks, where free water is allowed to settle and is drained off. The Dieso is then pumped through 5 μm pre-filters into two water coalescer-separator units (1 μm). Fuel enters through twelve coalescer elements in the lower half of the chamber, passes into the interior of the unit, and then out via ten separator elements in the upper half of the chamber. Coalesced water forms large droplets on the surface of the elements and sinks to the bottom of the chamber, where it can be drained off. The clean fuel is led off via an exit manifold to the fuel ring main. This serves four gas turbine engines; two Olympus engines forward and two Tyne engines aft.

The Type 42 fuel system is illustrated in Figure 1.3. It differs from the Type 21 system in that it also has sea water displaced tanks and centrifuges. Sea water is run in under the fuel to replace that which has been consumed, thereby maintaining

Figure 1.3

Type 42 Destroyer Fuel System



Fuel sampling points are indicated by 'X'.

Arrows show direction of fuel flow.

the ships centre of gravity. The fuel which is displaced passes through centrifuges, which reduce the water and particulate content. The undisplaced storage tanks are fitted with a stripping system which removes water bottoms before the fuel is transferred. Three water coalescer-separator units serve the fuel ring main. An emergency bypass system can be operated in the event of a sudden filter blockage, although this results in the engines receiving poor quality fuel. The undisplaced tanks are used in preference to the displaced tanks. After a time at sea, it is more practical to directly refuel the undisplaced tanks rather than transfer fuel and refuel the displaced groups. Nevertheless fuel has to be continuously transferred between tanks in order to trim the ship. This policy now also includes the fuel in the emergency tanks which is also recycled through the clean up systems.

Associated with the fuel system are engine lubricating oils. The slow speed diesel engine of merchant ships is particularly at risk as these engines run for long periods at a constant temperature conducive to microbial growth and are subject to continuous almost unavoidable water contamination from the cylinder jackets and/or the pistons (if the latter are water cooled)[87]. Hill[85] investigated a marine engine failure due to corrosion and attributed it to a massive microbial infection of the lubricating oil. The incidence of microbial infection and its consequence in turbine oil systems is still not clarified[87].

1.8.2 The Ships Fuel System as an Environment for Microbial Growth

As water is essential for growth in a fuel ecosystem[79,126] problems due to microbial contamination could be eliminated by preventing water coming into contact with fuel. In practice exclusion of water is almost impossible because fuels usually contain dissolved water which condenses as the temperature decreases. Also, on many ships water is deliberately added to the fuel storage tanks for ballast and to eliminate vapour space[126].

In fact providing the relative humidity was of at least 90%, kerosene fumes were found to adequately support growth of C. resinae[79]. The same is likely to be true for Dieso vapour, which may be significant when considering the growth of the fungus in fuel tanks that have been drained.

The sources of microbial contamination include the atmosphere, sea water and unclean fuel, all of which are difficult to control. For example C. resinae may enter the fuel supply system as airborne spores from soil, and sea water generally contains a range of microorganisms possibly including the sulphate reducing bacteria. Estuarine waters may be rich in sewage bacteria whereas highly saline deep sea water would be relatively free of microorganisms. Finally, freshly refined Dieso may be kept for long periods in underground storage tanks, where water can gain access by seepage from the ground or through condensation. While some discrete droplets of water can be expected on the tank wall, most of the water will settle to the bottom and develop into large pools which allows a full ecology of organisms to develop. Most facilities will drain the water from the tanks on a regular basis, but it is impossible to remove all the water and the organisms will remain as a mat or slime to reinitiate growth as more water enters. In time, growth by-products will develop a mineral scale that will serve to harbour both organisms and water when the tank is drained[126].

It is generally the case that growth is not limited by a shortage of oxygen. The fuel has a high dissolved oxygen level (often more than 300 ppm)[86] which is readily transferred to the aqueous phase[81]. The metabolic product, carbon dioxide, is also rapidly dissolved in fuel[154]. Although most fuel-water systems are therefore aerobic, stagnation and limited access of air, coupled with the rapid growth of organisms, may produce anaerobic conditions in storage tanks[82].

The temperature of the various components of the fuel system vary depending on their position in the ship. Temperatures will be found in fuel tanks away from engines and heating systems whereas temperatures in excess of 35°C will be found in centrifuges and coalescer-separators situated near the engines. Temperatures will

also vary depending on the geographic location of the ship. In most cases the temperature is favourable to growth.

Dieso contains at least 254 different compounds[208]. Analysis has shown that 47.5% of the hydrocarbons present are n-alkanes and that 73.6% of these alkanes have a chain length of between ten and eighteeen carbon atoms[208]. These have been shown to be the most readily and completely metabolised component of crude and refined oils[35,114].

Other nutrients, namely nitrogen, phosphorus, potassium and trace elements are less abundant in fuel, although their levels may be supplemented by fuel additives, rubber, paints, human waste and sea-water[93]. The concentration of nitrogen and phosphorus are major limiting factors in the biodegradation of oil in sea water[8]. The water soluble fraction of fuel oils contains variable amounts of benzene, toluene and xylene[217] which may be toxic to some microorganisms[70].

Turner et al.[209] imagined warship fuel systems to be divided into three basically different microbial environments. Firstly sea water displaced fuel tanks may contain a high ratio of water to fuel and offer a wide range of nutrients, in addition to those already in the fuel. This would depend on the type of water used to fill the header tanks. The extent of colonisation may be affected by such factors as sewage content and trace metal contamination. Secondly, undisplaced fuel tanks contain a low ratio of water to fuel and generally less particulate matter. Nutrient levels will be determined by the previous history of the fuel and availability from seepage and from deterioration of tank linings, seals, etc. Thirdly in coalescer elements a continuous flow of fuel and water passes over ensnared organisms, supplying their nutrient requirements and removing their metabolic products. Degradation of the cotton used in the construction of the coalescer elements may supply alternative carbon sources.

During the fuel's journey through the system, the levels of metabolic and cell lysis products will increase, the pH may be lowered by the production of acids[82] and oxygen will be consumed. However, conditions are likely to remain aerobic provided that there is

adequate fuel turnover[86].

Little published information regarding ship fuel system contamination is available and many reports are difficult to access because they are unpublished accounts of work undertaken in Service Establishments or in laboratories of industrial concerns. A major problem of corrosion, enhanced by anaerobic sulphate reducing bacteria growing in sea water ballasted fuel tanks in ships of the United States Navy has been studied[9,113]. Bailey and May[9] showed that the heavy fungal growth found in diesel fuel and a centrifugal purifier was almost entirely C. resinae though they rarely found C. resinae in entrained ballast water and failed to isolate it from sea water ballast. The predominant fungus in entrained water was a Fusarium species. Few bacteria and no sulphate reducing bacteria were detected in the fuel or the purifier, but sea water ballast in one ship contained large numbers of sulphate reducing bacteria.

In a report on the microbial infection of fuel used in the marine industry, Hill[86] drew attention to the severity of the problem in sea water displaced systems. He considered that of the organisms present in the water phase under light fuel oils, the most troublesome was C. resinae at normal temperatures for fungal growth and Aspergillus fumigatus at temperatures favouring thermophiles. He concluded that the growth of anaerobes was encouraged by high levels of organisms, and/or slow fuel turnover together with the presence of sea water.

In 1978 Burrows and Eaton[54] working on a Ministry of Defence supported research contract investigated the ecology of C. resinae and associated organisms in Dieso fuels. Organisms were isolated from fuel and/or sludge samples taken from a refinery, land storage tanks, refuelling tankers, sea water displaced tanks and coalescer and prefilter elements. With the exception of the refinery sample, from which only Fusarium aquaeductum and Alternaria alternata were isolated, C. resinae, Penicillium spp. and yeasts were found in all types of sample. Bacteria were not generally detected in fuel samples but were present in sludge, coalescer and filter samples; Pseudomonas fluorescens was the most frequently isolated bacterium.

Examination of fuel samples and filters from ships by staff at the Admiralty Research Establishment, Eastney, indicated that a diverse microbiological flora existed with C. resinae being the most predominant organism (D. Powell, pers. comm.).

Kuo[117] showed that in mixed populations bacteria were first to grow followed by yeasts and moulds. Generally C. resinae was not detected for 4 weeks. This was because neutral pH's favoured bacteria whereas the lower pH's favoured yeasts and moulds.

1.8.3 Effects of Microbial Contamination

Microbial growth in the fuel tank environment is initiated in water bottoms, in discrete droplets on the tank walls and in pools of water trapped behind ledges and strengthening girders. Plate 1.1 shows a contaminated fuel tank. Hydrocarbon utilisers will begin to grow at the fuel-water interface developing into a slime layer a few millimetres thick. Within six months this mat may reach a thickness of one centimetre or more depending on the prevailing conditions[96]. As the mat of growth develops it tends to act as a trap for any particulate matter such as rust and paint flakes leading to a rapid increase in depth. The breakdown of hydrocarbons into various metabolic products then allows the growth of non-hydrocarbon utilising organisms which increases the thickness of the mat still further. Periodically pieces of this slime will detach from the interfacial mat and sink to the tank bottom where a thick growing layer will develop[96]. Rough weather or rapid turnover may induce sufficient turbulence to disperse organisms into the fuel[88]. Once the contaminants become mobile they are effective in blocking filters and water-separator systems. Replacement of filter elements should normally only be necessary every year but in cases where the fuel is dirty or has become badly contaminated replacement may be necessary every day (J. Gisborne, pers. comm.). This is both costly and time consuming.

Growth at the interface is likely to remain aerobic. The by products of hydrocarbon utilisation alter the environment in a number of ways. Lower molecular weight organic acids will lower the pH of

Plate 1.1

A Contaminated Ship's Integral Fuel Tank

Heavy brown coloured filamentous growth can be seen on the strengthening girders.

the aqueous phase thus making it more corrosive to metals. They may also react with specific mineral salts in solution to release more corrosive acids[120]. The corrosion of metals will increase the availability of metallic ions in solution[42] which may enhance further growth. If fuel turnover is frequent there are unlikely to be significant chemical changes but slower turnover allows the fuel to pick up microbial surfactants and possibly microbially produced sulphide or organic acids[88]. Corrosion may be generalised due to changes in the fuel but is more likely to be confined to the water phase. Displaced fuel tanks of several R.N. ships have suffered from microbial corrosion (J. Gisborne, pers. comm.).

Higher molecular weight organic acids are surface active and by concentrating at the fuel-water interface will reduce the interfacial tension and permit ready emulsification of fuel and water. Alcohols and esters will distribute themselves between the aqueous and fuel phases and by doing so will increase the solubility of fuel in the aqueous phase. This leads to an extended zone of growth[96,120]. The major problem concerned with surfactant production is the breakdown of coalescer action which allows water into the gas turbines. This results in the reduction of turbine power, leading to a loss in the vessels operational efficiency[96].

Under certain conditions oxygen may be rapidly consumed leading to an anaerobic environment. For example rapid growth of sludge at the tank bottom may lead to anaerobiosis, especially underneath the sludge. If at the same time the temperature is suitable and if sulphate is present conditions will be favourable for growth of sulphate reducing bacteria[120] which would cause pitting corrosion of the steel tank and corrosion of other susceptible components by the production of soluble sulphide which would be carried throughout the fuel system. Thus, the following symptoms as summarised by Hill[86] may be apparent in contaminated fuels:

1. Mat-like or slimy deposits in the water phase in the tank bottom, particularly at the interface.

2. Unusual filter plugging, particularly during increased agitation in bad weather.

3. Reduction in interfacial tension resulting in increased entrained water in the fuel and malfunction of water separating equipment such as coalescer cartridges.

4. Corrosion in the neighbourhood of the fuel water interface.

5. Severe corrosion pitting in the structure bounding the water phase; pits in steel may have a graphitic appearance.

6. Black staining or black deposits on copper containing alloys used in fuel pipework or equipment.

7. Injector fouling and malfunction.

A comparison between gas turbine powered ships with and without seawater displaced fuel tanks has shown that whilst the former are more likely to experience microbial contamination problems, the latter are by no means immune[6].

1.8.4 Prevention of Microbial Corrosion

It is preferable to prevent rather than cure corrosion as, apart from wastage of materials there is the cost of manpower in dismantling and reassembling affected ship components. In addition the loss of availability and efficiency could be disastrous in war time[96].

There is no universal approach to the prevention of corrosion by microorganisms and the problem is usually tackled more from the corrosion than the microbiological point of view. However, it is not sufficient to provide a tank coating which will prevent corrosion as the presence of growths, living or dead, present a corrosion hazard should the coating become damaged[153]. Thus, as well as taking protective measures, the level of

microbial contamination must be continuously monitored and active steps taken to eradicate it once established.

Prevention is often easier than cure. There can be no significant microbial growth without free water and every effort should be made to re-locate drain points at dead tank bottoms or install scavenge pumps with a suction at the lowest level of a tank or sump whatever the trim of the ship[86]. Thus, there is a need for cooperation between designers and microbiologists to ensure that problems are not built into machinery.

The need for good housekeeping is very important. In addition to regular removal of water, routine maintenance cleaning removes accumulated foreign matter and old slime masses that not only cause problems in themselves but provide an excellent environment for the development of inoculum levels that cause widespread microbiological corrosion[132,143].

There are a large range of protective coatings available, such as epoxy type paints, which if chosen and applied correctly provide a high standard of corrosion protection. However, problems can occur in inaccessible crevices which are inadequately protected (D. Houghton, pers. comm.). An attractive idea is the use of antimicrobial additives in coatings which would leach into the water phase. Such anti-fouling paints have been developed for ships hulls[165] but do not appear practical for fuel tank use[154]. Biocides are expensive and their continuous use purely in a preventative role is not considered to be economically viable in ships fuel systems. The use of non-metallic materials has been proposed. Glass reinforced plastic is being used in fuel tanks and for hulls of small boats.

Cathodic protection is often used as a means of controlling corrosion. Two techniques are commonly used; the use of sacrifical anodes such as magnesium, zinc or aluminium alloys which corrode preferentially and provide protection for the structure, and the use of impressed current from a rectifier via an inert anode. Steel is usually polarised to -850mV vs $Cu/CuSO_4$ electrode, or higher in the presence of sulphide[187]. Use of these methods is restricted depending on the demands of the system, site

conditions and the undesirable necessity of providing a maintained water bottom.

Because of the complexity of many microbial problems it is necessary to monitor the level of contamination aboard individual units and from fuel storage systems. Methods for monitoring corrosion itself are inadequate[132]. Most are subjective estimates based on measuring either corrosion rates or microbiological populations. The unique characteristics of microbiological corrosion and the fact that corrosion occurs independent of electrochemical factors make procedures used with conventional corrosion studies inadequate. Analysis of, for example, water chemistry, electronic instrumentation or corrosion coupons will not provide consistent data about rates or severity of corrosion caused by microorganisms[132]. Most recent methods described in the literature[54] deal with the use of a photometer to measure bioluminescent and chemiluminescent reactions. Using the firefly luciferase-luciferin reaction ATP can be rapidly assayed[71]. The correlation of high concentrations of ATP in injection water and high microbiological corrosion rates has been fairly well established[132]. However, the method is not useful in non-petroleum environments where ATP from sources other than corrosion causing bacteria are prevalent[91].

Sulphide corrosion can be detected by leaving a piece of polished copper in contact with the fuel and examining at intervals (twice weekly) for black staining[86]. This is the basis of a more sophisticated Institute of Petroleum Test (IP 154/69). There may be a normal slow reaction, depending on the quality of fuel used and it would be advisable to get advice on interpretation from the fuel supplier.

Monitoring of microbiological contamination from ships by the Admiralty Research Establishment, Eastney, is conducted in two ways[96]. Firstly, from certain ships, millipore filter pads through which a known quantity of fuel has been drawn are received from various parts of the fuel system. From counts of trapped hyphal fragments it is possible to make a semi-quantitative analysis of contamination in that part of the system, or in the

ship as a whole. The technique is slow taking 15 minutes per filter, but possibly the best indicator at present[96]. Secondly, 'wet fuel' and 'sludge' samples may be examined, though currently samples containing water are not demanded and no effort is made to monitor for corrosion hazards (J. Gisborne, pers. comm.).

There are no simple on board tests to assay fuel or sludge samples for the presence of microorganisms. However, a water sample which would contain the majority of microorganisms, may be obtained from a tank drain or coalescer unit. Subsequent tests, could include 'Easicult', M-slide (for fungi), TTC slide (for aerobes) or S-test (for anaerobic sulphide producers). Heavy growth and khaki colouration on the M-slide and counts of greater than 10^4 on the TTC slide indicate heavy contamination and any positive reaction in the S-test gives cause for concern[86].

The Easicult kits are calibrated using standard models to which actual results may be compared thus giving quantitative data. It has been suggested[64] that for aqueous fluids results are normally within a log unit of the true count. However, this calibration does not hold if the fluid under test is more or less viscous than water, or does not 'wet' the agar gel on the slide. Thus they may not be suitable for fuel and sludge samples. There can also be false negative results if biocides are present. 'Sig-tests' can be used as on-site tests and will convey significance rather than numbers[90].

Having determined the level of microbiological contamination three options may be considered[96],

a) The level is not serious and is unlikely to impair the operational efficiency of the ship, so that no further action is necessary.
b) The system needs to be cleaned and drained at the earliest opportunity.
c) Contamination is serious and it is necessary for it to be drained, cleaned and treated with biocide dosed fuel.

The Royal Navy trys to avoid the latter option since

biocide is expensive and a period of three days is required for exposure to effectively eliminate microorganisms from the system[96]. Only in severe cases of contamination are biocides used, for example, occasionally this may be a warship where contamination has become serious over a period, or more commonly, a Royal Fleet Auxiliary tanker where the problems can be particularly acute, and the tanks difficult to clean.

At present an organoboron compound, "Biobor JF", is the biocide used by the Fleet. Besides its expense, it has the disadvantages of requiring a high dosage (270 ppm) and in tanks with more than a 1% water bottom its efficiency is considerably reduced[66,86,96,192].

Other biocides[88], e.g. Benomyl[192], Bodoxin, Grotan OX and Grotan OD are active in marine fuel at a few ppm, but again a fuel:water ratio of 400:1 or more is required for successful application. Of these only Benomyl satisfies fleet requirements.

Thus water compensated fuel storage systems are not amenable to control by current fuel biocides leading to active research into screening of both new compounds[66,167] and compounds from commercial sources, for their activity against microorganisms, especially C. resinae[122,192]. A rapid slide technique to study the germination and growth of C. resinae[191] in biocide treated fuel[192] has been developed.

Littman[126] has outlined the features of an ideal biocide and for fleet use it should be economic, effective against all organisms between 1-5 ppm and retain its efficiency in water bottoms as high as 10%. It is important that it is approved by the engine builder to be non-corrosive and otherwise compatible with the turbine machinery considering the fuel in use[86].

Because biocide treated ballast water would necessarily be off-loaded into a marine environment when a tank is refueled it is also necessary to determine the effect of the biocide on marine life. A biocide photodegraded by sunlight would cause less damage to the environment and selective biocides have been evaluated[144]. In simulated shipboard fuel tanks, a photodegradable mercaptopyridine compound was effective at the lowest

concentration.

Alternative methods for the removal of microbial contamination includes the installation of centrifuges, and filters. These have a limited capacity, increase cost and weight and require extra space, though by reducing the level of organisms they will make other treatments more effective[208].

The use of heat sterilisation has been considered. There are obvious logistical problems to be considered including the risk of explosion, cost and weight of equipment and increased chemical oxidation of the fuel at high temperatures[92]. It has been shown that spores of C. resinae in dry fuel could withstand 110°C for one minute and that an in-line treatment would have to raise the fuel temperature to at least 80°C for 20 seconds. However heat may be useful in the presence of biocides, for example, the effectiveness of ethylene glycol monoethyl ether was increased by raising the temperature to 50°C[179]. Sterilisation of jet fuel by gamma radiation has been proposed[112], but undesirable chemical changes may occur[84] and legislative problems are likely.

Thus there is no established procedure for dealing with microbiological corrosion. A combination of preventative techniques, monitoring and eradication of microbial contamination are carried out. Having thoroughly cleaned a contaminated system of loose debris, deposits and microorganisms, badly corroded parts may need to be replaced. Prior to putting the systems back into service it is necessary to pre-treat or passivate them to prevent the re-occurrence of corrosion. Passivation may be achieved by treating the system with the routine maintenance corrosion inhibitor at concentrations three to five times the maintenance treatment concentrations[132].

In fuel systems effective corrosion control may ultimately be based on the control of microorganisms using two biocidal systems. The first may be based on the incorporation of a high concentration of biocide into fuel oil. This biocidal oil could then be used as a disinfecting agent, to be pumped through the fuel system and then returned to store to be used again. The second would be compounds which may only be fungistatic but active

at very low concentrations of between 0.1 and 1.0 ppm. These would be added to all fuel oil thereby preventing the recolonisation of fuel tanks and fuel systems[191].

1.9 Corrosion Problems of some Metals and their Alloys in Naval Ships and Equipment

Naval departmental laboratories routinely examine various metals in simulated service conditions looking for good corrosion resistance. Promising samples may then be used in ship trials.

One of the problems in setting up simulated service conditions is that sea water varies widely in its corrosion properties according to the extent to which it is contaminated by either effluents or the products of microbial metabolism. The use of synthetic sea water has the advantage of providing a reproducible medium but obviously cannot simulate natural sea water. Use of recirculated natural seawater is more representative but its composition soon changes. Facilities for 'once-through' circulation of sea water are necessary for true simulation.

The choice of the four metals and alloys in the following section reflects their importance in Naval service, particularly in the fuel/seawater environment.

1.9.1 Stainless Steel

Most stainless steels are expensive and susceptible to some degree of corrosive attack in chloride contaminated environments. Thus their use in marine construction is limited to special circumstances where high strength and low cross sectional thickness are required, particularly in high temperature environments e.g. parts of oil sprayers for marine boilers and fittings and some pump and blower shaftings[187]. They are also used in many of the controls used to monitor fuel feed to gas turbines.

The stainless steels are notorious for their tendency towards crevice corrosion and pitting in sea water. Their use should be avoided if deposits, cracks or crevices are likely to be present as these allow a small area to remain in the active state when in contact with a larger area in the passive state. A technique to predict resistance to this type of attack has been developed[145].

Stainless steels however have good resistance to impingement attack up to about 30 ft/sec[170]. They have been successfully used for propellers on ships and the impellors of pumps, but only in circumstances where there is plenty of turbulence to carry oxygen to the surface of the metal so that the passive film may be maintained.

Corrosion in sea water is affected by the marine organisms present. Stainless steel forms a tightly adherent passivating film in sea water. Microbes and other fouling organisms produce a crevice in which regions of the normally passive film damaged by mechanical means or through halide attack go unrepaired; perhaps because the consumption of oxygen by the biofilm prevents the oxygen in the external environment from reaching the interior of the crevice. There have been several reports of biofouling and associated crevice corrosion[11,31,198], a particularly severe problem when the water velocity is low thus allowing attachment of fouling organisms and other solid matter[118].

Bacteria which oxidise ferrous to ferric ions or manganous to manganic ions (e.g. Gallionella, Sphaerotilis spp.) probably cause the co-accumulation of chloride ions in the region. This will cause the formation of acidic ferric chloride and manganic chloride solutions which are highly corrosive to stainless steels[157] and are often evident in the form of severe pitting[115,98]. Sulphur oxidising and sulphur reducing bacteria have also been found to be associated with pitting failures of stainless steel pipelines[115,198]. Twenty five isolates of both fungi and bacteria, particularly the former were shown to increase the corrosion of stainless steel 321[22] although neither the

mycelium nor the metabolites of C. resinae were found to enhance corrosion of a different stainless steel[72].

1.9.2 Mild Steel

Mild steel is the most commonly used metal in the ship building industry and because of its cheapness, strength, formability and weldability it is likely to remain so. It is used for ships hulls, buoys, containers, retaining walls, pipes, piles and underwater construction members of all types. All steels have a poor resistance to corrosion in sea water and salt laden atmospheres and their use often depends on secondary factors such as some form of barrier coat (e.g. paint) or cathodic protection. Although fuel tanks are often painted, pipelines and machinary used for fuel where corrosion is not expected are not. Plate 1.2 shows the complete perforation of a mild steel fuel supply pipe due to contaminating water.

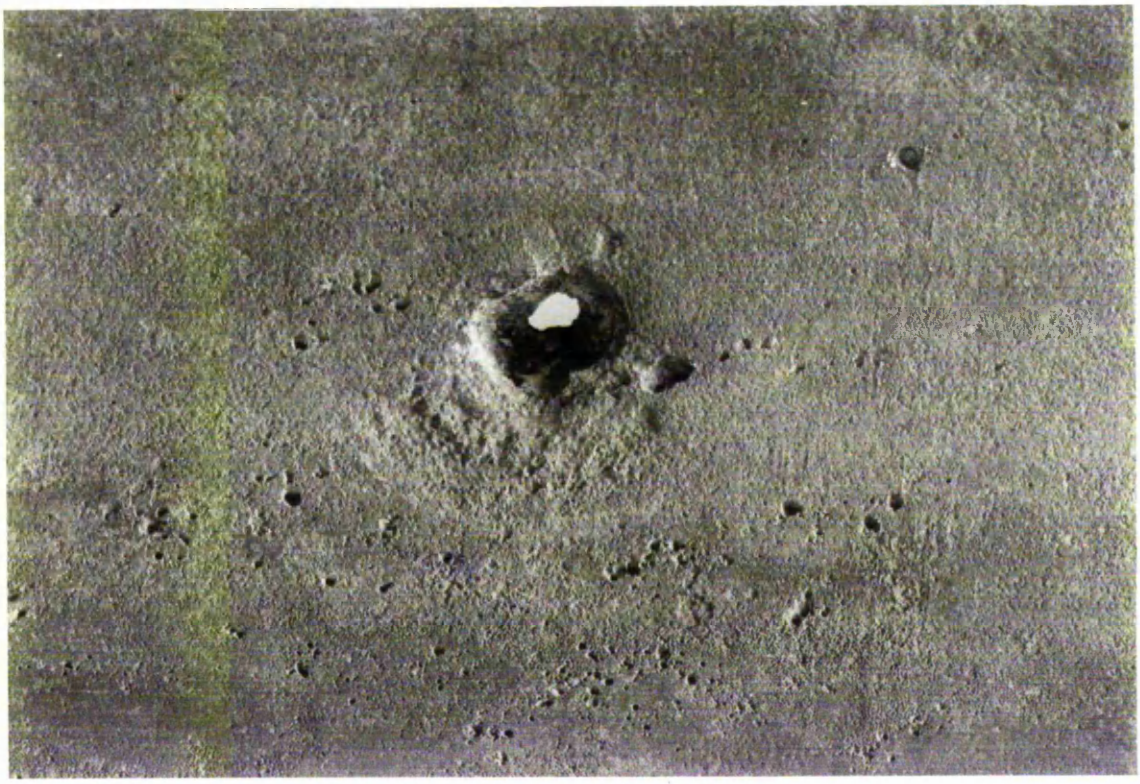
A loosely adherent voluminous corrosion product[31] is formed on mild steel which, under favourable conditions in unpolluted sea water, becomes increasingly protective during long exposures[118,178]. Disturbance of the rust with increasing water velocity increases the rate of attack until a critical velocity is reached[170]. The presence of fouling organisms or biological slimes may decrease the effect of velocity by isolating the metal from the water or may increase the corrosion by setting up areas of higher velocity with local turbulence[170]. Eventually, the most frequent cause contributing to an increased failure rate in ship steel piping carrying sea water is deposit attack under dead organic matter[170].

A cyclic sloughing off of the corrosion product has been reported[31]. With it went any biofouling which was attached. This rust/biofouling layer would be particularly troublesome in pipelines and intake systems where it may be subsequently carried to other parts of the seawater system where flow obstruction may occur, for example in heat exchangers.

Ferrous metals are probably more affected by marine

Plate 1.2

A Perforated Mild Steel Fuel Transfer Pipe



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organisms than any other class of metal, yet these biological influences are practically ignored by corrosion scientists[178]. Microbiologists have studied the role of sulphate reducing bacteria[169] and other organisms in the corrosion of mild steel in the marine environment but very little work has attempted to elucidate the role of fuel utilising microorganisms. Data obtained from long and short term exposures of structural carbon steel in different ocean locations showed a very high corrosion rate, up to 16 mpy during the initial period of exposure. After a year most of the test specimens had become covered with a mat of fouling organisms. These natural covers, although different in composition at the different sites all offered considerable protection to the steel panels. This beneficial effect was diminished once the sulphate reducers became active. It was suggested that if these bacteria could be selectively controlled, low corrosion rates for bare steel in sea water could be attained by the maintenance of a healthy fouling cover[178].

1.9.3 Cupronickel

Cupronickel is widely used in sea water systems because of its known corrosion resistance[108]. It is used for tubing in heat exchangers and steam condensers, for pipework and sometimes for fuel tanks which might contain sea water contaminated water bottoms.

Cupronickels are decreasingly susceptible to impingement attack as the proportion of nickel to copper increases. Thus 70/30 cupronickels are used for condensers which are narrow bore and have fast water speeds and 90/10 cupronickel is used for large bore pipework, for example fuel transfer pipes.

Copper nickel iron alloys suffer three main types of corrosion attack. Firstly, impingement attack or 'corrosion-erosion' is due to the erosion of the protective film by the impingement effect of collapsing entrapped air bubbles followed by corrosion of the exposed anodic areas giving a characteristic horse-shoe appearance. Its severity increases with increasing

amounts of entangled air and with higher water speeds. Poor fitments or entrapped foreign matter will also increase local turbulence and hence water speed. Secondly, deposit attack takes place under deposits of foreign matter or of loose corrosion products which have formed on the surface of the metal. This is most likely to occur in the presence of solid matter in the circulating water, with low water speeds or when the system is lying idle. Finally, pitting takes place due to the localised breakdown of the protective film. The rate of pitting increases in the presence of sulphide[196,197] and ammonia[164].

The case for microbiologically influenced corrosion of copper alloys is less well documented than for steels and aluminium. This may have been due to the misconception that copper ions and salts formed by copper alloy corrosion were lethal to all microorganisms[157]. However, pitting of cupronickel has been observed under microbial colonies[169] and Thiobacillus thiooxidans has been found to withstand copper concentrations as high as 2%[17]. C. resinae was found to tolerate 150 ppm copper sulphate[32] which is commonly toxic to other organisms.

The 90/10 copper nickel iron alloy is considered to be less prone to microbial fouling than the 70/30 cupronickel because it leaches more copper[170]. Alloys such as the 90/10 copper nickel iron alloy, because of their marine non-fouling properties are being considered for sheathing boats and fixed structures and for fish farming enclosures[29]. A test panel exposed to seawater for 18 months showed no evidence of biofouling attachment[31] a fact attributed to the toxicity of the cuprous oxide which forms as an adherent protective film in seawater.

1.9.4 Aluminium Alloys

Since World War II there has been a vast increase in the use of aluminium in the ship building industry. Many aluminium alloys exhibit a relatively high degree of resistance to corrosion by sea water and several have been primarily developed for marine use. Suitable alloys may be found for masts, ship

superstructures, life boats, funnel enclosures, davits, deckhouses, all-aluminium small craft and for the construction of minesweepers and the hulls of a number of boats, sloops and yachts[67,118,187]. They have been used for many years for the structural members, fuselage and fuel tanks of jet aircraft[119]. The strength/weight ratios required for ship construction are not so critical as those in aircraft. Hence alloys (aluminium - magnesium BS1470) are selected which exhibit high corrosion resistance with moderate strength levels. No previous work has been undertaken on these particular alloys which do not contain a grain boundary precipitated phase.

The majority of work on microbial corrosion has been concerned with that associated with jet fuel tanks[74,75,76,79,136,146]. The effect of C. resinae was most often studied and to a lesser extent Ps. aeruginosa, Desulfovibrio desulfuricans, and Aspergillus niger. In almost all of the systems tested aluminium suffered accelerated attack in the presence of microorganisms. This was generally observed as a weight loss with features of corrosion including exfoliation, blistering and pitting[98]. Attack by C. resinae has been shown to differ depending on whether the aluminium was subject to fungal growth or fungal metabolites[72]. The electrochemical behaviour of aluminium alloys in the presence of C. resinae, Candida sp. and Ps. aeruginosa has been followed. It was found that the pitting potential decreased due to the activities of the microorganisms[43,172,173,174].

The corrosion of aluminium alloys in single phase aqueous systems has not been studied nearly as often, though corrosion by sulphate reducing bacteria has been shown to accelerate the corrosion rate by 3 to 100 times that found in sterile medium[111]. A similar acceleration of weight loss by these organisms has been shown in seawater test systems[218]. In both these tests, pitting occurred rather than the general surface corrosion observed with iron in similar cultures.

The good performance of aluminium and its alloys in many corrosives is attributed to a protective, tightly adherent,

invisible oxide film on the surface. Oxygen or oxidising substances in many solutions and in the atmosphere instantaneously develop this protective film which is generally stable between pH 4.5 and 8.5[118]. Any adherent biofouling will serve to reduce oxygen access to the metal surface and can result in localised corrosion[31]. Microorganisms can alter the pH, CO_2 and O_2 content of seawater all of which have been shown to effect the corrosion rate of aluminium[50,51,171].

1.10 Aims of Thesis

Many examples of microbially induced corrosion have been published though further study is required as the specific mechanisms are poorly defined. Such corrosion processes have economic significance and a greater understanding is required in order to implement effective control measures. At present, adequate methods of dealing with it, short of replacement with resistant alloys, are largely unavailable[111, 137].

C. resinae has frequently been identified as the predominant organism in ships' fuel systems. Although the activity of a mixture of fuel contaminants was examined briefly, the Thesis was designed to examine the corrosion mechanisms involved and the susceptibility of Service and related alloys to attack by C. resinae and to determine the nature and severity of corrosion.

CHAPTER 2

MATERIALS AND METHODS

2.1 Isolation, Maintenance, Growth and Identification of Microorganisms

2.1.1 Culture Media

Several different culture media were used during the course of this work and the recipes were as follows:

Nutrient broth. Difco nutrient broth (8 g l^{-1} containing 3 g beef extract and 5 g peptone) was adjusted to pH 6.8, dispensed into suitable containers and autoclaved at 121°C for 15 min.

Nutrient agar. Oxoid nutrient agar (CM 3, 28 g l^{-1} containing 1 g Lab-lemco, 2 g yeast extract, 5 g peptone, 5 g NaCl and 15 g agar number 3) was adjusted to pH 7.4 and sterilized in 500 ml quantities at 121°C for 15 min. On cooling to about 55°C plates containing 15 ml agar per plate were prepared.

Malt extract agar. Oxoid malt extract agar (CM 59, 50 g l^{-1} containing 30 g malt extract, 5 g peptone and 15 g agar number 1) was adjusted to pH 5.4 and sterilized in 500 ml quantities at 114°C for 10 min. On cooling to about 55°C plates containing 15 ml agar per plate were prepared.

Malt extract broth. Malt extract broth (l^{-1}, 0.78 g Difco peptone, 2.35 g glycerol, 12.75 g maltose and 12.75 g glucose) were adjusted to pH 5.4; dispensed into suitable containers and autoclaved at 114°C for 10 min.

Bushnell-Haas mineral salts solution. Quarter strength modified Bushnell-Haas mineral salts solution, post autoclave pH 6.95, contained the following (g l^{-1}): $MgSO_4$ 0.05, $CaCl_2$ 0.005, KH_2PO_4 0.25, K_2HPO_4 0.25, NH_4NO_3 0.25 and half drop conc. solution of $FeCl_3$.

Turner's solution. Turner's solution was prepared as four separate solutions as shown in Table 2.1. After the ingredients had dissolved, solutions A and D were sterilized at 121°C for 15 min, solution B at 114°C for 15 min and solution C by filtration through a 0.45 μm Millipore pad. When all of the solutions were cool, they were mixed in the proportions indicated in the Table and the pH was adjusted to 5.8 with sterile NaOH.

This mineral solution was developed by Turner[207] and is hence referred to as Turner's solution.

Sea water. Sea water was prepared to BS 3900 specification and contained the following (g l^{-1}): NaCl 26.5, $MgCl_2$ 2.4, $MgSO_4$ 3.3, KCl 0.73, $NaHCO_3$ 0.2, NaBr 0.28, $CaCl_2$ 1.1. The pH was adjusted to 8.2 and sterilization was at 121°C for 15 min.

In many experiments modifications were made to the sea water, for example, 20, 30, 35 or 40% (v/v) sea waters were prepared by making suitable dilutions of concentrated sea water with distilled water before autoclaving. In the majority of experiments phosphorous (28 μg l^{-1}) was added. This was achieved, by adding to 1 litre of sea water, 1 ml of a 1/1000 dilution of a solution containing 1.23 g KH_2PO_4 per 10 ml. In addition nitrogen (0.5%, w/v) was added by adding 23.4 g $(NH_4)_2SO_4$ per litre of sea water. In each case the medium was pH'd to 7.0 with H_2SO_4.

Carbon source in Bushnell-Haas, sea water or Turner's solution. In the majority of experiments either Dieso or particularly n-undecane were supplied as the carbon source. Dieso (Naval diesel fuel, Defence standard 91-7/2) was obtained from RN Dockyard Laboratory, Portsmouth and n-undecane, henceforth called undecane from Sigma (>99% pure). Dieso was sterilised by filtration and undecane by autoclaving at 114°C for 15 min. They were gently layered onto the media generally before inoculation. The ratio of aqueous to non-aqueous material varied but was usually 10:1 unless otherwise indicated.

Table 2.1

Ingredients contained in Turner's Solution

Solution	Constituent	Concentration of solution prepared ($g\ l^{-1}$)	Amount added per litre of complete medium (ml)	Final Concentration ($g\ l^{-1}$)
A	KH_2PO_4	13.889		12.5
	$Na_2HPO_4 \cdot 12H_2O$	3.194	900	2.875
	NH_4NO_3	6.667		6.0
B	$MgSO_4 \cdot 7H_2O$	2.128	94	0.2
	$CaCl_2 \cdot 2H_2O$	0.213		0.02
C	$FeCl_3 \cdot 6H_2O$	1.67	5	8.85×10^{-3}
D	$ZnSO_4 \cdot 7H_2O$	0.09		9×10^{-5}
	$CuSO_4 \cdot 5H_2O$	0.08		8×10^{-5}
	$MnSO_4 \cdot 4H_2O$	0.08	1	8×10^{-5}
	$CoCl_2 \cdot 6H_2O$	0.09		9×10^{-5}
	H_3BO_4	0.05		5×10^{-5}
	$NaMoO_4 \cdot 2H_2O$	0.15		1.5×10^{-4}

2.1.2 Assessment of the Growth of C. resinae in Turner's or Sea Water Media

Growth of C. resinae in Turner's medium always occurred, at least initially, at the interface between aqueous medium and non-aqueous carbon source. This was also generally true for growth of the fungus in sea water. A variety of methods to estimate growth was tried and the visual assessment shown in Table 2.2 was found to be the most suitable.

Dry weight determinations were determined as follows: Whatman Number 1 or Millipore (0.22 μm) filter papers were numbered, dried at 55°C overnight and weighed to 0.1 mg accuracy. Residues of cultures filtered through the papers were dried to a constant weight at 55°C. The filter paper's weight was subtracted from the final weight to give the microorganism dry weight.

Spore counts of C. resinae inocula were carried out using an improved Neubauer haemocytometer and counting the spores using the x 40 objective of the microscope. Routinely five of the 25 0.004 mm^3 squares (the four corners plus the centre square) of a suitably diluted spore suspension were counted and the numbers per ml found by multiplying the count by 5×10^4 and correcting for dilution.

2.1.3 Isolation and Maintenance of Pure Cultures

Samples were occasionally provided from fuel systems of RN ships. When received these samples were processed quickly in an attempt to isolate as many species as possible.

A typical procedure was, as follows: a 1 ml sample of sludge from a RN ship's fuel tank was transferred to 10 ml of Bushnell-Haas mineral salts solution overlaid with Dieso. Incubation was at 30°C under static conditions. Samples were removed at 24 hour intervals and streaked onto nutrient or malt extract agar plates.

Isolates were examined microscopically and divided into

Table 2.2

Visual Assessment of the Growth of C. resinae in either Turner's or Sea Water media with Dieso or undecane as Carbon Source

a) Growth in Turner's Solution

0	No growth
1	Barely visible growth
2	Small amount of growth in isolated colonies
3	Easily visible growth, colonies tending to join giving semi-continuous cover
4	Continuous growth over the surface
5	Heavy growth. Thick mat covering whole of aqueous surface
6	Very heavy solid mat with growth up sides of flask and down into aqueous medium

b) Growth in Sea Water Medium

0	No growth
1	Barely visible growth
2	Slight growth at interface and/or base of flask
3	Still slight but easily visible growth. Still discrete colonies
4	Thin but continuous growth covering aqueous layer

bacteria and either unicellular or filamentous fungi. These were all maintained on either nutrient or malt extract agar slopes, subcultured as required and checked regularly for contamination. The possibility of contamination was of particular concern when using C. resinae for long term experiments. Thus, before inoculating large numbers of flasks for lengthy experiments the inoculum was prepared and was used initially to inoculate nutrient and malt extract agar plates. These grew readily within two days and were examined for purity. The remaining inoculum meanwhile was maintained at $4^{o}C$. Storing inoculum under these conditions, even for several days, was shown experimentally not to affect the viability of C. resinae.

2.1.4 Preliminary Studies on Isolated Microorganisms and Partial Identification of Five Bacterial Isolates

Eleven stable isolates were initially cultured on malt extract agar in an attempt to study culture characteristics and comparative growth rates. Four organisms would not grow on malt extract agar but did grow on nutrient agar.

Each isolate was then transferred to either malt extract or nutrient broth where the following tests were carried out:

Microscopic appearance.
Presence or absence of motility.
Growth in aerobic and/or anaerobic conditions.
Gram stain.

A wide range of biochemical tests were carried out to attempt to identify the five bacterial isolates, at least to the generic level. The tests were initially selected arbitrarily though the choice became more specific. The tests employed were as follows, each test being carried out several times and, unless indicated, always giving the same result.

a) Range of growth temperatures.

Each isolate was inoculated onto fourteen agar slants and these were incubated in pairs at $4^{o}C$, $25^{o}C$, $30^{o}C$, $37^{o}C$, $42^{o}C$, $45^{o}C$ and $55^{o}C$. Slants were examined daily for growth for seven days.

b) Indole production.

Each isolate was grown in tryptone water at $30^{o}C$ for 48 hours and the presence or absence of indole was determined using Kovac's reagent.

c) Methyl Red and Voges Proskauer Test.

Each isolate was grown in glucose phosphate broth at $30^{o}C$ for four days. To half of the broth was added 1.0 ml methyl red; an immediate red colouration indicating acid production and thus MR +ve.

To the second half of each broth was added alkaline α -naphthol. A red colour within two hours indicating acetoin production and thus VP +ve.

d) Citrate utilization.

Each isolate was grown in Koser's citrate medium for several days. The presence of growth and a blue colouration indicating the use of citrate as a carbon source in these organisms.

e) Hugh and Liefson's Test.

Each isolate was inoculated by a straight wire into two tubes of Hugh and Liefson's medium containing 1% glucose as carbon source. Liquid paraffin was added to the surface of one of each pair of tubes. After incubation at $30^{o}C$ the tubes were examined

daily for growth and indicator colour. A yellow colour in the open (aerobic conditions) but not in the closed tube indicated oxidative growth. A yellow colour in both tubes indicated fermentative growth.

f) Oxidase activity.

A colony of each organism was added to a filter paper together with 2 to 3 drops of 1% tetramethyl - ρ -phenylenediamine dihydrochloride. A dark purple colour within 10 sec is termed oxidase +ve indicating the ability of organisms to oxidize the dye via a cytochrome oxidase system.

g) Catalase test.

Each isolate was grown in nutrient broth for 48 h at 30°C. A capillary tube was dipped into a hydrogen peroxide solution and then a broth culture and the presence of gas bubbles within two minutes was termed catalase +ve.

h) Carbohydrate utilization.

Each isolate was inoculated into several tubes containing Andrade's peptone water, Durham tubes and one of a variety of carbohydrates. After incubation at 30°C the tubes were examined for gas production and a pink colour indicating acid production.

i) Gluconate oxidation.

Each isolate was grown at 30°C for 4 days in a peptone, yeast extract gluconate broth. Addition of Benedict's solution and formation of an orange colour after ten minutes boiling indicated the presence of 2-ketogluconate and hence gluconate oxidation.

j) Starch hydrolysis.

Each isolate was grown at 30°C for several days on starch agar plates. A blue colour after flooding the plate with iodine indicates the presence of starch whereas a brown colour indicates that starch has been hydrolysed by amylase enzymes.

k) Growth on MacConkey agar.

Each isolate was grown at 30°C for 14 days on MacConkey agar plates. This is a selective medium containing bile salts and a negative result was one in which no growth had occurred within 14 days.

l) Casein hydrolysis.

Each isolate was grown on skimmed milk agar at 30°C for at least one week. The ability of organisms to hydrolyse casein was denoted by a cleaving of the opaque agar around the colonial growth.

m) Gelatin hydrolysis.

Each isolate was grown in nutrient gelatin at 30°C. Every two or three days the tubes were refrigerated for two hours and examined for liquefaction. No liquefaction within 30 days was indicated a negative result.

n) Egg yolk test.

Each isolate was grown on nutrient agar containing egg yolk emulsion at 30°C. Plates were examined daily for a cleaving of the opaque agar around the colonial growth.

o) Arginine dihydrolase.

Each isolate was grown in arginine broth at 30°C for 48 h. A positive result was indicated by production of a brown colour on addition of Nessler's reagent.

p) Nitrate reduction.

Each isolate was grown in nitrate broth containing Durham tubes at 30°C for 5 days. Each broth was then examined for gas production and also the presence of nitrite, indicating nitrate reduction.

q) Nitrogen fixation.

Each isolate was grown on Ashbey's nitrogen free medium at 30°C for 10 days. Any growth, indicating the ability of organisms to fix nitrogen was considered to be a positive result.

r) Tween 80 hydrolysis.

Each isolate was grown on agar containing Tween 80 at 30°C for several days. The ability of organisms to hydrolyse Tween 80 was denoted by a clearing of the opaque agar around the colonial growth.

s) Presence of fluorescent pigments.

Plates of each organism were placed under an ultraviolet light source and examined for fluorescent pigment production.

t) API (10) Strip.

The API strip is essentially for help in identifying Enterobacteriaceae. It contains reagents for twelve tests which are carried out quickly in microtubules already containing

dehydrated media and other necessary reagents. The tests involved were:

i) Galactosidase
ii) Glucose utilization
iii) L(+)arabinose utilization
iv) Lysine decarboxylose
v) Ornithine decarboxylase
vi) Citrate utilization
vii) H_2S formation
viii) Urease production
ix) Tryptophan deaminase
x) Indole production
xi) Cytochrome c oxidase
xii) Nitrate reductase

2.1.5 Investigation of the Individual Growth Characteristics of Isolated Microorganisms under Varying Conditions

(a) A preliminary study of 15 isolates grown in either distilled water, sea water or Turner's solution with either Dieso, undecane or glucose as the carbon source was undertaken. Into 100 ml Erlenmeyer flasks were placed 30 ml of mineral solution, 1 ml of inoculum and either 3 ml of Dieso, undecane or glucose (final concentration 10 g l^{-1}). The flasks were statically incubated at 30°C for eight weeks. Visual growth assessments were made after 3, 4 and 8 weeks and pH values taken after 4 weeks.

(b) The growth characteristics of the five partially identified bacteria (isolates 1, 3, 4, 10 and 11), a yeast (isolate 7), a fungus (isolate 8) and C. resinae were investigated. Each organism was inoculated separately into eight 500 ml flasks which contained (in duplicate) the following media:

125 ml Turner's solution plus 15 ml Dieso
125 ml Turner's solution plus 15 ml undecane
125 ml modified sea water plus 15 ml Dieso
125 ml modified sea water plus 15 ml undecane

Flasks were examined regularly, visually for growth and, less often, pH estimations and viable counts using the Miles and Misra technique were carried out.

(c) Growth of C. resinae and isolates 1, 3, 4, 10 and 11 at initial pH's of 3 to 11 was assessed. The pH of sterile malt extract broth plus nutrient broth (50 : 50) plus 1% glucose (ME/NB/Glu) was aseptically modified using either 0.2M NaOH/Boric acid or 0.2M citric acid/K_2HPO_4 and distributed as 5 ml volumes in duplicate tubes. The tubes were inoculated and incubated statically at 30°C. Growth was assessed after 3 and 6 days. In addition, the effect of an initial pH of 6.5 or 8.2 for concentrated or 35% sea water on growth of C. resinae with undecane as the substrate was investigated.

2.1.6 Investigation of the Growth Characteristics of Selected Mixed Cultures of Microorganisms

An attempt was made to compare the growth of four microorganisms individually, altogether and altogether in the presence of a fifth organism, C. resinae. Twelve 500 ml flasks containing 200 ml of a malt extract broth/nutrient broth mixture (1:1) were prepared. Four organisms were selected, two yeasts (isolates 6 and 7) and two bacteria (isolates 4 + 12). Flasks were inoculated (in triplicate) with 1.5 ml inoculum and 6.0 ml sterile distilled water in each case. Three further flasks were inoculated with 1.5 ml of each of the four organisms plus 1.5 ml of sterile distilled water. Finally, three more flasks were inoculated with 1.5 ml of each organism and 1.5 ml of C. resinae. The flasks were incubated in an orbital shaker (150 rpm) at 25°C.

Samples were removed at intervals over a seven day period and viable counts of organisms made (except in the case of C. resinae where only a visual estimate of growth was made). During counting from mixed cultures organisms were differentiated by colony characteristics and on antibiotic-selective plates. Preliminary experiments had shown that the yeasts could be separated on MEA + 50 μ g ml^{-1} streptomycin and the bacteria on NA + 0.25 mg ml^{-1} cycloheximide.

2.1.7 An Investigation of the Growth Characteristics of C. resinae

Parbery[149] has shown that growth conditions profoundly affect the growth of C. resinae and it is reported that different isolates of the organism have different growth rates and produce varying amounts of acids. In view of these findings it was important to characterize the C. resinae isolates available and hopefully to select one for use in the study of microbial corrosion.

Initially, the growth rates of four C. resinae strains on solid media were studied; three had been isolated from contaminated tanks of HMS Battleaxe and the fourth was ATCC 34066. The isolates were grown on malt extract agar plates for 1 week at 30°C. A central 9 mm diameter plug was carefully removed and aseptically transferred to a fresh malt extract agar plate from which a 9 mm plug had been removed. The plates were incubated at 30°C and the diameter measured periodically over a nine day period.

Results were expressed as radial growth, that is (diameter - 9 mm) divided by two.

The growth rates of C. resinae strains in Turner's solution with undecane were also investigated. Flasks (500 ml) containing Turner's solution (250 ml) and undecane (25 ml) were inoculated with C. resinae spores and growth was observed at intervals over a 76 day period.

The effect of both static and shaking incubation conditions and also ratios of aqueous to non-aqueous phases on the growth of C. resinae were investigated. Flasks were incubated at 30°C over a ten day period and growth observed visually. The uptake of oxygen by aqueous solutions underneath varying depths of non-aqueous material was measured using a Clarke oxygen electrode assembly connected to a Servoscribe single channel recorder.

In preliminary experiments, two types of inoculum were used. Firstly, a damp swab or sterile glass spreader was streaked several times over a malt extract agar plate containing a seven day old C. resinae culture to which had been added approximately 5 ml of sterile distilled water. The suspensions of spores and broken mycelia from several plates were pooled and shaken to separate clumps of material. The suspension was then used to inoculate Turner's solution overlaid with undecane. In the second method of inoculum preparation sterile Turner's solution supplemented with 1% glucose replaced the distilled water. The suspension was incubated for 24 hours at 30°C in an orbital incubator and then centrifuged at 9,000 rpm for 10 min. The pellet was washed twice in water, and resuspended in water. This final suspension was then used to inoculate a second set of flasks.

Both sets of flasks were incubated statically at 30°C and growth and pH were measured at intervals.

The growth of C. resinae in sea water of varying concentrations was investigated. Concentrated sea water prepared to BS 3900 specification was used undiluted and also at 20, 30 and 40% concentrations. Duplicate flasks of each of the four sea water strengths together with two control flasks containing Turner's solution and undecane were inoculated with C. resinae and incubated statically at 30°C. Periodically flasks were examined for growth and pH change.

In a second experiment, growth of C. resinae in Turner's

solution and undecane suplemented with either 3% or 1% NaCl was compared to growth in 30% sea water containing either 0.5% nitrogen and 28 $\mu g\ l^{-1}$ phosphorous. Experimental conditions were as above.

2.2 Analysis of Culture Filtrates

2.2.1 General Methods

2.2.1.1 pH Measurements

A Russel micro pH electrode connected to a Pye Unicam series 292 meter was used.

For the experiments in which samples could not be withdrawn without permanently disturbing the interfacial position aseptic monitoring was practised. The electrode was soaked for ten minutes in 70% absolute alcohol, thoroughly rinsed in sterile distilled water and carefully inserted into first the control systems and then the test systems, rinsing with sterile distilled water between measurements.

Most experiments were not adversely affected by the withdrawal of a sample. A volume of only 0.7 ml was needed to obtain a reading.

2.2.1.2 Nitrate Concentration

An EIL 7030 meter with nitrate and reference electrodes was calibrated using nitrate standards in the range 20 to 200 ppm. They were made from a 1,000 ppm stock solution of potassium nitrate in saturated calcium sulphate. The nitrate concentration in the culture filtrates was read directly, usually after dilution in saturated calcium sulphate solution.

2.2.1.3 Redox Potential Measurements

The oxidation (or reduction) capacity of a solution is

characterised by the free electron activity in it. A solution containing only one reversible system, at sufficient concentrations of oxidised and reduced forms, acquires a stable value of electron activity and, consequently, a stable oxidation potential. Such solutions are buffers in regard to redox. However, a thermodynamically steady equilibrium state for solutions of microbial cultures is not characteristic. The interpretation of such redox potentials is difficult because their value is interdependent on reversible redox couples, irreversible reductors and the action of free oxygen and free hydrogen[102,103]. The potential characterises the occurrence of a stationary state dependant on the various kinetic factors. This so called 'stationary potential' has a value which is intermediate between the oxidation potentials of the reversible systems contained in the solution. In contrast to the oxidation potential the stationary potential cannot be defined in a strictly thermodynamic way but it can provide information on the redox characteristics of the solution and its changes.

According to Jacob[103] the start of growth is characterised by a shift to a more negative potential and the potential only becomes more positive due to diminished metabolism resulting from the beginning of lysis. The negation was explained by a reduction in the pressure of dissolved oxygen caused by the respiration of microorganisms. He further concluded that hydrogen generated during metabolism of facultative anaerobes caused strong redox negation. The role of extracellular metabolic products and the activity of intracellular enzymes (oxidases, hydrogenases) was considered "not to give a satisfactory explanation of the characteristic redox potentials".

A Pye-Unicam Series 292 meter was used as a millivolt meter. Its accuracy was checked by immersing a platinum indicator

and a saturated calomel reference electrode into a 0.1N HCl solution saturated with quinhydrone. This, when dissolved in an aqueous solution less than pH 8, yields equal concentrations of benzoquinone and benzoquinol. The potential of the quinhydrone electrode (Equ) is 620 mV with respect to the calomel electrode (Ecal) which is 230 mV at 37°C. Thus, the electromotive force (emf) is equal to

Ecal - Equ
= 230 - 620
= -390 mV

Therefore, the millivolt meter was set at -390 mV. Potentiometry has shown that the calomel electrode is positive with respect to the platinum electrode. The emf measurement at any given time is therefore represented by the expression

$$Emf = Ecal - Eh$$

Thus, Eh (stationary potential) may be calculated from Emf.

To measure Emf of the culture media the undecane layer was removed from the aqueous phase. The electrodes were immersed for five minutes before a reading was taken.

2.2.1.4 Analysis of Metal Ions

(i) A Perkin-Elmer model 103 atomic absorption spectrophotometer was used to analyse Fe, Cr, Cu, Ni, Mn, and Zn metals.

Calibration curves were prepared by plotting absorbance versus concentration of suitable standard solutions of each metal, diluted in modified sea water and Turner's solution.

The samples were acidified with 10N HCl (0.5 ml in 9.5 ml) to free the metal from any organic acid - metal complexes. The sample was diluted, if necessary, to fall within the linear concentration range.

(ii) Aluminium concentration was determined using a Perkin-Elmer Model 1000 Fluorescence Spectrophotometer.

A Standard Aluminium Solution (2 μg/ml) was prepared by dissolving 17.6 g $Al_2(SO_4)_3 K_2SO_4 \cdot 24H_2O$ in 1 litre of water and diluting this solution 1 in 500; A 0.1% solution of a 0,0'-dihydroxyazo dye was prepared by dissolving 0.1 gram of "Mordant Red 5" in 100 ml ethanol and finally ammonium acetate solution was freshly prepared by dissolving 50 g of the salt in distilled water and diluting to 500 ml.

To 50 ml volumetric flasks were added 5 ml of ammonium acetate, 0.5 ml 4N sulphuric acid and 1.5 ml of 0.1% Mordant Red 5 dye solution. Standards were prepared by adding 0, 2, 4, 6, 8 and 10 ml of the 2 μg/ml aluminium solution to the 50 ml flasks and diluting to 50 ml with distilled water. The pH of each solution was adjusted to 4.6 ± 0.2 before dilution.

Acidified sea and Turner's solution samples were generally used in volumes of 3 and 1 ml respectively instead of the standard addition above.

The solutions were allowed to stand for 1 hour. The fluorescence was measured at 541 nm using a 518 nm primary sample filter.

The concentration (ppm) of aluminium in the samples was calculated from the standard calibration curve.

2.2.2 Organic Acid Analysis by Temperature Programmed Gas Liquid Chromatography

2.2.2.1 Instrument Specifications

Perkin-Elmer Gas Chromatograph Model F33
Glass column - 61 cm length, 6.4 mm outside diameter
Column material - 10% Reoplex 400 on acid washed Chromosorb W (60 - 80 mesh)

Injection port temperature - 200°C
Temperature programming rate - 5°C/minute (range 50° - 210°C)
Nitrogen flow rate - 88 ml/min
Hydrogen pressure = 1.4 kg cm^{-2}
Air pressure = 1.6 kg cm^{-2}

The column was conditioned at 215°C to remove background noise.

The gas chromatograph was attached to a Spectrophysics SP 4100 integrator which printed out and/or depicted retention times and peak areas.

2.2.2.2 Preparation of Standards

Purified acids were purchased from Sigma Chemical Company. The methyl esters of lactic, pyruvic, citric, cis-aconitic, isocitric, α-ketoglutaric, succinic, fumaric, malic, maleic and itaconic acids were prepared using the following standard methylation procedure[73].

The acids (10 - 100 mg) were dissolved in 3 ml of 51% BF_3-methanol (Aldrich Chemical Company) in a capped universal bottle. Methylation was carried out by shaking the bottles overnight in a 28°C water bath shaker. The BF_3-methanol complex was then hydrolysed by the addition of 5 ml of distilled water and within 5 min the esters were extracted twice with 2 ml of chloroform by shaking vigorously for 2 min each time. The combined extracts were dried over anhydrous sodium sulphate and were injected directly into the gas chromatograph. An attenuation of 32 and an injection volume of 2 μl were used.

2.2.2.3 Preparation of Samples

A 20 ml sample was acidified to pH 2.0 or below using 5M H_2SO_4. This converted the acids from the salt form (R-COONa) to the free acid form (R-COOH) to facilitate esterification. The sample was taken to dryness using a rotary evaporator at 40°C and

methyl esters were prepared as described in the previous section. An attenuation of 2 and an injection volume of 5 µl were generally used.

2.2.2.4 Conversion of Standard Acid Peak Areas to Quantity of Acid in the Sample

Arbitrary peak areas corresponding to 50 mg of each acid were assigned by the integrator. For example a peak area of 3007500 units was obtained for 2 µl of a 50 mg preparation of succinic acid injected at an attenuation of 32. This standard value was converted to that which would correspond to the peak area had 5 µl and an attenuation of 2 been used:

$$3007500 \times \frac{32}{2} \times \frac{5}{2} = 1.203 \times 10^{8}$$

$$\text{mg per unit peak area} = \frac{50}{1.203 \times 10^{8}} = 4.156 \times 10^{-7}$$

This conversion factor was divided by 20 to account for the sample which was dried down from 20 ml. It was also multiplied by 10^{3} to allow conversions into µg/ml:

$$\frac{4.156 \times 10^{-7} \times 10^{3}}{20} = 2.08 \times 10^{-5}$$

Such conversion factors were calculated for each standard acid enabling the sample peak areas to be converted into µg/ml acid.

2.2.3 Electrochemical Measurements

2.2.3.1 Polarisation Resistance Technique

A Step-Polariser, Wenking Model SPO 80, was used to

measure the current density variation within a potential range of 10 mV around the rest or corrosion potential of the sample. The steepness of the current density zero crossing at the rest potential, the polarisation conductance (or reversely the polarisation resistance), rises and falls in correspondence with the corrosion rate of the sample.

The corrosion potential, E_{corr}, was read from a built in meter. The cell current was recorded on an XT single channel Servoscribe RE541 potentiometric recorder, connected to the terminals for current.

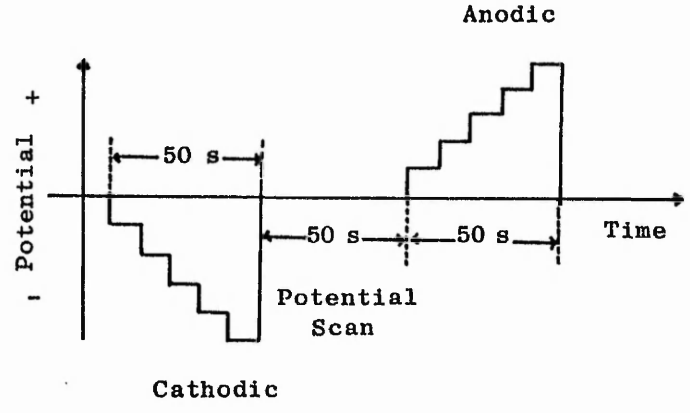
In connection with an electrochemical cell (following section) the SPO 80:

a) Measures and samples the rest potential of the working electrode versus the reference electrode before starting up the step-polarising programme.

b) Holds the sampled rest potential for control when starting the potential control programme at zero cell current i.e. E_{corr}.

c) Polarises the potential controlled working electrode deviating from the rest potential in small selectable steps (2 mV), 5 steps cathodic, 5 steps anodic polarisation in selectable succession, after an accurately settled potential versus time programme shown in Figure 2.1.

Figure 2.1
Potential Scan



The duration of each step is 10 s. Considering an initial polarisation of 10 s at the rest potential and a pause of 50 s between cathodic and anodic polarisation the complete programme is over within 160 s (non-repeating).

The linear polarisation technique is based on the fact that within about 10 - 20 mV of the corrosion potential the overvoltage varies linearly with applied current. Stern and Geary[194] derived the following mathematical relationship necessary for the application of the experimental data to calculate corrosion rates:

$$I_{corr} = \frac{\beta a \,.\, \beta c \,.\, \Delta i}{2.3\ (\beta a + \beta c).\ \Delta E} \qquad (1)$$

where βa = anodic Tafel slope

βc = cathodic Tafel slope

$\Delta i/\Delta E$ = admittance (reciprocal slope of the potential versus current)

Equation 1 is only valid if both reactions are under activation control. This is not the case in Turner's solution and so equation 2 was used which is valid when concentration polarisation prevails.

$$I_{corr} = \frac{\beta a \,.\, \Delta i}{2.3 \,.\, \Delta E} \qquad (2)$$

For absolute accuracy the Tafel constants, βa and βc, should be determined for each metal and each aqueous environment. A cathodic and anodic polarisation curve would have to be produced by polarising the specimen -300 mV from Ecorr and scanning up to +300 mV from Ecorr at the rate of 0.1 mV/sec.

Metals in Turner's solution were found to exhibit diffusion limited current density. The Tafel constants could not

be determined because the linear region did not extend over at least one decade of current. In any case a distinct disadvantage of having to measure the Tafel constants would be the time involved which would counteract the main advantage of the measurement of polarisation resistance, that is, it's speed.

For a large majority of metal-electrolyte systems βa varies from 0.06 to 0.12 V/decade and βc is greater than 0.06 V/decade and within these limits corrosion rates can be estimated within a factor of 2 when only an approximation of the beta values and the measured admittance are substituted into the equation[3]. Values of βa = 60 mV and βc = 120 mV were thought to be most applicable (pers. comm. Dr. D. Barker, Portsmouth Polytechnic).

The corrosion rates (mpy) were found using equation 3;

$$mpy = \frac{0.13 \times I_{corr} \times EW}{\rho} \qquad (3)$$

where EW = equivalent weight (g) of metal

ρ = density (g cm^{-3}) of metal

The metal specifications can be found in Section 2.3.1.

2.2.3.2 Preparation of the Electrochemical Cell

The solution whose corrosivity was to be determined was placed in a 250 ml beaker which was itself placed in a 30°C water bath. Using a series of clamps three electrodes were lowered into the solution in such a way that they were located close to, but not touching each other. The electrodes were as follows:

a) Calomel reference electrode (Russell 300 series).
b) Platinum counter electrode.
c) Metal working electrode.

When ready, the electrodes were left to equilibrate for 5 minutes before the step polarising programme was started.

The platinum counter electrode was prepared from high purity platinum foil (0.025 mm thick) and platinum wire (0.3 mm diameter) obtained from Goodfellow, Cambridge.

The foil was cut into 1.25 cm diameter squares. The wire was pushed through one corner of this and coiled around, ensuring good contact. The other end of the platinum wire was coiled around a crocodile clip. Before use the platinum electrode was cleaned in N HCl and rinsed in distilled water.

Working electrodes of either stainless steel, mild steel, cupronickel and aluminium metals were used (Section 2.3.1). Each metal was cut into a square with the top surface having an area of 1 cm^2. This top surface was polished to 600 grit. Obtaining as large a contact area as possible, copper extra flex (Radio Spares Components Ltd) was attached to the abraded under surface of each metal using silver loaded epoxy adhesive (Radio Spares Components Ltd). The flex was threaded through a moulded glass tube and the assembly was set in a cylindrical mount such that only the upper metal surface would be exposed. A metset mounting kit, type FT was used from Metaserv Metallurgical Services Laboratories Ltd. The working electrode was washed in absolute alcohol before use.

2.3 Corrosion Characteristics of Stainless Steel, Mild Steel, Cupronickel and an Aluminium Alloy

2.3.1 Metal Specifications

2.3.1.1 Stainless Steel

Density = 7.92 g cm^{-3}

Equivalent weight = 27.575 g

Element	% Detected	BS 970 pt 4 (321.512) (%)
Carbon	0.03	0.08 max
Silicon	0.38	0.2 - 1.0
Sulphur	0.01	0.03 max
Phosphorus	0.027	0.04 max
Manganese	1.52	0.5 - 2.0
Nickel	9.03	9.0 - 12.0
Chromium	17.63	17.0 - 19.0
Molybdenum	0.28	-
Titanium	0.37	5 x carbon
Iron	-	remainder

2.3.1.2 Mild Steel

Density = 7.84 g cm^{-3}

Equivalent weight = 27.92 g

Element	% Detected	BS 4360 (43A) (%)
Carbon	0.12	0.3 max
Silicon	0.33	-
Sulphur	0.02	0.06 max
Phosphorus	0.035	0.06 max
Manganese	0.82	-
Iron	-	remainder

2.3.1.3 Copper-Nickel-Iron Alloy (Cupronickel)

Density = 8.94 g cm^{-3}

Equivalent weight = 31.42

Element	% Detected	BS 2871 (CN 102 (%)
Nickel	11.08	10.0 - 11.0
Manganese	0.84	0.5 - 1.0
Iron	1.54	1.0 - 2.0
Copper	-	remainder

2.3.1.4 Aluminium Alloy

Density = 2.66 g cm^{-3}

Equivalent weight = 13.42 g

Element	% Detected	BS 1470 (NS8) (%)
Zinc	0.12	0.2 max
Silicon	0.18	0.4 max
Manganese	0.34	0.5 - 1.0
Copper	0.04	0.1 max
Iron	0.26	-
Magnesium	4.88	4.0 - 4.9
Aluminium	-	remainder

2.3.2 Preparation of Metal Coupons

Each metal was supplied in sheet form from the Royal Naval Dockyard in Portsmouth.

Using a lathe the rough surfaces were removed. Each metal was cut into coupons of dimensions 10.0 mm x 35.0 mm. A hole, 1/16 inch wide, was drilled centrally at the top of the coupon. The back of each coupon was engraved with an

identification mark.

Using a flat bed wet and dry hand grinder (Metaserv) one surface of each coupon was ground through 240 - 320 - 400 to 600 grit. A fresh abrasive sheet was used for each type of metal to prevent contamination and subsequent deposition/galvanic corrosion. Aluminium was further polished with 6 µdiamond paste.

The coupons were thoroughly rinsed in water followed by methanol. They were blow dried with cool air and stored in a dessicator. Using an analytical balance (Mettler AC 100) dried specimens were weighed to an accuracy of ± 0.1 mg.

2.3.3 Cleaning of Corroded Metal Coupons

Further attack on the specimens during the cleaning methods was unavoidable. Quantitative assessment by weight loss was required and the estimated corrosion had to be corrected for this error. The metal lost from triplicate control specimens was determined for the following cleaning methods. This weight loss was subtracted from that of the exposed specimens before corrosion rates were calculated.

The specimens were lightly rubbed to remove excess corrosion products. After chemically cleaning they were thoroughly rinsed in water, methanol and finally absolute alcohol. They were dried with warm air and stored in a dessicator prior to weighing and further examination.

Stainless steel

Immersed in 30% nitric acid at room temperature for 5 minutes. Control coupons lost 0.05 ± 0.05 mg of metal.

Mild steel

Immersed in 50% HCl and 5 g l^{-1} hexamine at room temperature for 1 minute.

Control coupons lost 1.8 ± 1.0 mg of metal.

Cupronickel

Immersed in 48 g Ferric sulphate dissolved in 475 ml water and 25 ml H_2SO_4 at room temperature for 1 minute.

Control coupons lost 1.3 ± 0.5 mg of metal.

Aluminium alloy (henceforth abbreviated to aluminium)

Immersed in a boiling mixture of 5 ml l^{-1} phosphoric acid and 7.5 g l^{-1} chromic acid for 30 minutes.

Control coupons lost 19.4 ± 1.4 mg of metal.

2.3.4 Calculation of Corrosion Rate from Weight Loss

Corrosion rates may be given in a variety of units. Milligrams per square decimetre per day (mdd) was considered to best represent the weight loss of the exposed coupons.

$$\text{Corrosion rate (mdd)} = \frac{\text{weight loss (mg)}}{\text{Area (dm}^2\text{) x exposure time (days)}}$$

Although each metal coupon was cut to the same size their surface areas were expected to differ slightly. Using a metric micrometer (Moor & Wright, Sheffield, England) the length, breadth and depth of 15 coupons of each metal were accurately measured. The initial weight (g) of the coupons was on average 3, 14.5, 10 and 7 for stainless steel, mild steel, cupronickel and aluminium respectively. Accurate weights for individual coupons were used in the calculation below. Knowing that volume is equal to both mass ÷ density and surface area x thickness, the surface area of each side of the coupon could be calculated from the equation:

$$\text{Surface area (cm}^2\text{)} = \frac{\text{mass (g)}}{\text{density (g cm}^{-3}\text{) x thickness (cm)}}$$

The surface area of the coupon was calculated by adding up the individual surface areas from each side. The mean and standard deviation of the 15 surface areas were calculated for each metal:

Stainless steel	=	$7.65\ (\pm\ 0.22) \times 10^{-2}\ dm^2$
Mild steel	=	$11.41\ (\pm\ 0.24) \times 10^{-2}\ dm^2$
Cupronickel	=	$9.67\ (\pm\ 0.06) \times 10^{-2}\ dm^2$
Aluminium	=	$13.44\ (\pm\ 0.36) \times 10^{-2}\ dm^2$

Corrosion rates quoted in the literature and those calculated using electrochemical methods are often expressed in milli-inches per year (mpy). In order for comparisons to be made rates calculated in mdd can be multiplied by the following factors to give rates in mpy[27].

Iron alloys	0.19
Cupronickel	0.16
Aluminium	0.53

Results of weight loss experiments were rarely expressed in SI units in past and current literature. For comparative reasons conventional units were used in the present work. However, conversions to SI units may be made[3] as follows:

1 mg/(dm^2) (day)	=	1.16 μg/(m^2) (sec)
1 milli-inch/year	=	0.805 picometers per second

2.3.5 Assessment of Effects on the Metal

2.3.5.1 Visual Examination

The appearance of the coupons before cleaning was recorded with attention to colour and texture of corrosion product. After cleaning the extent of general and localised corrosion was recorded and colour photographs were taken of

representative specimens.

2.3.5.2 Microscopic Examination

A Leitz binocular microscope was employed to examine the cleaned surface of the metal coupons. At each magnification the microscope was calibrated using an eyepiece graticule against a standard gauge.

The magnification most often used was x 80 (eyepiece x 10 x 0.8 and objective x 10), thus, one unit on the graticule was equal to 16 μm and the diameter of the field of view was 1.92 mm (area = 2.9 mm^2).

The depth of any localised attack was measured using a calibrated focussing knob. By focussing first on the lip of the pit and then on the pit bottom, pit depth was found by the difference.

Representative features of corrosion were photographed using a Leica camera with black and white Kodak plus X pan film.

Bearing in mind the appearance of the uncorroded metal an assessment of the effects of corrosion on the exposed metal was made. In the field of corrosion, microscopic examination and recording is primarily qualitative. Besides general and local corrosion the occurrence of blistering, exfoliation, crevice and intercrystalline corrosion were searched for.

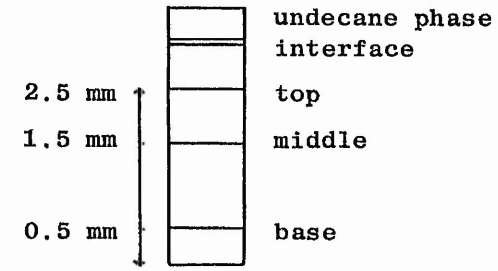
In order to provide some quantitative information of the corrosion, the pit frequency, depth and diameter were measured. The pit frequency was taken as the number counted in a scan from one side of the coupon to the other. At a magnification of x 80 such a scan was 10 mm x 1.92 mm in area. In some cases the number per field of view was recorded. For each scan the depth and diameter of the five deepest pits were recorded. The most common diameter and the diameter of the widest pits were also recorded.

Metal coupons which had been suspended through an undecane/aqueous phase interface were examined in the five areas shown in Figure 2.2. The interfacial area was examined with particular care in order to assess the effects of C. resinae

growth.

Figure 2.2

To show the position of areas examined on the coupon



2.3.5.3 Scanning Electron Microscopy

The resolution of photographs taken through the Leitz Microscope was not always good. Where greater depth of field facilitated the expression of representative features of corrosion a scanning electron microscope was used (ISI 40). The clean, dry specimens were stuck onto a stub using 'Uhu', a low vapour pressure glue. An electrically conducting path was made between the sample and the stub using the conducting paint, aqua-dag, a carbon based colloid. The stub mounted specimens were coated with a thin film of gold using an Emscope SC500 sputter coater.

In most cases a better image was obtained using the ISI-Robinson backscattered electron detector than the secondary electron detector. The resolution capability was not much less and the advantages were elimination of charging artifacts, reduced edge highlighting and easier imaging of specimens.

Found at the bottom right hand corner of the photographs is a micron marker. The first bar is equivalent to the following lengths

—— = 0.1 μm

—— – 1.0 μm

—— – – 10.0 μm

—— – – – 100.0 μm

In photographs depicting dual magnification the marker is only related to the lower magnification on the left hand side.

2.3.6 Measurement of Surface Texture

A Talysurf surface texture measuring instrument was used, the method conforming to BS 1134.

Basically a sharp stylus was drawn across the metal surface. The stylus was attached to an arm which also carried a skid. As the arm moved across the surface the skid resulted in an electrical signal. This was processed and ultimately gave a trace of the surface measured. A digital readout gave the centre line average (CLA) value i.e. the arithmetical mean deviation of the departure of the profile above and below the reference line.

Knowledge of the vertical magnification enabled pit depths to be assessed. However, the accuracy of this method depended on the shape of the pit with respect to the stylus, i.e. a wide stylus would not penetrate a narrow pit. This would result in depths being underestimated.

2.4 Experimental Procedures

2.4.1 Chapter Four - Corrosion of Metals Suspended Through an Aqueous/Hydrocarbon Two Phase System in the Presence or Absence of C. resinae

2.4.1.1 Preliminary Experiment

The corrosion of mild steel in the following two phase systems was examined;

1) Turner's solution/undecane
2) Turner's solution/Dieso
3) Sea water/undecane
4) Sea water/Dieso

Metal coupons were prepared (Section 2.3.2), suspended by nylon thread and sterilised in 70% ethanol for 15 minutes. They were dried in a sterile air cabinet under UV light and suspended in a 500 ml Ehrlenmeyer flask containing 250 ml of aqueous solution. The thread was secured to the neck of the flask in such a way as to allow as accurately as possible 3 mm of the coupon to protrude from the solution. Two flasks of each two phase system were inoculated with C. resinae spore suspension and a third remained uninoculated. Using aseptic technique 25 ml of either Dieso or undecane were overlaid in each of two inoculated and one control flask, so as to completely immerse the coupon. The flasks were incubated statically at $30^{o}C$ for 28 days.

At intervals pH values and visual assessment (Table 2.2) of growth and corrosion were recorded. After 28 days the coupons were removed, cleaned (Section 2.3.3), weighed and stored in a dessicator until examination using the light microscope and the scanning electron microscope. The surface texture of the coupon was examined using a Talysurf (Section 2.3.6).

The aqueous phase was filtered under vacuum through Whatman No. 1 filter paper of known dry weight and the dry weight

of C. resinae subsequently found (Section 2.1.2).

The culture filtrate was subject to the following analyses;

1) pH (Section 2.2.1)
2) Acid analysis by GLC (Section 2.2.2)
3) Corrosivity to mild steel using the polarisation resistance method (Section 2.2.3).

2.4.1.2 Long Term Corrosion Test in a Turner's Solution/Undecane System

The corrosion of stainless steel, mild steel, cupronickel and aluminium by C. resinae was examined.

A metal coupon assembly was designed to have more rigidity than in the preliminary experiment. The prepared metal coupons were threaded with nylon which was then threaded through a hollow glass tube. The tube was plugged with cotton wool about 2 cm from its base. This conferred stability to the coupon without allowing the plug to become wet. The entire assembly was sterilised in a hot air oven at 165°C for 3 h.

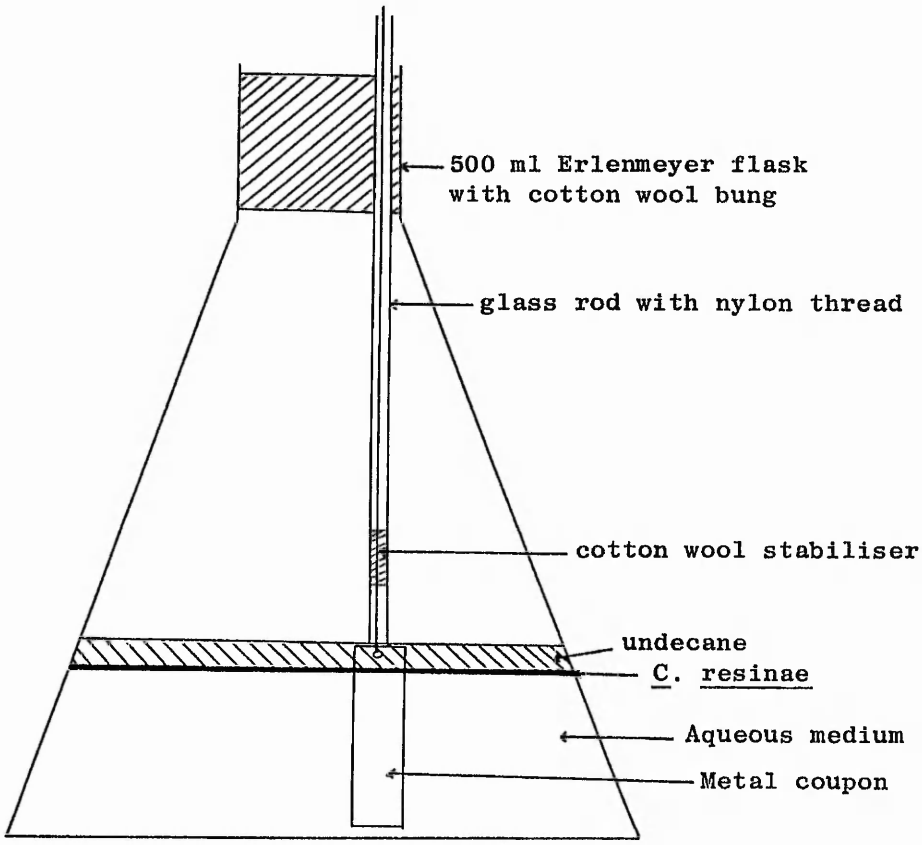
The following procedure was repeated for each metal; of 28 identical 500 ml Erlenmeyer flasks each containing 250 ml of Turner's solution, 14 were inoculated with C. resinae and 14 were left uninoculated. A metal coupon assembly was suspended in the solution so that about 3 mm were protruding. This was supported while 30 ml of n-undecane were overlaid so as to fully immerse the coupon. The coupon assembly was held in place by a cotton wool bung pressing the glass tube against the neck of the flask (Figure 2.3).

The flasks were incubated statically at 30°C for periods of 4, 9, 13 and 18 weeks. Four inoculated (test) and two uninoculated (control) flasks were incubated for 18 months.

Each week for eight weeks, pH measurements, using a sterile electrode, were taken for one test and one control flask of each metal.

Figure 2.3

Diagram of Long Term Corrosion Test System



After each exposure time the sampled flasks were separated into four components. The metal coupon was cut free from the assembly; the undecane was taken from the top of the aqueous phase and C. resinae was filtered from the aqueous phase. Each component was examined in the following manner:

1. Metal coupon
   - weight loss (Section 2.3.4)
   - microscopic examination (Section 2.3.5)

2. *C. resinae* - dry weight (Section 2.1.2)

3. Culture filtrate
   - pH (Section 2.2.1)
   - Metal ion analysis (Section 2.2.1)
   - Acid analysis by GLC (Section 2.2.2)
   - Corrosivity using the linear polarisation technique (Section 2.2.3)

The filtrate was divided into two volumes before analysis. A 170 ml volume was used for analysis of pH and corrosivity. The remaining 70 ml was used for acid and metal ion analysis.

2.4.1.3 Long Term Corrosion Test in a Modified Sea Water/Undecane System

The test was set up in exactly the same way as for Turner's solution. However an additional no-metal series of test and control flasks were included. This was because it became apparent that the activity of *C. resinae* caused more corrosion products to be present in the test filtrates than the control filtrates such that a comparison between a test and its control for any one parameter was not just analysing the effect of *C. resinae* and its growth products but also the subsequent effect of corrosion products.

An unforeseen problem arose in that growth of *C. resinae* was totally inhibited in the presence of mild steel, cupronickel and aluminium. The pH of these systems had become alkaline as a result of corrosion. After nine weeks in an attempt to salvage the experiment, the pH was reduced to 6.5 using H_2SO_4 and the system was reinoculated with fresh *C. resinae*. Growth was still poor after 31 weeks and a second reinoculation step was performed.

Growth was so variable with each metal that samples were not always taken at each exposure time of 7, 14, 26, 31 and 46 weeks and 72 weeks.

In addition to the measurements taken in the previous

experiment, redox potential values were calculated (Section 2.2.1).

2.4.2 Chapter Five - Corrosion of Stainless Steel, Mild Steel, Cupronickel and Aluminium by Either C. resinae, a Known Mixed Culture or an Unknown Fuel Sludge Culture in Turner's Solution or Modified Sea Water With n-Undecane as Sole Carbon Source

Materials

1. Turner's solution or modified sea water (225 ml per flask) and undecane (25 ml per flask).

2. Coupons of stainless steel, mild steel, cupronickel and aluminium were cut to 10 x 3.5 cm, ground to 400 grit, cleaned, weighed and sterilised in a hot air oven at 165°C for three hours.

3. C. resinae inoculum containing 10^7 spores/ml; 2 ml per flask.

4. Sludge inoculum from fuel tanks of HMS Liverpool and HMS Gibraltar. A cursory check indicated the presence of several bacteria; brown, pink, cream, white and red yeasts; brown, grey, white, green, lime coloured fungi. Anaerobic culture methods yielded no growth. Five ml were added per flask.

5. Mixed inoculum of 13 organisms isolated from HMS Gibraltar fuel sludge which were tentatively identified as:-

			cells x 10^7/ml	volume added (ml)
Bacteria	1.	Aeromonas	155	2
	2.	Pseudomonad	20	2
	3.	Pseudomonad	55	2

	4.	Micrococcus (orange)	?	2
	5.	Micrococcus (yellow)	?	2
	6.	Gram -ve rod	12	2
Yeasts	7.	Cream colony	33	2
	8.	Cream colony	7	2
	9.	Cream colony	?	2
	10.	Pink colony	8	2
Fungi	11.	Penicillium	?	2
	12.	Penicillium	?	2
	13.	Cladosporium resinae	10^7 spores/ml	6
			MADE UP TO 45 ml with water	

Method

Using aseptic technique a metal coupon was immersed in the aqueous solution. This was overlaid with undecane and inoculum (1, 2 or 5 ml) was added to the flask which was gently swirled to allow the organisms to rest at the interface. The flasks were statically incubated at 30°C for periods of 12, 16 or 21 weeks.

The following variables were examined in triplicate:

	no metal	stainless steel	mild steel	cupronickel	aluminium
no inoculum	T12/T21/S16	T12/T21/S16	T12/T21/S16	T12/T21/S16	T12/T21/S16
C. resinae	"	"	"	"	"
Mixed	"	"	"	"	"
Sludge	"	"	"	"	"

where, T12 = 12 wks in Turner's solution
T21 = 21 wks in Turner's solution
S16 = 16 wks in modified sea water.

For each variable the pH of one flask was taken at regular intervals. A microelectrode was used to cause minimal

disturbance.

At the end of the exposure time the undecane was discarded and the following measurements taken:-
Dry weight organisms - Section 2.1.2; pH, redox + nitrate concentration - Section 2.2.1; polarisation resistance and corrosion potential of cupronickel - Section 2.2.3; weight loss of clean metal coupons - Section 2.3.4.

2.4.3 Chapter Six - Continuous Monitoring of the Corrosivity of *C. resinae* at the Undecane/Aqueous Phase Interface and in the Culture Media Using the Linear Polarisation Technique

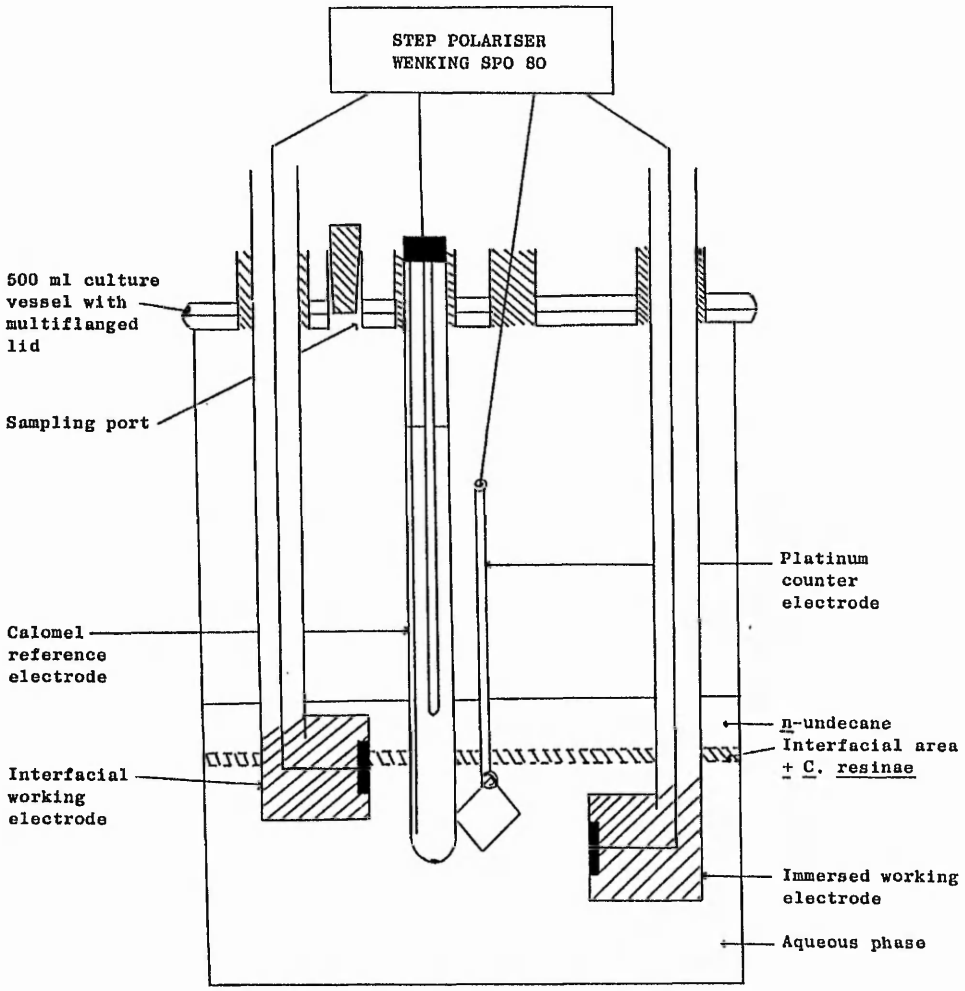
A polarisation cell was set up as shown in Figure 2.4. Two hundred millilitres of aqueous solution were added, the electrodes placed in position and 25 ml undecane overlaid ensuring complete coverage of the interfacial electrode. One of two vessels was inoculated with a spore suspension (1.9×10^7 spores in 2 ml) of *C. resinae*. The spores were allowed to fall through the undecane layer and generally settled just below it. A small magnetic flea was inserted at the base of the vessels to allow for a one minute slow agitation every 24 hours for the first week to allow the inoculum to reach the substrate at the interface. The control was treated in the same manner so as not to introduce variation of for example aeration and fluid flow. Such stirring was done after measurements had been taken so as not to disturb the electrodes prior to polarisation. The polarisation cells were maintained in a $30^{o}C$ waterbath.

The electrodes were step polarised in turn as described in Section 2.2.3. Measurements were taken at intervals for up to 40 days. The pH of the aqueous phase was taken aseptically at regular intervals during this time.

The method was followed for each of stainless steel, mild steel, cupronickel and aluminium in both Turner's solution and modified Sea Water.

Figure 2.4

Polarisation Cell for Continuous Measurement of Corrosion Rate by Linear Polarisation



2.4.4 Experimental Procedures in Chapter Seven

2.4.4.1 The Effect of Individual Organic Acids on the Corrosion Rate of Stainless Steel, Mild Steel, Cupronickel or Aluminium in Either Modified Sea Water or Turner's Solution

The following acids were obtained from Sigma Chemical Co., London, UK; Pyruvic, lactic, fumaric, succinic, itaconic, maleic, cis-aconitic, citric and isocitric acid.

Forty-four sterile weighed coupons of each of the following metals were obtained, stainless steel, mild steel, cupronickel and aluminium.

Method

The coupons were cleaned, dried, weighed, and dry air sterilised in their individual glass bottles. Each acid was made up to 1,000 ppm in both modified sea water and Turner's solution and then filter sterilised. Fifteen ml of each were added to duplicate bottles such that the coupons were completely immersed. A mixed acid treatment was included where a 1/9 dilution was made of a solution containing 1,000 ppm of all 9 acids, such that the mixture was equivalent to a total concentration of 1,000 ppm. Duplicate controls were set up using modified sea water or Turner's solution only. The bottles were incubated at $30^{o}C$ for 36 weeks.

The coupons were cleaned as described in Section 2.3.3. Their dry weight was recorded and weight loss calculated. The corrosion rate was calculated as described in Section 2.3.4.

2.4.4.2 The Effect of Metals on the Growth, Acid Production and Enzyme Activities of C. resinae

2.4.4.2.1 The Effect of Differing Metal Concentrations on the Growth of C. resinae in Turner's Solution plus Dieso

Materials

Filter sterilized stock solutions (μ g ml^{-1} metal in parentheses) were prepared and diluted as indicated: $CuSO_4.5H_2O$ (41,000), $FeCl_3$ (43,040), $NiCl_2.6H_2O$ (41,200) and Al $(SO_4)_3.16H_2O$ (22,500).

Method

The stock solutions were diluted in double strength Turner's Solution and sterile distilled water as appropriate to give 125 ml aliquots of normal strength Turner's solution containing 1/10, 1/100 and 1/1000 dilution of stock metals (1/5, 1/50, and 1/500 in the case of aluminium). The flasks were overlaid with 12.5 ml Dieso and incubated statically for 8 weeks during which time growth was assessed on a 0 - 5 scale.

2.4.4.2.2 The Effect of Metal Coupons on Extracellular Citric Acid Production by C. resinae in Turner's Solution and Modified Sea Water with Undecane as the Sole Carbon Source

Method

A mild steel coupon was placed in each of three flasks containing 225 ml Turner's solution (pH 7.0). Two of each set of three flasks were inoculated with 1 ml of C. resinae suspension and the third was left as an uninoculated control. The experiment was repeated using either stainless steel, cupronickel or aluminium coupons. For each solution a set free of metal coupons was made up. Each solution was overlaid with 26 ml of undecane and incubated

statically at 30°C. At fortnightly intervals growth was assessed on a scale of 1 - 5 and a 1.5 ml sample was taken aseptically. The pH was recorded and citric acid content estimated using a Citric Acid Analysis test kit (UV method) from Boehringer Mannheim. The absorption of NADH was measured at 340 nm on a Pye Unicam series SP1800 UV spectrophotometer.

2.4.4.2.3 Estimation of Selected Enzyme Activities from Cell Extracts Prepared from C. resinae Grown in the Presence of Various Metals

Method

To triplicate 250 ml Erlenmeyer flasks were added 27.5 ml double strength Turner's solution, 5 ml of 10% glucose solution, 4 ml of C. resinae inoculum and a volume of metal stock solution and/or distilled water so as to make the total volume 50 ml and the final metal concentrations as follows: Fe or Cu 1,000 $\mu g\ ml^{-1}$; Ni 412 $\mu g\ ml^{-1}$ and Al 450 $\mu g\ ml^{-1}$. All flasks (including a no metal control) were incubated in an orbital shaker (100 rpm) at 30°C. After 2 days all but the Ni containing flasks were harvested the latter being left for 7 days due to slow growth.

The triplicate cultures were combined and spun down in an MSE 18 centrifuge at 7,500 rpm for 5 minutes. After several washes to remove metal compounds the extracts were subjected to Mickel disintegration for 10 x 1 m, sonication for 10 x 1 m and finally to 20 cycles of a hand homogeniser. Washings and disintegration were carried out in a buffer containing 10 mM Tris-HCl (pH 7.2), 2 mM $MgCl_2$ and 0.25 M sucrose. They were then centrifuged at 2,500 rpm for 5 min in the MSE 18 centrifuge and the supernatants, termed cell extracts, were retained for protein estimation, acid phosphatase, alkaline phosphatase, malate dehydrogenase, catalase and cytochrome c oxidase assays.

Protein was estimated by the Lowry method[128] using Folin Ciocalteau reagent. The standard protein was bovine serum albumin

(0.4 mg ml^{-1}) diluted to give a range of 0 to 0.2 mg per assay. Five standard concentrations and each cell extract were all estimated in triplicate.

Acid and alkaline *p*-nitrophenyl phosphatases were estimated using the method of Torriani[206]. A final Triton X-100 concentration of 0.2% (w/v) was used routinely in all assays. The molar extinction coefficient of *p*-nitrophenol at 420 nm was taken to be 16×10^3.

Malate dehydrogenase activity was estimated using the method of Kitto[111] and followed the disappearance of NADH during the conversion of oxaloacetate to malate. The molar extinction coefficient of NADH at 340 nm was taken to be 6.22×10^3.

Cytochrome c oxidase was estimated using the method of Smith[189] following the oxidation of previously reduced cytochrome c at 550 nm. The molar extinction coefficient of cytochrome c at this wavelength being 18.5×10^3.

Catalase was estimated by the method of Lück[129] and followed the disappearance of H_2O_2 at 240 nm. The molar extinction coefficient of H_2O_2 at this wavelength being 43.5×103.

2.4.4.3 Examination of Growth, Adherence and Corrosive Effects of a Drop of *C. resinae* Spore Suspension on Stainless Steel, Mild Steel, Cupronickel and Aluminium

Sheets of stainless steel, mild steel, cupronickel and aluminium were cut into 2.0 x 2.0 cm squares and one surface was polished to 600 grit as described earlier. The metal specimens were sterilised in 70% alcohol for 15 minutes and dried in a sterile cabinet. Spore suspensions of *C. resinae* were made in Turner's solution and modified sea water. The spore count was 7.2×10^6 spores/ml.

For each metal, three squares were placed in each of four sterile petri dishes. Two dishes were used for Turner's spore

suspensions and two for modified sea water spore suspensions. A 0.1 ml drop of spore suspension was placed in the centre of two of the squares in each dish and an uninoculated drop was placed on the third square. Each dish was then gently filled with sterile undecane so that the immersed drops remained on the metal surface. The dishes were incubated at 30°C.

2.4.4.3.1 Examination of Growth and Corrosion

At intervals of one, three and six weeks growth was examined visually and or microscopically. Colour photographs were taken of some of the drops. Black and white photographs of representative features were taken using both a light and scanning electron microscope.

The nature of the adhesive sheath around the hyphae was determined by staining for 1 hour with 0.015% Ruthenium Red (BDH Chemicals, UK). The stain is indicative of an anionic polymer with polysaccharide-like characteristics[63,130,155].

2.4.4.3.2 Measurement of pH Change Within the Drop

A sterile 2.0 x 2.0 cm^2 square of stainless steel was placed polished face upwards in a small sterile beaker. A sterile micro pH electrode (Microelectrodes Inc., California, USA) was positioned about 1 mm from the metal surface. A 0.2 ml drop of spore suspension (1.3 x 10^6 spores/ml) in modified sea water, pH 6.5 was placed around the electrode tip. The drop was gently overlaid with sterile n-undecane. The pH was taken regularly for 14 days. The pH of an uninoculated drop was monitored under the same conditions.

2.4.4.4 The Sequestration of Metal Ions by C. resinae

A 100 ppm stock solution of each of the following metals were used:

$NiCl_2.6H_2O$
$CrCl_3.6H_2O$
$MgCl_2.6H_2O$
$MnCl_2.4H_2O$
$FeCl_3$
$CuCl_2$
$ZnCl_2$

C. resinae was grown in static 500 ml Erlenmeyer flasks containing 225 ml Turner's solution and 25 ml undecane at 30°C. Flasks were harvested after 7, 14, 18 and 25 days.

The mycelium was gently washed with 5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) to remove as much undecane as possible. The mycelium was suspended in 5 mM HEPES at pH 7.0 and gently teased apart. After 25 days the mycelial mat was too thick to wash and break open. It was therefore cleaned by gently pressing between several filter papers to absorb undecane. It was cut into 1 cm squares and presented to the metal solutions in this way. Representative aliquots (1 ml or $1cm^3$) equivalent to 12 mg ± 5% dry weight were taken and placed in 25 ml beakers containing 8 ml HEPES plus 1 ml of 100 ppm metal solution. The incubation time was generally 100 minutes at 30°C. The mycelium was removed and washed in 10 ml of 0.1 mM Ethylenediaminetetra-acetic acid (EDTA) for 5 minutes. The mycelium was again removed and acid digested in 10 ml 75% nitric acid at 70°C for 45 min in a capped tube. Accurate values were recorded and the metal content of the three solutions was then analysed using atomic adsorption spectroscopy, or fluorimetry for Al.

The following experiments were carried out:

a) C. resinae (7, 14, 18 and 25 days old) challenged with all 8 metals at 10 ppm (100 μg total) for 100 min.

b) C. resinae, 18 days old, challenged with 10 ppm of all 8 metals for 50,100 and 200 min.

c) C. resinae, 18 days old, challenged with either 20, 100 or 500 ppm of each metal for 100 min.

CHAPTER 3

PRELIMINARY EXAMINATION OF FUEL TANK MICROORGANISMS

3.1 Experimental Aims

The aims of this chapter were:

i) to gain experience in the culture and maintenance of microorganisms isolated from contaminated fuel tanks,

ii) to investigate the properties of and identify as far as possible several bacterial isolates,

iii) to determine the growth characteristics of microorganisms in pure and mixed cultures, and

iv) to find the response of C. resinae to incubation in several different types of liquid and solid culture media and to find the optimum physical parameters for growth.

3.2 Isolation of Microorganisms

Sludge samples from fuel tanks showed a lag phase in excess of 24 hours when transferred to Bushnell-Haas overlaid with Dieso. Once growth was established the relative numbers of bacteria to yeasts remained fairly constant over the first week. After this time the fungal species Penicillium and Cladosporium became apparent.

Initially 15 microorganisms were isolated though four were subsequently lost. Assessment of growth in sea water, Turner's solution or distilled water with either glucose, Dieso or undecane as the carbon source over a period of eight weeks showed that in all cases Turner's solution and glucose supported the best growth and distilled or sea water with Dieso supported the least growth. It was evident that several microorganisms were capable of reducing the pH.

3.3 Preliminary Studies on Microorganisms

Of the remaining eleven isolates, two (numbers 10 and 11) grew very slowly and showed little visible growth after 48 hours. Several

isolates showed prolonged lag phases of several days if they had been stored in a refrigerator for any length of time.

Table 3.1 shows the colony characteristics of each of the eleven isolates after growth on nutrient or malt extract agar. Table 3.2 shows the results of several preliminary tests and also attempts to classify the organism into bacteria, yeast or fungi.

Of the eleven isolates, two were filamentous fungi, probably genus Penicillium.

Four isolates were found to be unicellular budding yeasts which could be differentiated from each other on colony colour, cell size and arrangement.

The five remaining isolates were bacteria and were subjected to a range of tests, mainly biochemical, in order to identify them further.

3.4 Partial Identification of Five Bacterial Isolates

The standard reference book used throughout this section was the 8th Edition of Bergey's Manual of Determinative Bacteriology[23].

During the analysis of results each test was deemed to be equal to all other; though there was some choosing of the order in which data were considered.

Preliminary analysis of these data (Tables 3.3 and 3.4) confirmed that none of the five isolates could easily be assigned to a genus. Thus, the usual procedure was to eliminate as many genera as possible after considering tests such as Gram stain, shape, motility and growth conditions. Gradually it became possible to eliminate all except three or four likely genera though it was never possible to assign an isolate to a particular species.

Isolate 1 was interesting in that it has a strong fermentation reaction but it was not possible to grow the organism anaerobically on any of several media. It was quickly eliminated on several grounds from aerobic genera and was tentatively classed as a facultative anaerobe. The nearest genera which would fit the data, allowing at least one discounted result, were: Vibrio (which do not produce gas from glucose), Plesiomonas (which do not produce gas from

Table 3.1

Colony Characteristics of Eleven Organisms Isolated from Contaminated Fuel Tanks

Isolate	Colony Characteristics
1	Circular, cream colonies. Convex elevation. Where confluent growth large slimy mass with sticky texture and characteristic smell.
2	Circular, creamy white colonies with raised edges. Shiny.
3	Circular, off-white colonies, with feathery edges. Where confluent growth large glistening, slimy mass. Strong foul odour.
4	Similar appearance and smell to (3), though greenish-white colour.
5	Circular, cream, shiny, convex colonies.
6	Tiny punctiform creamy white colonies. Prolonged growth gave longer irregular colonies with hard, crusty appearance.
7	Circular, bright pinkish-red colonies.
8	Green/cream filamentous fungus.
9	Similar to (8).
10	Very small circular, orange colonies with smooth shiny surface.
11	Very small punctiform yellow colonies with distinctive smell.

Table 3.2

Results of Preliminary Identification Tests of Eleven Microorganisms Isolated from Contaminated Fuel Tanks

Key

* On malt extract agar unless stated (NA = nutrient agar).

** + 1, Gram positive; - is Gram negative; V is Gram variable; ? is not relevant; 1, 1+ --- 4,4+ represents increasing amounts of growth

Isolate Number	Relative growth at 30°C for 48 h*	Microscopic Appearance	Motility	Capability of:- Aerobic Growth	Anaerobic Growth	Gram Stain**	Conclusion
1	4+	Short small rods in chains	+	+	- or weak+	-	Bacterium
2	4+	Branched yeast	-	+	-	v	Unicellular yeast
3	3+ (NA)	Rods in chains	+	+	-	-	Bacterium
4	3+ (NA)	Rods in long chains	+	+	-	-	Bacterium
5	3+	Cells in chains. Mainly buds on individual mother cells.	-	+	-	v	Unicellular yeast
6	2+	Branched chains. Many rod shaped cells.	-	+	-	v	Unicellular yeast
7	3+	Branched yeast	-	+	-	V	Unicellular yeast
8	3+	Mycelial fungus	-	+	-	?	Probably
9	2+	Mycelial fungus	-	+	-	?	Penicillium spp.
10	+ (NA)	Tiny cocci in clumps	-	+	-	+	Bacterium
11	+ (NA)	Very small coccobacilli	-	+	-	V	Bacterium

Table 3.3

Results of Biochemical Tests used in Partial Identification of Bacterial Isolates from Contaminated Fuel Tanks

	Test	Isolate				
		1	3	4	10	11
a)	Range of growth temperature -					
	4°C	3+	4+	4+	-	-
	25°C	4+	4+	4+	4+	4+
	30°C	4+	4+	4+	4+	4+
	37°C	3+	1+	1+	3+	1+
	41°C	3+	-	1+	-	3+
	55°C	-	-	-	-	-
b)	Indole production	-	-	-	-	-
c)	Methyl Red test	-	-	-	-	-
	Voges Proskauer test	+	-	-	-	-
d)	Citrate utilization	-	-	-	-	-
e)	Hugh and Leifson test	F	0	0		
f)	Oxidase	+	+	+	-	-
g)	Catalase	+	+	+	+	+
h)	Carbohydrate utilization			See Table 3.4		
i)	Gluconate oxidation	+	+	+	-	-
j)	Starch hydrolysis	-	-	-	-	-
k)	Growth in MacConkey	+	+	+	-	-
l)	Casein hydrolysis	+	+	+	-	-
m)	Gelatin hydrolysis	-	-	-	-	-
n)	Egg yolk test	+	+	+	+	-
o)	Arginine dihydrolase	-	+	+	-	-
p)	Nitrate reduction	+	-	-	+	+
q)	Nitrogen fixation	-	-	-	-	-
r)	Tween 80 hydrolysis	-	-	+	+	-
s)	Fluorescent pigment production	+	+	+	-	+

(Cont'd)

Table 3.3 (Cont'd)

t)	API strip test -				
	Galactosidase	-	-	-	-
	Glucose utilization	+	-	-	-
	L(+) arabinose utilization	-	-	-	-
	Lysine decarboxylase	+	-	-	-
	Ornithine decarboxylase	+	-	-	-
	Citrate utilization	-	-	-	-
	H_2S formation	-	-	-	-
	Urease	-	-	-	-
	Tryptophan deaminase	-	-	-	-
	Indole	-	-	-	-
	Cytochrome oxidase	+	+	+	+
	Nitrate reductase	+	-	-	+

Key: F Fermentative
O Oxidative
\+ Positive
\- Negative

Table 3.4

Results of Carbohydrate Utilization Tests used in partial identification of Bacterial Isolates from Contaminated Fuel Tanks

Carbohydrate	Isolate				
	1	3	4	10	11
D-arabinose	+	-		W	
L-arabinose	-	-	-	-	-
D-ribose	+g				W
D-xylose		+	+g		
L-xylose	+	-	-	-	-
L-Rhamnose	+g				-
Fructose	+g	+	W	W	+
Galactose	+g	+	W	-	+
Glucose	+g	+	+	+	-
Mannose	+g	+	W	-	
Cellobiose	+			-	-
Lactose	-	-	-	+	-
Maltose	+g	-	-	-	-
Sucrose	+	W	+	W	+
Trehalose	+g	-	+		-
Salicin	-	-	+g	-	-
Adonitol	+				-
Glycerol	+	-	-		-
Mannitol	+g	-	-	-	-

Key: + Positive
- Negative
W Weakly positive
g Gas production

carbohydrates) and the most likely, Aeromonas (which do not produce acid from adonitol).

Isolates 3 and 4 looked identical and were initially considered to be the same organism. However, the full range of tests showed that organism 3 produced acid from glycerol, salicin and trehalose; hydrolysed Tween 80; and would not grow at 41°C. Organsim 4 gave opposite results in each case. Both organisms were fairly easily eliminated from all genera except Pseudomonas, Alcaligenes, Acetobacter and Bordetella. The most likely genus to which both organisms belong is Pseudomonas, particularly 4 which, apart from lacking the ability to hydrolyse gelatin, is identical to P. aeruginosa.

Isolate 10 was quickly eliminated from all genera except Micrococcus, Corynebacterium, Arthrobacter and Microbacterium. After the completed tests the latter three genera were eliminated; the organism appeared to be very similar to M. roseus.

Isolate 11 was considered to be a member of one of the following genera: Brucella, Bordetella, Micrococcus, Corynebacterium and Arthrobacter. The two most likely genera were Micrococcus and Corynebacterium though further work would be needed to ascertain the actual genus.

Thus, identification of five bacterial isolates proved to be difficult. Their fuel/sea environment was so specialised that they may have mutated in order to survive or may not yet be characterised. As might be expected from the demands of their environment they were all psychrophilic and grew in a wide pH range.

3.5 Growth Characteristics of Isolated Microorganisms

Much variation occurred in duplicate flasks using the method described in Section 2.1.5 part b. It was decided that in the absence of further replicates the data should be examined qualitatively and not quantitatively.

The viable cells of isolate 1 were halved after seven days. Growth was not observed in Turner's solution for 28 days nor in sea water/undecane for 36 days. No growth occurred in sea water/Dieso.

After 57 days best growth was supported by sea water/undecane followed by Turner's/undecane. A pH fall from 7.5 to 7.1 was observed in the former 15 days growth.

Isolate 3 increased in numbers up to day 8 then, except in the sea water/undecane system, declined to below the starting level after 51 days. The pH fell from 7.5 to 6.8 in sea water/undecane in this time.

The numbers of isolate 4 increased dramatically over the first seven days and remained fairly constant until day 57. This organism appeared best equipped of the bacteria to grow in the four conditions. Most growth occurred in sea water/undecane and least growth where Dieso was the substrate. The pH had fallen to 7.15 in sea water/undecane after 11 days.

Viability was lost from isolates 10 and 11 within 8 days except in sea water/undecane systems in which the isolates continued to grow, though after 49 days isolate 11 lost viability. After 27 days both had reduced the pH of sea water/undecane to 6.55.

Isolate 7 showed a large increase in growth after 14 days in Turner's solution (undecane being better than Dieso) though sea water supported only modest growth increases. After 57 days viable counts had increased in Turner's solution but had declined in sea water. After 11 days the pH had fallen to 7.2 in sea water/undecane.

Growth of isolate 8 was not supported in sea water/Dieso and was only slight in sea water/undecane after 23 and 38 days. It grew quickly in Turner's solution (particularly with undecane) and was substantial after 23 days. A pH fall from 5.8 to 5.4 was observed in Turner's/undecane after 7 days and from 7.5 to 6.2 in sea water/undecane after 37 days.

Growth of C. resinae was apparent after 3 days in Turner's solution and 7 days in sea water. A substantially higher growth rate and yield was supported by Turner's solution and undecane. After 23 days sea water/undecane had supported more growth than Turner's/Dieso which in turn had supported more growth than sea water/Dieso. After 37 days the pH of Turner's/undecane had fallen to 5.3 and that of sea water/undecane to 4.6.

In conclusion the bacterial isolates, 1, 3, 4, 10 and 11 grew

best in sea water/undecane and were often inhibited by Dieso. They all reduced the pH of sea water to some extent. The yeast (isolate 7) and fungi (isolate 8 and C. resinae) grew best in Turner's solution/undecane with sea water and/or Dieso supporting significantly less growth. The fungi produced sufficient acid to reduce the pH of the well buffered Turner's solution.

It was evident that all of the isolates were hydrocarbon utilisers. Surprisingly Dieso was found to support less growth than undecane. The wide variety of microorganisms in contaminated fuel tanks are likely to utilise Dieso by means of co-oxidation processes. However, pure isolates may only be able to utilise limited components of Dieso and may be susceptible to inhibition by others. The growth of C. resinae, the organism selected for the study of microbial corrosion, was particularly poor with Dieso as the substrate. Since good growth was of paramount importance in the investigation undecane was chosen as the substrate for further work.

It was found that whilst bacteria grew best in modified sea water, yeast and fungi did not. Thus, in a contaminated fuel tank bacteria may be expected to colonise first, yeast and fungi needing a modified environment. This modification may simply be a reduction in pH or it may be the provision of a more easily utilisable substrate. All the bacteria were capable of reducing the pH of sea water and hence could make it more conducive to the growth of yeasts and fungi. In reducing the pH these bacteria may also play a role in acid-related corrosion. Better growth of the yeast and fungi in Turner's solution than in sea water may have been due to the lower initial pH as well as the more complete mineral composition. The effect of the initial pH of sea water was examined and was found to support more growth of C. resinae at pH 6.5 than pH 8.3 when both concentrated or at 35% strength. As described in the following section other modifications of sea water were found to be necessary to promote growth. The modifications of natural sea water by biological, physical and chemical reactions in the fuel system make it impossible to provide a fully representative aqueous phase. Superior growth of C. resinae was supported by Turner's solution and so it was chosen as the electrolyte for corrosion experiments.

An experiment was carried out to determine the growth and pH reduction of ME/NB/glu medium at various initial pH's. After 6 days all isolates were found to reduce the pH from initial values of 7 to 11. In particular isolates 1 and 10 reduced the pH from 8.0 or 11.0 to 4.8 and 5.0 or 5.6 and 5.2 respectively. Some isolates reduced an initial pH of 5.0 or 6.0 further and no isolates reduced an initial pH of 3.0 or 4.0. Only C. resinae was capable of growth at pH 3.0, however its growth was not supported at pH's 10.0 and 11.0. After three days acidic pHs supported more growth of C. resinae than pH's 6.0 to 9.0. However, assessment after six days showed more growth for pH 7.0 - 9.0 than for the more acidic pH's. Thus, it would appear that an acidic pH facilitated germination whereas an alkaline pH induced a lag phase. However, once germination had proceeded growth occurred at pH's 3.0 to 9.0, the lower pH's possibly becoming inhibitory.

3.6 Growth Characteristics of a Selected Mixed Culture of Microorganisms

The growth curves were compared for isolates in pure culture and in mixed culture. Although isolate 11 (bacterium) had been viable at the start of the experiment it did not grow in the seven day incubation period. A longer transition period in the liquid seed medium from the refrigerated slant may have circumvented this problem. In pure culture the remaining isolates grew exponentially over the first 24 hours. However, isolate 7 (yeast) declined and died over the next two days. The other bacterium (isolate 4) and yeast (isolate 6) each entered and remained viable in the stationary phase over the next six days.

When the organisms were grown together the growth curves of isolates 6 and 4 were relatively unaffected. However, isolate 7 remained viable for an extra day. The presence or absence of C. resinae did not appear to affect the growth curves of any of the organisms. However, had the experiment been allowed to continue the microorganisms would probably have been succeeded by C. resinae.

Further work on mixed cultures is reported in Chapter 5.

3.7 Growth Characteristics of C. resinae

It is generally believed that C. resinae is the most widely and commonly found species in the ships' fuel system. As the predominant organism it may play a major role in the microbial corrosion of these systems. Indeed, corrosion of aluminium by C. resinae has been widely reported[79,146,149,174] for aircraft fuel systems. Although the probable role of the many other contaminants was borne in mind, mechanisms of corrosion were considered to be complex enough without the interactions of mixed cultures. Hence initially it was considered more constructive to examine the role of a pure culture of C. resinae in corrosion and therefore, preliminary experiments were undertaken to find suitable growth conditions for a fast growing, acid producing strain of C. resinae.

The growth rates of C. resinae isolates on malt extract agar were 2.8, 2.0, 2.1 and 3.1 mm/day for isolates A, B, C and ATCC 34066. Although isolate A had a similar growth rate to ATCC 34066 its lag phase was between 24 and 48 hours compared to less than 24 hours for the latter organism. However, before selecting ATCC 34066 for further study its growth in Turner's solution/undecane was investigated. The growth of C. resinae isolates A and C had assessment levels of 1, 2 and 2 after 7, 38 and 76 days and no pH fall was recorded. ATCC 34066 had growth assessment levels of 1, 2, 3, 4 and 5 after 3, 7, 14, 21 and 27 days respectively. A pH fall from 5.8 to 5.4 was observed after 17 days.

C. resinae is an aerobic organism which grows at the interface of hydrocarbon-aqueous mixtures. As several workers have reported different growth rates under static versus shaken incubation conditions, the affect of this parameter was investigated in C. resinae ATCC 34066 together with the effect of varying the amount of hydrocarbon. It was important for the proposed corrosion system that good growth could occur under static conditions with at least 3 - 4 mm

of hydrocarbon overlay. The results showed that static growth conditions gave far better growth than shaken conditions irrespective of ratio of aqueous : hydrocarbon phases. In fact the 1 : 1 hydrocarbon : aqueous ratio gave a better growth than a 1 : 2 mixture. This suggests two things, firstly that shaken conditions, even if very slow and gentle cause disruption of the mycelium which severely disrupts growth. Secondly, at least within the limits of the experiment, oxygen diffusion through the static hydrocarbon phase is not limiting. This latter fact was confirmed by measuring the rate of oxygen uptake by aqueous solutions overlaid by varying depths of Dieso or undecane. At a depth of 5 mm of the non-aqueous material, oxygen diffusion into the aqueous phase was still 75% of maximum and this did not interfere with growth. This would allow the use of 25 ml undecane or Dieso on 250 ml aqueous medium; a ratio generally used in future experiments.

The lag of C. resinae varied depending on whether a fresh spore suspension or one that had been incubated in Turner's solution plus glucose for 24 hours was used. It was expected that glucose would accelerate germination, however a shorter lag phase and maximal yield was achieved in Turner's plus undecane within 20 as opposed to 24 days in glucose. In addition pH fall was more rapid, achieving its lowest level (pH 4.2) after 35 days as opposed to 53 days. In future experiments fresh spores were used to inoculate flasks.

Preliminary experiments showed that C. resinae grew very poorly in laboratory sea water even after prolonged periods of incubation. Although sea water is the aqueous phase most likely to be present in ship's fuel systems its composition varies enormously. For example, the ship takes on sea water from regions with differing extents of pollution; this will be further modified by the ingress of rust, paint, bilge, condensate and the growth of microorganisms. Hence, modification of laboratory sea water to improve growth is justified. Nitrogen and phosphorous may be regarded as major limiting factors to growth of C. resinae. A supplement of 0.5% nitrogen and 28 $\mu g\ l^{-1}$ phosphorous was chosen, firstly because Parbery[146] found this

amount of nitrogen (as $(NH_4)_2SO_4$) allowed good growth and acid production of C. resinae and secondly because Turner[208] found an average of 28 µg l^{-1} phosphorous in nine ship's fuel systems. The latter was added as KH_2PO_4 because Parbery[152] found K_2HPO_4 inhibited growth of C. resinae. Besides a lack of essential elements sea water may not support good growth because of inhibition by one or several of its components. The major component of sea water is NaCl (26.5 g l^{-1}), a salt concentration which may be inhibitory to growth. Thus sea water was diluted to 20, 30 and 40% in an attempt to reduce possible inhibition. In addition, Turner's solution which normally supports good growth was supplemented with 1% and 3% NaCl to examine any inhibitory effects.

In the first experiment growth of C. resinae in Turner's solution plus undecane was compared to growth in concentrated, 20%, 30% or 40% sea water. In Turner's solution plus undecane rapid growth occurred within 7 days and the usual thick mat of mycelium was evident at the interface within 20 days. Only after 24 days did the pH begin to fall significantly from the initial pH of 5.85, eventually reaching 4.0 by 53 days.

There was little growth in any of the sea water containing flasks though 40% showed slightly better growth than 30% which was better than either 20% or concentrated. In all three diluted sea waters the pH dropped from 8.0 to 7.0 after 10 weeks.

Table 3.5 illustrates the results for modified sea water and Turner's solution. Sodium chloride was not inhibitory to growth or acid production at 1% and 3%. Growth in 30% sea water was quite poor; the presence of either added nitrogen or phosphorous improved growth and when both elements were added the amount and particularly the rate of growth improved further. The pH dropped markedly over the first 35 days in sea water containing flasks and except when unsupplemented remained fairly constant through to day 56. The pH in Turner's solution fell initially but rose again in NaCl containing flasks. Cell lysis or utilisation of acids may explain this observation.

Table 3.5

Growth of C. resinae in Various Liquid Media

Medium	Growth Assessment							pH		
	Day									
	6	9	15	20	28	35	56	0	35	56
30% Sea water	1*	1*	1*	1*	1	1	2	8.0	5.0	7.9
30% Sea water + 0.5% Nitrogen	2	2	2	2	2	2	2	6.0	5.1	5.0
30% Sea water + 50 μg l^{-1} Phosphorous	1*	1*	1	1	2	2	2	8.4	5.6	4.7
30% Sea water + 0.5% Nitrogen and 50 μg l^{-1} Phosphorous	3	3	3	3	3	3	3	6.0	4.5	4.5
Turner's Solution	1	3	4	5	5	5	5	5.8	4.9	4.0
Turner's Solution + 1% NaCl	3	4	4	4	4	5	5	5.0	3.1	3.6
Turner's Solution + 3% NaCl	1*	2	4	4	5	5	5	5.0	3.0	3.8

Growth assessment is based on the scales shown in Table 2.2.

*Indicates growth at the bottom of the flask other than of the interface.

3.8 Conclusions

The sludge samples tested contained a wide variety of microorganisms, many of which were relatively easy to maintain in the laboratory. However, caution was needed as several isolates were quickly lost during subculture and no doubt the conditions employed did not support the growth of many potential isolates at all. No strict anaerobes and only a few species of facultative organisms were discovered. Organisms in the latter category grew poorly anaerobically in the conditions employed.

Conventional methods of identifying the five bacterial isolates failed to fit the organisms into a known species. Since the species conveys clinical significance and possible treatment medical microbiology requires that bacteria are isolated in pure culture and identified. However, isolation and identification of fuel system contaminants may tell us little about their role in corrosion. For example, it cannot be assumed that because an isolate reproduces rapidly on laboratory media to give a visible colony it can also metabolise actively in the environment from which it has been isolated: common laboratory media do not mimic the natural environment nutritionally or physically. In addition, tests such as indole production have little relevance to corrosivity. Thus a new philosophy to that of medical microbiologists might be in order in such highly specialised environments as the ships fuel system. For example, it is essential that the 'identity' and relative numbers of the most dominant spoilage strains are established. The media and growth conditions must be adapted to suit the sample, thus information on the history of the problem, the chemical and physical environment and the exact source of the sample must be known. All too often a sample of doubtful age is provided in a dubious container from an ill defined source. Conventional identification may be disregarded in favour of a 'numerical profile' based on several groups of informative tests[90]. Such a numerical profile may help clarify the organisms significance in corrosion processes. This method would also be able to determine the relative importance of the most numerous organisms. However, organisms still have to be isolated when in fact spoilage or

corrosion undoubtedly occurs as a result of the many species acting together.

Generally bacteria were found to grow better in sea water plus undecane systems whereas yeasts and mycelial fungi grew better in Turner's solution plus undecane. Thus bacteria may be the primary colonisers in contamination of fuel systems. Growth curves showed bacteria grew quickly and soon entered the stationary phase whereas yeasts had a lag phase and a slower growth rate but produced a higher yield. Penicillium and Cladosporium sp. had a longer lag phase, up to a week, but the latter became the predominant species.

Although C. resinae was considered the most prolific organism it was not alone in producing acidic metabolites and hence involved in pH related corrosion. The bacterial isolates 1 and 10 also reduced the pH, the latter lowering its culture medium to 5 from between 6 to 11. Penicillium spp. also reduced the pH of its culture medium but the yeast isolates were found to produce little acid.

As a major contaminant of fuel systems the corrosivity of C. resinae was to be examined. For this reason good growth of a suitable isolate had to be achieved. ATCC 34066 was chosen because it reduced the pH of Turner's solution more and grew faster than the other isolates. Better growth of C. resinae was established under static rather than shaken conditions. Presumably any advantage in oxygen availability was counteracted by damage to the mycelia. This was fortuitous since the following corrosion experiment involves the suspension of metal coupons through growth at the interface. Static conditions enabled a well defined interfacial area to be compared to the top and bottom of the metal coupon. Although C. resinae is a strict aerobe the Dieso or undecane overlay was not found to inhibit growth. However, the virtual lack of growth in sea water systems was a problem to be overcome. A concentration of 35% sea water supplemented with nitrogen and phosphorous was found to support growth, though still not to the same extent as Turner's solution.

In studying the mechanisms of microbial corrosion the effects of growth on the metal were of prime importance. For this reason Turner's solution was chosen as the aqueous medium for growth. However, as well as interactions between C. resinae and the metal,

those between the aqueous environment and the metal had to be considered. Although modified sea water supported less growth than Turner's solution its composition was more likely to represent that found in the ships fuel system. Hence in most of the future experiments the corrosive effects in the presence and absence of microorganisms were compared in both aqueous phases.

CHAPTER 4

CORROSION OF METALS SUSPENDED THROUGH AN AQUEOUS/HYDROCARBON TWO PHASE SYSTEM IN THE PRESENCE OR ABSENCE OF *C. RESINAE*

4.1 Preliminary Experiment

Before embarking on a long term study of the corrosive effects of C. resinae a preliminary experiment to determine an effective laboratory system was carried out. Although best growth was achieved in Turner's solution and undecane (Chapter 3) the inherent corrosion characteristics of Turner's solution were expected to differ from those of sea water. For example, the buffering action of phosphate anions in Turner's solution would prevent the localised acidification necessary for pit growth[219]. Moreover, anions of weak acids act as inhibitors of the pitting process and modify the type of film formed on the metal surface. Turner's solution also contains ammonium nitrate which alone is particularly corrosive to mild steel[211] though it may inhibit corrosion of aluminium[13]. Sea water contains a high concentration of aggressive chloride ions which leads to pitting due to the breakdown of the protective film[145].

The experiment examined growth, corrosivity of the filtrates and corrosion characteristics of mild steel in both sea water with Dieso or undecane and Turner's solution with Dieso or undecane.

There was no growth in the sea water flasks. Growth was more prolific in Turner's solution with undecane than with Dieso, that is, after 28 days the dry weight of C. resinae was 208 mg and 117 mg respectively. The pH fell from 5.9 to 5.5 and 5.7 respectively though the pH of undecane did not change from 6.0.

Acid analysis by GLC showed citric, isocitric and α-ketoglutaric acids to be present in culture filtrates of C. resinae in Turner's solution with both undecane or Dieso. Lactic acid was also found in the former and succinic acid plus several unidentified peaks in the latter.

Thus, not only did Dieso support less growth and less acid production than undecane but different acids were produced. This difference may have been a consequence of differing growth rates and yields.

As the effect of growth of C. resinae on the corrosion of metals was important in this experiment, undecane was chosen as substrate because it supported better growth. However, the different

acids produced may alter the corrosion characteristics. Results in section 7.1 show the differing effects of the various acids produced.

The corrosion rate in Turner's solution as calculated from weight loss of the exposed metal coupons showed the control value to be 4.1 mdd and the test values with undecane or Dieso to be 18.3 or 10.5 mdd respectively. Thus the effect of growth was to increase the corrosion rate by a factor of 4.5 and 2.6 respectively. The corrosion rate in sea water was 13.7 mdd indicating that it was 3.3 times more corrosive to mild steel than Turner's solution.

The corrosivity of the filtrate as measured by the linear polarisation resistance of mild steel was greater where undecane had been the substrate. The corrosion rate in sterile Turner's solution was 0.3 compared to 0.5 mdd in the presence of C. resinae. When Dieso replaced undecane the corrosion rate dropped to 0.2 mdd.

The corrosion rate in the control as monitored by the linear polarisation technique was approximately 10-fold less than that determined by the weight loss method. The values were not expected to be the same because the former method was only true at a particular point in time, while the weight loss method was subject to changing conditions over the month's exposure time. The traditional weight loss method was considered to have provided the more accurate corrosion rate, the linear polarisation technique yielding more arbitrary values which nevertheless were still very useful in comparing the corrosivity of the various filtrates. Other workers[190] have also found a poor correlation between the corrosion rates found by weight loss and polarisation data for carbon steel in contaminated sea water. Thus in future experiments, rates measured by the linear polarisation technique were used in a semi-quantitative manner for direct comparisons.

The type of corrosion product differed in the two aqueous phases. The typical red rust of steel was apparent in sea water though a fine cream coloured precipitate was loosely adhered to a dark grey film in Turner's solution. This corrosion product may be more protective and account for the lower weight loss in Turner's solution.

The acid cleaned coupons were examined microscopically. The sea water and Turner's solution controls were examined for differences

in corrosion characteristics. The Turner's solution test and control samples were examined for the effects of growth and indicated that there was not always an obvious interfacial area probably because the supportive thread was unstable. This was corrected in future experiments. No comparison was made between the sea water test and control because of the lack of growth.

In the sea water controls there was a 2 μm deep shelf at the interface. In the middle of the coupon there were a few 2 μm deep, 1 μm diameter pits amidst general corrosion. In contrast, the Turner's controls had many pits 1 - 5 μm deep of 1 μm diameter and a few 3 μm deep of 2 - 3 μm diameter. The general corrosion appeared even and there was no obvious interface.

The Turner's solution test coupons had a line of pits at the interface which averaged 7 μm depth and 4 μm diameter. The middle of the coupons had much uneven general corrosion. The average depth of pits was 10 μm, the deepest recorded was 28 μm. They varied from 3 - 10 μm in diameter.

Thus Turner's solution appeared to promote more pitting than sea water and the presence of C. resinae further enhanced this. C. resinae exerted most effect at the interface where growth occurred and Plate 4.1 illustrates this for mild steel in Turner's solution/ undecane. The occurrence of strips of corrosion 2 x 17 μm in area and pits 2 - 5 μm in diameter can be seen. The occurrence of a bright halo around the pits was due to penetration of the electron beam through this area of metal. This indicated that a 'lip' protruded over the pit hiding the true extent of pitting below.

As a further indication of the coupon's surface texture a Talysurf instrument was used to measure the centre line average (C.L.A.) values. Figure 4.1 shows an amplified graphical plot aross the interfacial area. Pit depths were calculated from the plot. C.L.A. values were obtained for the interfacial area, the middle and base of the coupons and the results are shown in Table 4.1. Generally, the uninoculated controls had lower values than the inoculated tests. The interfacial area was not necessarily "rougher" than the middle and base even though C. resinae was in close contact with the metal at this point. The base of the coupon was often

Plate 4.1 (x 1140)

Electron Micrograph of the Interfacial Area of Mild Steel Exposed to C. resinae in Turner's Solution and Undecane for 28 Days

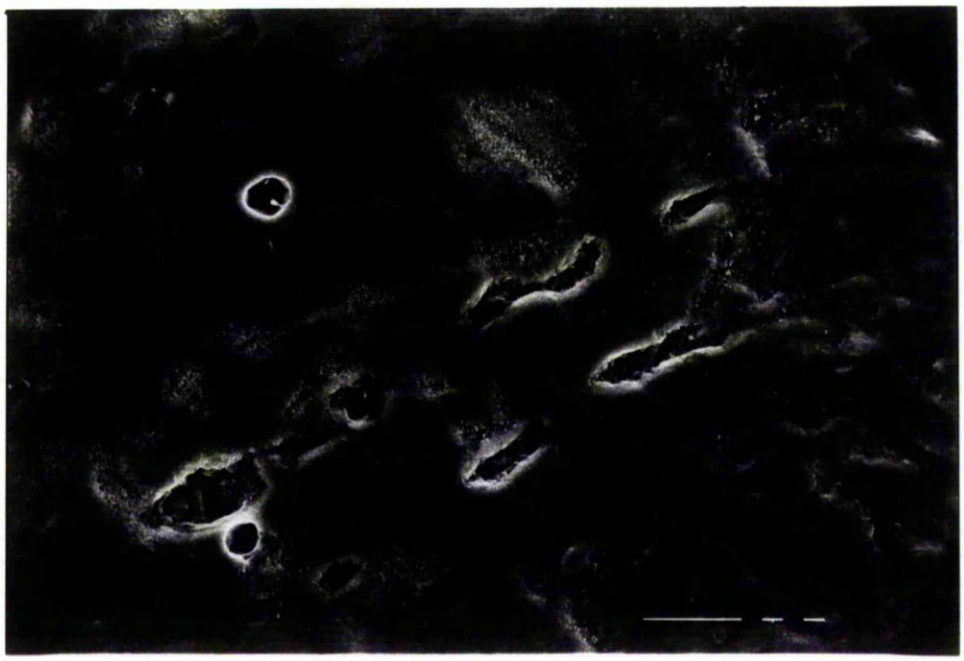
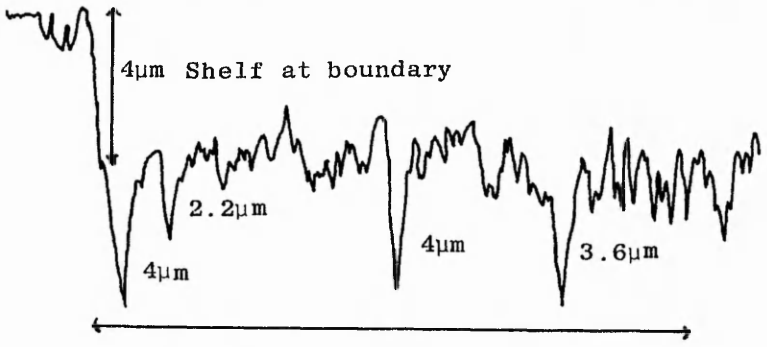


Figure 4.1

To show the Surface Texture of Mild Steel Exposed to Turner's Solution/Undecane and C. resinae for 28 days



Vertical magnification = 5000

Horizontal magnification = 100

Table 4.1

To show the C.L.A. Values of Mild Steel Exposed to Sea Water or Turner's Solution in the Presence or Absence of C. resinae for 28 days

System	C.L.A. Value		
	Interface	Middle	Base
Controls			
Sea/undecane	0.15	0.1	0.35
Sea/Dieso	0.15	0.15	0.80
Turner's/undecane	0.21	0.15	0.14
Turner's/Dieso	0.06	0.06	0.09
Tests			
Turner's/undecane	0.85	0.7	0.7
Turner's/undecane	1.6	0.84	1.0
Turner's/Dieso	0.55	0.5	0.3
Turner's/Dieso	0.33	0.65	0.95

rougher than the other areas.

Thus the presence of C. resinae increased the surface roughness of mild steel and it's effect was exerted all over the coupon due to the excretion of metabolites into the aqueous phase. This method was time consuming and only examined small sections of the coupon at any one time thus its use was rejected in future experiments.

In summary, a difference was found in the corrosion characteristics of mild steel in sea water compared to Turner's solution. In Turner's solution the corrosion rate was three times less and the corrosion that occurred had pitting tendencies.

Sea water did not support good growth of C. resinae which made it unsuitable for study of microbial corrosion. The effects of C. resinae on mild steel in Turner's solution were more easily observed and it was therefore chosen as an aqueous phase for the next experiment. Although Turner's solution was chosen it was considered necessary to carry out a further experiment with modified sea water, which although not fully representative of natural sea water may provide more realistic corrosion characteristics.

4.2 Long Term Corrosion Test

The method using Turner's solution and modified sea water have been described previously and as they were essentially similar experiments, the parameters examined for each will be discussed together. As explained in Chapter 2 inhibition of growth in modified sea water necessitated reinoculation. Growth after reinoculation was still slight but the experiment continued to provide useful information. A further inoculation step after 31 weeks was considered justified to enhance growth and to simulate partly the probable continuous supply of fresh inoculum contaminating fuel systems. This introduction of new variables made direct comparisons with microbial corrosion in Turner's solution less meaningful. In addition, exposure times in the two media were different to accommodate the different

amounts of growth.

4.2.1 Continuous Monitoring of pH Values

The pH of one test and one control flask were monitored for eight weeks.

pH Change of Turner's Solution

Growth of C. resinae caused a pH fall in Turner's solution which was dependent on the metal present (Figure 4.2). Alone, the corroding metals did not modify the pH (results not presented). Mild steel did not lower the growth yield but may have altered the metabolism of C. resinae with regard to acid and/or alkali production. Alternatively metal acid complexes may have been formed thus negating some of the pH fall.

pH Change of Modified Sea Water

The addition of a no-metal control in this experiment illustrated very clearly the effect of corroding metals on pH (Figure 4.3). The effects were more obvious than in Turner's solution because sea water does not have the same buffering capabilities. Over the eight week period the pH fell dramatically in the no metal and corrosion resistant stainless steel test systems. Slightly more growth being observed in the latter. In the presence of cupronickel, mild steel and aluminium the pH rose to 8.2 and faint growth was only observed after the eighth week. Thus, not only did the corroding metals increase the pH they also inhibited the growth of C. resinae. It was shown earlier that C. resinae will grow at such pH's thus implicating the corrosion product as being inhibitive to growth. Such inhibition led to reinoculation after 9 weeks. This led to some growth, especially in aluminium systems, though it was never as much as in Turner's solution. However, the experiment was continued in order to obtain as much information as possible.

The toxicity of the corrosion product may have differed in

Figure 4.2: The pH fall of Turner's Solution due to the Growth of *C. resinae* in the presence of Stainless Steel, Mild Steel, Cupronickel and Aluminium

Figure 4.3: The pH Fall of Modified Sea Water due to the Growth of *C. resinae* in the Presence of No Metal, Stainless Steel, Mild Steel, Cupronickel and Aluminium

Key for Figures 4.2 and 4.3

⊙ - No metal
● - Stainless steel
■ - Mild steel
▲ - Cupronickel
⊡ - Aluminium

Figure 4.2

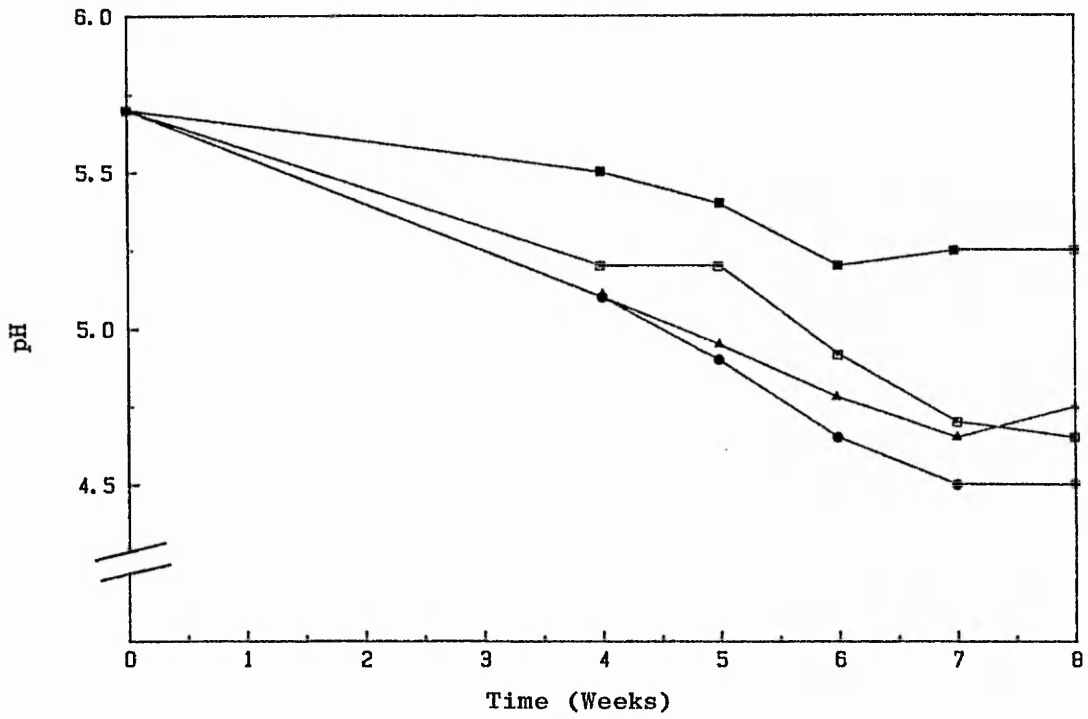
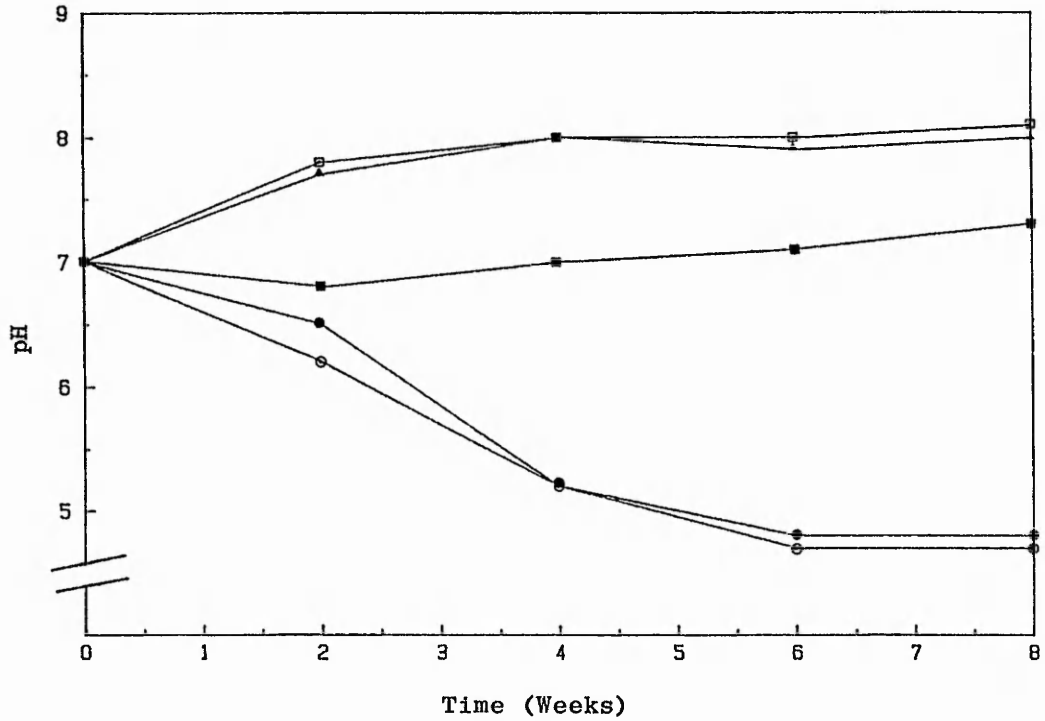


Figure 4.3



Turner's solution and modified sea water. Alternatively, the combination of rapid growth and slow corrosion in Turner's solution compared to slow growth and rapid corrosion in sea water would allow germination and growth of C. resinae in the former, but not the latter, before the level of inhibitive corrosion products built up. This ought to render the role of C. resinae in corrosion of metals in sea water systems unimportant though its proliferation in service conditions illustrates that germination is not inhibited; probably as a result of protection by other organisms or painted surfaces.

4.2.2 Final pH value of culture filtrates

The average pH values of Turner's solution and modified sea water after exposure to C. resinae in the presence of metal alloys are presented in Tables 4.2 and 4.3 respectively.

pH Values in Turner's Solution

There was virtually no pH change in the control solutions from 5.7 except in the presence of mild steel after 72 weeks.

After 4 and 9 weeks the pH had fallen in the test solutions, the amount depending on the metal present. As explained earlier this may have been due to either the corrosion process directly or the effect of the corrosion process on the metabolism of C. resinae. Solutions exposed to stainless steel and C. resinae showed the greatest pH fall of 1.3 units.

In the 13, 18 and 72 week test solutions the pH became progressively more alkaline. This was true also in the presence of the relatively passive stainless steel, indicating alkaline metabolic products possibly were excreted by C. resinae.

pH Values in Modified Sea Water

The pH's of the no metal and stainless steel control filtrates remained near the original value of 7.0 throughout the treatment time. The pH's of the remaining metal control flasks were

Table 4.2

To Show the Final pH of Turner's Solution After Exposure to Metal Alloys in the Presence and Absence of *C. resinae*

System Weeks	Stainless Steel		Mild Steel		Aluminium		Cupronickel	
	Test	Control	Test	Control	Test	Control	Test	Control
4	5.1	5.6	5.5	5.7	5.2	5.6	5.1	5.6
9	4.5	5.7	5.1	5.8	5.1	5.7	4.8	5.7
13	-	-	5.5	5.9	5.7	5.7	5.6	5.8
18	5.7	5.8	6.0	6.0	5.9	5.8	5.8	5.9
72	6.4	5.7	5.9	7.0	6.5	5.7	6.3	5.7

Key:

- No sample taken

The initial pH was 5.7

Table 4.3

To Show the Final pH of Modified Sea Water After Exposure to Metal Alloys in the Presence and Absence of *C. resinae*

System	No Metal		Stainless Steel		Mild Steel		Aluminium		Cupronickel	
Weeks	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
7	4.7	7.0	4.5	6.9	-	-	-	-	-	-
14	5.1	7.1	4.7	7.1	5.5	6.4	5.0	6.7	6.7	7.1
26	5.6	6.9	4.8	7.0	6.1	6.3	5.4	6.5	5.7	7.1
31	5.4	7.1	6.2	7.0	-	-	6.5	6.4	-	-
46	3.8	6.9	3.8	6.7	6.0	6.2	6.8	6.8	6.5	7.2
72	4.0	6.8	3.5	6.8	6.0	6.3	7.2	6.8	6.4	7.1

Key:

- No sample taken

The initial pH was 7.0

the same as their respective inoculated flasks due to lack of growth.

The pH of the test filtrates had fallen 2.7 and 2.5 pH units over 7 weeks. Over the following sample times the pH rose until, due to reinoculation after 31 weeks, it had fallen again by the 46 week sample. The original fall in pH was due to the production of extracellular acids and the subsequent rise was at least in part due to their catabolism. Results later confirm that acid concentration falls during this period. Excretion of alkaline metabolites may also account for the pH rise. After reinoculation further acid excretion was expected to be the cause of the subsequent pH fall. However, results in Section 4.2.4 show that this was not the case. In the absence of corroding metal the only explanation was the metabolism of the alkaline products excreted by the ageing cultures.

As explained in the previous section, inhibition of growth by alkaline corrosion products, occurred in the presence of mild steel, aluminium and cupronickel and the solutions were reinoculated and/or acidified to pH 6.5. After 14 weeks the control filtrate pH was slightly lower in the presence of mild steel and higher in the presence of aluminium and cupronickel. It did not alter significantly after this treatment time indicating that the corrosion process had slowed to a rate which no longer exhibited itself as a pH rise.

Reinoculation of the test systems gave rise to growth of C. resinae, especially where aluminium was present. A pH fall of 1.5 units was recorded with a subsequent pH rise. Reinoculation after 31 weeks did not result in a further fall as in the no metal and stainless steel systems. Similar results were obtained for mild steel. In the presence of cupronickel, reinoculation did not give rise to a pH fall after 14 weeks. Possibly continuing corrosion was responsible for masking acid production. A pH fall was observed after 26 weeks when the corrosion process may have stabilised. As before the pH rose again by the 46 week sample.

4.2.3 Dry Weight of C. resinae

The dry weight was calculated as described earlier though it was often difficult, especially in the case of mild steel, to separate the microorganism from the corrosion product. Table 4.4 and 4.5 indicate the average dry weight of C. resinae grown in Turner's solution and modified sea water respectively.

Table 4.4

Average Dry Weight (mg) of C. resinae grown in Turner's Solution in the presence of various Metals for Periods of 4 to 72 Weeks

	Average Dry Weight (mg)			
Weeks	Stainless Steel	Mild Steel	Aluminium	Cupronickel
4	214	237	228	203
9	691	880	491	655
13	-	1095	941	631
18	1376	1436	1375	978
72	2902	2668	3912	4138

In the absence of a no-metal control results were compared to the passive stainless steel and indicated no inhibition of growth after 4 weeks. After 9 weeks there was lower yield in aluminium containing systems though after 13 and 18 weeks cupronickel lowered the yield, possibly due to the formation of inhibitory corrosion products. However, after 72 weeks no such inhibition was apparent and the yield was significantly greater than in the presence of stainless steel. The presence of aluminium also stimulated growth, whereas that of mild steel inhibited it. This fact may prove significant when considering the corrosion of mild steel fuel tanks by C. resinae. Indeed, a visual inspection of several rusty tanks showed little growth (D. Powell, pers. comm.).

The presence of voluminous corrosion products made it difficult to obtain true values of the small growth yield observed in the presence of mild steel, aluminium and cupronickel. Table 4.5 therefore only illustrates the dry weights in the presence of stainless steel and no metal. After 46 weeks cupronickel and aluminium containing systems were estimated to have supported 71.0 and 82.3 mg dry weight of C. resinae respectively.

Table 4.5

Average Dry Weight (mg) of C. resinae grown in Modified Sea Water ± Stainless Steel for Periods of 7 to 72 Weeks

	Average Dry Weight (mg)	
Weeks	Stainless Steel	No Metal
7	26.4	21.3
14	40.0	26.7
26	47.2	40.9
31	56.9	60.8
46	61.6	36.4
72	50.5	32.5

Growth of C. resinae was enhanced by the presence of stainless steel. Although the metal was not visibly corroding the provision of small quantities of metal ions may have stimulated growth. Analysis of metal ions in the culture filtrate, reported later, showed increased amounts of iron, chromium, manganese and nickel.

4.2.4 Acid Analysis by Temperature Programmed Gas Liquid Chromatography

The retention times, sensitivity to 50 mg of acid and conversion factors are illustrated in Table 4.6 and the preparation of the standards and means of calculating conversion factors were described in Section 2.2.2. A linear response was observed for up to 100 mg acid. The sensitivity of the method to 50 mg of each acid varied considerably (Table 4.6).

Isocitric and α-ketoglutaric acids gave two peaks under the conditions used. Possibly they decomposed either during methylation or after injection due to high temperatures and retention times. Only the first of the two peaks is considered in the discussion of the results.

Samples were prepared as described earlier; the culture filtrates of duplicate test systems which had contained stainless steel were examined for both Turner's solution and modified sea water unless indicated.

At each sample time controls for sea water and Turner's solution with stainless steel contained no acids.

Standard preparations of acids dissolved in Turner's solution, sea water and distilled water, prior to evaporation and esterification had the same retention times and response as the esterified dry acid without pre-treatment.

Several acids were not identified (A-H, Table 4.7). Given the large number of possibilities (for example, TCA cycle + related acids, various chain length fatty acids, dioic acids and products of subterminal oxidation of n-undecane) it was not feasible to attempt complete identification of the range of acids produced. From the literature[124,136,188] possibilities might be acetic, glycollic, glyoxylic, oxaloacetic, adipic and mono C10, di C7, C10, C11 and C12 acids. It was found that C and D had retention times similar to those dodecanoic acid and 1,7 heptanedicarboxylic acid respectively. They were not quantified. The other unknowns were found not to be capric, malonic or glutamic acids.

The peak areas of the unknowns were simply corrected to be

Table 4.6

To Show the Retention Time, Response and Conversion Factor from Peak Area to $\mu g\ ml^{-1}$ of the Standard Acids

Acid	Retention Time (Min)	Peak Area for 50 mg ($\times 10^{-5}$)	Conversion Factor ($\times 10^{5}$)
Pyruvic	2.94	22.857	2.73
Lactic	3.88	18.605	3.36
Fumaric	6.47	9.535	6.55
Succinic	7.85	30.075	2.08
Itaconic	9.93	41.885	1.49
Maleic	11.12	25.397	2.46
Malic	15.32	19.512	3.20
α-ketoglutaric	16.71/17.61	35.714/6.623	1.75/9.41
Cis-Aconitic	19.70	11.299	5.53
Citric	23.14	22.663	2.76
Isocitric	25.24/27.28	8.782/5.357	7.12/11.68

Table 4.7

To Show the Retention Time of the Eight Unidentified Peaks, Named A to H

Peak	Retention Time (Min)
A	0.40
B	1.78
C	12.96
D	18.52
E	21.46
F	24.55
G	28.70
H	30.67

equivalent in terms of volume injected and attenuation used. A 1,000-fold reduction in the arbitrary value was made to facilitate tabulation. It should be remembered that A to H are not intercomparable because the same concentration of each may elicit a different response in terms of peak area. This was made apparent by the known standards.

The concentrations of acids may be of a semi-quantitative nature as it was assumed that the boron trifluoride-methanol esterification procedure gave 100% recovery for the esters of the acids. However, it may be as low as 50 - 55% for the methyl and trimethyl esters and 90 - 100% for the dimethyl esters. As long as such a % recovery is the same for the standard and the unknown sample such an effect may be eliminated during direct comparison. Harmon and Doelle[73] indicated that as long as there was no more than 100 mg of acid such recoveries were consistent. Thus, in samples whose total acid content multiplied by 20 was greater than 100 mg the value may be an underestimate. Such a value of 5,000 µg/ml was only found to be the case in the 18 and 72 week samples of Turner's solution.

The results are presented in Tables 4.8 and 4.9. A higher concentration of acid was found in the Turner's filtrates than in the sea water filtrates and was probably related to the greater amount of growth supported by the former. Comparing the 9 week Turner's samples (average 4,067 µg/ml) and the 14 week sea water samples (average 205 $\mu g/ml^{-1}$) there is a 20-fold difference and the difference in dry weight of C. resinae at these times was found to be 17-fold.

In both Turner's solution and sea water the duplicate culture filtrates at each time interval varied significantly in several respects, for example, the total acid content; the presence or absence of an individual acid; and, the concentration of an individual acid. Work by Parbery[150] has shown growth of C. resinae to be variable even under essentially similar conditions. This work conforms to those findings. For true comparisons to be made many more replicates were required.

Table 4.8

To Show the Concentration ($\mu g\ ml^{-1}$) of Standard Acids and an Aribtrary Concentration of Unidentified Acids for Duplicate Culture Filtrates of C. resinae Grown in Turner's Solution and Undecane at Five Sampling Times

	$\mu g ml^{-1}$ - 4 Weeks		$\mu g\ ml^{-1}$ - 9 weeks		$\mu g\ ml^{-1}$ - 13 weeks		$\mu g\ ml^{-1}$ - 18 weeks		$\mu g\ ml^{-1}$ 18months
Acid	1	2	1	2	1	2	1	2	Mixture
Pyruvic	-	76.5	46.6	76.6	22.5	32.1	12.0	9.4	-
Lactic	67.8	-	14.7	38.2	5.4	8.9	131.2	182.4	569.3
Fumaric	-	100.9	143.9	168.5	37.6	26.3	-	-	153.8
Succinic	10.6	-	-	36.4	-	-	15.7	-	20.7
Itaconic	-	-	1.2	-	-	-	70.2	12.9	10.7
Maleic	-	-	7.2	78.3	-	-	24.8	10.0	16.4
Malic	-	-	-	22.9	-	-	6.9	-	19.7
α-ketoglutaric	-	-	7.4/ 15.6	-	-	-	2.4/ 13.3	-	-
Cis-Aconitic	-	-	49.0	94.8	48.6	53.3	12.4	539.0	3653.9
Citric	148.2	353.4	891.6	1400.0	372.6	126.5	83.8	751.5	519.2
Isocitric	17.7/ 3.0	511.1/ 121.5	2067.7/ 1089.6	2988.7/ 1532.5	551.5/ 529.1	1120.2/ 769.2	2016.8/ 391.2	12884.1/ 4479.9	17886.7 7596.5
Total	244.3	2598.4	3229.2	4904.8	1038.1	1367.3	2376.2	14384.3	22850.3
A	785	700	712	171	242	313	355	119	52
B	-	-	4	679	51	47	62	94	14
C	-	-	-	1	0.5	1	1	-	1
D	-	-	1	235	1	23	-	-	1
E	-	-	-	-	1	2	1.2	1.6	2.3
F	-	-	0.5	0.5	1.5	2	1.5	3.8	9.3
G	0.5	-	4.5	1.5	3.2	1.7	79.3	63.8	1.6
H	-	-	21	6.4	-	-	-	-	-

NB: Stainless steel culture filtrates except at 13 weeks when those of mild steel were used.

Table 4.9

To Show the Concentration ($\mu g\ ml^{-1}$) of Standard Acids and an Arbitrary Concentration for Unidentified Acids for Duplicate Culture Filtrates of C. resinae Grown in Modified Sea Water and Undecane at Five Sampling Times

	$\mu g\ ml^{-1}$ - 7 Weeks		$\mu g\ ml^{-1}$ - 14 Weeks		$\mu g\ ml^{-1}$ - 26 Weeks		$\mu g\ ml^{-1}$ 31 Weeks		$\mu g ml^{-1}$ - 46 Weeks	
Acid	1	2	1	2	1	2	1	2	1	2
Pyruvic	-	-	7.7	-	-	-	-	-	1.5	-
Lactic	49.2	-	-	-	-	-	9.3	11.8	4.3	-
Fumaric	98.9	23.2	26.2	29.4	-	-	20.4	-	3.9	8.2
Succinic	3.6	18.9	3.4	28.7	9.6	13.3	16.9	30.7	2.2	6.7
Itaconic	95.4	17.0	2.8	-	9.1	15.6	-	-	-	-
Maleic	13.2	3.3	13.8	-	0.1	-	43.6	-	-	-
Malic	7.6	-	-	-	-	-	-	-	1.1	-
α-ketoglutaric	20.4/ 700.5	1.5/ 28.0	-	-	-	-	-	-	-	-
Cis-Aconitic	17.2	32.6	12.6	-	-	-	86.0	32.5	2.7	-
Citric	73.2	42.9	31.0	59.0	38.0	29.7	64.7	31.9	2.8	4.6
Isocitric	15.4/ 175.2	154.9/ 310.9	92.7/ 149.0	103.1/ 32.4	135.9/ 72.8	137.2/ 70.4	73.4/ 65.8	92.3/ 70.6	29.2/ 19.2	36.1/ 32.8
TOTAL	394.0	294.1	190.1	220.2	192.6	195.8	314.3	199.1	47.7	55.6
A	724	707	206	786	380	484	516	666	806	732
B	-	9	-	-	-	-	12	118	3	21
C	12	-	-	-	-	-	-	-	3	-
D	-	-	5	-	-	-	0.3	-	-	-
E	1	2	0.2	-	-	4	-	-	-	-
F	13	-	-	-	0.2	-	0.8	0.8	-	-
G	2.2	1.5	-	-	-	-	-	-	-	-
H	2	-	4	6	2	-	4	-	12	-

Acid Analysis in Turner's Solution

The total concentration of acid in Turner's solution rose as the time before sampling increased. The 13 week Turner's sample did not fit into the pattern possibly because filtrates from mild steel tests rather than stainless steel tests had to be used. The acids may have formed insoluble complexes with the corrosion products thus escaping analysis.

The predominating acid was isocitric followed in most instances by citric acid. Pyruvic, lactic, fumaric and aconitic acids were usually present, their relative amounts varied depending on the sample time.

The following summary shows that the final pH is not indicative of acid content. For example the average pH from 9 to 18 weeks rose by 1.3 units with a concomitant increase in dry weight of *C. resinae*, from 691 mg to 1,376 mg and a doubling of total acid. Presumably *C. resinae* also forms alkaline products. The removal of hydrogen ions by the cathodic corrosion process was unlikely in the presence of resistant stainless steel.

Sample Time (Weeks)	4	9	13	18
Dry wt. *C. resinae* (mg)	214	691	941	1376
pH	5.1	4.5	5.5	5.8
Total acid ($\mu g\ ml^{-1}$)	1421.4	4067.0	1203.1	8382.8

Acid Analysis in Modified Sea Water

In modified sea water culture filtrates the predominant acid was again isocitric and although citric acid was always present it was not always in the second highest concentration occasionally being superceded by aconitic or fumaric acids. Succinic acid was always present under these conditions which represented a distinct difference from its variable presence in Turner's solution. In addition, lactic

and particularly pyruvic acids which were nearly always present in Turner's solution were rarely present here.

The differences in rate of growth of C. resinae in sea water and in Turner's solution may explain these variations.

A summary of the results is as follows:-

Sample Time (Weeks)	7	14	26	31	46
Dry wt. C. resinae (mg)	26.4	40.0	47.2	56.9	61.6
pH	4.5	4.7	4.8	5.4	3.8
Total Acid $\mu g\ ml^{-1}$	344.1	205.2	194.2	256.7	51.7

An unexpected observation was the four-fold reduction in acid content after 46 weeks. Due to reinoculation after 31 weeks there had been some increase in weight and most significantly a decrease in pH. In these growth conditions the utilisation of excreted acid products was apparently preferable to further utilisation of the hydrocarbon source. A concurrent utilisation of alkaline products may account for the fall in pH which was incongrous with the fall in acid concentration.

General Discussion

The literature shows that a variety of acids have been found to be excreted by C. resinae. The types and relative concentrations of the acids have been found to vary with carbon source[124,136,188] and culture time[188]. McKenzie et al.[136] found only citric and isocitric acid in the media of n-alkane grown C. resinae. They reported 5.9 moles/ml (1,240 μg/ml) of citric acid in Bushnell and Haas mineral salts solution[26] supplemented with n-tridecane after 8 weeks incubation of C. resinae. This amount was similar to that found in the current work from Turner's solution after 9 weeks growth of C. resinae on undecane. However, they found higher concentrations of

citric acid from other n-alkanes and the concentration of isocitric acid was always lower.

Lin et al.[188] grew a Cladosporium sp. on glucose and several alkanes, including n-undecane and they recorded the presence of acids as a percentage of the total carboxylic acids present. After 11 days growth they found citric acid to be 81.5% of the total, followed by cis-aconitic at 4.6%. This was not supported in the current work where isocitric followed by citric or aconitic were present in the greatest concentrations though sampling did not begin until after 4 weeks.

Siporin and Cooney[188] found the predominant fatty acid on glucose and n-alkanes to be dodecanoic acid (> 60%). They found growth to be more rapid on glucose and that more acid accumulated in the medium. Acetic, glycolic, glyoxylic and an unknown acid were found in the glucose medium.

In the three reports cited[124,136,188] the authors implied that the differences in acid production could be directly attributed to the type of substrate. However, the current work using two mineral media suggests that the rate of growth and growth yield of C. resinae on these substrates may contribute to the varying types of acid production.

Given sufficient time this hypothesis could be tested by restricting the growth of C. resinae on a single substrate in various ways. The provision of a sub-optimum temperature would be more useful than a sub-optimum pH or the use of a growth limiting medium. The latter may have secondary effects on the acid produced. Such manipulation of type and quantity of acid produced would be useful in reducing corrosivity of C. resinae. However, outside a controlled laboratory situation it would be difficult to apply.

4.2.5 Analysis of Metal Ions in Culture Filtrates

The aims of this analysis were two-fold. Firstly, as corrosion of stainless steel in Turner's solution was too low to be measured by weight loss the demonstration of an increase in the concentration of metal ions in the test filtrate above that of the control filtrate could be taken to indicate that C. resinae had accelerated the corrosion process of stainless steel.

Secondly, any changes in the relative proportions of the major cations from the metal alloy would indicate specific uptake by C. resinae.

The concentrations (ppm) of the metal ions in culture filtrates containing stainless steel can be seen in Table 4.10. The total of the four metals analysed was taken as 100% enabling the percentage content of the individual metals to be calculated.

Stainless Steel in Turner's Solution

The test filtrates contained 2.6, 4.3 and 4.0 times the total metal content of the control filtrates after 4, 9, and 18 weeks respectively illustrating the ability of C. resinae to break down the inherent resistance of stainless steel in Turner's solution. The results showed that the presence of C. resinae increased the concentration of each metal ion though their relative proportions changed. In the uninoculated controls the percentages of Fe, Cr, Ni and Mn were 34.6, 32.5, 22.6 and 10.3 respectively though the percentage of each metal in the alloy was very different, i.e. 72.5, 17.0, 9.0 and 1.5 respectively. This may partly have been due to selective phase attack. The effect of C. resinae was to vary the control proportions in such a way that the presence of Fe was increased to 76.7% and that of Cr, Ni and Mn were decreased to 10.9, 9.6 and 2.8% respectively. Using the limited information available the two most likely corrosive activities of C. resinae were considered to be as follows:- firstly, C. resinae may actively extract only Fe from the alloy, and secondly, C. resinae may accelerate the dissolution of all the metal components but then may sequester Cr, Ni

Table 4.10

To Show the Concentration (ppm) and Proportion (%) of Fe, Cr, Ni and Mn in Solutions Exposed to Stainless Steel ± *C. resinae* for Various Lengths of Time

		Fe		Cr		Ni		Mn		Total	
		Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Turner's Solution											
4 Weeks	ppm	1.9	0.3	0.3	0.3	0.2	0.2	0.0	0.1	2.4	0.9
	%	77.1	34.8	12.9	32.6	8.3	22.5	1.7	10.1	100	100
9 Weeks	ppm	3.4	0.3	0.4	0.3	0.3	0.2	0.1	0.1	4.2	1.0
	%	80.8	34.7	10.2	32.7	5.9	22.4	3.1	10.2	100	100
18 Weeks	ppm	4.0	0.5	0.5	0.5	0.8	0.3	0.2	0.2	5.5	1.4
	%	72.3	34.3	9.6	32.1	14.5	22.9	3.6	10.7	100	100
% Range		76.7	34.6	10.9	32.5	9.6	22.6	2.8	10.3		
		±3.5	±0.2	±1.4	±0.3	±3.6	±0.2	±0.8	±0.3		
Modified Sea Water											
7 Weeks	ppm	0.3	0.2	0.3	0.1	0.3	0.3	0.1	0.0	0.9	0.6
	%	32.6	28.6	29.1	15.9	32.6	47.6	5.8	6.3	100	100
14 Weeks	ppm	0.6	0.4	0.3	0.3	0.3	0.4	0.1	0.1	1.3	1.0
	%	48.0	35.0	24.0	25.0	24.0	35.0	4.0	5.0	100	100
26 Weeks	ppm	0.8	0.4	0.2	0.2	0.3	0.5	0.1	0.1	1.3	1.2
	%	61.5	37.4	11.5	19.1	23.1	39.1	3.8	4.3	100	100
31 Weeks	ppm	1.4	0.7	0.3	0.3	1.0	0.7	0.1	0.1	2.8	1.8
	%	50.0	38.9	10.7	16.7	35.7	38.9	3.6	5.6	100	100
46 Weeks	ppm	1.8	1.2	0.6	0.7	1.3	1.1	0.2	0.2	3.8	3.0
	%	47.4	40.0	14.5	21.6	34.2	36.7	3.9	5.0	100	100
% Range		47.9	36	18	19.7	29.9	39.4	4.2	5.2		
		±9.2	±4.1	±7.3	±3.3	±5.3	±4.4	±0.8	±0.7		

and Mn leaving Fe in solution. The ability of C. resinae to sequest metal ions from solution will be examined later. It is possible that the altered environment inhibits the dissolution of Cr, Ni and Mn and/or accelerates the dissolution of Fe.

In conclusion the presence of C. resinae does increase the total metal ion concentration in solution and hence accelerates corrosion of stainless steel.

Stainless Steel in Modified Sea Water

The total metal ion concentration in the test filtrates was greater than that in the control filtrates but not by a large amount though increased corrosion rate was apparent as measured by weight loss (reported later). The low total concentration in sea water may therefore be explained by sequestration by C. resinae. Often the presence of Ni and Cr metal ions were lower in the test filtrates indicating that these two metals may be particularly susceptible to sequestration. Any biological function of these two metals to C. resinae is unknown, though they may be required for specific metabolic processes by certain organisms[156].

The corrosion characteristics of stainless steel were different in modified sea water as indicted by the relative proportions of Fe, Cr, Ni and Mn in the controls, i.e. 36.0, 19.7, 39.4 and 5.2% respectively which was modified to 47.9, 18.0, 29.9 and 4.2% by C. resinae showing a similar, though less obvious pattern to that in Turner's solution.

Other Metal Alloys

Examination of filtrates which had contained mild steel, aluminium or cupronickel coupons ± C. resinae further supported the proposal that C. resinae or its metabolic products sequester metal ions. Thus, although the corrosion of these metals was accelerated by C. resinae the relative proportions of the metal components was altered. Manganese was a component of each metal and its % presence was reduced in each case. Where applicable, Ni and Al were also

reduced. Iron was a component of each metal and its % presence was increased in each case. The % of Cu was also increased. Therefore, of the six metals analysed, the evidence suggests that Mn, Ni, Cr and Al were sequestered by C. resinae and that Fe and Cu were not.

Sequestration of metal ions by C. resinae was examined in Section 7.4.

4.2.6 Redox Potential Measurements

Redox potential and other electrochemical parameters, for example corrosion potential, can be of importance in assessing whether the oxidising conditions of the medium can reach the values needed to initiate localised corrosion by virtue of breaking down the passive oxide film. The direction and intensity of microbiological synthesis depends considerably on redox conditions in the culture[10] such that the demonstration of strong oxidising characteristics in a fuel tank may indicate that highly corrosive sulphate reducing bacteria would be absent as they require strong reducing conditions to grow[14]. In addition, the redox potential, by affecting the form of some inorganic anions and the valence of some heavy metals, can influence the solubility, mobility and toxicity of some pollutants[195].

In the absence of metal coupons growth of C. resinae caused the redox potential of modified sea water to increase from 0.33V to 0.40V after 7 weeks and to 0.44V after 31 weeks (Table 4.11). The shift to a more positive potential indicated that the affect of oxygen consumption was counteracted by the production of metabolites and the incorporation of products of cellular lysis in the solution. There was only likely to have been a slight O_2 depletion during growth of C. resinae as a consequence of the high solubility of oxygen in n-undecane[208]. Overall the solution became more oxidising, i.e. there was an increase in the amount of reducible species existing in the medium (H^+ and/or unknown metabolic products). The reduction in pH and the production of extracellular acids have been shown to accompany growth of C. resinae. Salvarezza et al.[173] showed that increasing acidity due to citric, oxalic and sulphuric acid resulted in more positive redox potentials. This linear response was not followed by

Table 4.11

Redox Potential (V) of Modified Sea Water Exposed to Metal Alloys in the Presence and Absence of C. resinae for up to 31 Weeks

		Redox Potential (V)			
Filtrate		7 wks	14 wks	26 wks	31 wks
No Metal	Test	0.40	0.42	0.43	0.44
	Control	0.33	0.34	0.33	0.33
Stainless	Test	0.40	0.45	0.45	0.46
Steel	Control	0.32	0.32	0.32	0.32
Mild Steel	Test	-	0.42	0.39	-
	Control	-	0.36	0.38	-
Aluminium	Test	-	0.44	0.42	0.43
	Control	-	0.40	0.36	0.34
Cupronickel	Test	-	0.49	0.49	-
	Control	-	0.42	0.37	-

Key:

- No determination

cultures of C. resinae at equivalent pH values illustrating the complexity of the living system.

The presence of corroding metal coupons provided further complexity of oxidation-reduction reactions. There was little difference in the sterile solutions containing stainless steel coupons. Their presence in the C. resinae inoculated solutions enhanced growth which probably accounts for the more pronounced shift in the positive direction.

The presence of mild steel, aluminium and cupronickel all shifted the potential to a more positive value compared to the no metal control. This was surprising since the corrosion process tends

to evolve H_2 (as previously explained depletion of oxygen may not be a problem in this system). The corrosion products must have counteracted this effect or alternative cathodic processes may have taken place. For each metal the redox potential of the controls varied with time, presumably due to the changing corrosion rate. In each case the presence of C. resinae caused a further shift in the positive direction as was the situation when no metal was present.

Provided a suitable control value could be obtained the determination of redox potential in ships' fuel tanks would provide useful information. However, contaminants other than C. resinae would abound and a more positive value may not be realised. Chapter 5 discusses the role of mixed cultures in altering redox potentials of both Turner's solution and modified sea water.

4.2.7 Corrosivity of the Filtrates

The corrosion potential (E_{corr}) and the corrosion rate (m.p.y.) were found for stainless steel, mild steel, cupronickel and aluminium in the various culture filtrates. The aqueous phase was separated from the undecane and filtered prior to the electrochemical determinations.

For Turner's solution filtrates the determinations were carried out for both the metal which was present in the original system and for cupronickel in each system. The former allowed a comparison with corrosion rates found using the weight loss method while the latter allowed comparisons to be made between each set of filtrates. The average values were recorded in Table 4.12.

With modified sea water filtrates it was not possible to determine values for aluminium. The corrosivity of each filtrate to stainless steel, mild steel and cupronickel was determined. This allowed a direct comparison of the susceptibility of each metal to corrosion by the extracellular products of C. resinae with or without the soluble corrosion products of other metals. Table 4.13 shows the average corrosion rates and corrosion potentials for stainless steel, mild steel and cupronickel in modified sea water filtrates.

To facilitate discussion of the results the following

abbreviations have been used:-

SS = stainless steel,

MS = mild steel,

CN = cupronickel, and

Al = aluminium.

A numerical prefix indicated the number of weeks treatment time, e.g. 9 CN = a 9 week cupronickel sample.

Determination of Corrosion Potentials and Corrosion Rates with Turner's Solution as the Aqueous Phase

The results in Table 4.12 show that except for some MS samples all E_{corr} values were more negative in the test filtrates than the control filtrates which was indicative of the presence of C. resinae and its corrosive effects. It was not considered to be a simple pH effect because E_{corr} was no more negative in the 4 and 9 week test samples (when the pH difference between the test + control was up to one unit) than in the 13, 18 and 72 weeks samples when there was little or no pH difference.

The corrosion rates for the metal which had previously been present in the sample showed them to be greater in the culture filtrates than the control filtrates. Thus, the growth of C. resinae increased the corrosivity of the environment to each type of metal. However, there was some fluctuation in the values of the control filtrates with time indicating that the presence of corrosion products also influenced the subsequent corrosion rate.

For SS test filtrates the corrosivity increased 35-fold from 4 to 18 weeks and had decreased after 72 weeks. The greatest increase in the test compared to the control was after 18 weeks when the test filtrate was 19.4 times more corrosive. The presence of C. resinae evidently increased the corrosion rate of stainless steel. Considering that the pH had risen to 5.7 by this time mechanisms other than pH change were responsible for accelerated corrosion.

The corrosion rate of mild steel was not accelerated in MS culture filtrates of C. resinae between 4 and 18 weeks. This may have been due to inhibition from existing corrosion products. Such results

Table 4.12

To Show the Corrosion Potential and Corrosion Rates of Stainless Steel, Mild Steel, Cupronickel and Aluminium in Turner's Solution in the Presence or Absence of *C. resinae* Plus Corrosion Products of the Said Metals

		Working Electrode = Metal in Test					Working Electrode = Cupronickel				
		E_{corr} (mV)		Corrosion Rate (mpy x 10^3)		Test mpy ÷ Control mpy	E_{corr} (mV)		Corrosion Rate (mpy x 10^3)		Test mpy ÷ Control mpy
		T	C	T	C		T	C	T	C	
Stainless Steel											
Weeks:	4	-74	+23	0.10	0.09	1.1	-	-	-	-	-
	9	-85	-50	1.00	0.22	4.5	-68	-52	15.05	4.59	3.3
	13	-	-	-	-	-	-	-	-	-	-
	18	-400	-80	3.50	0.18	19.4	-115	-51	25.44	20.54	1.2
	72	-88	-10	0.27	0.08	3.4	-130	-24	10.05	3.45	2.9
Mild Steel											
Weeks:	4	-690	-700	45.30	41.28	1.1	-	-	-	-	-
	9	-690	-690	29.29	27.42	1.1	-92	-50	10.29	6.01	1.7
	13	-710	-700	81.54	77.92	1.0	-110	-30	11.29	6.15	1.8
	18	-700	-710	6.53	88.58	0.1	-250	-50	2.06	19.67	0.1
	72	-700	-660	76.96	16.76	4.6	-72	-18	27.75	4.95	5.6
Cupronickel											
Weeks:	4	-93	-43	29.12	6.68	4.4	-	-	-	-	-
	9	-64	-50	22.24	7.09	3.1	-	-	-	-	-
	13	-80	-50	24.82	8.34	3.0	-	-	-	-	-
	18	-96	-46	14.06	14.25	1.0	-	-	-	-	-
	72	-72	-18	27.92	4.98	5.6	-	-	-	-	-
Aluminium											
Weeks:	4	-280	-180	0.50	0.26	1.9	-	-	-	-	-
	9	-290	-120	1.90	0.65	7.3	-96	-44	43.63	6.26	7.0
	13	-250	-150	0.80	0.76	1.1	-50	-48	1.04	6.11	0.2
	18	-	-	-	-	-	-180	-50	3.08	6.41	0.5
	72	-	-	-	-	-	-135	-40	10.50	3.15	3.3

were not upheld in the next section where corrosion rates from weight loss determinations showed that C. resinae increased corrosion between 2 and 7-fold.

The corrosion rate of cupronickel was increased 3 - 4 fold by C. resinae, a fact also observed from weight loss determinations. Again more than a simple pH effect was apparent as a pH difference between test and control filtrates did not correlate with differences in corrosion rate. For example 4CN control and 13CN test filtrates had the same pH of 5.6 yet their corrosion rates were 6.7 and 24.8 m.p.y. respectively. Thus, it would appear that the composition rather than the pH of the environment dictated the corrosion rate within it.

The corrosivity of C. resinae filtrates to aluminium was greatest after 9 weeks when it increased the corrosion rate 7-fold.

Using the corrosion of cupronickel as a standard to compare all the filtrates it was apparent that the rate was very variable. Depending on the treatment time its response in the test or control filtrates of any one metal coupon varied erratically. Generally speaking the corrosion rates were higher in the test filtrates irrespective of metal present or treatment time.

Initially the sensitivity of this method to system fluctuations was considered to be advantageous in monitoring the effects of C. resinae. However, in service conditions where the aqueous composition would be far from predictable it may not be of use. The provision of an adequate set of control samples would be the major problem. Depending on the control composition the effects of contamination would differ. This, together with the types, extent and prolongation of contamination would affect the electrochemical determination of corrosion rate. From the results old established contamination was less obviously corrosive than fresh contamination. Thus, if electrochemical determinations were to be used for in service monitoring they would have to be performed alongside other tests. For example, the total count and viability of contaminants would be of much importance in analysis of results.

Determinations with Modified Sea Water as the Aqueous Phase

Table 4.13 showed that in the absence of corrosion products the effect of C. resinae on modified sea water caused accelerated corrosion of stainless steel and cupronickel electrodes but reduced corrosion of mild steel. Thus, at the treatment times indicated C. resinae did not increase corrosion of mild steel. This was in spite of the fact that pH fell from 6.5 to as low as 3.8 in the test (Table 4.3). Thus, it would appear that C. resinae may have excreted products which were protective towards mild steel.

However, in the presence of corrosion products (except those of aluminium) an acceleration of corrosion was recorded at most treatment times. This may indicate that the excreted product responsible for protection may have combined with the corrosion products to form a non-protective complex.

The corrosion of stainless steel tended to be accelerated to a greater extent than that of cupronickel. Thus, when discussing the corrosivity of C. resinae to metals it is imperative to specify the corroding metal. Much of the literature on corrosivity of C. resinae is related to aluminium because of its importance in aircraft fuel tanks. However, it should not be assumed that C. resinae is generally corrosive. The prevalence of C. resinae in ships' fuel systems together with it's ability to corrode aluminium led to the assumption that it was probably responsible for the corrosion encountered in mild steel fuel tanks. However, these results and those in Chapters 5 and 6 show that C. resinae was not wholly responsible for accelerated corrosion and may even reduce corrosion.

Although the pH was less and corrosion rate of stainless steel and cupronickel was more in the no metal filtrate tests than in the controls, the corrosion potential was not always more negative. Thus E_{corr} could not be used as an indication of contamination in ship's fuel systems. Generally speaking the corrosion rates in the test filtrates fell with time which indicated that the most corrosive and/or least protective products were formed in the first 14 and 31 weeks with reference to stainless steel and cupronickel respectively. After this time the fall in corrosivity may be due to cell lysis,

Table 4.13

To Show the Corrosion Potential and Corrosion Rate of Stainless Steel, Mild Steel and Cupronickel in Modified Sea Water ± C. resinae ± the Said Metals Including Aluminium

	Stainless Steel Working Electrode					Mild Steel Working Electrode					Cupronickel Working Electrode				
	E_{corr} (mV)		Corrosion rate (mpy x 10^3)			E_{corr} (mV)		Corrosion rate (mpy x 10^3)			E_{corr} (mV)		Corrosion rate (mpy x 10^3)		
Sample	T	C	T	C	T mpy ÷ C mpy	T	C	T	C	T mpy ÷ C mpy	T	C	T	C	T mpy ÷ C mpy
NM															
Weeks															
7	-240	-180	1.63	0.27	6.0	-510	-690	13.58	18.11	0.75	-185	-185	3.52	1.54	1.64
14	-360	-300	1.18	0.44	2.7	-660	-685	27.35	28.24	0.97	-170	-195	3.12	1.71	1.82
26	-180	-290	0.51	0.40	1.3	-490	-695	17.61	29.35	0.60	-155	-190	2.92	1.32	2.21
31	-250	-220	0.39	0.22	1.8	-650	-685	23.58	24.78	0.95	-160	-155	3.09	1.60	1.93
46	-210	-270	0.58	0.24	2.4	-660	-660	29.65	30.25	0.98	-180	-180	1.20	1.12	1.07
72	-180	- 80	0.20	0.15	1.3	-610	-720	14.78	14.08	1.05	-210	-260	0.82	1.15	0.71
SS															
Weeks															
7	-245	-210	0.47	0.41	1.1	-530	-680	30.78	25.05	1.23	-185	-190	3.99	1.60	2.49
14	-310	-275	0.55	0.37	1.5	-650	-670	29.55	30.50	0.97	-165	-200	3.37	1.85	1.82
26	-170	-305	0.43	0.31	1.4	-470	-685	29.56	25.28	1.17	-155	-205	4.14	1.57	2.64
31	-190	-310	0.37	0.29	1.3	-655	-690	22.95	27.56	0.83	-135	-185	3.95	1.46	2.71
46	-275	-320	0.52	0.60	0.9	-670	-680	34.04	30.25	1.13	-180	-185	1.68	0.98	1.71
72	-300	-460	0.77	0.93	0.8	-650	-680	18.11	20.32	0.89	-225	-230	1.07	0.54	1.98
MS															
Weeks															
14	-350	-320	1.69	0.51	3.3	-695	-680	39.30	28.61	1.37	-195	-200	3.10	1.71	1.81
26	-140	-355	0.36	0.34	1.1	-660	-685	28.71	27.46	1.05	-170	-200	2.08	1.83	1.14
46	-315	-350	0.29	0.51	0.6	-675	-645	32.90	28.74	1.14	-190	-210	1.65	1.25	1.32
72	-310	-140	0.17	0.23	0.7	-680	-620	30.18	27.67	1.09	-290	-260	0.37	0.56	0.66
CN															
Weeks															
14	-200	-275	2.14	0.35	6.1	-645	-680	28.92	30.81	0.93	-140	-195	8.90	2.30	3.87
26	-135	-315	0.60	0.44	1.4	-425	-660	52.19	27.41	1.90	-160	-205	19.29	2.82	6.84
46	+ 52	-320	0.71	0.39	1.8	-645	-640	43.11	29.26	1.47	-105	-140	9.34	2.32	4.03
72	- 13	- 46	0.45	0.27	1.7	-620	-760	44.64	19.43	2.30	-150	-215	0.79	0.65	1.22
Al															
Weeks															
14	-270	-340	0.57	0.25	2.0	-670	-660	28.67	29.55	0.97	-175	-200	2.30	1.30	1.77
26	-215	-305	0.62	0.41	1.5	-445	-695	18.36	27.67	0.66	-160	-250	3.07	1.83	1.68
31	-330	-400	0.72	0.35	2.1	-610	-685	23.27	30.18	0.77	-180	-195	1.26	1.04	1.21
46	-375	-285	1.00	0.53	1.9	-630	-645	29.50	35.93	0.82	-175	-260	1.36	1.86	0.73
72	-295	-280	0.55	0.30	1.8	-760	-740	17.86	25.02	0.71	-240	-230	3.18	1.25	2.54

Key:

NM = No Metal; SS = Stainless Steel; MS = Mild Steel; CN = Cupronickel; AL = Aluminium; C = Control; T = Test

utilisation of corrosive products, and/or excretion of 'protective' or non-corrosive products.

The corrosion rate of stainless steel was similar in all the metal control filtrates indicating that the presence of the various corrosion products did not affect the rate of corrosion. The corrosion rates at the various treatment times did differ in the test filtrates indicating that either C. resinae alters the corrosion product or that the corrosion product alters the growth and/or subsequent extracellular products of C. resinae. Chapter 5 and 7 show that metals have a pronounced effect on the growth of C. resinae. After 14 weeks the corrosion rates of stainless steel in the test filtrates were no metal - 1.18, SS - 0.55, MS - 1.69, CN - 2.14 and Al - 0.57 with pH values of 5.1, 4.7, 5.5, 6.7 and 5.0 respectively. Thus low pH did not correlate with high corrosion rate. In fact the opposite was true. It is generally believed[160] that increased acidity leads to increased corrosion of stainless steel though as previously suggested, other factors must be operating in the mechanism of corrosion.

The most obvious effect of corrosion products on thecorrosion rate of cupronickel were those of cupronickel itself, where their presence increased the rate above that of the no metal control. It was also reflected in high rates in the CN test filtrates, however, the tests were significantly greater than the controls as was illustrated (for example 6.84 times after 26 weeks). For the stainless steel filtrates the corrosion rate for cupronickel was generally doubled though it was less than this for MS and Al filtrates. Thus the interaction of C. resinae with various corrosion products is of significance to the corrosivity of the aqueous phase. Again the importance of media composition when monitoring by electrochemical methods was illustrated. Although the test filtrate was generally more corrosive than the control filtrate a realistic control would be difficult to simulate in field conditions.

4.2.8 Weight Loss and Subsequent Corrosion Rate of Metal Alloys

The weight loss of each metal in both Turner's solution and modified sea water at each exposure time in the presence and absence of C. resinae is presented in Tables 4.14 and 4.15; the subsequent corrosion rates calculated from the average weight loss are illustrated in Figures 4.4 to 4.7.

4.2.8.1 Stainless Steel

No weight loss was recorded for stainless steel in either sterile Turner's solution or in the presence of heavy C. resinae growth. This was probably due to the natural resistance of stainless steel combined with the buffering capacity of Turner's solution preventing the pH fall usually associated with C. resinae growth. Although no weight loss was recorded, results in section 4.2.5 showed an increase in metal ions in the test filtrates and the following section discusses tarnishing found in the area of adherent growth. However, enhancement of corrosion by C. resinae was insufficient to cause a demonstrable weight loss under the experimental conditions.

Sterile modified sea water was not corrosive to stainless steel until after 26 weeks exposure when 0.55 mg in weight was lost from the coupon, probably due to the breakdown of the passive film. Although weight loss increased with exposure time the subsequent corrosion rate stopped rising after 31 weeks and after the 46 week sample it began to fall. The reformation of a protective film would account for this.

The presence of C. resinae promoted both the onset and extent of corrosion of stainless steel in modified sea water. That is weight loss was recorded 7 weeks earlier and, like corrosion rate, was always greater in the control. The corrosive activity of C. resinae was most significant after 26 weeks when it accelerated the corrosion rate three times. Over the weeks this acceleration declined indicating that C. resinae wasn't capable of inhibiting the natural passivating process of stainless steel.

Table 4.14

To Show the Average Weight Loss (mg) Standard Deviation and Value of Test Divided by Control Weight Loss for Mild Steel, Cupronickel and Aluminium Exposed to Turner's Solution in the Presence or Absence of C. resinae

	Weight Loss (mg)								
	Mild Steel			Cupronickel			Aluminium		
	$\bar{x}$	σ	Test÷ Control	$\bar{x}$	σ	Test÷ Control	$\bar{x}$	σ	Test÷ Control
4 Weeks									
Test	45.8	18.5	3.4	2.6	0.3	2.6	7.1	0.1	2.3
Control	13.4	2.5		1.0	0.6		3.1	0.6	
9 Weeks									
Test	144.8	2.7	2.7	7.5	1.1	3.0	28.6	1.1	3.1
Control	54.1	1.0		2.5	0.9		9.2	3.8	
13 Weeks									
Test	222.2	23.5	4.1	11.6	0.1	2.5	40.0	3.7	3.3
Control	54.6	8.3		4.6	0.4		12.1	2.2	
18 Weeks									
Test	407.4	185.4	6.9	22.2	1.0	4.0	57.6	2.7	3.1
Control	59.3	7.0		5.6	1.1		18.3	3.6	
72 Weeks									
Test	416.7	159.3	2.4	60.0	21.2	9.2	119.7	20.8	5.2
Control	172.5	19.3		6.5	0.4		22.9	1.8	

Key: Average weight loss = $\bar{x}$; Standard deviation = σ

Table 4.15

To Show the Average Weight Loss (mg), Standard Deviation and Value for Test Divided by Control Weight Loss for Stainless Steel, Mild Steel, Cupronickel and Aluminium Exposed to Modified Sea Water in the Presence or Absence of C. resinae

	Weight Loss (mg)											
	Stainless Steel			Mild Steel			Cupronickel			Aluminium		
	$\bar{x}$	σ_{n-1}	Test ÷ Control	$\bar{x}$	σ_{n-1}	Test ÷ Control	$\bar{x}$	σ_{n-1}	Test ÷ Control	$\bar{x}$	σ_{n-1}	Test ÷ Control
7 Weeks												
Test	0	0	0	ND	-	-	ND	-	-	ND	-	-
Control	0	0		ND	-		ND	-		ND	-	
14 Weeks												
Test	0.25	0.35		408.4	7.2	1.1	64.15	3.0	1.6	15.9	50.92	2.0
Control	0	0		377.3	14.7		39.40	4.8		8.15	2.90	
26 Weeks												
Test	1.65	0.49	3.0	583.9	48.6	1.3	80.80	3.3	1.2	24.60	5.0	1.7
Control	0.55	0.35		461.1	74.8		66.40	10.9		14.57	3.0	
31 Weeks												
Test	3.05	0.07	1.7	ND	-	-	ND	-	-	35.45	4.7	1.7
Control	1.80	0.70		ND	-		ND	-		21.45	5.3	
46 Weeks												
Test	3.25	0.21	1.2	1308.9	110.5	1.2	92.75	7.7	1.2	50.20	3.8	1.7
Control	2.65	0.07		1079.2	105.2		78.45	2.6		29.15	1.2	
72 Weeks												
Test	3.60	0.28	1.3	1815.1	193.1	1.3	154.50	37.6	1.6	58.90	7.0	1.6
Control	2.85	0.35		1433.2	81.1		95.12	15.1		36.20	3.6	

4.2.8.2 Mild Steel

The corrosion of mild steel in Turner's solution was significantly increased in the presence of C. resinae. The greatest difference in weight loss was observed after 18 weeks when the test samples lost up to seven times more metal than the controls.

After 9 weeks the corrosion rate of the control coupons began to fall probably due to the formation of a protective corrosion product but in contrast the corrosion rate of the test coupons continued to rise until week 18. From the steepness of the graph (Figure 4.5) it was probable that had further samples been taken the rate would have continued to increase for several weeks before stabilising and decreasing to the rate found after 72 weeks exposure.

It was evident that C. resinae increased the corrosion rate and inhibited the passivation of mild steel in Turner's solution.

The weight loss and corrosion rate of mild steel in modified sea water was greater in the presence of C. resinae. However, the results deviated sufficiently from the mean to cast doubts over the significance of this difference. Modified sea water was inherently very corrosive to mild steel. The formation of corrosion products inhibited the growth of C. resinae thus reducing its own corrosive effect. The corrosion rate increased rapidly over the first 13 weeks (Figure 4.5) then fell and rose again between the next two sample points. This may be accounted for by the formation of loosely adhered protective 'rust' which was subsequently shed allowing the rate to rise again.

4.2.8.3 Cupronickel

Turner's solution was not inherently corrosive to cupronickel. The weight loss was only 6.5 mg after 72 weeks and the maximum corrosion rate recorded was only 0.6 mdd after 18 weeks (Figure 4.6).

The presence of C. resinae increased the weight loss and subsequent corrosion rate 4 times after 18 weeks and 9.5 times after

Figure 4.4: To show the Corrosion Rate of Stainless Steel in Turner's Solution and Modified Sea Water in the Presence and Absence of C. resinae

Figure 4.5: To show the Corrosion Rate of Mild Steel in Turner's Solution and Modified Sea Water in the Presence and Absence of C. resinae

Key for Figures 4.4 and 4.5

▲ - Turner's Solution plus C. resinae

△ - Turner's Solution minus C. resinae

● - Modified Sea Water plus C. resinae

⊙ - Modified Sea Water minus C. resinae

Figure 4.4

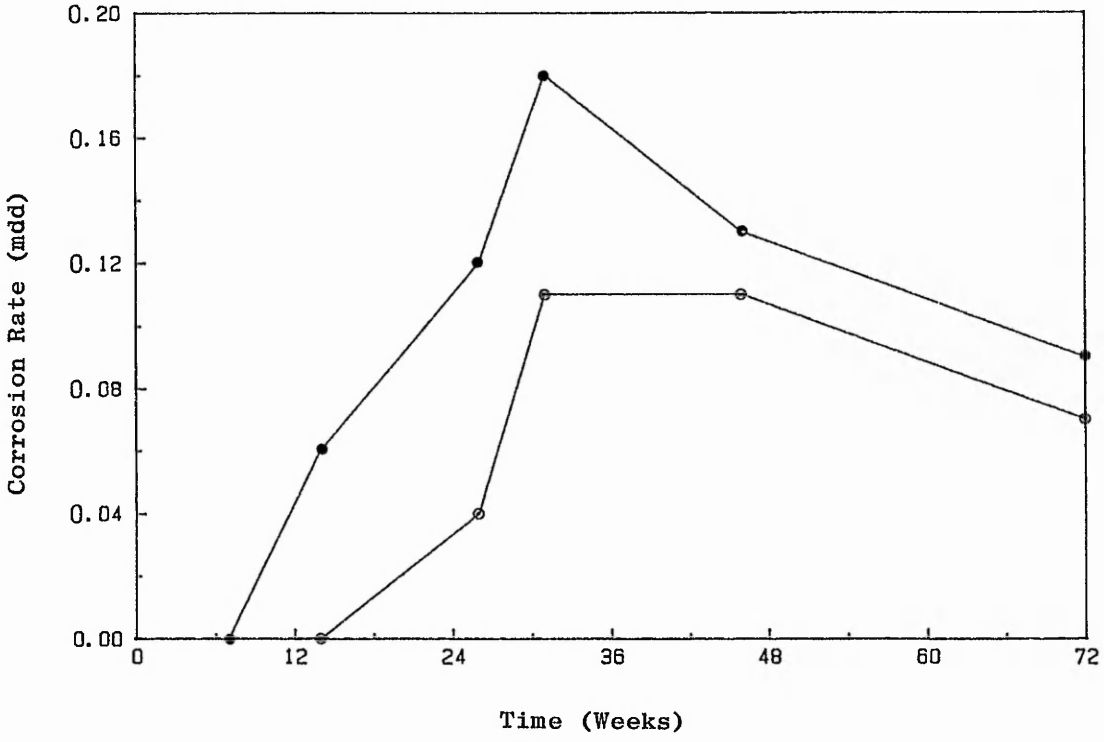
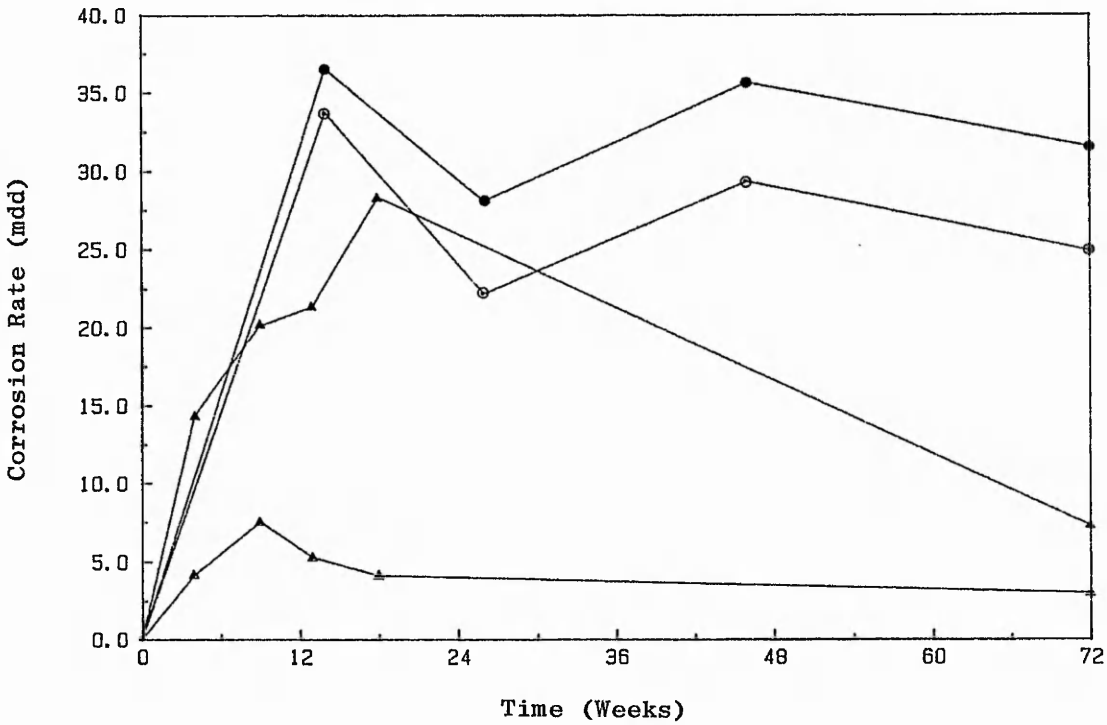


Figure 4.5



72 weeks. The rise in corrosion rate between the 13 and 18 week samples, seen in Figure 4.6, was steep enough to indicate that a further rise could be expected before the decrease in the rate observed after 72 weeks.

Modified sea water was inherently corrosive to cupronickel. Although growth of C. resinae was inhibited by the corrosion products of the metal it was still sufficient to significantly increase it's weight loss and subsequent corrosion rate (Figure 4.6). The corrosion rate of cupronickel increased rapidly in the first 14 weeks. A passivating film may have then formed which reduced the corrosion rate so that by 26 weeks it had reached a low almost steady state.

4.2.8.4 Aluminium

Weight loss of aluminium in sterile Turner's solution reached its peak soon after the 18 week sample was taken. Probably due to the formation of a stable passivating film little further weight loss was recorded.

The presence of C. resinae significantly increased the weight loss, generally 3-fold, which continued to rise between the 18 and 72 week period. The corrosion rate rose rapidly during the first 9 weeks when it stabilised and then after 18 weeks began to fall.

Figure 4.7 shows that modified sea water is inherently corrosive to aluminium. It's weight loss was significantly increased by C. resinae even though growth was initially inhibited. The corrosion rate increased steadily during the first 31 weeks, stabilised until 46 weeks, and then declined until 72 weeks. The same pattern was followed for both test and control.

4.2.9 Corrosive Effects of C. resinae on the Metal Alloys

Observations are reported for coupons immersed in Turner's solution for 4, 9, 13 and 18 weeks or in modified sea water for 7, 14, 26 and 31 weeks.

Figure 4.6: To show the Corrosion Rate of Cupronickel in Turner's Solution and Modified Sea Water in the Presence and Absence of C. resinae

Figure 4.7: To show the Corrosion Rate of Aluminium Alloy in Turner's Solution and Modified Sea Water in the Presence and Absence of C. resinae

Key for Figures 4.6 and 4.7

▲ - Turner's Solution plus C. resinae

△ - Turner's Solution minus C. resinae

● - Modified Sea Water plus C. resinae

⊙ - Modified Sea Water minus C. resinae

Figure 4.6

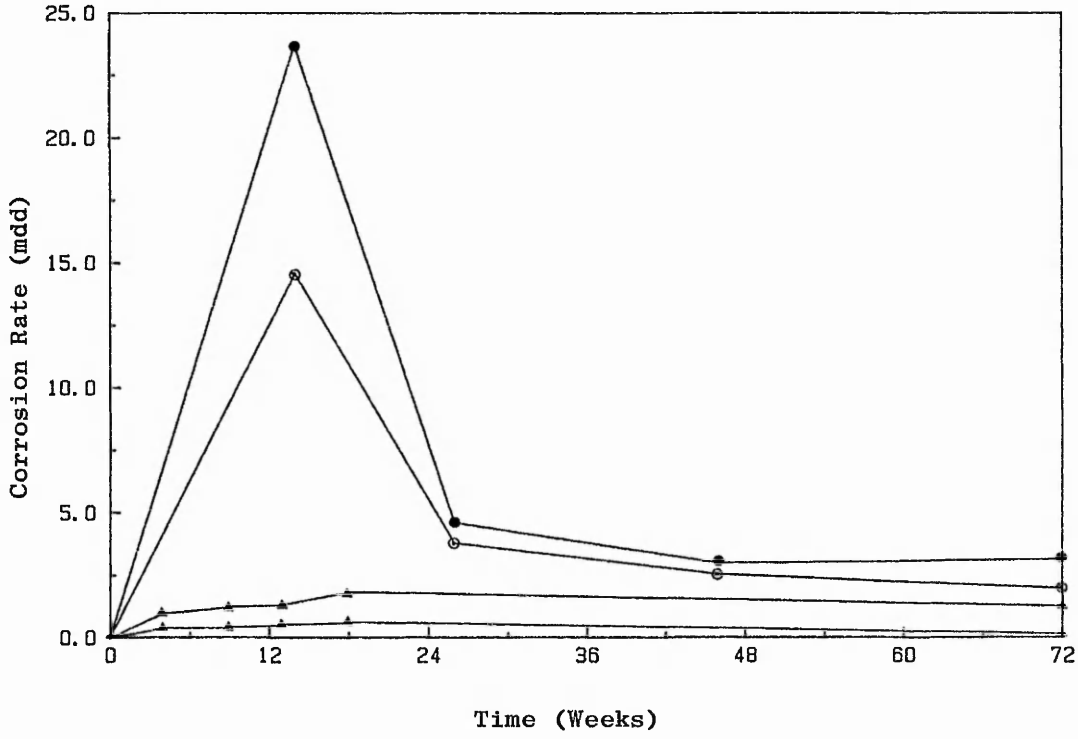
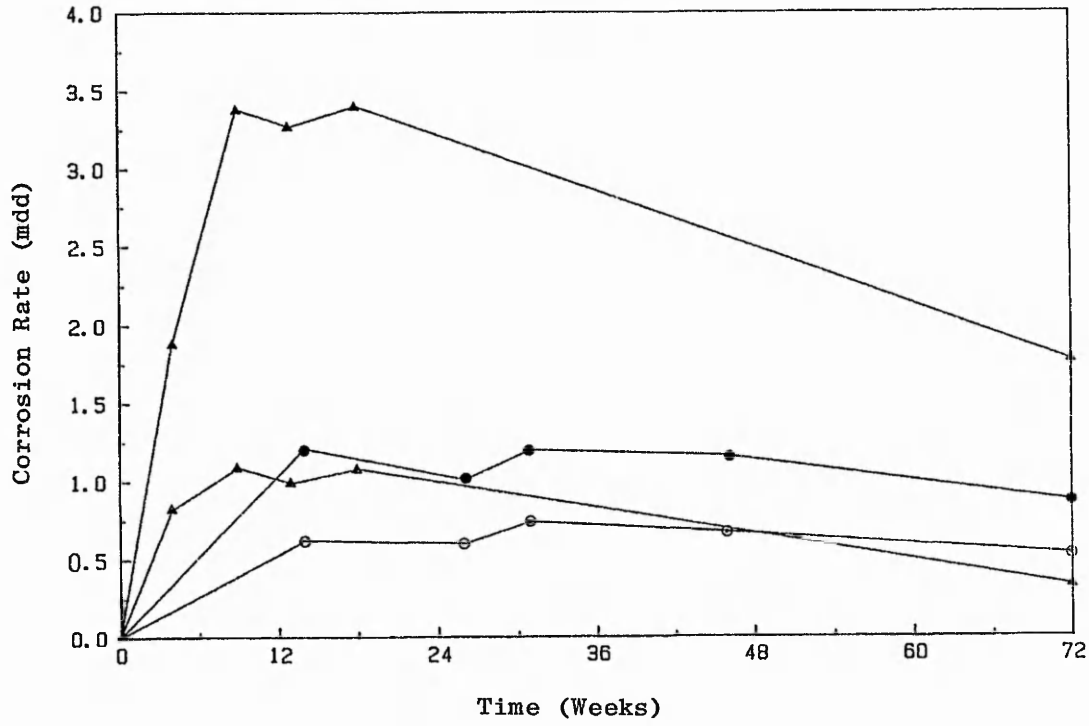


Figure 4.7



4.2.9.1 Stainless Steel in Turner's Solution

Visual Examination

In the aqueous phase, control and test coupons showed slight tarnishing after 9 and 4 weeks respectively. This tarnishing worsened on the test coupons and appeared to be associated with increased growth and subsequent adherence of C. resinae at the interface. Plate 4.2 shows a control and test coupon after 13 weeks exposure. After cleaning only test coupons exposed for 9 weeks exhibited tarnishing and this effect was only visible at the interfacial area where there had been close contact between C. resinae and the metal.

Microscopic Examination

There was no sign of corrosion until after 18 weeks in the control coupons when a small number of randomly spaced corroded spots became apparent, the shallow depth and separation of which did not facilitate measurement. After 9 weeks the test coupons exhibited slight pitting at the interfacial area and at the base of the coupon. Plate 4.3 shows staining at the interface after 18 weeks exposure. The main corrosive effect of C. resinae was to enhance the appearance of the polishing marks as compared to the control coupons.

4.2.9.2 Stainless Steel in Modified Sea Water

The visual and microscopic appearance of the samples was much the same as described for coupons immersed in Turner's solution. The staining exhibited beneath adherent growth may be seen in Plate 4.4 and was less obvious than in Turner's solution probably due to the lesser amount of growth. Plate 4.5 illustrates growth adhering to stainless steel.

Plate 4.2 (~x 2)

Stainless Steel Control and Test Coupons after 13 Weeks Exposure to Turner's Solution/Undecane

Plate 4.3 (x 80)

The Interfacial Area of a Stainless Steel Coupon after 18 Weeks Exposure to *C. resinae* in Turner's Solution/Undecane

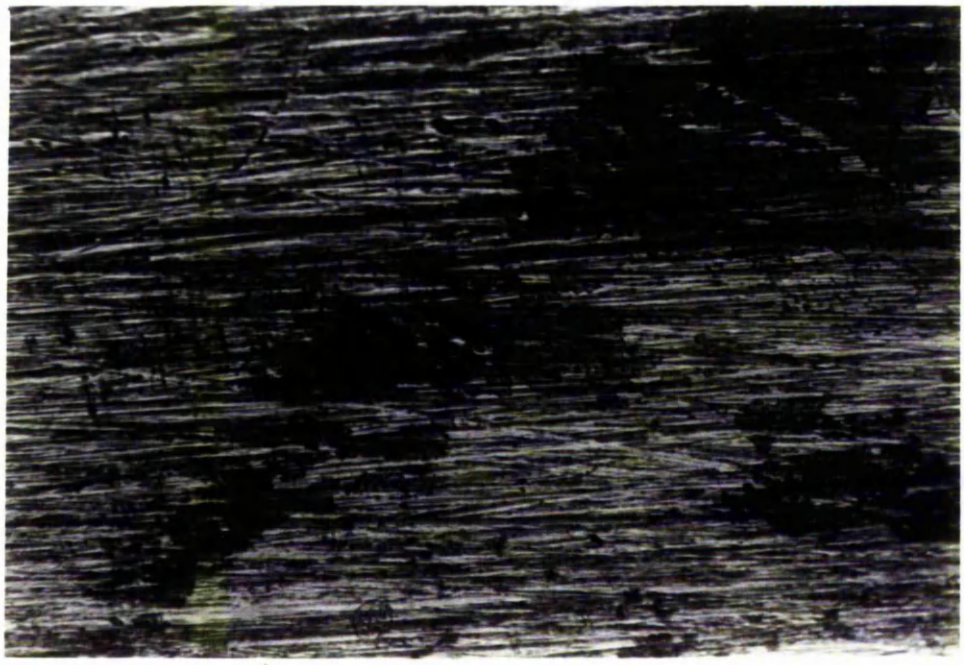


Plate 4.4 (~x 2)

Stainless Steel, Control and Test Coupons (Cleaned) After 26 Weeks Exposure to Modified Sea Water/Undecane

Plate 4.5 (~ x 2)

Adherence of *C. resinae* at the Interfacial Area of a Stainless Steel Coupon, During Exposure to Modified Sea Water/Undecane for 26 Weeks

Plate 4.4

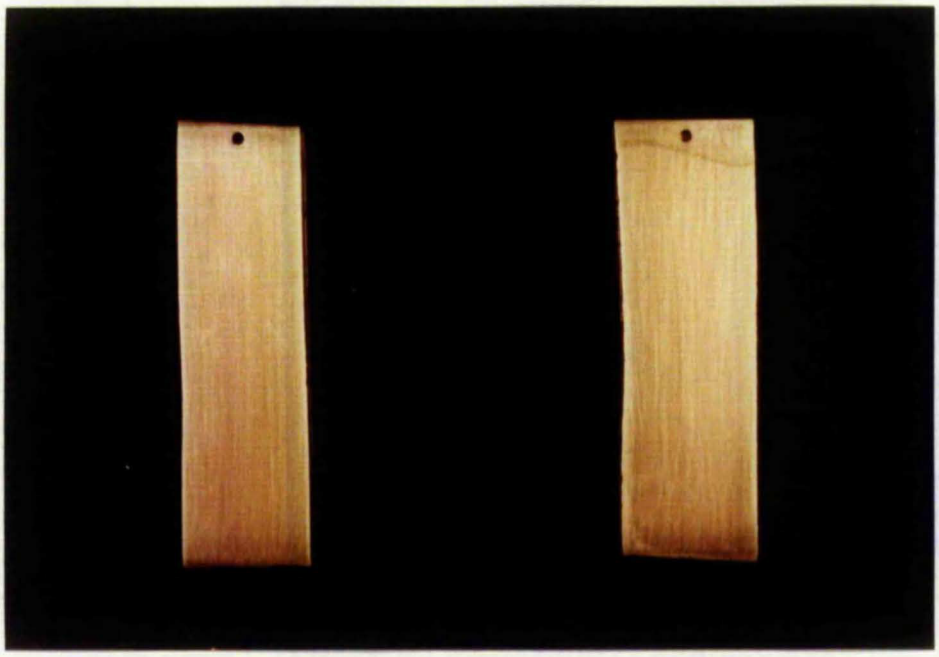
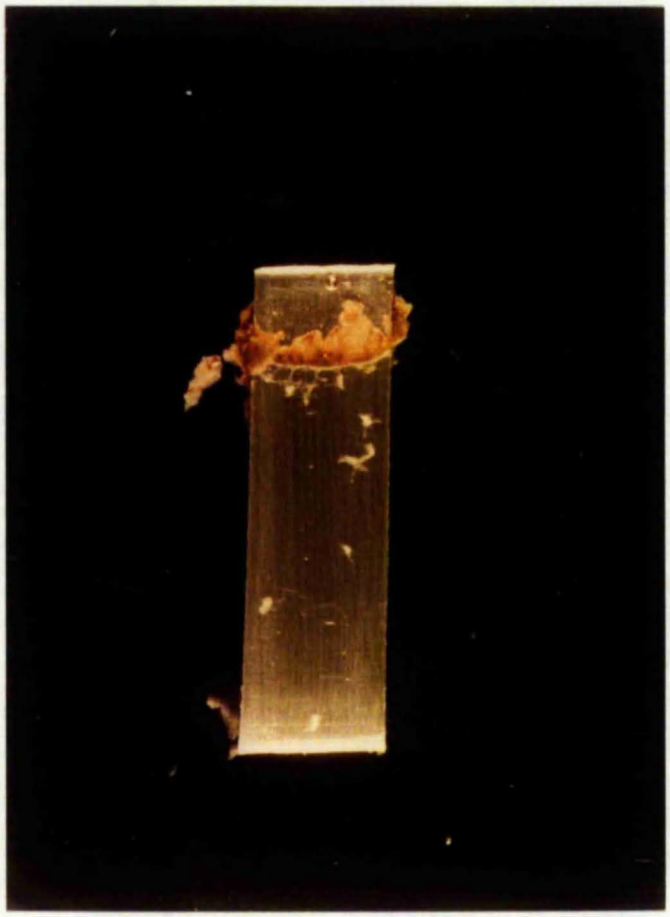


Plate 4.5



4.2.9.3 Mild Steel in Turner's Solution Visual Examination

The corrosion product of the test and control coupons differed. In the aqueous phase the controls were covered with a granular yellow precipitate which thickened to between 2 - 4 millimetres after 18 weeks. Below the easily removed yellow precipitate was found a hard green one which overlaid a thin black film. In the undecane phase the coupons, both test and control, developed a dark grey film. After 4 weeks the test coupons exhibited adhered growth at the interface which after 13 weeks had grown around the top of the coupons. In the aqueous phase, a black film was speckled with a yellow precipitate, which developed a greenish hue near the interfacial area.

After cleaning both the test and control coupons exhibited extensive general corrosion. Although the top of the 4 weeks coupons was not as severely corroded as the rest (Plate 4.6) the interface was not a well defined line. The type of corrosion differed greatly between the test and control coupons the former exhibiting a rougher surface than the latter. Increased exposure gave rise to more severe corrosion. The control coupons were slightly corroded above the interface and showed extensive 'speckled' corrosion below. The test coupons exhibited some localised corrosion above a heavily pitted interfacial area. The base appeared more pitted than the middle of the generally corroded coupons.

Microscopic Examination

In the control coupons corrosion above the interface was slight. The interface wass visible as a row of small pits which joined up to produce a 30 ± 10 m and 42 ± 10 μm deep shelf after 13 and 18 weeks respectively. The middle of the coupons exhibited small closely packed corrosion pits numbering more than 1,000 per 2.8 mm^2. The average range of their diameters and depths increased during the 4, 9, 13 and 18 week exposure times as follows:
9 - 18, 16 - 32, 18 - 34 and 20 - 40 μm^2, and 6 ± 2, 10 ± 4, 12 ± 4 and 15 ± 5 μm respectively.

Plate 4.6 (~x 2)

Mild Steel, Test and Control Coupons (Cleaned) After 4 Weeks Exposure to Turner's Solution/Undecane

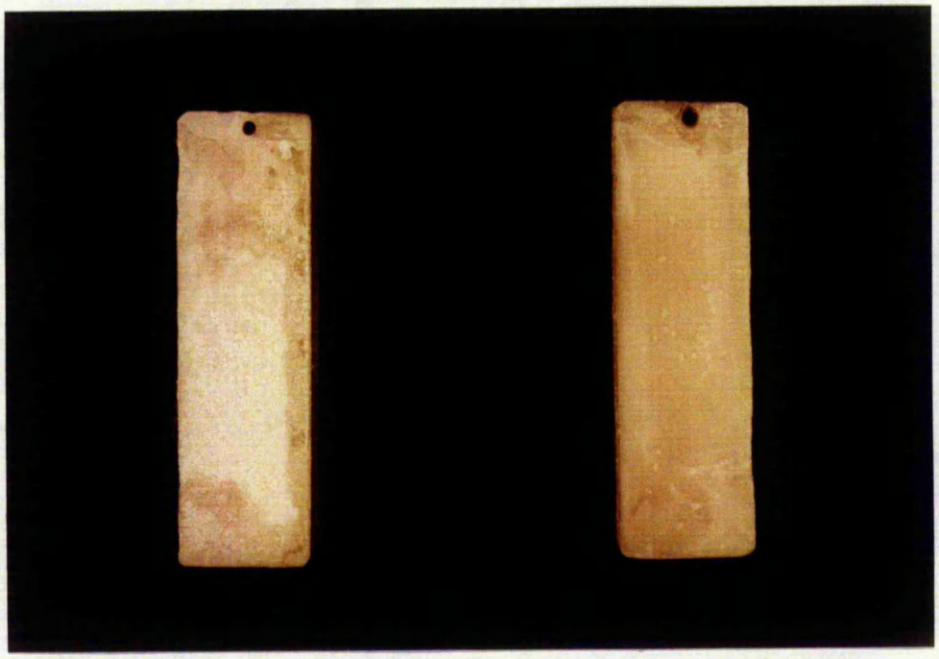
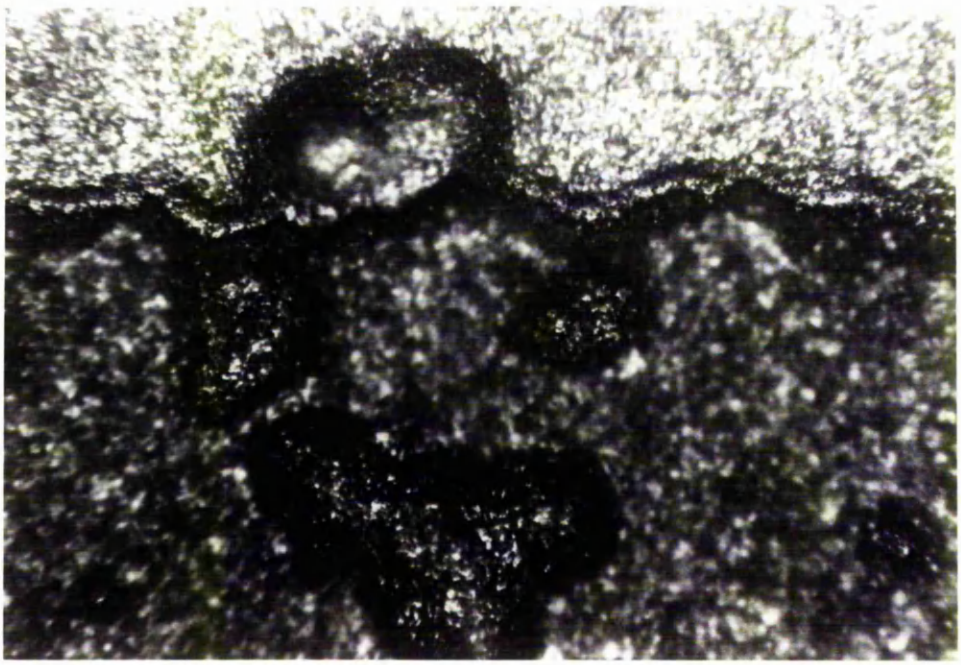


Plate 4.7 (x 400)

The Interfacial Area of a Mild Steel Coupon After 9 Weeks Exposure to *C. resinae* in Turner's Solution/Undecane



The corrosion exhibited by the test coupons was very different to that of the controls. After 4 and 9 weeks the top was relatively unaffected. The interface was visible as a continuous 'shelf' with several large pits along it's length (Plate 4.7). After 4 weeks the shelf depths of duplicate coupons were 12 and 17 μm with the largest pits having a 135 μm diameter and a depth of 24 μm. After 9 weeks the shelf depths were 15 and 18 m with the largest pits having a 240 μm diameter and a depth of 38 μm. The 13 and 18 week coupons were similar except that there was no longer an obvious interfacial shelf. The growth of C. resinae was noted to have spread around the interfacial area thus masking the well defined corrosive effect.

Below the interface the coupons exhibited extensive general corrosion which manifested itself as a labyrinth of interwoven humps and hollows. In the 4 week sample it was difficult to assess the frequency, area and depth of pits against such a background. After 9 weeks some pitting was visible, the shapes of the pits being either circular or roughly rectangular. Some 'strips' of corrosion may be the result of several joined circular pits (Plate 4.1). In the 9 week sample the numbers of such pits were 21 ± 4 and 27 ± 3 per 2.8 mm^2 with varying dimensions from 160 x 240 μm to 160 x 960 μm. The circular pits had diameters between 80 and 320 μm.

Table 4.16 shows measurements of pits found in the middle and base of the mild steel coupons after 18 weeks exposure to C. resinae. The frequency was obtained by counting the number of pits in 5 random fields of view and the depths of the deepest pit in each field of view was recorded to obtain an average value. There was a great variation in the diameters of pits and only the widest were recorded in the table.

The depths and areas were far in excess of those found in the control coupons. The table shows that although the frequency of pitting was much greater at the base of the coupon than in the middle the depths were very similar. The increased frequency of pitting may be representative of the action of C. resinae some of which settled around the base of the coupons.

Table 4.16

The Frequency, Depth and Diameter of Pits Found on Mild Steel Exposed to Turner's Solution Plus C. resinae for 18 Weeks

Sample / Parameter		Coupon A		Coupon B		Coupon C		Coupons A + B + C	
		Middle	Base	Middle	Base	Middle	Base	Middle	Base
Frequency per 2.8 mm^2	$\bar{x}$	23.4	37.6	20.2	22.2	15.6	2.6	19.7	28.6
	σ	3.2	5.7	4.6	5.2	7.0	7.3	3.9	8.0
Depth (μ m)	$\bar{x}$	41.6	44.0	56.4	54.4	69.8	73.8	55.9	57.4
	σ	11.9	11.9	9.3	7.0	10.3	31.8	14.1	15.1
Diameter (μ m)									
Maximum		240	320	240	320	800	240	-	-

4.2.9.4 Mild Steel in Modified Sea Water

Visual Examination

Both the test and control coupons were covered with an orange/red precipitate in the aqueous phase. There was no growth in the seven week sample. After gentle cleaning, electron microscopy showed growth after 14 weeks to be adherent to the corroded surface (Plate 4.8). The metal above the interfacial area appeared relatively unaffected though beneath this area both the test and control coupons had a similarly roughened surface.

Microscopic Examination

No comparisons were made either after 7 weeks because of lack of growth in the tests or after 26 weeks because severe general corrosion masked any pitting effects. However, measurement of an interfacial shelf, the result of severe corrosion in sea water and relatively no corrosion in undecane, was possible. After 26 and 46 weeks the control values were 30 - 50 and 55 - 75 μm, and the test values were 30 - 80 and 75 - 120 μm respectively.

For the 14 week samples the interface and a line across the top, middle and base of the coupon below the interface was scanned. The presence of general corrosion masked the pits making frequency values inaccurate. However, the depths and corresponding diameters of the five deepest pits were recorded in Table 4.17.

The diameters of the pits varied enormously and there was no obvious correlation between depth and diameter nor between position on the coupon and diameter. In general the pit areas appeared to be larger on the test coupons though insufficient measurements were taken to confirm this observation.

Statistical analysis of the pit depths showed that there was much variability between the same and between different positions on any one coupon. Such variability within the same treatment demanded careful interpretation of results when comparing test to control values. However, duplicate samples have provided sufficent data to

Table 4.17

To Show the Depth and Diameter of Pitting at the Interface, Top, Middle and Base of Mild Steel Coupons Immersed in Modified Sea Water in the Presence or Absence or Absence of C. resinae for 14 Weeks

Sample	Parameter (μm)	Interface	Top	Middle	Base
Test A	Depth	42,55,40,42,60	54,56,78,64,34	15,45,35,30,50	50,54,65,30,40
	元	47.8	57.2	35.0	47.8
	σ_{n-1}	9.1	16.0	13.7	13.4
	Diameter	80,96,128,112,112	112,112,160,80,96	80,80,64,96,48	80,96,160,96,80
Test B	Depth	42,48,45,30,60	45,45,35,35,45	15,15,42,30,35	50,50,45,30,50
	元	43.4	41.0	27.4	45.0
	σ_{n-1}	8.9	5.5	12.1	8.7
	Diameter	32,64,80,128,160	48,64,80,96,128	48,48,64,80,80	80,80,64,48,64
Test A/B	Average Depth σ_{n-1}	46.4 9.5	57.2 16.0	27.4 12.1	46.4 10.7
	Range	36.9 - 55.9	41.2 - 73.2	15.3 - 39.5	35.7 - 57.1
Control A	Depth	33,40,40,20,35	40,40,50,50,54	45,40,35,40,35	32,28,36,24,38
	元	33.6	46.8	39.0	31.6
	σ_{n-1}	8.2	6.4	4.2	5.7
	Diameter	64,80,64,96,80	80,48,64,32,64	32,64,48,48,64	48,64,96,64 x 48, 64 x 28
Control B	Depth	25,20,35,45,60	45,40,35,35,30	30,30,35,30,30	40,40,40,45,35
	元	37.0	37.0	31.0	40.0
	σ_{n-1}	16.0	5.7	2.2	3.5
	Diameter	32,48,80,64,64	80,64,96,80,80	48,80,80,96,64	64,80,96,96 x 28, 80 x 28
Control A/B	Average Depth σ_{n-1}	35.3 12.1	39.2 14.3	35.0 5.3	35.8 6.3
	Range	23.2 - 47.4	24.9 - 53.5	29.7 - 40.3	29.5 - 42.1

conclude that with the exception of the middle position the test coupons suffered deeper pitting than the control coupons.

On average the pit depths of the various areas on the mild steel control coupons were found to be similar. Thus, the position of the metal within the flask did not cause a differential effect. Conversely there were large variations in the pit depths found on the test coupons. Deeper pitting at the base of the test coupon may have been due to the close association of hyphae which had descended from the interfacial mat. However, the middle of the test coupon was less deeply pitted than the control indicating that the growth modified environment, despite a pH fall, was not particularly corrosive and in this instance may have been protective.

Although growth of C. resinae was concentrated around the interfacial position the pit depths were found to be greatest at the top position, i.e. just below the interface. Plate 4.9 shows an electronmicrograph of the interfacial area of mild steel exposed to C. resinae in modified sea water. In conclusion, C. resinae was found to exert a corrosive effect towards mild steel in the area of adherent or closely associated growth.

4.2.9.5 Cupronickel in Turner's Solution

Visual Examination

The control coupons were unaffected above the interface and developed a smooth blue-green coloured precipitate below it. After 4 weeks the test coupons were unaffected above the interface and had developed a smooth salmon-pink coloured precipitate below it. Growth did not appear to be adhered to the coupon. After 9 weeks a dark brown film was loosely attached to the pink precipitate. Interfacial growth was closely associated with but not firmly bound to the metal. After 13 + 18 weeks the area above the interface appeared tarnished. Plate 4.10 shows the different corrosion products of the test and control coupons after 18 weeks exposure.

After cleaning the control coupons were observed to be corroded at and below the interface but not above it. The test

Plate 4.8 (x 260)

Electron Micrograph Showing Adherence of C. resinae to Mild Steel After 14 Weeks Growth in Modified Sea Water/Undecane

Plate 4.9 (x 120)

Electronmicrograph of the Interfacial Area of Mild Steel After 14 Weeks Exposure to C. resinae in Modified Sea Water/Undecane

coupons were more severely corroded below the interface than the controls; the surface both looked and felt rougher. After 9 weeks a second interfacial line, not considered to be due to evaporation because of its absence in the control coupons, was visible. It was likely to have been due to the expanding mat of growth into the undecane phase at the interfacial area.

Microscopic Examination

The interfacial area of the test and control coupons was vastly different and illustrated the corrosive effect of C. resinae. After 4 weeks there was no corrosion above the interface of both the test and control coupons, the interface being visible as a continuous line of pits with average depths of 4.1 ± 0.96 μm and 3.6 ± 0.95 μm respectively. The diameters of the pits varied substantially, larger examples being up to 111 x 45 μm in diameter, with average values of 18 and 45 μm at the control and test interface respectively. A major difference on the test coupons was the occurrence of intergranular corrosion immediately below the interfacial area which was considered to be due to preferential corrosion of the grain boundaries by extracellular products of C. resinae. After 9 weeks there was little change at the interface of the control coupons except the pits had deepened to an average of 16 μm. However, the test coupons had developed a second more severely corroded interface above that of the first. Pits at the second interface were 20.8 ± 4.2 μm in depth, up to 160 μm in diameter and were often joined up. The area between the two interfaces was not severely corroded and assuming that the second, more heavily pitted interface was due to adherent growth of C. resinae, the following reasoning applies. The first interface, as in the control, was mainly due to the presence of the aqueous phase.

Growth of C. resinae was not adherent after 4 weeks though its presence did enhance corrosion in the interfacial area. Between 4 and 9 weeks the mat of hyphae moved into the aqueous phase and eventually adhered to the metal. This upward movement may have been due to dispersion of carbon dioxide, or even undecane, throughout the mat of growth causing it to float upwards. The metal between the

interfaces may have remained unaffected because of a protective layer of undecane lying between the mat of growth and the aqueous phase.

After 13 weeks the area above the interface of the control had become corroded with small, <16 μm diameter, and 10 ±5 μm deep pits. Pitting at the interface formed an almost continuous line with 309 ± 42 pits, which, after 18 weeks, had depths of 36 ± 5, 21 ± 4 and 16 ± 3 μm (average 24 ± 10 μm) and diameters in the range 16 - 48 μm in the triplicate control coupons. Corrosion of the test coupons was more severe with pitting above the second interface after 13 weeks being innumerable, <16 μm diameter and 15 ± 5 μm deep. The secondary interface tended to be a band of large joined up pits rather than a line of discrete pitting their depths being 43.3 ± 11.1 μm with diameters up to 240 x 480 μm. Corrosion between the two interfaces resembled that of above the secondary interface. The lower interface consisted of a discrete line of pits, depths 30 ± 5 μm and diameters between 16 and 64 μm. After 18 weeks the band between the two interfaces was 800 64 μm wide and suffered from many flecks and small pits. Pit depths at the secondary interface had become 28 ± 5, 40 ± 8 and 26 ± 4 μm (average 31 ± 8 μm) in the triplicate test coupons. Intergranular corrosion had become apparent around the secondary interface as illustrated in Plate 4.11. The selective corrosion was 25 μm deep and was thought to be a direct effect of C. resinae.

Cupronickel exposed to the aqueous phase was also found to be more severely corroded in the presence of C. resinae. The control coupons tended to exhibit localised patches of corrosion rather than pits. After 9 weeks pitting was 15 - 70 and 19 ± 8 μm deep in the middle of the test and control coupons respectively. After 13 weeks the middle and base of the test and control coupons had depths of 30 7 and 28 ± 8, and 17 ± 5 and 14 ± 6 μm respectively. There were innumerable pits in both test and control with diameters being approximately the same and up to 80 μm in diameter.

After 18 weeks many pits had joined up on the test coupons giving rise to large circular pits and strips of corrosion. Strips of corrosion were also apparent on the control coupons but generally localised patches of corrosion were visible. Table 4.18 illustrates

Plate 4.10 (~x 1.3)

Cupronickel Test and Control Coupons After 18 Weeks Exposure to Turner's Solution/Undecane

Plate 4.11 (x 12 (x 10))

Electronmicrograph Showing Intergranular Corrosion at the Interfacial Area of Cupronickel After 18 Weeks Exposure to C. resinae in Turner's Solution/Undecane

Plate 4.10

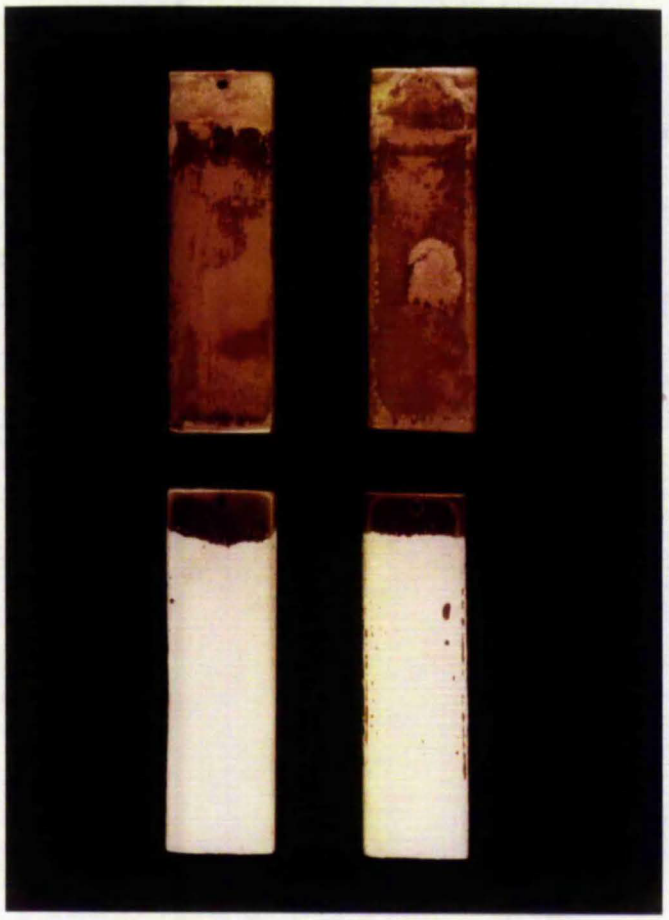


Plate 4.11

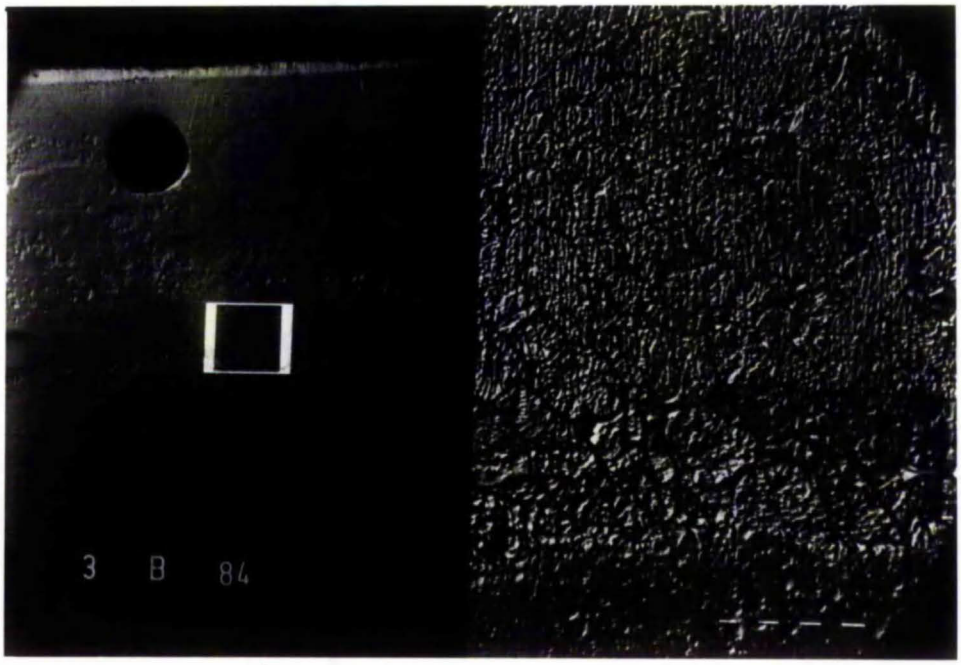


Table 4.18

To Show the Frequency, Depth and Diameter of Pits on Cupronickel Exposed to Turner's Solution in the Presence and Absence of *C. resinae*

	CONTROL COUPON								TEST COUPON							
	1		2		3		Average		1		2		3		Average	
Frequency																
Top	281	18	163	21	111	12	185	87	TMTC		TMTC		TMTC		TMTC	
Middle	231	14	185	9	57	9	158	90	TMTC		TMTC		TMTC		TMTC	
Base	196	44	138	9	35	8	123	82	TMTC		TMTC		TMTC		TMTC	
							155 ± 79								TMTC	
Depth																
Top	31.8	6	19.6	12	15.1	3	22.1	9	26.6	7	40.3	8	25.7	4	24.2	2
Middle	31.5	3	28.5	5	25.0	9	28.3	3	26.1	3	30.2	6	24.7	5	27.0	3
Base	24.8	6	29.3	7	25.5	2	26.5	2	25.8	11	31.8	8	28.2	4	28.6	3
							25.7 ± 6								26.6 ± 3	
Diameter																
Top	32 x 48, 32-48		32 x 64, 48 x 64		10-30, 32 x 48				16-48, 240		16-48, 160		15-25, 15 x 40			
Middle	32-80, 40 x 50		15 x 40, 40 x 20		15-35, 32 x 64				16-32, 160,80		16-48, 24 x 32		10-25, 16 x 18			
Base	16-64, 16 x48		5-25, 30 x 64		10-40, 15 x 40				32 x 160, 32 x 64		24 x 64, 240		24 x 72, 32 x 64			

Key:

TMTC - Too many to count (>300 per field of view);

Frequency - Pits per 2.8 mm^2 (average of 5 random fields of view)

Depth - Average of 5 deepest pits in each of the 5 fields of view.

the average frequency and depth of pits. Examples of the range of diameters were recorded

The frequency of pits was always greater in the presence of C. resinae indicating that the extracellular products promoted the onset of pitting. However, the depths of the pits at the top and base of the test coupon were only slightly greater than in the control and the depths in the middle were less. Thus, there was no evidence to suggest that extracellular products of C. resinae accelerated the development of pits after their initiation.

4.2.9.6 Cupronickel in Modified Sea Water

Visual Examination

The coupons were unaffected in the undecane phase and had a green precipitate in the aqueous phase. There was no growth until after reinoculation, when a thin interfacial film formed. It did not appear to be adhered to the coupons. Removal of the corrosion product revealed a roughened surface below the interface. The test coupons generally exhibited two interfacial lines (Plate 4.12), the upper one having the greatest depth.

Microscopic Examination

Corrosion was found to be quite variable between the duplicate coupons. The average depth of the interfacial line or pitting below the interface was summarised in Table 4.19. Further information regarding frequency and the five deepest pits and largest areas in a scan across the width of the top, middle and base of the test coupons was recorded in Table 4.20.

Generally speaking the control coupons exhibited light general corrosion below the interface with many small (4 - 20 μm diameter) shallow pits. After 46 weeks such pits were central to localised patches of corrosion, their frequency being 120 ± 20 per 19.2 mm^2. With the exception of coupon B after 14 weeks, the interface was a continuous band (64 ± 8 μm wide) it's depth

Table 4.19

To Show the Average Depth of the Interfacial Line or Pits Below the Interface(s) of Cupronickel in Modified Seawater in the Presence and Absence of C. resinae for 14, 26 and 46 Weeks

		Average Depth (μm) of Interfacial Line or Pits					
		14 Weeks		26 Weeks		46 Weeks	
		A	B	A	B	A	B
Control	Interface	15	29.9 ± 7.6	25	21	22	38
	Below Interface	5 ± 2.5	10 ± 4.1	21 ± 6.3	16 ± 2.2	32.2 ± 7.4	46.1 ± 10
Test	Secondary Interface	69.5 ± 23.3	81.8 ± 33.3	125	88	117.5 ± 66.7	70.5 ± 27
	Primary Interface	21.9 ± 5.5	40	20	22	38	25
	Below Interface	39.0 ± 11.9	17.6 ± 4.1	46.3 ± 14.6	33.6 ± 11.1	46.9 ± 15.9	48.3 ± 9.2

increasing with exposure time.

The primary interface of the test coupons was generally a continuous band of corrosion of variable depth and 120 ± 30 μm width. In some instances it was associated with intergranular corrosion. The secondary interface was more heavily and deeply pitted with a width increasing to 480 μm. After 46 weeks the diameters of the pits ranged from 96 - 560 μm and are illustrated in Plate 4.13. In addition intergranular corrosion was often apparent around the secondary interface. General corrosion with some pitting (16 - 32 μm diameter; 2 - 10 μm depth) was apparent between the two interfaces, the resulting band being up to 2 mm wide.

A summary of the type of pitting observed on the test coupons below the interfacial band was recorded in Table 4.20. After 14 weeks the top position of coupon A had heavy general corrosion as well as a large number of pits. The middle position was only lightly corroded making the pits easier to see. The base of the coupon was in the main generally corroded with fewer but deeper pits. The area of the pits was very variable throughout these positions. Coupon B was similar except that the pits were only half as deep. After 26 weeks the average depth of pitting was greater than after 14 weeks. The frequency of pitting was least in the middle position and after 46 weeks was greatest at the base. Table 4.20 shows the largest and deepest pits, though on average pits in the middle position tended to be smaller and those at the base were surrounded by heavy general corrosion and were ill defined.

Despite the variability of the duplicate coupons it was still apparent that the tests suffered more general and localised corrosion than the controls. Below the interfacial area pitting was of a greater frequency and generally of a greater depth than in the control. It was concluded that C. resinae modified the sea water environment and initiated pitting during the first 14 weeks, the pits being of a greater depth in the 26 week sample but no greater after 46 weeks. Initiation of pitting in the control coupons was slower and it was not until the 26 week sample that localised patches of corrosion often centred with a pit were observed. After 46 weeks their depth was only slightly less than the 'older' test pits indicating that C.

Plate 4.12 (~x 3.5)

Cupronickel Control and Test Coupons (Cleaned) After 14 Weeks Exposure to Modified Sea Water/Undecane

Plate 4.13 (x 20 (x 5))

Electronmicrograph of the Interfacial Area of Cupronickel After 46 Weeks Exposure to *C. resinae* in Modified Sea Water/Undecane

Plate 4.12

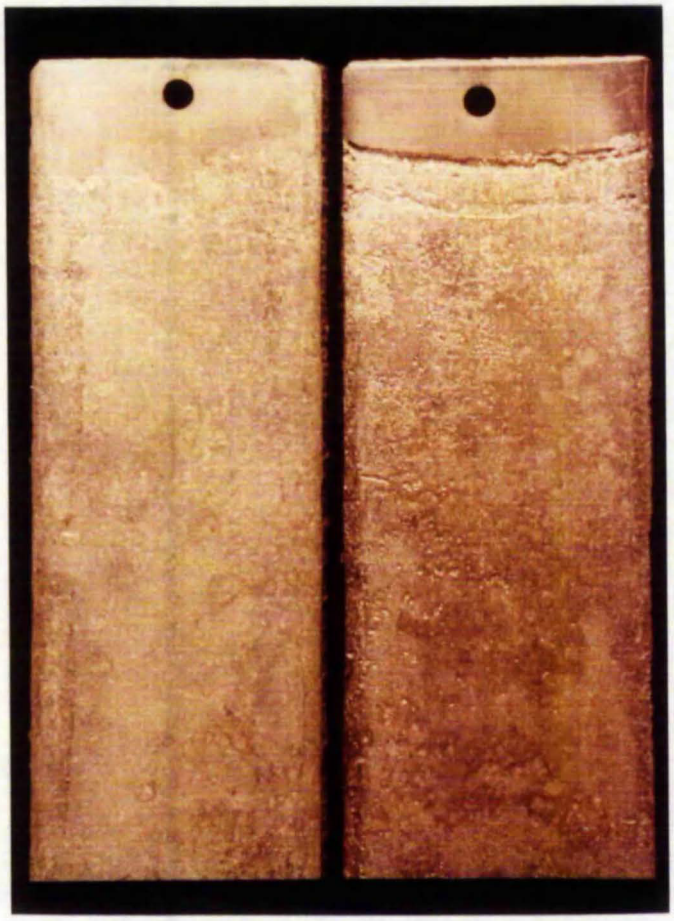


Plate 4.13

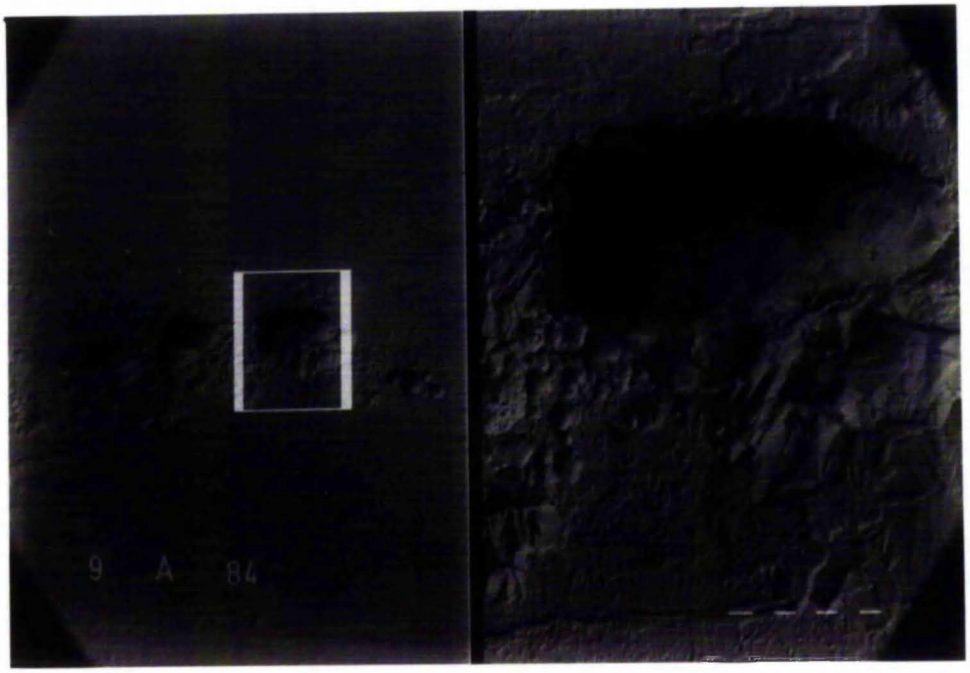


Table 4.20

To Show the Frequency, Depth and Diameter of Pits Below the Interfacial Area of Cupronickel in Modified Sea Water in the Presence of *C. resinae*

	TEST COUPON A															TEST COUPON B														
	TOP					MIDDLE					BASE					TOP					MIDDLE					BASE				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
14 Weeks																														
Frequency (per 19.2 mm^2)	88					80					50					84					63					42				
Depth (μm)	58	39	30	35	30	38	30	20	40	52	35	56	50	22	50	25	26	24	27	21	15	12	16	11	16	16	22	18	20	15
$\bar{x}$	38.4					36.0					42.6					20.6					14.0					18.2				
σ_{n-1}	11.6					11.9					13.9					4.0					2.3					2.9				
Diameter	192x	112x	112x	192x	112x	140x	96x	112x	80x	64x	128x	80x	96x	112x	96x	128x	112x	128x	68x	80x	92x	80x	100x	140x	140x	80x	160x	100x	140x	112x
(μm)	60	132	88	152	120	180	120	120	168	128	100	80	100	120	160	128	120	140	50	100	112	100	120	100	160	80	128	100	180	92
26 Weeks																														
Frequency (per 19.2 mm^2)	60					33					44					21					10					42				
Depth (μm)	35	28	24	34	40	48	43	70	35	60	50	45	65	48	70	19	18	25	30	22	50	25	30	50	50	42	40	30	33	40
$\bar{x}$	32.2					51.2					55.6					22.8					41.0					37.0				
σ_{n-1}	6.3					13.9					11.1					4.9					12.4					5.2				
Diameter	48x	80x	80x	80x	80x	160x	112x	96x	128x	160x	96x	160x	160x	80x	160x	160x	96x	80x	112x	96x	160x	128x	112x	128,	96x	96x	112x	112x	128x	148x
(μm)	80	96	64	128	160	80	112	160	80	128	112	148	80	64	128	80	20	64	80	48	80	128	80	96	96	80	120	112	160	112
46 Weeks																														
Frequency (per 19.2 mm^2)	32					13					80					38					32					73				
Depth (μm)	38	25	70	65	45	50	32	25	37	27	55	65	45	65	60	35	50	45	40	38	68	59	58	50	38	54	55	48	38	45
$\bar{x}$	48.6					34.2					58.0					41.6					54.6					48.0				
σ_{n-1}	18.8					10.0					8.4					5.9					11.3					7.0				
Diameter	80x	112x	120x	128x	160x	64x	112x	160x	112x	160x	160x	288x	160x	240x	560x	160x	96x	80x	112x	96x	128x	112x	80x	112x	64x	160x	80x	96x	80x	80x
(μm)	96	96	96	112	80	112	160	176	160	128	160	192	192	128	96	128	80	80	160	112	128	96	64	112	48	80	112	128	160	80

resinae had further modified the environment making it less aggressive. The major effect of C. resinae was at the interfacial area where a secondary interface developed with pits 2 - 4 times deeper than in the control and where the primary interface was generally more severe than the control interface. In addition, close contact with C. resinae caused intergranular corrosion of cupronickel.

4.2.9.7 Aluminium in Turner's Solution

Visual Examination

The control coupons were unaffected in the undecane phase but appeared tarnished in the aqueous phase with black spots near the interface. After 4 weeks immersion the test coupons had growth adhered to their interface and sides, with a grey closely adhered corrosion product in the aqueous phase. Plate 4.14 shows a control and test coupon after 4 weeks exposure. After 9 weeks a cap of growth surrounded the coupon in the undecane phase; the interfacial area had become pitted with black spots and the metal in the aqueous phase had developed a smooth, dark grey, loosely adherent precipitate overlayering a thin, pale grey, strongly adherent precipitate.

The corrosion product proved difficult to remove, however, after cleaning the control coupons retained their shiny appearance above the interfacial area but below it general corrosion and pitting was apparent. The test coupons became increasingly corroded with time. The area above the interface became pitted and tarnished after 9 weeks which corresponded to the onset of growth in that area. The interface itself was heavily pitted and below this was general corrosion, blistering and pitting of the aluminium alloy. Corrosion appeared especially heavy at the base of the coupons.

Microscopic Examination

The surface of the control coupons differed considerably from that of the tests. After 4 weeks the interfacial area was visible as a transition from no corrosion in the undecane phase to a

tarnished area with small flecks and localised patches of corrosion which had often formed around an inclusion. Below the interfacial shelf of depth 20 μm, the frequency per 2.8 mm^2, depth and diameter of pits were 40 ± 5, 2 ± 0.3 μm and 30 ± 8 μm respectively. The extent of corrosion was more pronounced after 18 weeks with an interfacial shelf of depth 25 μm and patches corrosion with depths between 10 - 21 μm and diameters up to 250 μm wide in the aqueous phase.

After 4 weeks the surface of the test coupons, both at and below the interfacial area, had a blistered appearance, and there were many small shallow pits all over the coupons (Plate 4.15). The average values of measured pits were as follows:-

Area on Coupon	Frequency per 2.8 mm^2		Depth (μm)		Diameter (μm)	
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
Above the Interface	115	25	1.9	0.3	18	7
At the Interface	150	20	3.4	2.1	27	8
Below the Interface	290	60	3.2	0.2	22	6

Pitting in the area above the interface was indicative of a partitioning of corrosive extracellular products into the undecane phase, since no such corrosion was observed in the controls. However the frequency, depth and diameter of these pits was less than at and below the interface where adherent growth and/or extracellular products were concentrated. Although the frequency of pits was found to be less under adherent growth than due to extracellular products alone, the depth and diameter were greater. Compared to the control coupons the frequency and depth of pits were much greater but the areas were less, though this was due to the controls tending to have localised patches of corrosion rather than pits. The major effect of C. resinae was to cause a band of interwoven channels at the interfacial area; the band being 2.2 mm wide which was equivalent to the thickness of the mat of growth. Characteristics of corrosion were similar after 9, 13 and 18 weeks, the latter test sample having pits

Plate 4.14 (~x 2.5)

Aluminium Alloy Control and Test Coupons After 4 Weeks Exposure to Turner's Solution/Undecane

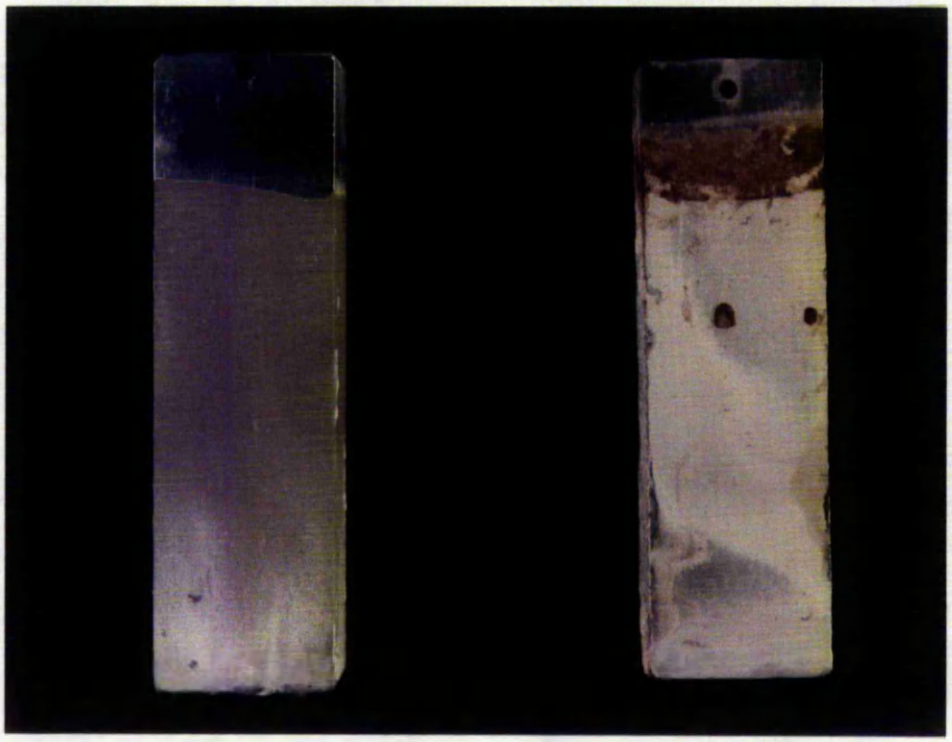
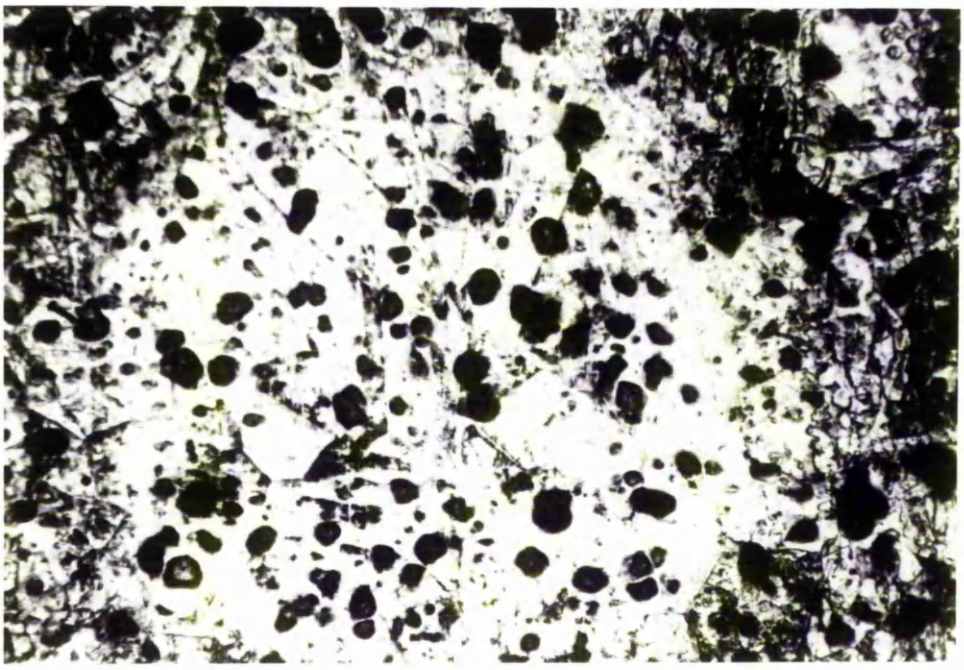


Plate 4.15 (x 140)

Middle Area of Aluminium Alloy Coupon After 4 Weeks Exposure to *C. resinae* in Turner's Solution/Undecane



up to 45 μm deep with diameters ranging from 50 - 200 μm. Several coupons exhibited blisters raised 15 μm above the surface just below the interfacial area. It was concluded that C. resinae encouraged theinitiation of pits in the aluminium alloy and enhanced their development by making the environment more corrosive. In addition, the effect of adherent C. resinae was to plough through the aluminium alloy causing the formation of channels beneath the hyphae. Such adherence and possible causes for it's effects are discussed in Chapter 7.

4.2.9.8 Aluminium in Modified Sea Water

Visual Examination

In the interfacial area the control coupons were covered with a thin film of strongly adhered white precipitate. There were patches of coarse white precipitate associated with gas formation on the sides of the coupon. A similar corrosion product with a pink tinge to it was observed in the test coupons. There was no effect in the undecane phase. After cleaning the test and control coupons were left with a tarnished appearance below the interfacial area, the former also having a pitted interface.

Microscopic Examination

Throughout the sampling time the control coupons were not corroded above the interfacial area as opposed to the test coupons, which after 14 weeks exhibited channels extending upwards from the interface (Plate 4.16). These appeared to be due to the hyphal growth of C. resinae into the undecane and onto the metal. In addition, pits developed at the interfacial area giving rise to a band of corrosion beneath adherent growth (Plate 4.17). After 14 weeks the control interface was visible as a generally corroded line of depth 12.5 ± 2.5 μm. Evaporation after 26 weeks had given rise to a second interface 640 μm below the first one with pit depths 13 ± 2 μm and diameters of 32 - 48 μm. In the test coupons the interfacial band was 80

Plate 4.16 (x 73 (x 5))

Electronmicrograph to Show the Channels of Corrosion on an Aluminium Alloy Coupon After 14 Weeks Exposure to C. resinae in Modified Sea Water/Undecane

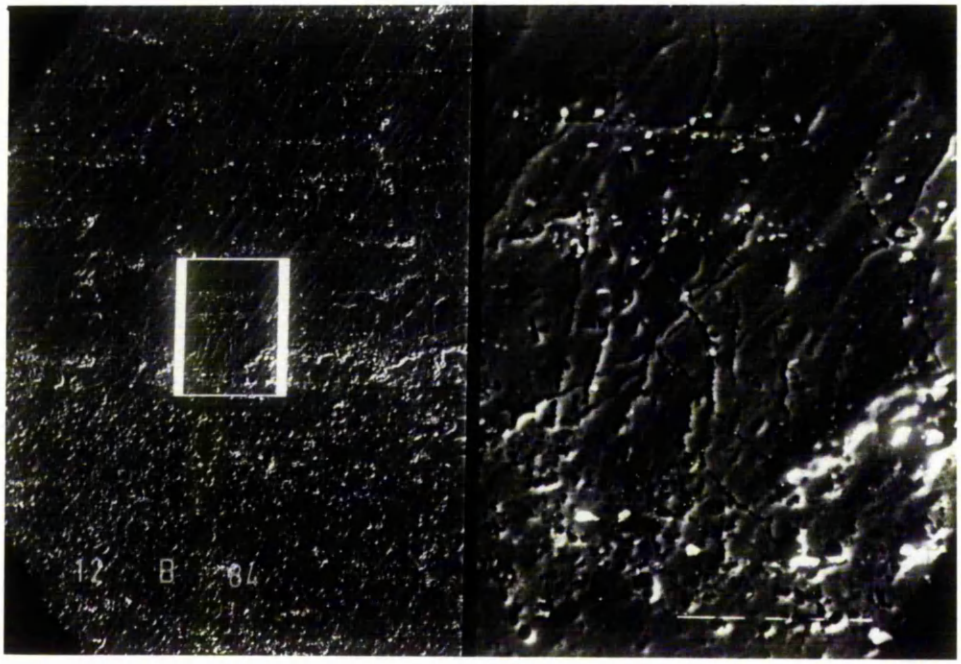


Plate 4.17 (x 55)

Electronmicrograph to Show the Interfacial Area of an Aluminium Alloy Coupon After 14 Weeks Exposure to C. resinae in Modified Sea Water/Undecane

± 16 μm with pits 22 ± 6 μm deep and 16 - 32 μm in diameter after 14 weeks. After 26 weeks the band of hyphal valleys in the test coupons was 1.8 ± 0.7 mm wide. The valleys were fairly shallow having a depth of only 4 ± 1 μm. Pits had depths of 43 ± 19 μm and diameters ranging from 48 to 160 μm. After 31 weeks the interfacial band of hyphal valleys was heavily pitted except at the outer edges of the undecane phase. The band was 3.7 ± 0.5 mm wide with valleys of 10 μm depth. Host pits had joined up to cause large holes (80 μm depth) though individual ones had depths of 37 ± 12 μm and diameters in the range 48 - 80 μm.

Below the interface the surface suffered from small flecks and shallow patches of corrosion often centred around a pit; these patches being better defined in the test than the control. After 14 weeks both the test and control were similar, the flecks being 16 m wide and 1 μm deep; the pits being 19 ± 6 and 18 ± 5 μm deep respectively. Table 4.21 shows the average frequency, depth and diameter of pits found on triplicate test and control coupons exposed to the aqueous water.

Table 4.21

To Show the Average Frequency, Depth and Diameter of Pits found on Triplicate Aluminium Alloy Coupons Exposed to Modified Sea Water in the Presence or Absence of C. resinae

Parameter	Frequency		Depth (μm)		Diameter (μm)	
Sample	(1 cm x 160 μm)					
	$\bar{x}$	σn-1	$\bar{x}$	σn-1	$\bar{x}$	σn-1
Control						
Top	8	3	43	7	88	28
Middle	18	10	36	11	96	13
Base	15	8	38	13	80	32
Test						
Top	53	9	46	18	64	25
Middle	25	14	32	8	53	27
Base	60	30	44	19	72	40

Compared to the controls pitting in the test coupons was generally of a greater frequency, similar depth and smaller area. The greatest difference between the test and control was the frequency of pits at the top and base of the coupon. This may be explained by the close association of growth from the interfacial area and from the bottom of the flask. Any extracellular products of C. resinae would be in a stronger concentration around these areas and would be diluted out in the middle position where frequency of pitting was least.

It was concluded that the extracellular products of C. resinae initiated pitting of aluminium in sea water but that adherent growth was necessary to promote an increase in their depth and diameter. In addition the adherence of C. resinae was found to promote the formation of channels, especially in the undecane phase. This may be the result of corrosive extracellular products remaining localised because of insolubility in undecane.

4.3 CONCLUSION

The following points have been raised:

1. The corroding metal affected the fall in pH caused by growth of C. resinae. This was at least in part due to alkaline corrosion products and was most obvious in modified sea water.

2. The corrosion products in modified sea water inhibited both germination and growth of C. resinae.

3. Between 7 and 14 weeks the initial fall in pH was counteracted, probably due to excretion of alkaline products, uptake of acidic products or cell lysis.

4. In the presence of stainless steel the dry weight of C. resinae was 1,376 mg and 47 mg after 18 weeks in Turner's solution and 26 weeks in modified sea water respectively. Thus the latter was a poor mineral solution for C. resinae.

5. The total concentration ($\mu g\ ml^{-1}$) of acid detected in Turner's solution filtrates after 9 weeks of growth was 20 times greater than that found in modified sea water samples after 14 weeks growth. This was considered to be a result of the 17-fold difference in dry weight found at these times.

6. The type and concentration of acids in duplicate samples varied significantly.

7. In Turner's solution the acid concentration increased with growth of C. resinae. A rise in pH after 9 weeks indicated that alkaline products were also excreted into the aqueous phase.

8. In modified sea water acid content did not increase with growth of C. resinae. Utilisation of excreted acid products due to the poor growth conditions was thought to be the cause. From the pH

values recorded it was apparent that there was also excretion and uptake of alkaline products.

9. The predominating acid in both Turner's solution and modified sea water was found to be isocitric. Notable differences in the presence of other acids were those of succinic acid not always found in Turner's solution, and pyruvic and lactic acids not always found in modified sea water. The differences in yield and rate of growth was thought to be responsible for this.

10. Analysis of metal ions in culture filtrates which had contained stainless steel showed that C. resinae enhanced corrosion of this metal and suggested that sequestration, especially of Ni and Cr, occurred.

11. A shift to a more positive redox potential during growth of C. resinae was indicative of the production of a more oxidising environment.

12. Growth of C. resinae in Turner's solution caused the corrosion potential to become more negative. However, this was not always the case in modified sea water.

13. Growth of C. resinae increased the corrosivity of Turner's solution to stainless steel, mild steel, cupronickel and aluminium electrodes. The presence of corrosion products modified this effect.

14. Corrosivity as measured by linear polarisation did not correlate with pH indicating the role of aggressive metabolites.

15. Growth of C. resinae increased the corrosivity of modified sea water towards stainless steel and cupronickel but not towards mild steel electrodes. However, the presence of corrosion products modified the corrosivity as measured by linear polarisation.

16. No weight loss was recorded for stainless steel exposed to C. resinae in Turner's solution but weight loss and subsequent corrosion rate were increased up to three times in modified sea water plus C. resinae.

17. The corrosion rate of mild steel was increased up to seven times by C. resinae in Turner's solution but less than two-fold in modified sea water.

18. The corrosion rate of cupronickel was increased up to nine-fold by C. resinae in Turner's solution but less than two-fold greater in modified sea water.

19. The corrosion rate of aluminium was increased up to five-fold by C. resinae in Turner's solution and doubled in modified sea water.

20. Growth of C. resinae in Turner's solution caused slight pitting at the interfacial area of stainless steel and tarnishing below that. There was no effect in modified sea water.

21. Mild steel was extensively corroded by C. resinae in Turner's solution. Pitting beneath adherent growth and general corrosion due to the excretion of metabolites were noted.

22. Growth of C. resinae in modified sea water increased the depth of pits when closely associated with mild steel.

23. Growth of C. resinae in Turner's solution caused intergranular corrosion when closely associated with cupronickel. The presence of extracellular metabolites increased the frequency of pitting but adherent growth was necessary to increase the depth.

24. Adherent growth of C. resinae in modified sea water caused heavy pitting corrosion of cupronickel. Extracellular metabolites enhanced the frequency though not necessarily the depth of pitting.

25. Adherent growth of C. resinae in Turner's solution caused channels of corrosion in aluminium. The frequency and depth of pitting was greater on metal exposed to extracellular metabolites. The presence of C. resinae also caused aluminium to blister.

26. Adherent growth of C. resinae in modified sea water caused channels of corrosion in aluminium. It was also found to increase the depth of pitting. Frequency of pitting increased in the presence of extracellular products.

CHAPTER 5

CORROSION OF STAINLESS STEEL, MILD STEEL, CUPRONICKEL AND ALUMINIUM BY EITHER *C. RESINAE*, A KNOWN MIXED CULTURE OR AN UNKNOWN FUEL SLUDGE CULTURE IN TURNER'S SOLUTION OR MODIFIED SEA WATER WITH UNDECANE AS SOLE CARBON SOURCE

5.1 Experimental Aims

It is considered that the use of C. resinae in pure culture probably does not represent the microbial population and gives rise to misleading data on the corrosive effects of contaminated fuel. The use of pure cultures precludes the possibility of cooxidation and synergism between the variety of fungi, algae, diatoms, yeasts and bacteria involved in corrosion processes. Depending on the many varied environmental conditions within and amongst ships' fuel systems the predominant organisms will differ, leading to difficulties in laboratory simulation.

In this study a pure culture of C. resinae was employed in order to both compare results to previously published data and to allow a direct comparison between pure and mixed cultures. A mixed culture containing partially identified organisms from fuel sludge samples was used to allow some assessment of the effects of known organisms and a fresh fuel sludge sample containing a variety of unknown organisms used for a more realistic representation.

Currently employed procedures for monitoring the control of microbiological corrosion are inadequate. Most are subjective estimates based on measuring either corrosion rates or microbiological populations. Hence there is a continuing need to find better methods of identifying microbial corrosion processes in order for them to be quickly eliminated. This experiment investigates the usefulness of monitoring various parameters associated with growth and subsequent corrosivity of the culture media. The effect of corroding metals on these parameters was also examined and is believed to be the first study of this kind. The large amount of data accumulated were considered in three ways:

1. The effect of the various organisms on the corrosion rate of each metal.
2. The effect of the various organisms on the culture medium.
3. The effect of the metal on the organism and/or culture medium.

5.2 Results and Discussion

Tables 5.1 illustrate the results for each treatment time and culture medium; those results referring to 12 weeks in Turner's solution in 5.1.1, 21 weeks in Turner's solution in 5.1.2 and 16 weeks in sea water in 5.1.3.

5.2.1 Visual Examination of Growth and Corrosion

Turner's Solution

C. resinae grew well in Turner's solution as confirmed by the dry weight results. It also grew well in the mixed culture amongst thick yeast and bacterial growth and flourished especially when mild steel was present. Stimulation of growth of C. resinae by iron may partly explain it's prevalence in mild steel fuel tanks. It was present in progressively lesser amounts with cupronickel, aluminium and stainless steel, indicating that the metal ions do indeed affect its growth in a mixed culture. The sludge cultures all grew prolifically. The mineral solution was a thick creamy colour with yeasts and bacteria and the interface was very thick (5 mm) and included C. resinae, Aspergillus, Fusarium and Penicillium species.

Stainless steel was not visibly corroded; mild steel coupons were coated with a yellow powdery product; cupronickel coupons were coated with a blue product in the control, a turquoise product with C. resinae, and little product for mixed and sludge cultures. Aluminium was coated with a closely adhered thin grey film in all but the mixed culture where a black film was visible.

Modified Sea Water

C. resinae grew less well in modified sea water than in Turner's solution. In the mixed cultures, thick yeast and bacterial growth was apparent. The presence of C. resinae was more abundant with stainless steel and cupronickel coupons than aluminium and mild

Table 5.1.1

To Show the Mean Result and Standard Deviation of the Six Parameters Examined in Filtrates from Turner's Solution Grown Cultures After 12 Weeks Growth

	Organism dry weight (mg)		pH		Redox potential (mV)		Corrosion rate (mpy) of cupronickel x 10^{-3}		Corrosion potential (Er)mV of cupronickel		Metal coupon wt. loss (mg)		Corrosion rate	
													mdd	mpy
System	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	$\bar{x}$
No Metal														
Control	-	-	5.85	0.0	320	16	5.2	1.1	- 60	9	-	-	-	-
C. resinae	541	80	5.70	0.2	295	12	2.6	0.4	100	11	-	-	-	-
Mixed	175	33	5.70	0.3	270	10	0.3	0.2	120	15	-	-	-	-
Sludge	500	100	5.90	0.3	255	4	0.6	0.3	230	18	-	-	-	-
Stainless Steel														
Control	-	-	5.85	0.0	318	6	6.6	2.8	- 59	10	0.13	0.05	0.02	0.004
C. resinae	311	237	5.20	0.6	297	25	2.6	1.6	-110	7	0.27	0.05	0.04	0.008
Mixed	350	64	5.50	0.0	292	14	5.8	0.2	-113	12	0.25	0.15	0.04	0.007
Sludge	1828	274	6.60	0.4	257	2	1.8	1.4	-145	14	0.20	0.20	0.03	0.006
Mild Steel														
Control	-	-	5.85	0.0	320	8	4.0	1.8	- 63	13	10.1	2.2	1.05	0.20
C. resinae	371	280	5.90	0.2	280	8	1.4	0.5	-120	29	20.9	9.4	2.18	0.41
Mixed	381	27	5.63	0.0	275	5	1.6	1.9	-115	35	30.7	0.9	3.20	0.61
Sludge	1345	185	6.70	0.1	267	5	0.9	0.4	-180	22	28.4	2.1	2.96	0.56
Cupronickel														
Control	-	-	5.85	0.0	347	5	7.8	0.7	- 57	2	9.0	1.3	1.11	0.18
C. resinae	704	128	5.63	0.6	310	14	2.8	0.3	- 97	10	9.2	0.1	1.13	0.18
Mixed	104	30	4.97	0.1	343	5	2.5	0.3	- 48	6	11.5	1.3	1.42	0.23
Sludge	1867	102	6.88	0.1	283	17	0.7	0.2	-172	13	7.0	0.6	0.97	0.16
Aluminium														
Control	-	-	5.85	0.2	317	19	3.4	0.6	- 68	2	5.0	0.7	0.41	0.22
C. resinae	849	594	5.75	0.2	290	14	2.2	0.8	112	43	4.3	0.2	0.34	0.19
Mixed	176	56	5.63	0.0	304	5	0.9	0.6	107	9	3.1	0.4	0.25	0.13
Sludge	1529	348	6.90	0.0	262	6	0.8	0.1	190	8	2.6	0.2	0.21	0.11

Table 5.1.2

To Show the Mean Result and Standard Deviation of the Six Parameters Examined in Filtrates from Turner's Solution Grown Cultures After 21 Weeks Growth

	Organism dry weight (mg)		pH		Redox potential (mV)		Corrosion rate (mpy) of cupronickel x 10^{-3}		Corrosion potential (Er)mV of cupronickel		Metal coupon wt. loss (mg)		Corrosion rate	
													mdd	mpy
System	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	$\bar{x}$
No Metal														
Control	-	-	5.80	0.1	250	32	4.0	0.7	- 70	10	-	-	-	-
C. resinae	369	279	5.80	0.6	270	20	1.5	0.2	133	43	-	-	-	-
Mixed	186	99	5.60	0.0	225	10	1.6	0.2	103	8	-	-	-	-
Sludge	2339	77	5.55	0.0	180	20	0.2	0.1	253	33	-	-	-	-
Stainless Steel														
Control	-	-	5.80	0.0	300	0	2.6	0.5	- 78	2	1.9	0.2	0.17	0.03
C. resinae	613	363	6.00	0.6	360	128	2.0	2.3	-180	57	1.8	0.2	0.16	0.03
Mixed	635	62	5.48	0.0	327	25	0.1	0.0	-143	5	2.9	0.1	0.25	0.05
Sludge	2779	148	6.80	0.1	257	26	0.2	0.0	-243	9	1.6	0.2	0.15	0.03
Mild Steel														
Control	-	-	5.80	0.0	295	0	4.1	0.6	- 65	11	13.3	0.6	0.79	0.15
C. resinae	785	403	5.76	0.1	275	19	0.8	0.3	-170	43	32.3	2.1	1.92	0.36
Mixed	564	229	5.50	0.1	288	2	0.5	0.2	-117	5	35.4	6.9	2.11	0.40
Sludge	1408	494	7.00	0.2	98	32	0.3	0.1	-273	21	37.6	7.4	2.24	0.43
Cupronickel														
Control	-	-	5.80	0.0	333	13	4.8	0.2	- 67	2	8.8	0.7	0.62	0.10
C. resinae	1093	243	6.10	0.5	300	15	3.2	2.0	-152	30	10.6	1.9	0.75	0.12
Mixed	988	137	3.23	0.2	378	2	2.4	0.2	- 40	4	14.1	1.9	0.99	0.16
Sludge	3030	538	6.88	0.1	187	9	0.5	0.3	-193	17	10.8	2.2	0.76	0.12
Aluminium														
Control	-	-	5.80	0.0	359	27	3.7	1.0	- 80	7	7.8	0.4	0.39	0.21
C. resinae	938	123	5.80	0.6	352	84	0.6	0.4	202	9	31.6	7.4	1.60	0.85
Mixed	504	127	5.50	0.1	368	79	0.1	0.1	132	6	14.8	0.7	0.75	0.40
Sludge	3167	695	6.70	0.1	210	16	0.2	0.1	263	17	8.9	1.4	0.45	0.24

Table 5.1.3

To Show the Mean and Standard Deviation of the Six Parameters Examined in Filtrates from Sea Water Grown Cultures After 16 Weeks

	Organism dry weight (mg)		pH		Redox potential (mV)		Corrosion rate (mpy) of cupronickel x 10^{-3}		Corrosion potential (Er) mV of cupronickel		Metal coupon wt. loss (mg)		Corrosion rate	
													mdd	mpy
System	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	$\bar{x}$
No Metal														
Control	-	-	6.60	0.3	375	15	0.8	0.1	-210	20	-	-	-	-
C. resinae	485	60	2.95	0.1	410	12	1.2	0.2	-190		-	-	-	-
Mixed	257	229	2.60	0.6	375	20	0.7	0.1	-218	8	-	-	-	-
Sludge	2111	32	2.10	0.0	440	15	0.3	0.1	-213	3	-	-	-	-
Stainless Steel														
Control	-	-	6.60	0.3	395	15	0.7	0.2	-215	5	0.75	0.05	0.09	0.02
C. resinae	184	17	3.00	0.0	445	5	0.7	0.1	-210	5	1.45	0.05	0.17	0.03
Mixed	519	65	2.60	0.0	315	5	0.5	0.1	-228	3	0.50	0.10	0.06	0.01
Sludge	2245	15	2.05	0.1	460	10	0.7	0.4	-203	8	0.90	0.20	0.11	0.02
Mild Steel														
Control	-	-	6.60	0.2	270	0	0.7	0.1	-225	11	316.3	22.1	24.75	4.70
C. resinae	117	14	6.00	0.5	357	13	0.6	0.1	-243	5	289.4	2.9	22.65	4.30
Mixed	80	25	6.90	0.1	337	9	0.9	0.3	-212	10	196.6	24.4	15.38	2.92
Sludge	1953	78	3.90	0.1	390	8	1.4	0.2	-177	5	166.5	5.9	13.03	2.48
Cupronickel														
Control	-	-	7.53	0.1	350	0	1.8	0.4	-210	11	42.6	7.2	3.93	0.63
C. resinae	269	369	7.80	0.5	420	22	17.2	14.2	-130	36	76.9	19.0	7.10	1.14
Mixed	675	283	5.80	0.1	400	16	92.5	13.7	- 48	9	861.6	49.3	79.55	12.73
Sludge	1714	29	5.92	0.1	475	11	33.0	8.0	- 78	3	628.0	43.7	57.98	9.28
Aluminium														
Control	-	-	7.87	0.2	273	9	0.7	0.1	-250	14	41.1	1.4	2.73	1.45
C. resinae	231	7	3.77	0.1	430	8	0.7	0.1	-223	5	76.1	9.2	5.06	2.68
Mixed	54	9	7.42	0.3	282	29	1.1	0.1	-233	10	39.8	12.3	2.64	1.40
Sludge	1122	411	4.05	0.1	340	8	1.2	0.3	-183	13	83.5	22.9	5.55	2.94

steel. The sludge cultures were thick with yeast and bacterial growth, the interface consisting mainly of C. resinae. Thus it would appear to be competitive against other fungi in sea water.

Stainless steel was not visibly corroded. Mild steel had typical orange rust except with sludge cultures where the deposit was black. Cupronickel had a loose green precipitate, the surface being especially rough and corroded by the mixed cultures. Aluminium had a thin grey corrosion film.

5.2.2 Organism Dry Weight

Figure 5.1 illustrates the dry weight of the organisms after growth in Turner's solution or modified sea water and undecane. The growth of C. resinae is renowned for being variable[149] even under identical conditions and the standard deviations calculated from sets of triplicate flasks were recorded in Table 5.1.

Turner's Solution

After 12 weeks in the absence of metals (Fig. 5.1a) the mixed culture grew only half as well as C. resinae and sludge. After 21 weeks the dry weight of sludge was at least six times greater than that of either C. resinae or the mixed culture, indicating when given sufficient time for ecosystem development, sludge organisms grew to a much greater extent. Thus, pure C. resinae and a laboratory mixed culture may not be expected to adequately represent a sludge sample.

The presence of metals had a stimulatory effect on the growth of sludge after 12 weeks and, except for mild steel, such stimulation continued over the following 9 weeks. The apparent inhibition by corrosion products of mild steel on sludge may be of relevance in the fuel tank. Should corrosion be directly related to amount of growth then it may be expected to be self-limiting, i.e. growth would enhance corrosion until the quantity of corrosion products became inhibitory.

After 21 weeks the presence of metals had stimulated growth of both C. resinae and the mixed culture. This was especially

Figure 5.1: To show the Dry Weights of C. resinae, Mixed Culture and Sludge After Growth with No Metal, Stainless Steel, Mild Steel, Cupronickel or Aluminium, for 12 and 21 Weeks in Turner's Solution and 16 Weeks in Modified Sea Water

Figure 5.1a: Turner's Solution - 12 Weeks.

Figure 5.1b: Turner's Solution - 21 Weeks.

Figure 5.1c: Modified Sea Water - 16 Weeks.

Key:

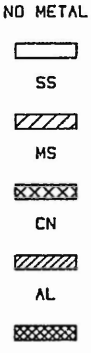


Figure 5.1a

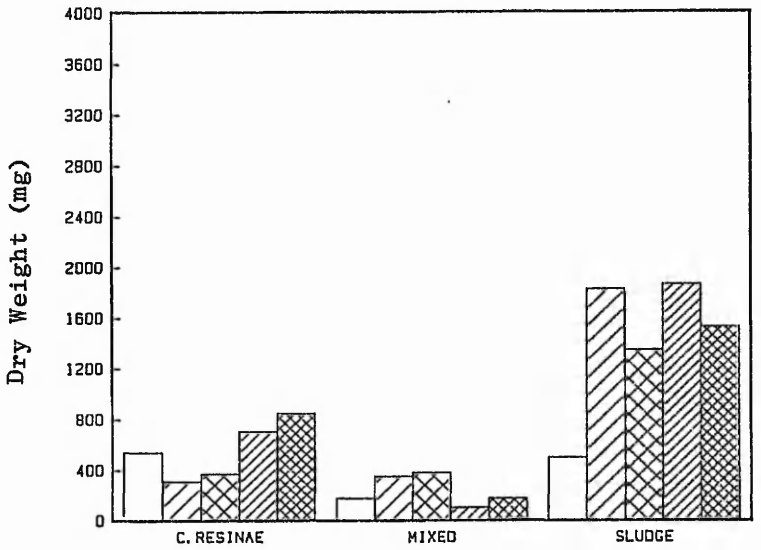


Figure 5.1b

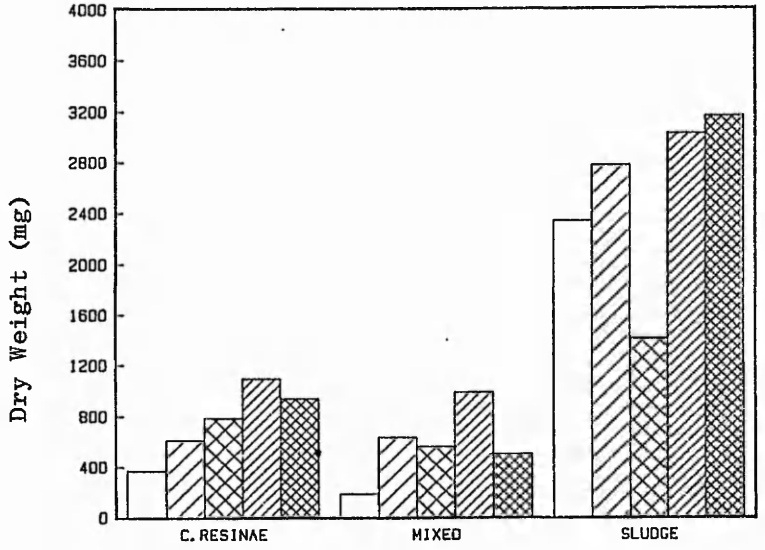
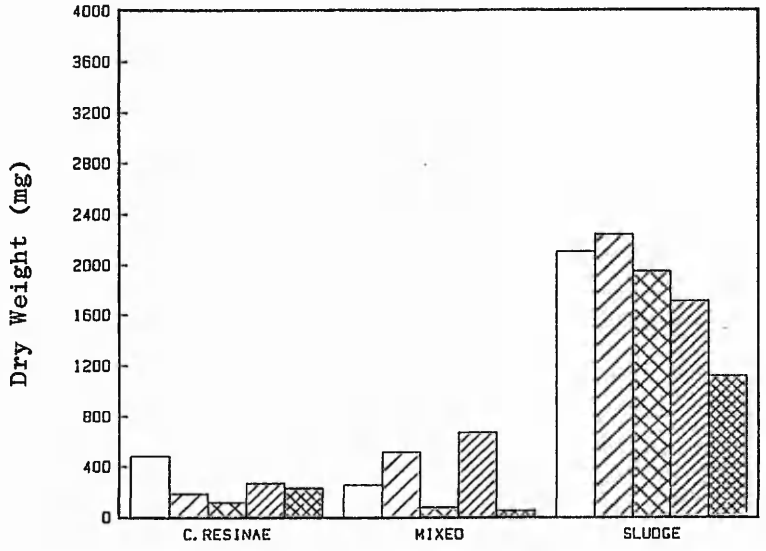


Figure 5.1c



interesting for two of the metals. Firstly, stainless steel showed little sign of corrosion yet was able to provide sufficient metal ions to stimulate growth, and secondly, cupronickel (often considered to be resistant to fouling because of its toxic properties) stimulated growth to the greatest extent. Turner's solution contains several trace metals in amounts considered to optimise growth of *C. resinae*[208] but this experiment indicates that the corroding metal coupons provided further metal requirements.

Modified Sea Water

In the absence of metal, growth of sludge was about five times greater than that of *C. resinae* or the mixed culture, again indicating that pure and laboratory mixed cultures were not representative of amount of growth and may therefore not represent subsequent microbial corrosion.

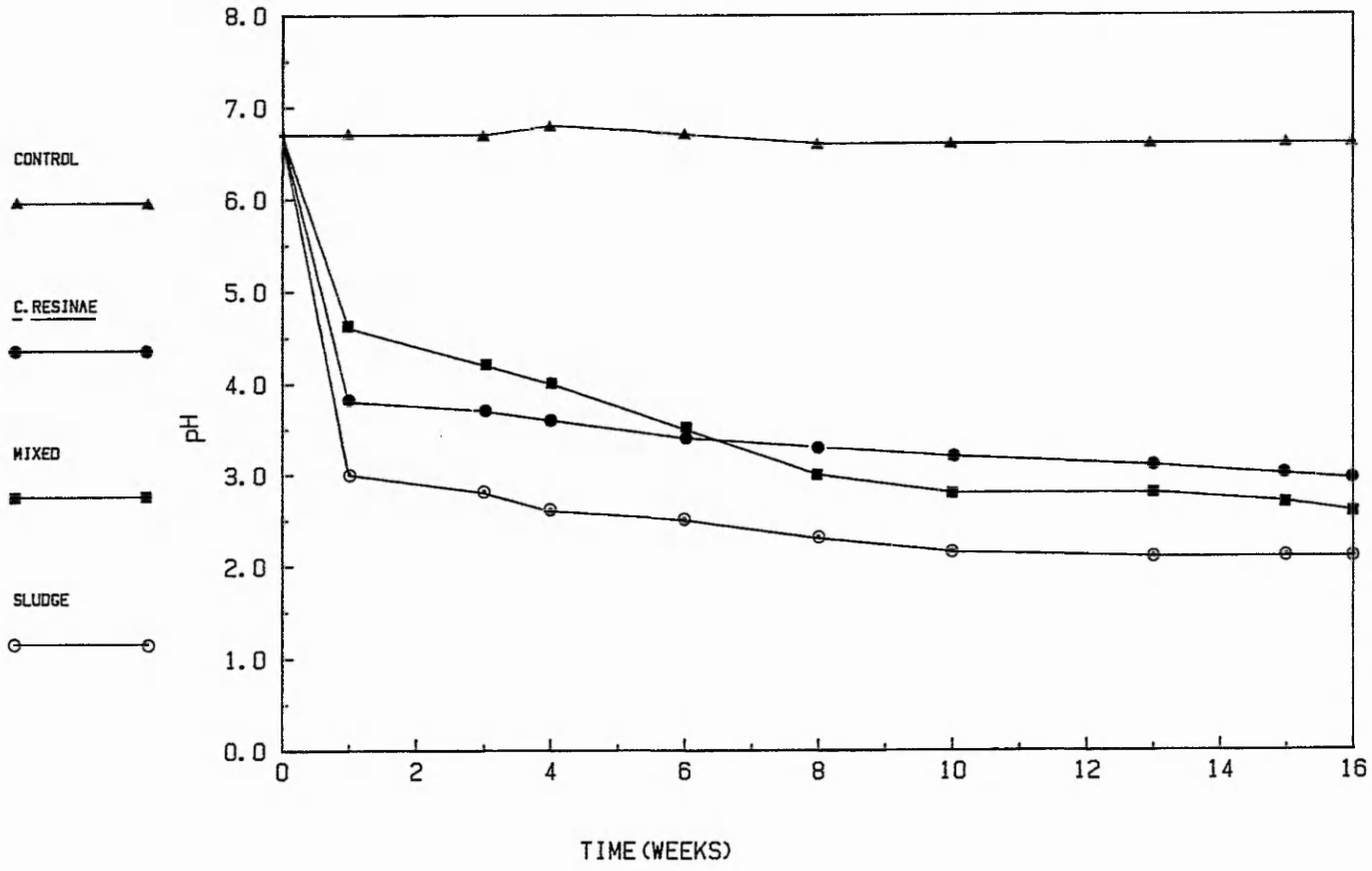
The fact that the presence of metals generally inhibited growth was surprising in view of the stimulation observed in Turner's solution. Evidently the effect of the metal coupons depended on the influence of the physicochemical characteristics of the recipient environment in modifying the corrosion product and/or the microbes response to it.

5.2.3 pH

The pH of the uninoculated and no metal flasks was taken at regular intervals throughout the experiment. Turner's solution showed little change in pH over 16 weeks probably due to its buffering capacity (results not shown). Figure 5.2 shows the change in modified sea water plus undecane systems by each type of inoculum. In the absence of metals, within a week the pH of all inoculated media had fallen significantly, *C. resinae* and sludge cultures remaining fairly constant over the next 15 weeks but the pH of the mixed culture flasks fell gradually from the first to the tenth week. The sludge culture media was always more acidic thus it may be expected to have the greatest effect on corrosion.

Figure 5.2

To show the Change in pH of Modified Sea Water due to Growth of C. resinae, Mixed Culture and Sludge Culture in the Absence of Metal



Turner's Solution

In Turner's solution the presence of metals have little effect on the initial pH and microbial growth only produced small pH changes. After 12 weeks a slight pH fall for C. resinae plus stainless steel systems and a larger drop in mixed culture plus cupronickel systems were observed. Only the pH drop of the latter example was maintained after 21 weeks (Fig. 5.3a). As cupronickel did not change the pH of the uninoculated medium and the mixed culture did not decrease the pH in its absence the metal may be expected to have either a direct effect on the metabolism of the microorganisms or it may be toxic to certain organisms, those it selects for being acid producers. Hence a toxic metal may actually increase microbial corrosion. This should be borne in mind when selecting biocides or antifouling paints. A relatively inocuous organism in a mixed culture may become a significant problem if selected for.

In Turner's solution the presence of metals exerted their greatest effect on the sludge cultures where the pH of the culture filtrate increased. From prior reasoning the metabolisms may have been modified or alkali producers may have been selected for. The production of a neutral pH may well give rise to non-corrosive conditions.

Modified Sea Water

Greater pH variations were observed in modified sea water after 16 weeks than in Turner's solution. Figure 5.3b shows that both the corrosive culture and the corroding metal were responsible for the ultimate pH value. Generally speaking the sludge reduced the pH to a greater extent than C. resinae or the mixed culture. The presence of stainless steel did not affect the final pH; probably because of its resistance to corrosion and subsequent lack of corrosion products. Except for the mixed culture plus mild steel and the C. resinae plus cupronickel filtrates all the inoculated filtrates were more acidic than their respective uninoculated controls. Mild steel did not affect the control pH and its effect on the mixed culture may be

Figure 5.3

To show the Final pH in Turner's Solution after 21 Weeks and in Modified Sea Water after 16 Weeks Growth of C. resinae, Mixed Culture or Sludge Culture ± Stainless Steel, Mild Steel, Cupronickel or Aluminium

Figure 5.3a: Turner's Solution - 21 Weeks

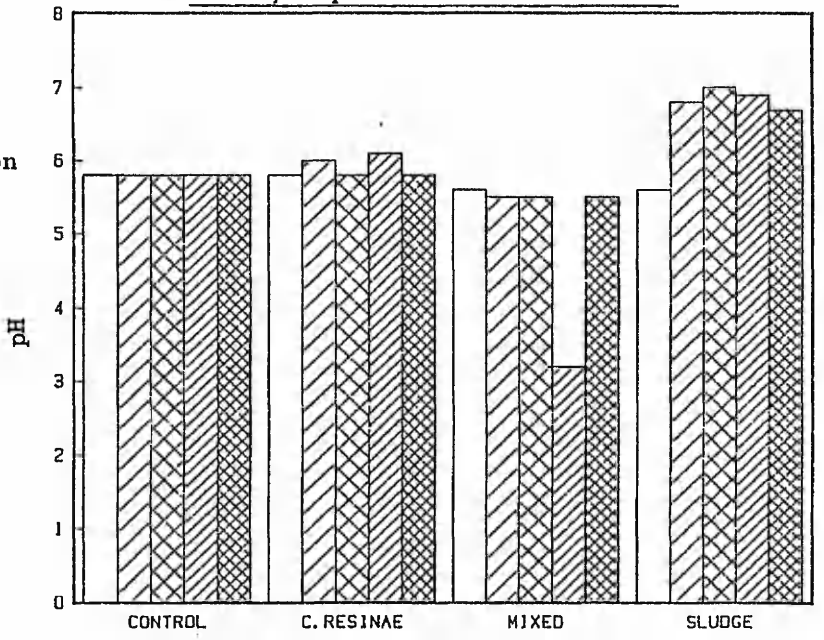
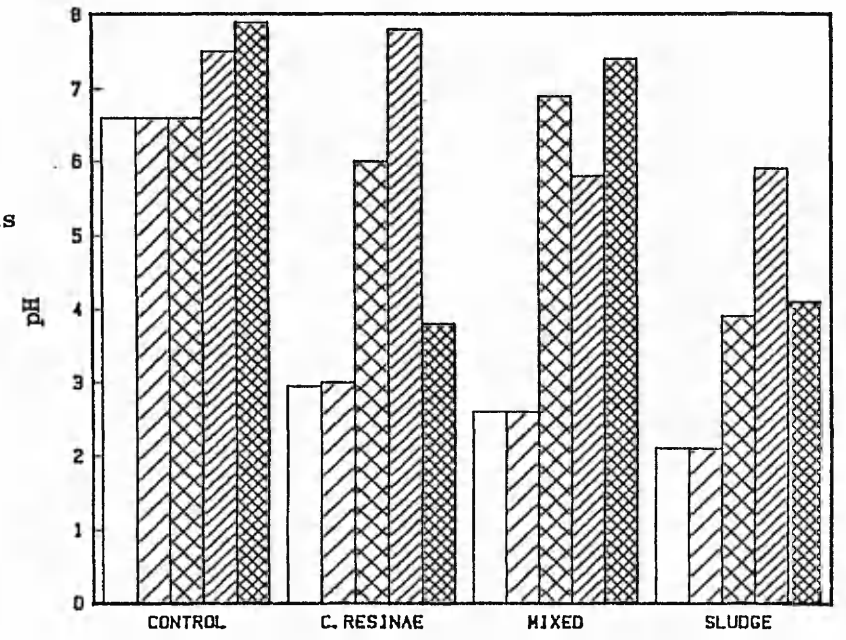


Figure 5.3b: Modified Sea Water - 16 Weeks



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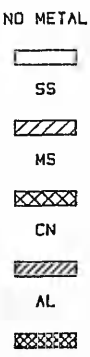


similar to that on sludge cultures in Turner's solution. Cupronickel, however, did increase the pH of the uninoculated control and the further increase in the presence of C. resinae may not have been due to any effect on the organism. That is, cupronickel may produce alkaline corrosion products in modified sea water or the corrosion reaction may utilise any free H^+ ions in the system thus raising the pH. In the presence of C. resinae the corrosion rate was found to be accelerated thus increasing alkalinity by these methods.

To summarise the results for modified sea water;

a) Most acid was produced by sludge then C. resinae then the mixed culture.
b) Stainless steel did not affect the final pH.
c) Mild steel, aluminium and cupronickel generally increased the final pH of the uninoculated control and reduced the acidity of the culture filtrates. This may have been due to a combination of the following factors;

1. Modification of microbial metabolism, i.e. decreasing production of acidic metabolites and/or increasing production of alkaline metabolites.

2. Selection for alkaline producers (in mixed and sludge cultures).

3. Formation of alkaline corrosion products in the microbial environment.

4. Utilisation of H^+ ions during corrosion.

5. Neutralisation of acidic products.

These results led to the conclusion that measurement of pH in a possibly contaminated and potentially corrodible system would not be indicative of the extent of growth and subsequent corrosion. For example, in the light of this experiment a low pH may not necessarily be expected in a contaminated mild steel fuel tank. That is, a

laboratory mixed culture in the absence of metal gave a low pH of 2.6 but in the presence of mild steel a pH of 6.9 was recorded. Thus, it may not be feasible to screen organisms for corrosive factors such as pH in the absence of a particular metal as has been the case in much of the literature. Similarly the monitoring of water samples in fuel systems must be subject to more vigorous tests than pH where microbial corrosion is suspected.

5.2.4 Redox Potential

As explained in Chapter 4, the onset of growth is characterised by a shift to a more negative redox potential due to either reduction of oxygen during respiration or the generation of hydrogen during metabolism. The shift to a more positive Eh is indicative of a more oxidising and hence more corrosive environment and may be due to the release of extracellular metabolites or products of cell lysis. The presence of corroding metals further complicates the redox characteristics of the system.

Turner's Solution

After 12 weeks (Fig. 5.4a) in the no metal control, the redox potential was most reduced by the sludge culture, then the mixed culture and finally by *C. resinae*. This pattern was generally followed when either stainless steel or mild steel were present. The presence of cupronickel or aluminium caused the reduction in redox potential by the mixed culture to be insignificant. The redox potential of the uninoculated controls was only increased by cupronickel, the other metals had no effect. The presence of this metal also caused the values of the culture filtrates to be raised above that of their respective no metal controls. Thus, after 12 weeks the cultures appeared to reduce the corrosivity of Turner's solution.

After 21 weeks the effect of the cultures was more variable (Fig. 5.4b). In the no metal control *C. resinae* had increased the redox potential (indicating an increase in corrosivity) whereas the

Figure 5.4: To show the Redox Potential in Filtrates of C. resinae, Mixed Culture, and Sludge Culture ± Stainless Steel, Mild Steel, Cupronickel or Aluminium Grown in Turner's Solution for 12 or 21 Weeks and Modified Sea Water for 16 Weeks

Figure 5.4a: Turner's Solution - 12 Weeks.

Figure 5.4b: Turner's Solution - 21 Weeks.

Figure 5.4c: Modified Sea Water - 16 Weeks.

Key:

CONTROL

C. RESINAE

MIXED

SLUDGE

Figure 5.4a

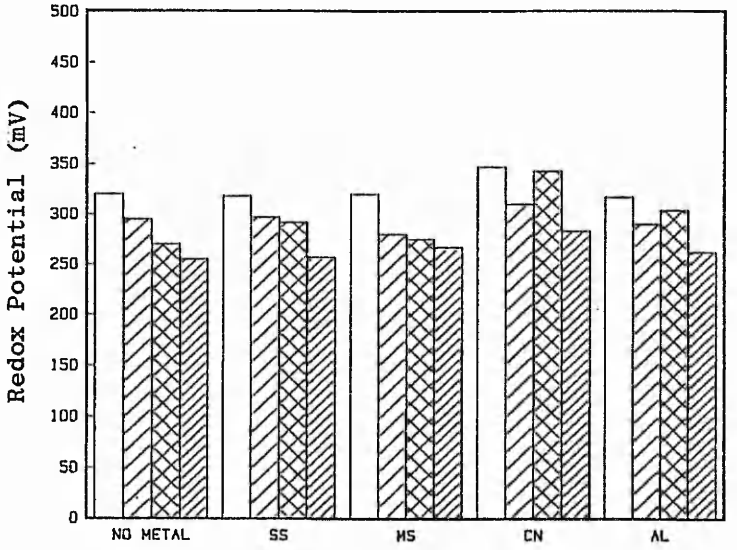


Figure 5.4b

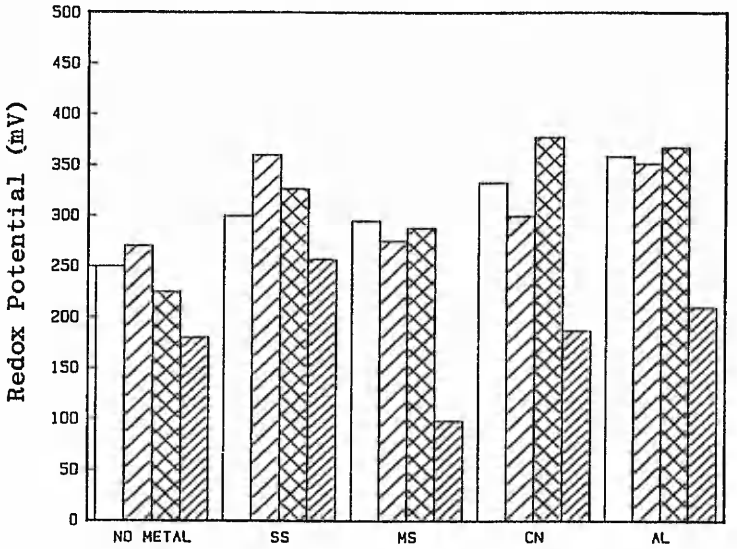
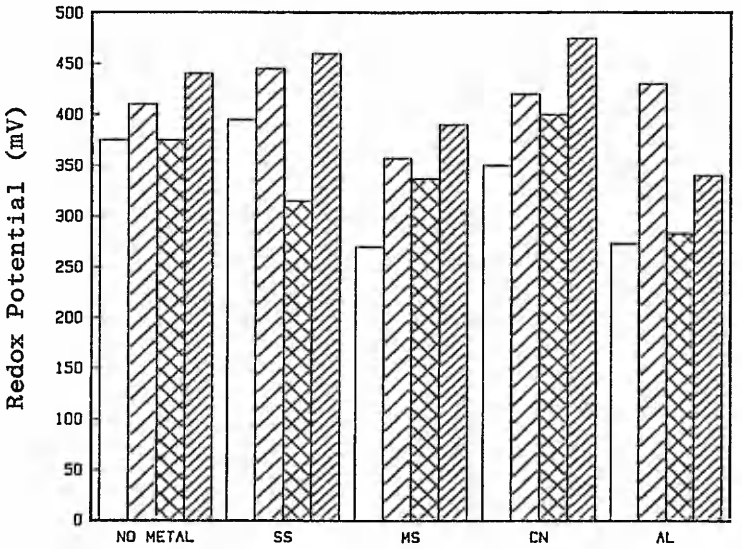


Figure 5.4c



mixed and sludge cultures both reduced Eh compared to the uninoculated control. In the presence of metals the Eh of C. resinae and the mixed culture were similar to the uninoculated control. The Eh of sludge was significantly reduced especially in the presence of mild steel. These results illustrate that in Turner's solution sludge did not promote corrosive conditions as monitored by redox potential. Reasons for the reduction in corrosivity were unknown. However, the complexity of the system and the small differences observed made this method inadequate for monitoring microbial contamination and subsequent corrosivity.

Modified Sea Water

In the no metal controls the growth of microorganisms increased the redox potential, this increase being negligable for the mixed culture (Fig. 5.4c). The presence of metals in the uninoculated controls generally reduced the Eh though this was not always apparent in the culture filtrates. Except for the stainless steel/mixed culture filtrate the Eh value was increased indicating a more oxidising and hence corrosive environment had developed. However, in most cases this difference was insufficient to recommend the use of Eh monitoring as a means of recognising corrosive conditions.

5.2.5 Nitrate Concentration

The concentration of nitrate remaining in Turner's solution after 21 weeks was measured. Table 5.2 and Figure 5.5 show the results obtained.

Turner's solution contains 6 g l^{-1} of NH_4NO_3. The average value of each of the control concentrations was 6013 $\pm$ 91 μ g ml^{-1}. Under conditions when no metal was present it can be seen that both C. resinae and particularly sludge reduce the NO_3^- content and the mixed culture increases it. It was rather surprising that the mixed culture was unable to reduce the NO_3^- concentration since it was made up of C. resinae and isolates 1, 10 and 11 (Chapter 3) all of which were able to reduce NO_3^-. Evidently this mechanism was not active in the mixed

culture and/or oxidation reactions took place, for example of NH_4^+ or NO_2^-. With the exception of mild steel the presence of metal coupons had no effect on this general trend. This metal caused the reduction of NO_3^- in the presence of C. resinae to stop completely. The reason for inhibition was unclear since both the growth yield of C. resinae as monitored by dry weight was unaffected and the amount of corrosion product as monitored by weight loss was similar to that of the sludge culture where NO_3^- reduction continued. Presumably a proportion of the multitude of organisms present in sludge could withstand the presence of corroding mild steel.

Nitrate reducing bacteria were likely to be present in sludge. Nitrate is used as a hydrogen acceptor under anaerobic conditions. The system under test was not anaerobic, though in certain areas, e.g. under corrosion films, below organism growth on the coupons, in the sludge layer at the base of the flask and in the centre of the mat of growth, conditions would tend towards anaerobiosis. Certainly, facultative organisms e.g. pseudomonads would be able to utilise NO_3^- under these conditions and further reduction of NO_2^- to N_2 may follow. Should any of the organisms present also possess a hydrogenase system, they could depolarise the cathodic reaction using NO_3^- as the hydrogen acceptor. Using a strain of Escherichia coli it was shown[133] that the anaerobic corrosion of ferrous metals was in accordance with the equation:

$$Fe + H_2O + NO_3^- \longrightarrow Fe(OH)_2 + NO_2^-$$

This mechanism of corrosion may be supported by this experiment because the sludge was seen to utilise $NO3^-$ for the oxidation of organics (no metal control) and more so for the oxidation of cathodic hydrogen in the presence of metals. However, sludge was only observed to increase the corrosion rate above that by C. resinae and the mixed culture in the case of mild steel. This indicates that other mechanisms of corrosion were important for the various metals. For example, it was expected that the removal of NO_3^-, a corrosion inhibitor of aluminium, may increase the corrosion rate of the metal. However, the sludge culture increased the corrosion rate of aluminium

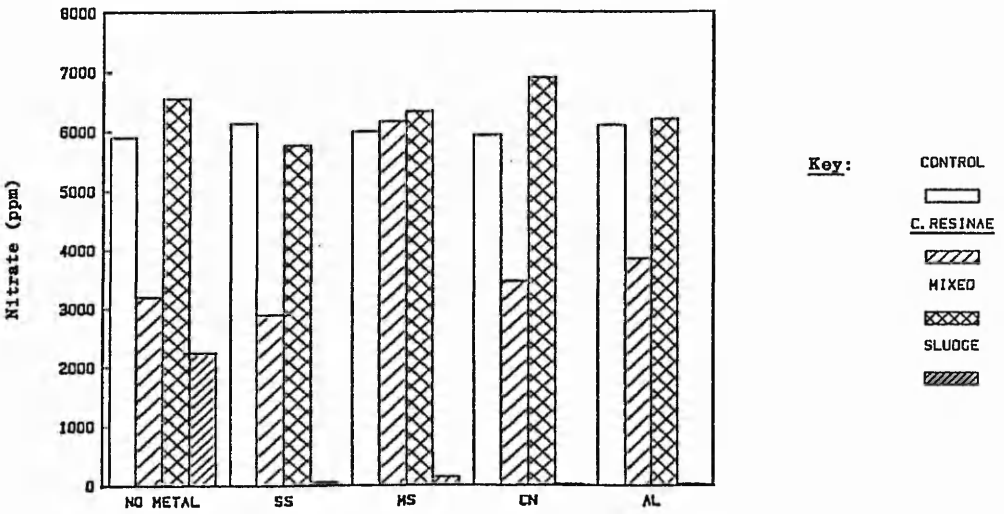
Table 5.2

To Show the Average Concentration and Standard Deviation of Nitrate ($\mu g\ ml^{-1}$) in Culture Filtrates and Sterile Turner's Solution after 21 Weeks Incubation

Metal in System		Nitrate concentration $\mu g\ ml^{-1}$			
		Control	*C. resinae*	Mixed	Sludge
No metal	$\bar{x}$	5900	3200	6550	2250
	s.d.	110	250	50	50
Stainless steel	$\bar{x}$	6133	2900	5767	61
	s.d.	262	300	377	56
Mild steel	$\bar{x}$	6000	6167	6333	142
	s.d.	497	403	170	83
Cupronickel	$\bar{x}$	5933	3467	6900	32
	s.d.	262	685	424	10
Aluminium	$\bar{x}$	6100	3850	6200	32
	s.d.	432	550	216	15

Figure 5.5

To show the Final Concentration of Nitrate in the Filtrates of *C. resinae*, Mixed Culture and Sludge Culture and Uninoculated Turner's Solution after 21 Weeks Incubation



between 12 and 21 weeks to a lesser extent than both the mixed culture and C. resinae, where NO_3^- was still present in the filtrates. Evidently other factors besides removal of corrosion inhibitors and the utilisation of nitrate as a hydrogen acceptor were more important as mechanisms of corrosion.

5.2.6 Corrosivity by the Linear Polarisation Method

Using polarisation measurements the corrosion rate of cupronickel was calculated for each culture filtrate. As explained in Chapter 4 the corrosion rates were not absolute because of the complexity of the filtrates but they allowed a comparison of the effects of metals and microorganisms on media corrosivity. It must be borne in mind that conclusions drawn may only pertain to cupronickel. The results are illustrated in Figure 5.6.

Turner's Solution

The overall effect of microorganisms was to reduce the corrosivity of Turner's solution to cupronickel. The reason for this reduction was uncertain though the removal of inherently corrosive components by utilisation or complexation is a possibility. The problem with using polarisation to measure corrosion rate is that it is only valid for one particular point in time. These results indicated that the culture filtrate was not corrosive to cupronickel at 12 weeks.

In the uninoculated controls, the corrosion rates were generally less after 21 than 12 weeks possibly indicating that corrosion products also reduce the corrosivity of Turner's solution. Again complexation with previously corrosive components may explain this observation.

Modified Sea Water

In the absence of metal only C. resinae increased the corrosivity of modified sea water after 16 weeks. The mixed culture had little effect and sludge reduced the corrosivity. With the

Figure 5.6: To show the Corrosion Rate of Cupronickel in Filtrates of C. resinae, Mixed Culture and Sludge Culture ± Stainless Steel, Mild Steel, Cupronickel or Aluminium Grown in Turner's Solution for 12 or 21 Weeks and Modified Sea Water for 16 Weeks

Figure 5.6a: Turner's Solution - 12 Weeks.

Figure 5.6b: Turner's Solution - 21 Weeks.

Figure 5.6c: Modified Sea Water - 16 Weeks.

Key:

CONTROL

C. RESINAE

MIXED

SLUDGE

Figure 5.6a

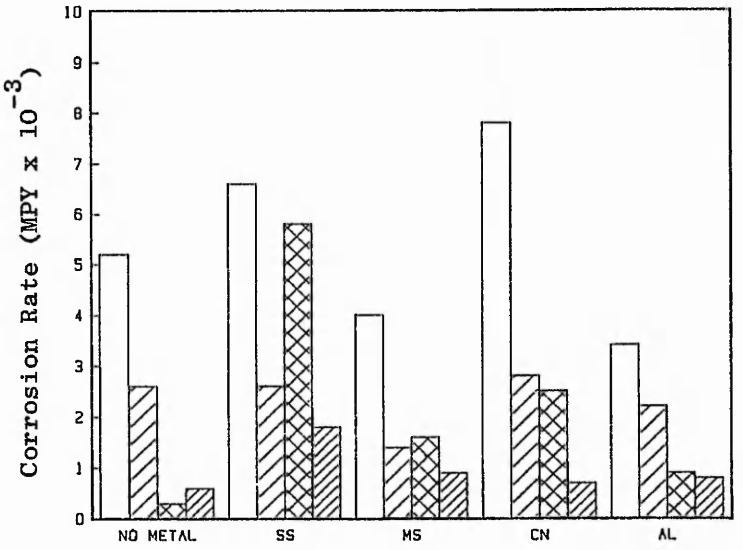


Figure 5.6b

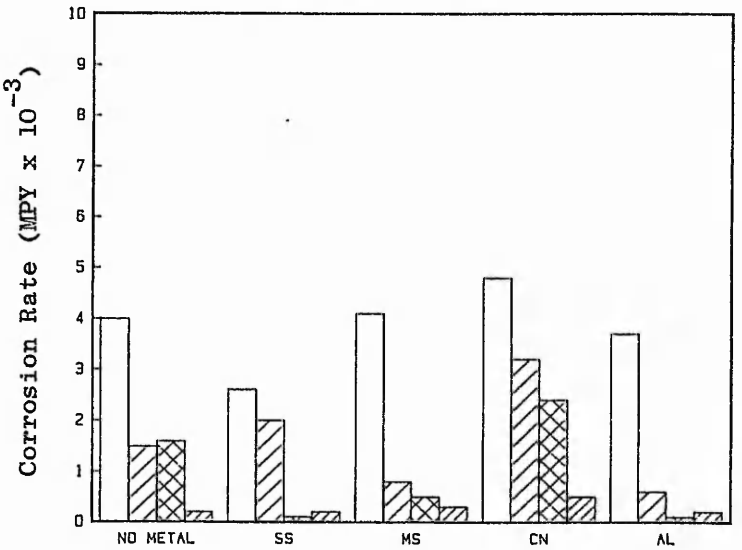
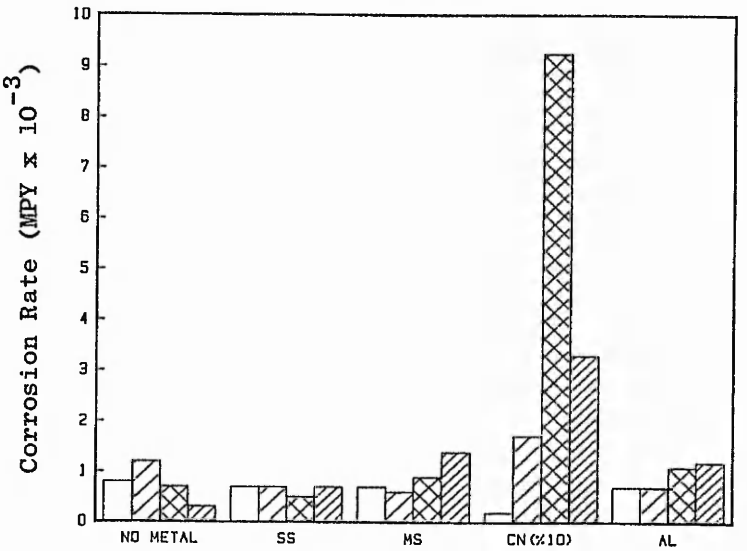


Figure 5.6c



exception of cupronickel the presence of metals generally had little effect. Cupronickel more than doubled the corrosivity of the uninoculated control indicating that the presence of it's own ions or corrosion products stimulated it's corrosion. In the presence of microbial cultures the corrosion rate was increased many fold, ie C. resinae x 14, mixed x 132 and sludge x 110, compared to the no metal controls. Thus the culture filtrates were especially corrosive to cupronickel. This was confirmed by weight loss experiments.

5.2.7 Corrosion Potential of Cupronickel

The results are shown in Figure 5.7; a shift to a more negative corrosion potential generally indicates a more corrosive environment.

Turner's Solution

In the no metal control filtrates the most negative potential was effected by sludge followed by the mixed culture and then C. resinae. This was generally true in the presence of metals where there was little difference between the effect of C. resinae and the mixed culture though the sludge culture had a much greater effect. Whilst metals alone did not alter the corrosion potential in the uninoculated controls, they did affect the potential-reducing activity of the sludge cultures. C. resinae and the mixed culture were little effected except in the case of the latter by cupronickel. After 21 weeks cupronickel again reduced the activity of the mixed culture. The other cultures had reduced the potential further than after 12 weeks and the presence of metals had little effect on this.

Measurement of corrosion potential may be a useful tool in predicting microbial contamination since it was significantly more negative in culture filtrates. However, possible anomalies, for example in mixed/cupronickel filtrates for cupronickel electrodes means that further supportive tests would be necessary.

Figure 5.7: To show the Corrosion Potential of Cupronickel in Filtrates of C. resinae, Mixed Culture and Sludge Culture ± Stainless Steel, Mild Steel, Cupronickel or Aluminium Grown in Turner's Solution for 12 or 21 Weeks and Modified Sea Water for 16 Weeks

Figure 5.7a: Turner's Solution - 12 Weeks.

Figure 5.7b: Turner's Solution - 21 Weeks.

Figure 5.7c: Modified Sea Water - 16 Weeks.

Key:

CONTROL

C. RESINAE

MIXED

SLUDGE

Figure 5.7a

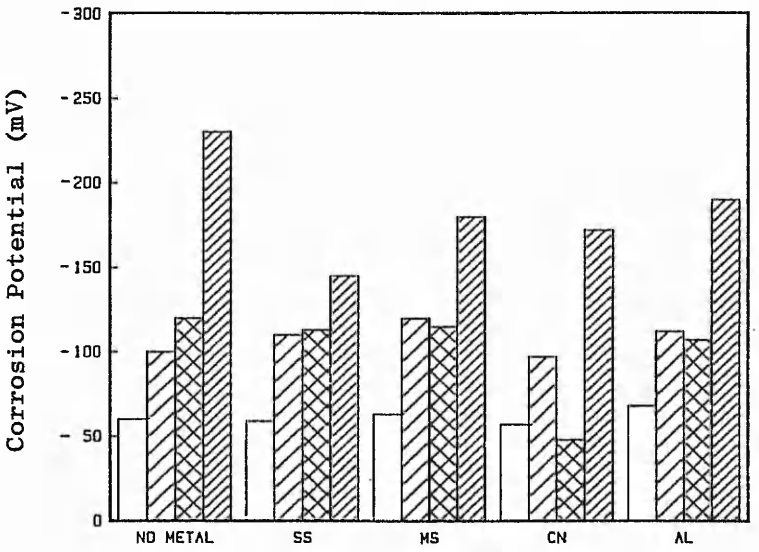


Figure 5.7b

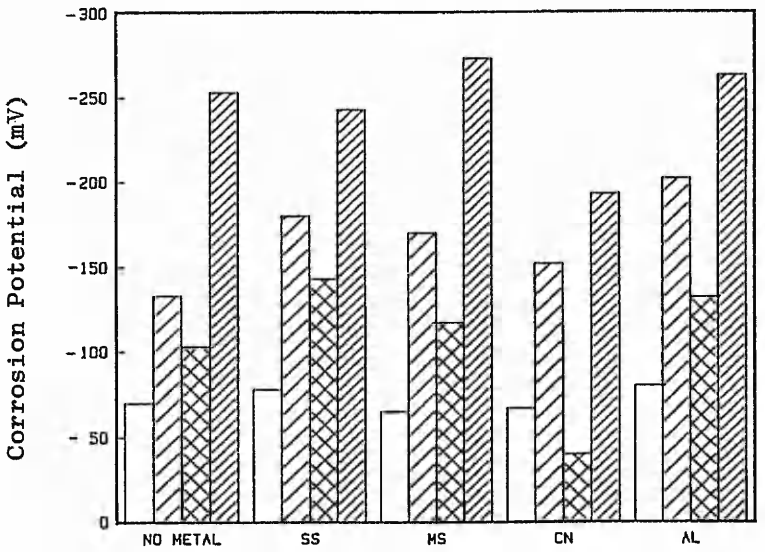
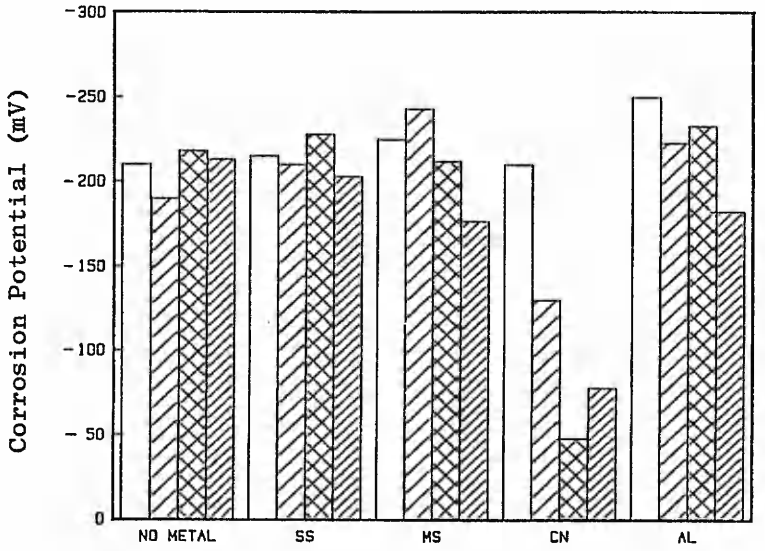


Figure 5.7c



Modified Sea Water

The value of the corrosion potential altered very little in modified sea water regardless of the presence of organisms and/or metals. An exception was in the presence of cupronickel where the potential of the culture filtrates increased. This may be explained by an effect of copper or nickel ions on the cupronickel electrode used for the measurements. It was not apparent in Turner's solution illustrating the importance of all environmental factors when choosing a test system. Evidently the use of a cupronickel electrode to monitor corrosion potential as an indication of corrosivity in modified sea water may not be ideal.

5.2.8 Corrosion Rate of Metal Coupons

The corrosion rate of the coupons was determined from their weight loss and the results are presented in Figure 5.8.

Turner's Solution

In Turner's solution the corrosion rates of the uninoculated metals were less after 21 weeks than after 12 weeks exposure. It was to be expected that weight loss per unit time would decrease as soon as a protective layer of corrosion products was formed on the surface.

After 12 weeks the presence of microorganisms had caused the corrosion rates of all the metals except aluminium to increase above that of the uninoculated control. The apparent resistance of aluminium to microbial corrosion over this time period was surprising in view of numerous literature reports to the contrary. However, after 21 weeks the corrosion rates of all the metals were increased by microorganisms. The most corrosive culture depended both upon the metal under test and also the exposure time. The latter was considered to be most important when screening for corrosive cultures whose growth and metabolism and hence subsequent effect on the metal under test would vary with time.

It cannot be assumed that lack of corrosivity by a culture towards one test metal indicates that it is non-corrosive towards

Figure 5.8: To Show the Corrosion Rates of the Metal Coupons (Stainless Steel, Mild Steel, Cupronickel and Aluminium) When Exposed to C. resinae, Mixed Culture, Sludge Culture, Uninoculated Turner's Solution for 12 and 21 Weeks and Modified Sea Water for 16 Weeks

Figure 5.8a: Turner's Solution - 12 Weeks.

Figure 5.8b: Turner's Solution - 21 Weeks.

Figure 5.8c: Modified Sea Water - 16 Weeks.

Key:

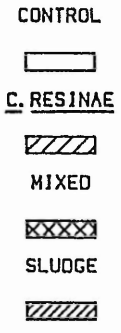


Figure 5.8a

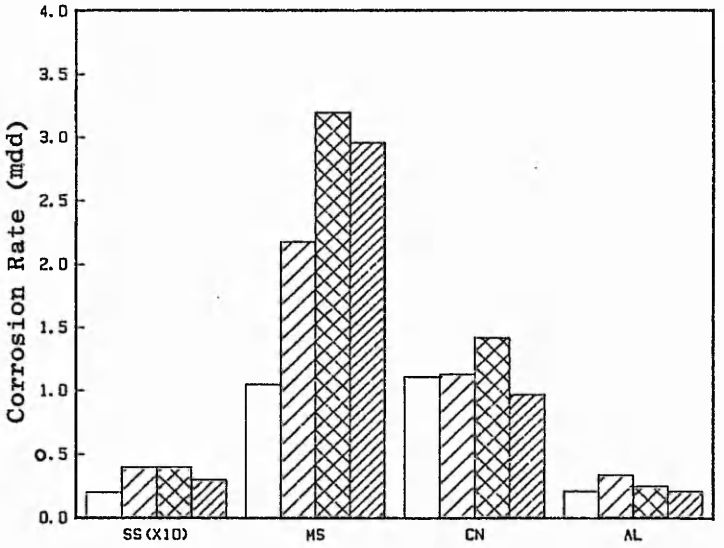


Figure 5.8b

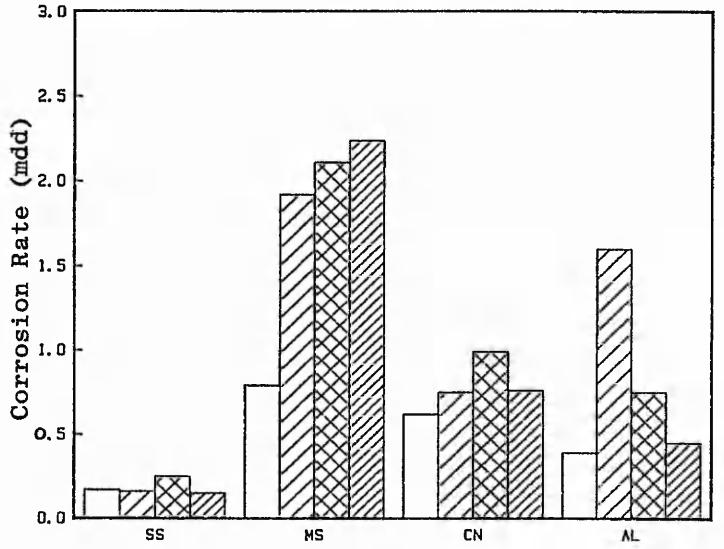
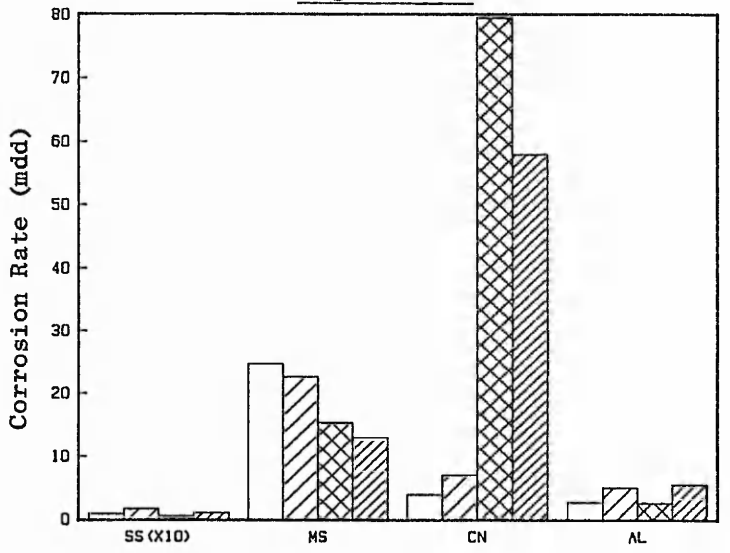


Figure 5.8c



metals in general. For example, sludge had little effect on aluminium and cupronickel but greatly enhanced the corrosion of mild steel. Similarly, C. resinae had little effect on cupronickel but greatly enhanced the corrosion of mild steel and aluminium. Corrosion rates caused by C. resinae and sludge were not comparable for aluminium and their comparability for mild steel depended on exposure time thus care has to be taken when interpreting data using atypical pure cultures.

Although stainless steel was relatively resistant to corrosion in Turner's solution its corrosion rate was doubled after 12 weeks exposure to microorganisms.

Aluminium was also fairly resistant to corrosion in Turner's solution; after 12 weeks the cultures exhibited a protective role, though after 21 weeks C. resinae had increased the corrosion rate four fold. It was doubled by the mixed culture and barely affected by sludge. As discussed earlier the removal of the corrosion inhibitor, NO_3^-, may have been a cause of the uncharacteristic increase in corrosion rate with time. Alternatively products corrosive to aluminium may have been only released in the latter stages of growth or during cell lysis.

The corrosion rate of cupronickel was not significantly enhanced by the activities of C. resinae or sludge and increased slightly by the mixed culture. It was probably of some significance that only the mixed culture lowered the pH of Turner's solution.

The metal most affected by the presence of microorganisms was mild steel. After 12 weeks its corrosion rate was doubled by C. resinae and trebled by sludge and the mixed culture. A summary of all the results monitoring the potential corrosivity of cultures towards mild steel did not predict this to be the case. That is the culture dry weight was relatively low, pH, Eh and Er were relatively unchanged, only sludge reduced the NO_3^- concentration and corrosivity by the linear polarisation method was low. Thus monitoring of the aqueous phase, in the case of Turner's solution, was misleading.

Modified Sea Water

Modified sea water was both inherently more corrosive to the

metals and also less able to support growth of cultures than Turner's solution. Thus microorganisms had to be particularly corrosive in order for a significant increase over controls to be seen. This was especially true for mild steel where the corrosion rate of the uninoculated control was 4.7 mpy. Figure 5.8c shows that the corrosion rate actually decreased in the presence of microorganisms. This protective role was surprising especially with sludge where growth was little affected and pH fell to 3.9. In Turner's solution the pH rose and the corrosion of mild steel was high, thus indicating that pH was not an important factor in the corrosion of mild steel.

Contrary to the results of Chapter 4, this experiment has shown that microorganisms do not enhance corrosion of mild steel in sea water. The difference may be explained by the exposure to both adherent growth and extracellular metabolites in the previous experiment but only to the latter in this case. This conforms to the observation that corrosion of unprotected mild steel fuel tanks is usually found only beneath adherent growth.

Cupronickel has often been considered to be fairly resistant to microbial corrosion because of the toxic effects of copper. This may be true for C. resinae but the corrosion rate was greatly enhanced, 21-fold and 15-fold, by the mixed and sludge cultures respectively.

Stainless steel was fairly resistant to corrosion by modified sea water; its corrosion rate was doubled by C. resinae but little effected by mixed and sludge cultures.

Aluminium was not affected by the mixed culture though its corrosion rate was doubled by C. resinae and sludge. Thus, for aluminium at least, C. resinae represented the corrosivity of fuel sludge flora.

Much of the published work on microbial corrosion has dealt with the problem of aluminium, mainly because it was used in aircraft fuel tanks, an area where serious research began. The style of research undertaken has propagated the idea that C. resinae is both the major fuel contaminant and also the major corrosive agent. However, this research has shown that C. resinae was not necessarily representative of fuel sludge flora and was not corrosive to all metals.

As regards cupronickel where microbial corrosion was most

evident, organism dry weight, redox potential and corrosion rate measured by linear polarisation all indicated greater corrosivity of the mixed and sludge cultures than of those of C. resinae. Although the various methods of monitoring the corrosivity of the aqueous phase were inadequate for Turner's solution they were more useful in modified sea water. Thus the aqueous environment is not only a major determinant of the growth and corrosive activity of the microorganisms but is also of ultimate significance to the type of monitoring system used to measure corrosivity.

5.3 Conclusions

1. Given sufficient time to develop sludge grew to a greater extent than the mixed culture or C. resinae in both mineral solutions. Thus the amount of growth was not adequately represented by laboratory cultures.

2. In general, the presence of metals stimulated growth in Turner's solution but inhibited it in sea water. Thus the physicochemical characteristics of the environment attenuates or potentiates the 'toxicity' to microbes of metals deposited in it.

3. Little pH change was observed in Turner's solution. In the absence of metals the pH of modified sea water fell rapidly; the sludge culture having the greatest effect.

4. Of the metals examined only stainless steel did not increase the final pH of the culture filtrates in modified sea water. Thus in the presence of corroding metals, pH may not adequately represent the acidity caused by contaminating microorganisms.

5. Redox potential measurements in Turner's solution did not differ significantly for control and culture filtrates and generally indicated a reduction in corrosivity. In modified sea water an increase in corrosivity was indicated but again the differences were insufficient to recommend the use of Eh monitoring.

6. Nitrate was removed from Turner's solution by C. resinae and sludge cultures to the extent of 35 and 92% respectively. A mechanism of corrosion may be the use of NO_3^- as a hydrogen acceptor thus depolarising the cathodic reaction. In addition the removal of NO_3^- as a corrosion inhibitor of aluminium may have accelerated its corrosion over the control.

7. After 12 and 21 weeks the corrosivity as measured by the corrosion rate of cupronickel from linear polarisation was shown to be less in the culture filtrates than in uninoculated Turner's solution. In modified sea water only C. resinae or cupronickel containing filtrates increased the corrosivity. This technique proved very sensitive to the physicochemical environment and unless suitable controls can be developed monitoring will not be reliable.

8. Corrosion potential values were much reduced in culture filtrates of Turner's solution indicating increased corrosivity. However, in sea water small differences were observed and they tended to be in the positive direction. This parameter may only be of use in certain aqueous environments, non-contaminated controls of which would be difficult to represent in the continually changing and variable fuel system.

9. The most corrosive culture in Turner's solution as regards weight loss of metal coupons depended on the exposure time and the metal under test. Mild steel suffered most from microbial corrosion, especially by sludge and the mixed culture. Thus C. resinae was not truly representative of fuel tank sludge flora.

10. Except in the case of cupronickel microorganisms did not significantly increase the corrosion rate of metals in modified sea water. Again the recipient environment into which metals and potentially corrosive metabolites are deposited was shown to be a major factor in the relative corrosivity of microorganisms.

5.4 Summary

The experiment has shown the difficulties of choosing a representative microbial system for studying corrosion. The corrosivity of a culture depended on the metal under test, the exposure time and the aqueous environment. The use of a pure culture of *C. resinae* to represent such a diverse flora as found in fuel tank sludge was questionable. As one of many contaminants it modifies and is modified by the growth and activity of other microbes. The choice of a mixed culture was also often not representative of the corrosivity of sludge. It was evident that corrosion studies of a particular metal system could only be meaningful if samples of in-service contamination were used.

The parameters for monitoring contamination were largely dependant on the aqueous environment and the effect of the corroding metal in it. In ship's fuel systems the aqueous environment is very variable. Thus the monitoring by any one of or a group of parameters as described in this experiment cannot be considered to be a reliable exercise. It seems probable that any laboratory test would not adequately represent in-service conditions, thus leading to the necessity for continuous protection of structures.

CHAPTER 6

CONTINUOUS MONITORING OF THE CORROSIVITY OF C. RESINAE AT THE UNDECANE/AQUEOUS PHASE INTERFACE AND IN THE CULTURE MEDIA USING THE LINEAR POLARISATION TECHNIQUE

6.1 Introduction

Despite several reports implicating C. resinae as a corrosive force little work has been done to distinguish the corrosive effect of extracellular products from that of mycelial contact. The study of corrosion using microscopy in Chapter 4 illustrated that the degree of corrosion was far more severe in the area of growth than in areas exposed solely to fungal metabolites. Increased weight loss was evidence of microbiological corrosion but, at the sampling times chosen, quite often little difference was observed between the corrosivity of the test and control filtrates using the linear polarisation technique.

An initial experiment, similar to that described in Section 2.4.3 except that only a fully immersed electrode was used, showed that cupronickel corroded much more rapidly in Turner's solution containing actively growing C. resinae than in sterile Turners solution. Thus, at least in the first few weeks of growth, the technique was a useful monitor of increasing corrosivity due to a microbially modified environment. It's use as a continuous monitoring system in ship's fuel systems was considered during the following experiments which were designed to allow a comparison between the increase in corrosion rate caused by adherent C. resinae and that caused by it's extracellular products. It was envisaged that the relative importance of the possible mechanisms of corrosion in each case might be evaluated.

6.2 Results and Discussion

Only one set of results are presented for each variable. Duplicate tests did not yield identical results largely due to the variable growth characteristics of C. resinae.

Preceding each linear polarisation resistance measurement the free corrosion potential was recorded. The following general trends were observed throughout the experiments:

1. The test corrosion potentials were more negative than those of the control indicating that C. resinae increased the corrosivity of it's environment.

2. The test corrosion potentials became increasingly more negative indicating increased corrosivity with growth.

3. The control corrosion potentials became increasingly more positive indicating that the metal was becoming less susceptible to corrosion probably because of the formation of a passivating film on it's surface.

4. Although the corrosion rates were always greater in sea water the first three observations were most significant in Turners solution.

The trend of the corrosion potential values was of particular interest because of work by Jones and Greene[105] who examined localised crevice corrosion of stainless steel in 3% NaCl by the linear polarisation method. They noted three significant features of localised corrosion

a) the total corrosion current increases

b) a significant active shift in corrosion potential occurs, i.e. more negative

c) erratic corrosion potential fluctuations occur in the early stages.

In the following experiment only the first of these features was observed on a regular basis. Often there was an associated dramatic increase in corrosion potential. No erratic fluctuations were noted though measurements were only taken every few days and the actual initiation of localised corrosion may have been missed.

The pH was recorded regularly to coincide with polarisation measurements. No pH fall was observed in Turner's solution though in modified sea water from an initial pH of 6.5, cupronickel raised the value to 7.2, mild steel reduced it to 6.1 and stainless steel had no effect. The growth of C. resinae reduced the pH to 3.1, 4.0 and 5.0 in 8 days, to 3.1, 4.0 and 3.1 in 17 days and to 3.4, 3.5 and 2.7 in 32 days for cupronickel, mild steel and stainless steel respectively.

During the first few hours of the experiment the corrosion rates were exceptionally high because the freshly prepared metal electrodes were very reactive; hence corrosion rates were only reported after 24 hours equilibration.

Table 6.1 shows the total range of percentage increase in corrosion rate of the test over the control for each variable. Although the mean value tells us little since the corrosion rate was being continually modified it was some indication of the average response to the growth of C. resinae.

In order to compare the corrosion rates caused by C. resinae at the interfacial (I) and fully immersed (FI) positions the intrinsic difference as exhibited by the uninoculated system had to be accounted for. Such an intrinsic difference may arise from both variation in the type of electrolytic environment and in the oxygen levels at the two positions in the system. Thus the control corrosion rate was subtracted from its respective test corrosion rate for each position and the final values, the corrected corrosion rates, were compared to each other. The corrosion rates in the test and control systems were recorded in Tables 6.2 - 6.8 and the effect on the fully immersed and interfacial metal electrodes illustrated in Figures 6.1 - 6.7. The results for each metal are considered separately.

Table 6.1

To Show the Average Percentage Increase in Corrosion Rate of the Test over the Control and it's Total Range for each Variable

Electrode System		Percentage Increase in Corrosion Rate			
		Turner's Solution		Modified Sea Water	
		Range	$\bar{\alpha}$	Range	$\bar{\alpha}$
Stainless steel:	FI	20 - 106	48	12 - 158	84
	I	3 - 100	44	8 - 142	58
Mild steel:	FI	6 - 1337	345	-24 - 124	21
	I	50 - 8700	1649	- 41 - 88	13
Cupronickel:	FI	-22 - 3200	854	-30 - 678	163
	I	-10 - 644	166	-34 - 1490	442
Aluminium:	FI	-51 - 800	287	-	-
	I	112 - 425	320	-	-

Key:

Percentage Increase =

$$\frac{\text{Test corrosion rate - Control corrosion rate x 100}}{\text{Control corrosion rate}}$$

6.2.1 Stainless Steel

At all times the corrosion rates in the test systems were greater than those in the respective control systems (Tables 6.2 and 6.3). In the test systems the fully immersed electrodes showed a greater average percentage increase than the interfacial electrodes (Table 6.1). In both positions C. resinae had its greatest effect in modified sea water.

In Turner's solution the corrected corrosion rate of the interfacial electrode, increased steadily for the first ten days when it became fairly stable (Figure 6.1). Peaks in this corrosion rate occurred on days 4 and 21 which may have corresponded to the onset of localised corrosion. The corrected corrosion rate of the fully immersed electrode rose fairly steadily from three to 19 days after which it fell rapidly.

In modified sea water the corrosion rate of the interfacial electrode peaked on day 7 and then dropped for the remainder of the experiment (Figure 6.2). The corrosion rate of the fully immersed electrode rose fairly steadily throughout the experiment.

Whether C. resinae exerted its major effect on either the interfacial or the fully immersed electrode depended both on the mineral solution supporting growth and corrosion, and on the exposure time. In Turner's solution the fully immersed electrode was almost always more severely corroded. In modified sea water the interfacial was greater than the fully immersed corrosion rate for the first 12 days when the position was reversed. Thus, it would appear that during the first few days the onset of germination and adherent growth at the interface increased corrosion in this area. Further growth did not accelerate the corrosion rate of the interfacial electrode; indeed it appeared to protect it. The steady rise in corrosion rate of the electrode fully immersed in modified sea water may have been due to the increasing amounts of corrosive metabolites, for example acids, being excreted into the mineral solution as the pH fell from 6.5 to 2.7 during the 35 day experiment. However, a similar though

Table 6.2

To Show the Test, Control and Corrected Corrosion Rates of the Stainless steel Electrodes at the Interface of and fully Immersed in Turner's Solution and Undecane ± C. resinae

Exposure Time (Days)	Fully Immersed Stainless Steel			Interfacial Stainless Steel		
	TEST mpy x 10^{-5}	CONTROL mpy x 10^{-5}	Corrected corrosion rate	TEST mpy x 10^{-5}	CONTROL mpy x 10^{-5}	Corrected corrosion rate
1	12.0	8.2	3.8	10.2	9.1	1.1
2	12.0	9.2	2.8	10.0	8.2	1.8
3	12.0	10.0	2.0	10.2	8.2	2.0
4	12.7	10.0	2.7	11.0	7.1	3.9
6	15.6	10.8	4.8	11.2	8.8	2.4
7	14.6	9.6	5.0	11.8	9.0	2.8
8	13.2	9.6	3.4	12.6	9.0	3.6
10	13.8	9.2	4.6	12.0	8.1	3.9
12	13.2	8.6	4.6	12.6	8.4	4.2
14	14.6	9.3	5.3	11.9	8.2	3.7
15	14.6	8.4	6.2	11.0	7.3	3.7
18	15.8	9.2	6.6	11.0	6.4	3.6
19	18.5	9.0	9.5	10.2	6.4	3.8
21	12.0	8.4	3.6	11.0	5.5	5.5
22	12.0	9.2	2.8	10.2	7.3	2.9
25	10.2	8.4	1.8	10.0	6.4	3.6

less pronounced rise in corrosion rate was observed in Turner's solution where no pH fall was apparent. Thus, the type and concentration of extracellular products and their effect,

Table 6.3

To show the Test, Control and Corrected Corrosion Rates of Stainless Steel Electrodes at the Interface of and Fully Immersed in Modified Sea Water and Undecane ± C. resinae

	Fully Immersed Stainless Steel			Interfacial Stainless Steel		
DAYS	Test mpy x 10^{-5}	Control mpy x 10^{-5}	T-C	Test mpy x 10^{-5}	Control mpy x 10^{-5}	T-C
3	10.3	9.1	1.2	16.0	8.0	8.0
4	8.5	6.9	1.6	15.0	8.0	7.0
5	10.3	6.9	7.4	14.4	9.2	5.2
6	13.7	11.0	2.7	16.4	10.0	6.4
7	18.5	13.0	5.5	21.9	8.8	13.1
10	15.8	12.3	3.5	12.6	5.0	7.6
13	11.6	6.2	5.4	11.6	7.2	4.4
15	17.8	8.2	9.6	10.3	6.8	3.5
17	15.8	6.2	9.6	10.1	6.2	3.9
21	15.8	7.5	8.3	11.6	4.8	6.8
24	19.9	10.3	10.2	11.6	6.2	5.4
26	21.9	8.9	13.0	13.0	10.6	3.6
32	27.2	10.8	16.4	17.8	13.2	4.6
35	29.4	12.3	17.1	15.1	14.0	1.1

Fig. 6.1

To Show the Corrected Corrosion Rates of a Stainless Steel Electrode at the Interface of and Fully Immersed in Turner's Solution and Undecane plus C. resinae

Fig. 6.2

To Show the Corrected Corrosion Rates of a Stainless Steel Electrode at the Interface of and Fully Immersed in Modified Sea Water and Undecane plus C. resinae

Key for Figures 6.1 and 6.2:

- ● Fully Immersed Electrode
- ▲ Interfacial Electrode

Figure 6.1

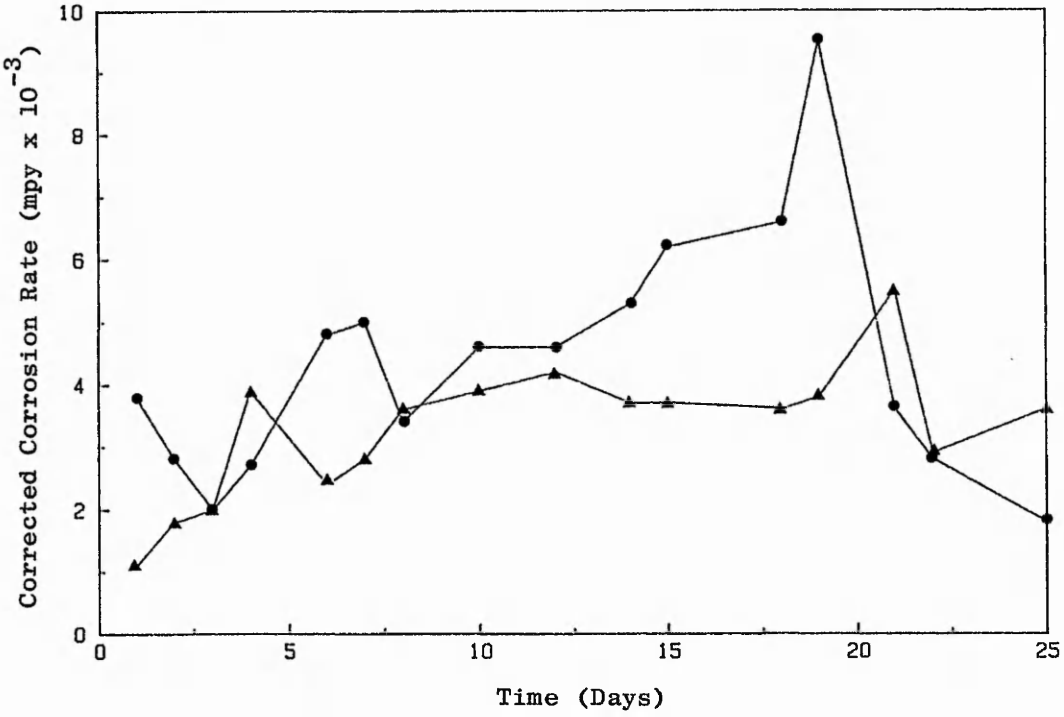
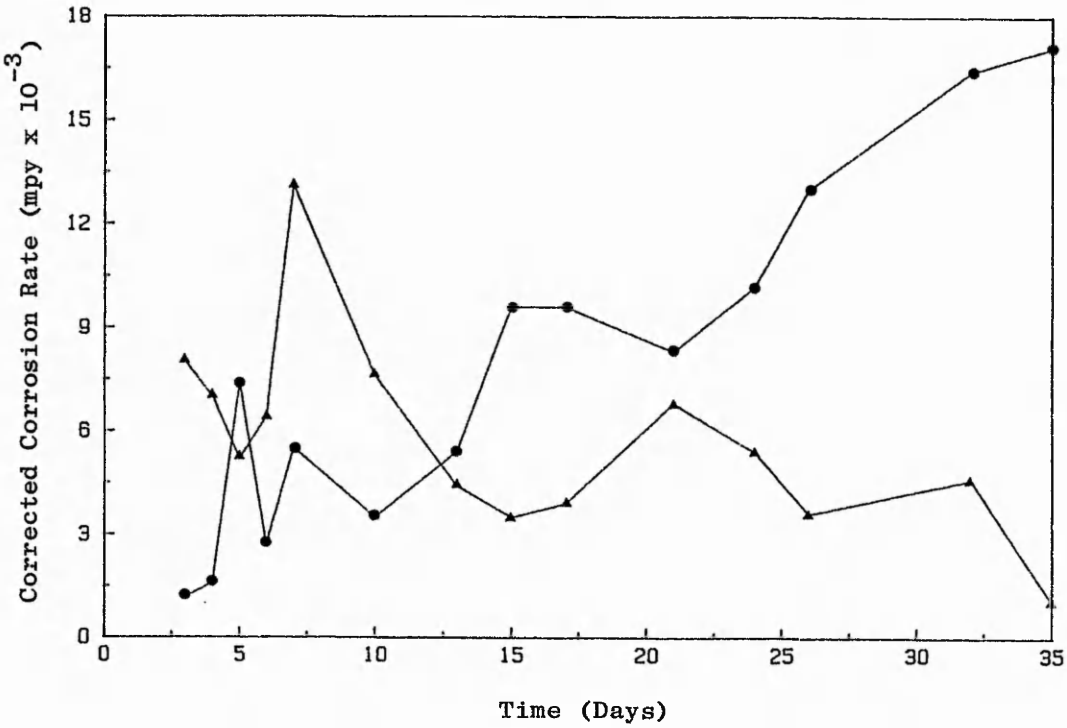


Figure 6.2



other than altering pH, on the recipient environment were important factors in corrosion. The sudden fall in corrosion rate of the fully immersed electrode in Turner's solution may be explained by the utilisation of corrosive metabolites. This may not have been observed in modified sea water because it supports a slower growth rate.

6.2.2 Mild Steel

Since the results from Turner's and modified sea water systems were dissimilar, the effect of C. resinae being much greater in Turner's solution, they will be discussed separately.

In Turner's solution the test corrosion rate was significantly greater than that of its respective control (Table 6.4). In the test systems the average percentage increase was greatest for the interfacial electrode (Table 6.1). Figure 6.3 shows that the general trend in corrosion rate was similar at both positions; the interfacial electrode being affected to a greater extent than the fully immersed electrode. The rates remained fairly steady until day 7 when they increased dramatically for the next 7 days after which time they decreased as rapidly as they had increased. The sudden surge may have been influenced by the development of a mat of growth causing adherence and excretion of corrosive products after 7 days. The subsequent reduction in corrosion rate of the fully immersed electrode, which was also observed for stainless steel, coincided with a pH rise from 5.8 to 6.8. It was hypothesised that the mere physical presence of C. resinae would enhance corrosion however it would seem that the corrosivity of adherent growth depended on localised excretion of corrosive products. The interfacial electrode however did maintain a corrosion rate somewhat greater than that of the fully immersed electrode indicating that adherent growth was more corrosive than the modified environment.

In modified sea water the test corrosion rate was not always greater than its respective control (Table 6.5). In the test systems the greatest % increase in corrosion rate was on average exhibited by the fully immersed electrode (Table 6.1). Within eight days growth

Table 6.4

To Show the Test, Control and Corrected Corrosion Rates of the Mild Steel Electrodes at the Interface of and fully Immersed in Turner's Solution and undecane ± C. resinae

DAYS	Fully Immersed Mild Steel			Interfacial Mild Steel		
	Test mpy x 10^{-4}	Control mpy x 10^{-4}	Corrected Corrosion rate	Test mpy x 10^{-4}	Control mpy x 10^{-4}	Corrected Corrosion rate
1	15.2	7.6	7.2	14.0	5.4	8.6
2	8.4	3.4	5.0	8.4	2.2	6.2
3	6.9	3.0	3.9	6.9	2.3	4.6
4	6.9	2.3	4.6	6.5	2.6	3.9
5	5.7	3.6	2.1	8.5	2.3	6.2
6	4.1	1.9	2.2	10.6	2.7	7.9
7	8.2	1.7	6.5	12.5	1.5	11.0
8	3.6	1.9	1.7	21.0	1.1	20.9
11	54.0	4.0	50.0	114.0	1.5	112.5
14	84.8	5.9	78.9	184.8	2.1	182.7
15	33.0	3.2	29.8	62.0	1.3	60.7
18	22.3	2.8	19.5	23.0	1.4	21.6
20	11.4	4.0	7.4	14.0	1.9	12.1
22	7.6	1.5	6.1	13.2	1.7	11.5
24	6.9	2.6	4.3	10.2	1.7	8.5
26	3.6	3.4	0.2	5.8	1.5	3.3
30	2.4	2.2	0.2	2.6	1.5	1.1

Table 6.5

To Show the Test, Control and Corrected Corrosion rates of the mild steel Electrodes at the Interface of and fully Immersed in Modified Sea Water and undecane ± C. resinae

	Fully Immersed Mild Steel			Interfacial Mild Steel		
DAYS	Test mpy x 10^{-3}	Control mpy x 10^{-3}	Corrected Corrosion Rate	Test mpy x 10^{-3}	Control mpy x 10^{-3}	Corrected Corrosion Rate
1	15.6	19.2	-3.6	19.6	16.2	3.4
2	16.4	18.4	-2.0	19.2	16.6	3.0
3	14.0	18.0	-4.0	18.8	16.0	2.8
4	14.8	17.2	-2.4	17.6	15.0	2.6
5	13.2	17.4	-4.2	20.8	14.0	6.8
6	12.8	16.2	-3.4	24.8	13.2	11.6
7	14.4	13.4	1.0	22.0	14.0	8.0
8	14.4	14.0	0.4	20.0	15.2	4.8
10	13.2	12.8	0.4	16.0	14.0	2.0
12	12.8	11.6	1.2	16.0	12.4	3.6
13	14.4	11.4	3.0	15.2	12.8	2.4
14	10.8	10.2	0.6	12.8	12.8	0
15	9.2	10.0	-0.8	10.6	12.6	-2.0
17	10.0	9.6	0.4	8.0	12.4	-4.4
20	10.2	10.4	-0.2	8.8	12.8	-4.0
21	10.4	10.6	-0.2	7.6	12.8	-5.2
24	16.2	10.6	5.6	10.6	12.4	-1.8
27	19.2	10.6	8.6	12.0	12.2	-0.2
29	18.0	9.6	8.4	11.2	11.4	-0.2
32	17.2	9.4	7.8	10.8	10.8	0
35	19.4	9.6	9.8	13.6	10.4	3.2
40	22.4	10.0	12.4	12.8	10.0	2.8

Fig. 6.3

To show the Corrected Corrosion Rates of a Mild Steel Electrode at the Interface of and Fully Immersed in Turner's Solution and Undecane plus C. resinae

Fig. 6.4

To Show the Corrected Corrosion Rates of a Mild Steel Electrode at the Interface of and Fully Immersed in Modified Sea Water and Undecane plus C. resinae

Key for Figures 6.3 and 6.4:

- ● Fully Immersed Electrode
- ▲ Interfacial Electrode

Figure 6.3

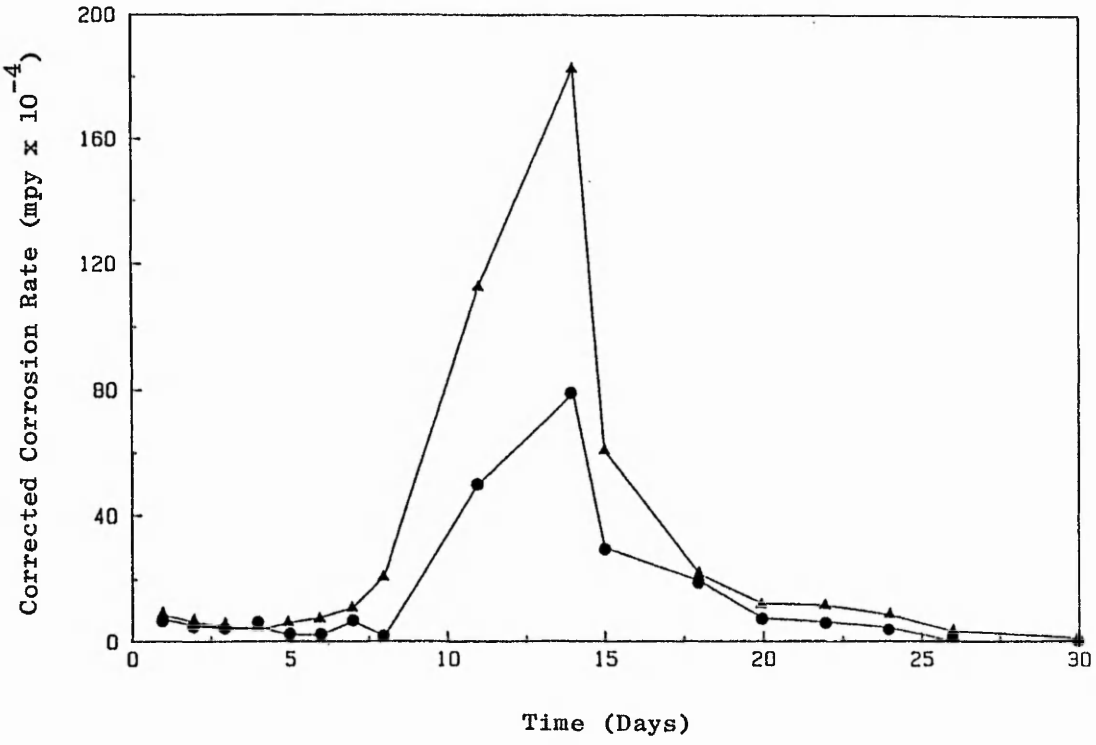
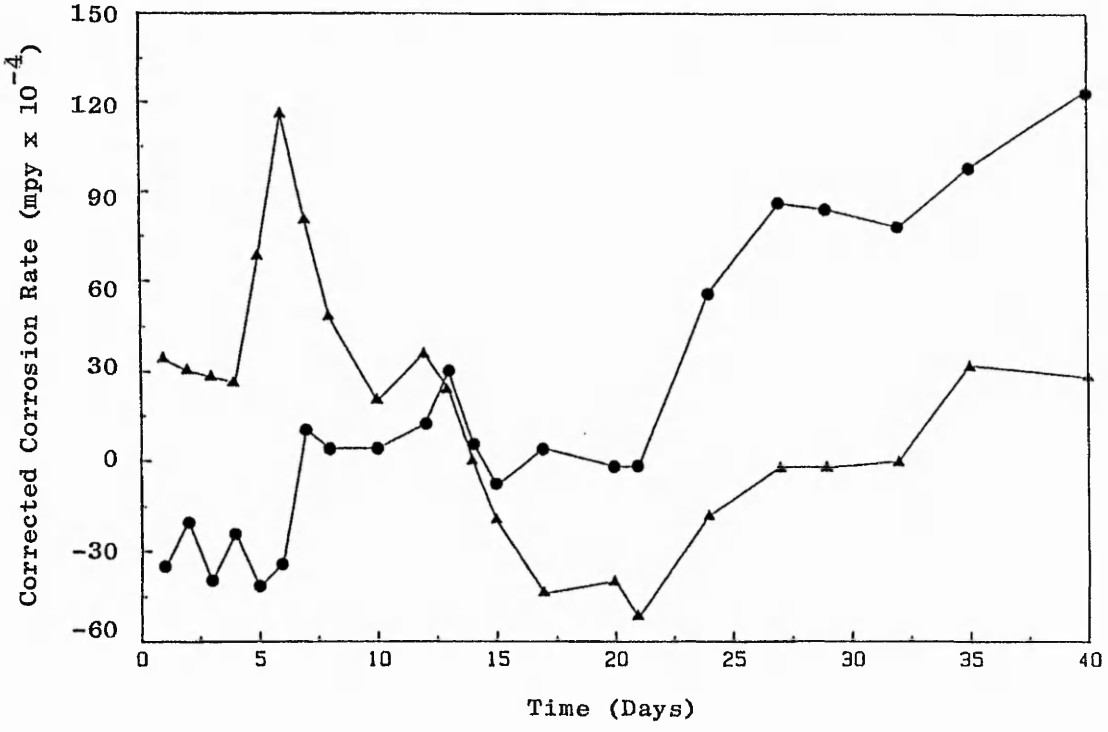


Figure 6.4



was apparent and the pH had fallen from 6.5 to 4.0. The corrosion rate of the interfacial electrode had begun to fall from its peak value by this time (Figure 6.4) indicating that the germination process had been important in accelerating corrosion but that subsequent growth was not as corrosive. In fact, as growth continued the corrosion rate of the test fell to below that of the control indicating a protective role of C. resinae towards mild steel in sea water. The corrosion rate of the fully immersed electrode was less than or approximately equal to that of its control for the first 21 days of exposure. Sea water was intrinsically corrosive to mild steel and the corrosive nature of C. resinae would have to be severe to significantly increase the corrosion rate. It would appear that after 21 days a corrosive product was excreted in sufficient quantities to cause enhanced corrosion of mild steel.

Overall, C. resinae was initially corrosive at the interface but it soon became non-corrosive and even protective. At about this time the environment had become sufficiently modified to increase the corrosion rate of the fully immersed electrode.

6.2.3 Cupronickel

After five days a mat of growth (factor 3) had developed in Turner's solution which caused the corrosion rates of the electrodes to rise above those of their respective controls (Table 6.6). On average the fully immersed electrode showed a greater percentage increase than the interfacial electrode (Table 6.1). For the first ten days the corrosion rate increased at the interface at which time it began to fall until by day 18 it was no greater than that of the control (Figure 6.5) though it began to slowly rise again after day 27. Again it was illustrated that germination and initial growth, with possible adhesion, enhanced corrosion but that further growth had no effect until perhaps cell disintegration allowed release of corrosive intracellular products.

The corrosion rate of the fully immersed electrode was slightly more than that of the control for the first 20 days when it began to rise rapidly until day 31 when it started to fall again. An

Table 6.6

To Show the Test, Control and Corrected Corrosion Rates of the Cupronickel Electrodes at the Interface of and fully Immersed in Turner's Solution and Undecane ± *C. resinae*

Days	Fully Immersed Cupronickel			Interfacial Cupronickel		
	Test mpy x 10^{-3}	Control mpy x 10^{-3}	Corrected corrosion rate	Test mpy x 10^{-3}	Control mpy x 10^{-3}	Corrected corrosion rate
1	0.84	1.02	-0.18	1.19	1.15	0.04
3	0.39	0.50	-0.11	1.26	0.74	0.52
6	0.77	0.25	0.52	1.34	0.43	0.91
8	0.79	0.22	0.57	1.94	0.39	1.55
10	0.62	0.17	0.45	2.34	0.34	2.00
12	0.82	0.20	0.60	2.26	0.32	1.94
14	0.84	0.15	0.69	1.87	0.30	1.57
15	0.92	0.10	0.82	1.04	0.24	0.8
16	0.62	0.17	0.45	0.64	0.28	0.36
17	0.56	0.07	0.49	0.36	0.32	0.04
18	0.56	0.22	0.34	0.26	0.29	-0.03
19	0.40	0.20	0.20	0.35	0.26	0.09
20	0.50	0.20	0.30	0.30	0.24	0.06
21	1.59	0.35	1.24	0.30	0.26	0.04
22	1.81	0.40	1.41	0.35	0.28	0.07
23	2.68	0.82	1.86	0.40	0.30	0.10
24	3.48	0.30	3.18	0.35	0.30	0.05
25	1.74	0.35	1.39	0.35	0.28	0.07
26	2.73	0.32	2.41	0.30	0.26	0.04
27	2.92	0.20	2.72	0.35	0.24	0.11
28	7.26	0.22	7.04	0.40	0.22	0.18
29	7.82	0.32	7.50	0.50	0.20	0.30
30	7.82	0.40	7.42	0.50	0.20	0.30
31	8.07	0.45	7.62	0.60	0.19	0.41
32	7.14	0.32	6.82	0.72	0.18	0.54
35	5.83	0.20	5.63	0.81	0.20	0.61

Fig. 6.5

To Show the Corrected Corrosion Rates of a Cupronickel Electrode at the Interface of and Fully Immersed in Turner's Solution and Undecane plus *C. resinae*

Fig. 6.6

To Show the Corrected Corrosion Rates of a Cupronickel Electrode at the Interface of and Fully Immersed in Modified Sea Water and Undecane plus *C. resinae*

Key for Figures 6.5 and 6.6:

- ● Fully Immersed Electrode
- ▲ Interfacial Electrode

Figure 6.5

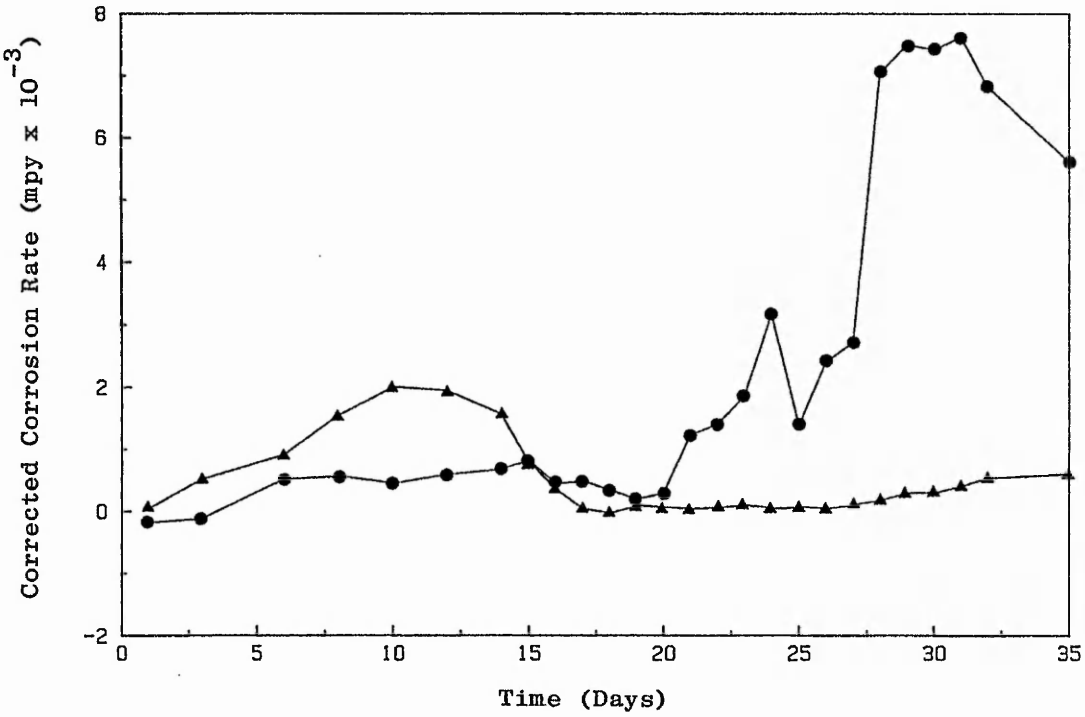
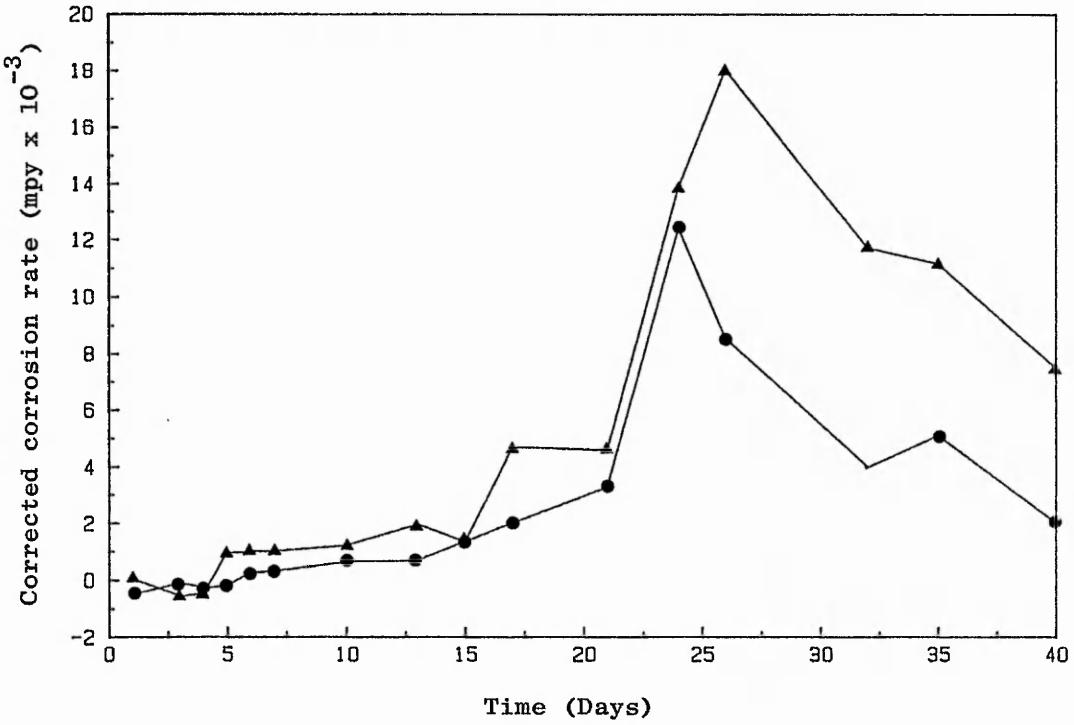


Figure 6.6



explanation might be that the concentration of extracellular metabolites did not build up to a sufficient level to cause corrosion of cupronickel until day 20. After this time increasing concentrations further enhanced corrosion. Subsequent utilisation of these products may have reduced the corrosivity of the environment.

In modified sea water it took six days for the test corrosion rates to exceed those of the control. The effect of growth was much greater than in Turner's solution (Table 6.7). In the test system the interfacial electrode corroded to a greater extent than the fully immersed electrode, though both exhibited a similar trend (Figure 6.6). A mat of growth was established within ten days, the pH having fallen to 3.2 after eight days. The effect of growth on cupronickel at the interface manifested itself after six days though it was not until day 15 that the corrosion rate began to rise rapidly. Close contact and subsequent localisation of products at the interface caused the corrosion rate to increase further and for a longer period than the immersed electrode. Tentative suggestions to explain the subsequent fall in corrosion rate have been described for other systems. It was evident that the type and concentrations of products were of more importance than the subsequent pH since this did not fall below 3.2, a value reached after only eight days. In addition, recent work[177] showed that bacterial contamination, particularly by pseudomonads, of cupronickels led to the loss of passivation, and that the corrosion rate was determined by the rate of oxygen diffusion through the slime. This may explain the fall in corrosion rate of the interfacial electrode as adherent growth increased.

6.2.4 Aluminium

Aluminium electrodes would not produce normal polarisation curves in modified sea water and no results are reported. In Turner's Solution the test electrodes had higher corrosion rates than their respective controls (Table 6.8). In the test system the interfacial electrode corroded much more rapidly than the immersed electrode. The corrosion rate of the interfacial electrode began to rise immediately and continued to do so throughout the exposure time (Figure 6.7).

Table 6.7

To Show the Test, Control and Corrected Corrosion Rates of the Cupronickel Electrodes at the Interface of and Fully Immersed in Turner's Solution and Undecane ± C. resinae

Days	Fully Immersed Cupronickel			Interfacial Cupronickel		
	Test mpy x 10^{-3}	Control mpy x 10^{-3}	Correctaed corrosion rate	Test mpy x 10^{-3}	Control mpy x 10^{-3}	Corrected corrosion rate
1	1.15	1.64	-0.49	1.12	1.09	0.03
3	1.34	1.44	-0.10	1.08	1.64	-0.56
4	1.15	1.42	-0.27	1.34	1.79	-0.45
5	1.04	1.21	-0.17	2.83	1.87	0.96
6	1.56	1.29	0.27	2.91	1.91	1.00
7	1.64	1.32	0.32	3.05	2.02	1.03
10	2.09	1.43	0.66	3.43	2.20	1.23
13	2.31	1.60	0.71	4.02	2.06	1.96
15	3.05	1.70	1.35	4.28	2.95	1.33
17	4.02	2.00	2.02	7.45	2.76	4.69
21	5.29	2.00	3.29	6.40	1.82	4.58
24	14.24	1.83	12.41	15.42	1.56	13.86
26	10.13	1.61	8.52	19.36	1.34	18.02
32	5.44	1.46	3.98	12.66	0.97	11.69
35	6.18	1.09	5.09	11.92	0.78	11.14
40	3.43	1.42	2.01	8.27	0.52	7.49

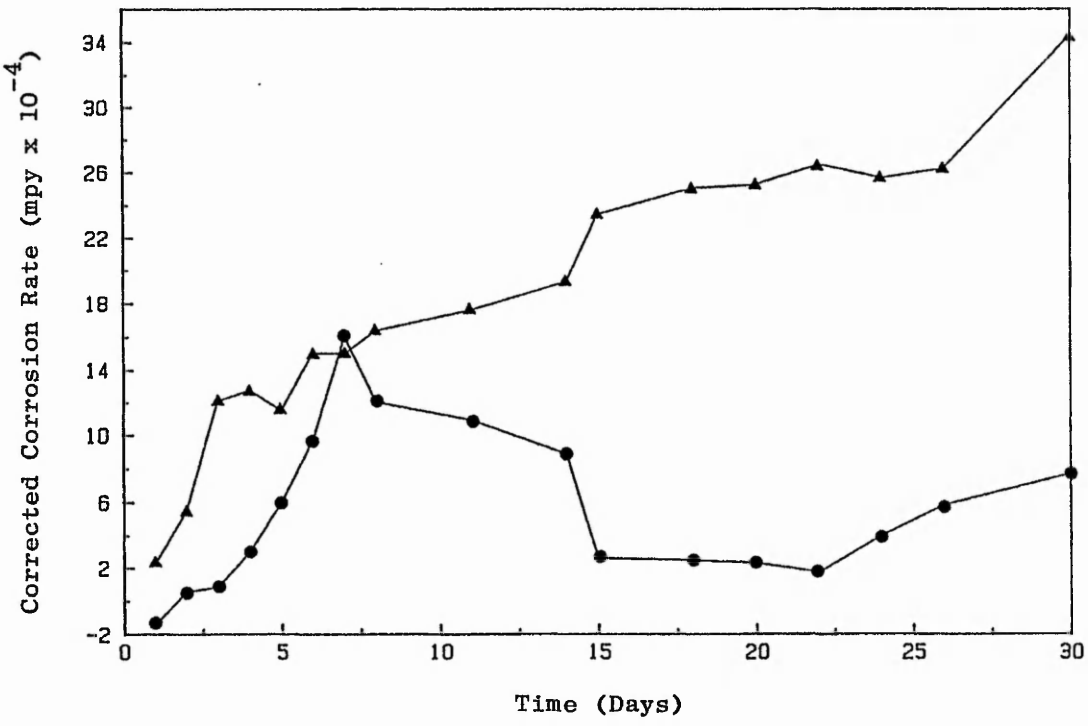
Table 6.8

To Show the Test, Control and Corrected Corrosion Rates of the Aluminium Electrodes at the Interface of and fully Immersed in Turner's Solution and undecane ± C. resinae

Days	Fully Immersed Aluminium			Interfacial Aluminium		
	Test mpy x 10^{-4}	Control mpy x 10^{-4}	Corrected corrosion rate	Test mpy x 10^{-4}	Control mpy x 10^{-4}	Corrected corrosion rate
1	1.32	2.72	-1.4	4.29	2.02	2.27
2	2.72	2.15	0.57	8.01	2.51	5.50
3	3.29	2.43	0.86	15.59	3.49	12.10
4	4.30	1.29	3.01	16.10	3.36	12.74
5	7.87	1.86	6.01	16.20	4.61	11.49
6	11.30	1.64	9.66	20.00	5.01	14.99
7	18.00	2.00	16.00	20.50	5.54	14.96
8	14.30	2.29	12.01	21.50	5.12	16.38
11	12.90	2.00	10.90	23.20	5.59	17.61
14	10.70	1.86	8.84	23.88	4.56	19.32
15	4.57	2.00	2.57	29.00	5.52	23.48
18	4.29	1.86	2.43	31.60	6.53	25.07
20	4.57	2.29	2.28	32.50	7.21	25.29
22	3.72	2.00	1.72	33.75	7.26	26.49
24	7.01	3.00	4.01	33.75	8.06	25.69
26	8.01	2.15	5.86	34.89	8.62	26.27
30	10.15	2.43	7.72	43.47	9.11	34.36

Figure 6.7

To Show the Corrected Corrosion Rates of a Aluminium Electrode at the Interface of and Fully Immersed in Turner's Solution and Undecane plus C. resinae



Key: ● Fully Immersed Electrode

▲ Interfacial Electrode

Germination, growth and subsequent adherence of C. resinae greatly accelerated corrosion of aluminium in Turner's Solution. The corrosion rate of the immersed electrode only increased until day 7 when it fell, stabilised between day 15 and day 22 and then rose again. The rather short optimal corrosivity period may have been due to the formation of a protective film which was subsequently broken down as the environment became increasingly corrosive.

6.3 Conclusion

The corrosive effect of C. resinae depended on both the metal under study and the aqueous environment. With the exception of mild steel/sea water its presence significantly increased the corrosion rate of both the interfacial and fully immersed electrodes. In addition, with the exception of stainless steel/Turner's solution, the interfacial electrode initially if not continually exhibited higher corrosion rates than the immersed electrode. Thus in the majority of cases the corrosion mechanisms may have been as follows:

1. Localisation of extracellular products causing a concentrated effect of corrosive metabolites possibly associated with a large pH change.

2. Localisation of extracellular (or intracellular upon cell lysis) enzymes which may catalyse electron requiring reactions and hence drive the cathodic corrosion reaction.

3. Differential aeration between interfacial growth and the remainder of the metal.

4. Accumulation of metal ions from the surface thus driving the anodic corrosion reaction.

In addition, recent work by Scotto et al.[180] showed that stainless steel surface colonisation by bacterial and algal populations altered the cathodic oxygen reduction process. They found

that this phenomenon facilitated the initiation of localised attack and heightened their progress and extension, corroborating the fact that natural sea water is more aggressive than sterile sea water of equivalent salt content.

The first two mechanisms may have occurred in the growth medium but due to the effects of dilution would take longer to become apparent. It was often the case that as the medium became more corrosive, the interface became less so. The adherent growth may have become less corrosive or even protective for one or more of the following reasons:-

1. Utilisation of localised metabolites.

2. Slight detachment as the culture aged.

3. A continuous layer of growth on the metal surface either giving protection from the external corrosive environment or acting as a barrier to oxygen diffusion.

4. Inhibition or death of C. resinae due to a toxic build-up of metal ions.

Further experimentation is required before the exact mechanisms of corrosion can be determined. It was difficult to make a complete distinction between the effect of physical contact causing, as any debris might, crevice corrosion and the effect of either localised products or differential aeration by growth in close contact. An inert substance could never simulate hyphal attachment and dead hyphae could still exert a localised effect due to cell lysis.

It was evident that physical contact was not necessary for microbial corrosion to occur but that in several instances such contact did enhance corrosion. It was significant that aluminium was one metal particularly susceptible to adherent growth. In general, laboratory experiments using aluminium have shown that C. resinae renders culture media more corrosive and examination of fuel tanks has shown areas under adherent growth to be particularly corroded. This

has led to a general belief that localisation of metabolites and differential aeration causes enhanced corrosion of all metals. This experiment has shown that each metal was susceptible to C. resinae in different ways at different times and that this susceptibility was influenced by the aqueous environment. Thus, the metal, C. resinae and the environment all interacted with each other. The overall corrosive effect depending on the dominant mechanism of corrosion under the prevailing conditions.

More information may have been gleaned by improvements in the experimental design. For example a horizontally placed interfacial electrode would have allowed growth across the entire surface and would have provided a better comparison with the fully immersed electrode. This is not without its problems since an upward facing immersed electrode would be prone to growth on its surface from detached C. resinae and one which faced downwards may suffer polarisation effects if the evolved hydrogen could not escape. More conclusive results may have been provided with the use of a larger vessel allowing duplicate electrodes to be placed in the same system. However, growth of C. resinae and hence its corrosive effect may be expected to vary considerably with flask size, ratio of undecane to aqueous phase and surface area of the interface. In addition the larger the aqueous phase the more diluted the extracellular products will become which in turn would affect the corrosion rate of the immersed electrode.

The usefulness of a continual linear polarisation system to monitor contamination and corrosivity in displaced fuel tanks is still not proved. The interfacial level is continually moving due to turbulence, fuel use, water ballast and refuelling. The method might prove more useful in non-displaced tanks with only a small and fairly constant water bottom particularly if a metal susceptible to microbial corrosion and which did not form a strongly protective film in the uncontaminated environment was chosen. Regular use of freshly prepared immersed electrodes may be less complicated though the results must be interpreted carefully since this experiment has shown that corrosivity of the aqueous phase was not necessarily indicative of the potential corrosion caused by adherent growth.

CHAPTER 7

THE INVESTIGATION OF FACTORS THOUGHT TO INFLUENCE THE CORROSION RATE OF VARIOUS METALS BY C. RESINAE

This Chapter further examines a variety of factors which, from the results of previous experiments, were postulated as having an effect on the corrosion rate of mild steel, stainless steel, cupronickel and aluminium.

There are four main sections which examine;

1) the role of individual organic acids in corrosion
2) the effect of the metal on C. resinae
3) the effect of adherence of C. resinae
4) the sequestration of metal ions by C. resinae

7.1 The Effect of Individual Organic Acids on the Corrosion Rate of Stainless Steel, Mild Steel, Cupronickel or Aluminium in either Modified Sea Water or Turner's Solution

7.1.1 Introduction

Previous experiments have implicated the role of extracellular products in the corrosion of metals. In several instances a fall in pH accompanied growth induced corrosion. As described in Chapter 4, acid analysis of C. resinae culture filtrates showed the presence of pyruvic, lactic, fumaric, succinic, itaconic, maleic, cis-aconitic, citric and isocitric acids. The aim of this experiment was to determine which, if any, of the acids were particularly corrosive to each metal. The relative concentrations of the acids detected in previous experiments were found to depend on the mineral solution and varied with time. Due to this variability and as the acids were to be examined alone and not in mixtures, an arbitrary value of 1,000 ppm was chosen for each acid. This was in view of the work by McCowen et al.[135] who regarded 1,000 ppm as the concentration necessary to activate organic acid-metal corrosion.

7.1.2 Results and Discussion

The corrosivity of the acids was compared by means of a multiplication factor which was calculated as the test corrosion rate divided by the control corrosion rate. Table 7.1 shows detailed

Table 7.1

To Show the Weight Loss, Corrosion Rate and Multiplication Factor Caused by Various Acids on Stainless Steel, Mild Steel, Cupronickel and Aluminium in Turner's Solution and Modified Sea Water

Metal/ Aqueous phase \ Acid	Control	Pyruvic	Fumaric	Aconitic	Maleic	Succinic	Itaconic	Lactic	Citric	Isocitric	Mixture
STAINLESS STEEL											
MODIFIED SEA WATER											
mg. wt. loss	0.8	4.0	3.1	5.8	11.5	10.0	6.5	3.7	10.6	2.4	4.1
Corrosion rate	0.04	0.21	0.16	0.30	0.60	0.52	0.34	0.19	0.55	0.12	0.21
Multiplication Factor	-	5.25	4.00	7.50	15.00	13.00	8.50	4.75	13.75	3.00	5.25
TURNER'S SOLUTION											
mg. wt. loss	0.6	8.0	3.2	2.7	2.6	2.8	3.5	3.0	2.7	2.1	4.3
Corrosion rate	0.03	0.41	0.17	0.13	0.13	0.15	0.18	0.16	0.14	0.11	0.22
Multiplication Factor	-	13.67	5.67	4.33	4.33	5.00	6.00	5.33	4.67	3.67	7.33
MILD STEEL											
MODIFIED SEA WATER											
mg. wt. loss	578.4	655.1	145.1	492.0	130.1	502.0	80.9	277.8	142.8	301.5	184.4
Corrosion rate	20.08	22.75	5.03	17.08	4.52	17.43	2.81	9.65	4.96	10.47	6.40
Multiplication Factor	-	1.13	0.25	0.85	0.23	0.87	0.14	0.48	0.25	0.52	0.32
TURNER'S SOLUTION											
mg. wt. loss	42.0	37.6	43.0	39.3	49.7	42.4	36.9	30.9	63.7	50.9	41.9
Corrosiion rate	1.46	1.31	1.49	1.36	1.73	1.47	1.28	1.07	2.21	1.77	1.45
Multiplication Factor	-	0.90	1.02	0.93	1.18	1.01	0.88	0.73	1.51	1.21	0.99
CUPRONICKEL											
MODIFIED SEA WATER											
mg. wt. loss	73.4	65.1	64.8	66.5	92.1	83.6	65.0	76.5	74.4	57.0	80.6
Corrosion rate	3.01	2.67	2.65	2.72	3.77	3.43	2.66	3.13	3.05	2.34	3.30
Multiplication Factor	-	0.88	0.88	0.90	1.25	1.14	0.88	1.04	1.01	0.78	1.09
TURNER'S SOLUTION											
mg. wt. loss	14.1	17.4	25.3	8.5	12.9	15.8	26.8	9.9	51.9	28.3	16.9
Corrosion rate	0.58	0.71	1.03	0.35	0.53	6.48	1.10	0.41	2.13	1.16	0.69
Multiplication Factor	-	0.37	1.78	0.60	0.29	11.17	1.90	0.71	3.67	2.00	1.19
ALUMINIUM											
MODIFIED SEA WATER											
mg. wt. loss	14.1	9.4	9.2	46.4	45.5	76.1	10.5	53.4	80.2	14.1	10.7
Corrosion rate	0.41	0.27	0.27	1.37	1.34	2.24	0.31	1.58	2.36	0.41	0.31
Multiplication Factor	-	0.66	0.66	3.34	3.27	5.46	0.76	3.85	5.76	1.00	0.75
TURNER'S SOLUTION											
mg. wt. loss	1.4	9.3	0.7	0.5	1.2	12.5	4.4	33.0	3.4	1.4	1.3
Corrosion rate	0.04	0.27	0.02	0.01	0.03	0.37	0.13	0.97	0.10	0.04	0.04
Multiplication Factor	-	6.75	0.50	0.25	0.75	9.25	3.25	24.25	2.50	1.00	1.00

Key: mg. wt. loss is the average value of duplicate coupons; Corrosion rate = mdd; Multiplication factor = test mdd ÷ control mdd.

results and Table 7.2 a summary by listing the three most and least corrosive acids and the average multiplication factor of the acids in each system.

Table 7.2

To Show the Three Most and the Three Least Corrosive Acids for each System

	Multiplication Factor of all acids		Most corrosive			Least corrosive		
System	$\bar{x}$	σ_{n-1}	1st	2nd	3rd	8th	9th	10th
StSt/Sea	8.0	4.4	Mal	Cit	Succ	Lac	Fum	Iso
StSt/Turner's	6.0	2.9	Pyr	Mix	Ita	Aco	Mal	Iso
MSt/Sea	0.5	0.3	Pyr	Succ	Fum	Fum/Cit	Mal	Ita
MSt/Turner's	1.0	0.2	Cit	Iso	Mal	Pyr	Ita	Lac
CuNi/Sea	1.0	0.1	Mal	Succ	Mix	Pyr/Ita	Fum	Iso
CuNi/Turner's	2.4	3.3	Succ	Cit	Iso	Mal	Lac	Aco
Al/Sea	2.6	2.0	Cit	Succ	Lac	Mix	Fum	Pyr
Al/Turner's	5.0	7.4	Lac	Succ	Pyr	Mal	Fum	Aco

Stainless steel in Turner's Solution was most severely corroded by the addition of pyruvic acid which caused a corrosion rate 13.67 times greater than the control. The mixed acids showed a corrosion rate 7.3 times greater than the control (Table 7.1) and all of the other acids were within the 3.7 to 6.0 value. There was a wide ranging effect of the individual acids on corrosion rate as indicated by the average increase being six times greater than the control with a standard deviation of 2.9. The effect of acids was generally more

severe in modified sea water (mean range 3.6 - 12.4) than in Turner's solution (mean range 3.1 - 8.9). The most corrosive acids were maleic, citric and succinic acids whose corrosion rates were 15.00, 13.75 and 13.00 times greater than the control. On average the corrosion rate was increased eight times with a standard deviation of 4.4, again indicating a wide ranging effect from the individual acids.

The acids had little effect on the corrosion rate of mild steel in either Turner's solution or sea water. In the former the average multiplication factor was 1.0 with a standard deviation of 0.2; citric acid being the most corrosive, increasing the rate 1.5 times. In the latter only pyruvic acid caused corrosion to a greater extent than the control (1.13) the values for the other acids ranging from 0.14 to 0.87. Such unexpected results must be considered in the light of the very high corrosion rate of mild steel in the absence of acids and the relatively small volume of solution surrounding the metal. In acid solutions the corrosion rate is usually quite high because insoluble corrosion products which could stifle the anodic reaction cannot form. With a small volume it was possible that the available hydrogen ions were quickly reduced at the cathode leaving a near neutral solution. Further corrosion would involve the reduction of oxygen.

$$O_2 + 2H_2O + 4e \longrightarrow 4OH^-$$

The presence of OH^- would increase the pH which would cause the corrosion rate to slow down by allowing the formation of insoluble hydroxides on the surface.

In the case of mild steel the voluminous rust production would eventually stifle the reaction. It was probable that this occurred rapidly in the presence of acids thus preventing further corrosion. If weight loss had been determined after a shorter exposure time the control coupons may have been less corroded than the test coupons.

The most corrosive acid towards cupronickel in Turner's solution was succinic which increased the corrosion rate by a factor

of 11.2. The least corrosive acid was maleic which reduced the corrosion rate to 0.3 of it's control value. Generally the addition of acids to seawater had little effect on the corrosion rate of cupronickel, the values varying between 1.25 and 0.78. Thus, there was an appreciable difference in the effect of the individual acids on cupronickel depending on the electrolyte used. In both cases succinic acid was amongst the two most corrosive acids. However, it's effect on cupronickel in Turner's solution was ten times more severe than it's effect in sea water. The composition of the recipient environment evidently plays a major role in corrosion processes. The metal/acid complexes may be affected by medium/metal or medium/acid complexes during their formation. The formation of an organic acid/metal complex which was insoluble and precipitated on the metal surface may protect the metal from corrosion. Such a passivating film could not form if the acid-metal complex was soluble.

The corrosion rate of aluminium in Turner's solution varied greatly depending on the acid present. The most corrosive acids were lactic and succinic which increased the corrosion rates by factors of 24.3 and 9.3 respectively. Fumaric, aconitic and maleic acids all reduced the corrosion rate.

The corrosion rate of aluminium in sea water was generally increased by a factor of 2.6 with a standard deviation of 2.0. The most corrosive acids were citric and succinic which increased the rates by 5.8 and 5.5 respectively and the least corrosive acids were pyruvic and fumaric which decreased the rates to 0.7 of the control value. There was some consistency in the effect of individual acids on the corrosion rate of aluminium in sea water and Turner's solution. In both cases succinic and lactic acids were among the three most corrosive acids and fumaric was within the three least corrosive acids though the degree of increased corrosion was quite different. Again the different mineral species and subsequent types of complexes formed within the two electrolytes must account for such variations in individual acid effects on aluminium.

In conclusion, it was evident that there were several complex interactions. The acids did not always increase the corrosion rate and their effect depended both on the metal and on the aqueous

solution. With the exception of stainless steel the corrosion rates were more severely affected in Turner's solution than in sea water though as the control corrosion rates show, Turner's solution was intrinsically less corrosive to each metal than sea water. In addition, for both solutions the control corrosion rate increased in the order stainless steel, aluminium, cupronickel and mild steel. However, Table 7.2 illustrates that the susceptibility to acid corrosion decreased in that order. Thus, in general the most corrodible control system was least accelerated by acid corrosion within the confines of this experiment.

It was apparent that some acids were more corrosive than others, the mixture falling somewhere in the middle. Table 7.3 showed it to only appear in the three most corrosive acids twice and the three least corrosive acids once. The average value of the multiplication factors for each acid was, except in two cases, found to be greater than that of the mixed acid alone. This might be expected since the mixture of acids was made up to 1,000 ppm by using 1/9 of the individual concentrations. However, the relative contributions to corrosivity were likely to differ as illustrated by the individual acids. The mixed acid solution was most likely to resemble a culture filtrate of C. resinae where it has been shown that increased corrosion rates do not always correspond to a fall in pH (Chapter 4). With the exception of pyruvic acid the pH of the mixture in sea water was less than for the individual acids. However, this did not make it more corrosive. As the following discussion indicates the type and concentration of acid was probably more important than the relative strengths of the acids.

For each metal/aqueous system examined the concentration of the acid was 1000 ppm. However the strength of the various acids differed due to the efficiency with which they acted as proton doners, or to the extent to which they increased the hydrogen ion concentration when dissolved in aqueous solution. This can be recorded as the dissociation constant, Ka, of the acid, more frequently expressed as pKa (pKa = -logKa). Table 7.4 shows pKa values for the acids used. A strong acid almost completely dissociates in dilute solution, it has a large Ka and a small pKa. A weak acid shows little disassociation and

Table 7.3

To Show the Frequency of Occurrence of Individual Acids Within the Most Corrosive and Least Corrosive Three Acids

ACID	Frequency of occurrence within three most corrosive acids			Frequency of occurrence within three least corrosive acids		
	Sea	Turner's	Overall	Sea	Turner's	Overall
Pyruvic	1	2	3	2	2	4
Fumaric	1	0	1	4	0	4
Cis-Aconitic	0	0	0	0	3	3
Maleic	2	1	3	1	3	4
Succinic	4	2	6	0	0	0
Itaconic	0	1	1	2	1	3
Lactic	1	1	2	1	2	3
Citric	2	2	4	1	0	1
Isocitric	0	2	2	2	1	3
Mixture	1	1	2	1	0	1

has a large pKa. Thus, the table shows that in water at 25°C maleic is the strongest and succinic the weakest acid.

Such strength is affected by the proton accepting or proton donating properties of the medium in which the acid is dissolved. Thus one cannot necessarily expect the relative acid strengths to be the same in sea water or Turner's solution as in water. The simplest way of recording the strength of an acid is as the pH of a dilute solution of stated concentration. Table 7.4 shows the pH values of 1000 ppm acid in sea water and Turner's solution. The order of strength was approximately the same in each; pyruvic > fumaric > aconitic > maleic > succinic > itaconic > lactic > citric > isocitric. The only similarity with pKa was that the four strongest acids were at the top of both lists.

Table 7.4

To Show the pH of 1000 ppm Acid in Modified Sea Water or Turner's Solution and the Standard pKa Value

Acid	pH - Sea Water	pH - Turner's	pKa (water 25°C)
Control	7.0	6.0	Water = 14
Pyruvic	2.6	3.9	2.49
Fumaric	3.2	4.4	3.02/4.38
Cis-Aconitic	3.5	4.8	2.80/4.46
Maleic	3.6	5.2	1.97/6.24
Succinic	3.8	5.2	4.21/5.64
Itaconic	3.8	5.2	3.82/5.55
Lactic	3.9	5.5	3.86
Citric	4.0	5.4	3.13/4.76/6.40
Isocitric	7.8	6.3	3.29/4.71/6.40
Mixture	3.0	4.8	-

It was envisaged that the strongest acids would cause the greatest degree of corrosion, that is by greater dissociation they would provide protons to act as an electron sink thus driving the cathodic reaction. The anions which would then be available to combine with the free metal ions forming organometallic compounds.

Tables 7.2 and 7.3 illustrated that there was no correlation between corrosivity and pH in Turner's solution or sea water. For example pyruvic was the strongest acid but it was only found amongst the top three corrosive acids three times out of eight and, more significantly, was amongst the three least corrosive acids four times out of eight. Furthermore, although fumaric and aconitic acids were at the top of the list as regarded strength, the former in sea water and the latter in Turner's solution, they appeared in the least corrosive acids four and three times respectively and in the most corrosive acids only once and not at all respectively.

Such results lead one to the conclusion that corrosion rates of the four metals in both sea water and Turner's solution have not

been dictated by acid strength in the form of pH value. However, the method used was probably not sensitive enough to distinguish beween acids of similar strength, for example, cis-aconitic, maleic, succinic, itaconic, lactic and citric acids. However, a comparison between pyruvic, pH 2.6 and 3.9 and lactic pH 3.9 and 5.5 should have been possible, since their respective pH values were quite different. It was also convenient to compare these two acids because of their similar property in only having one carboxylic group. However, Table 7.3 shows that both pyruvic and lactic acids appear in the most corrosive three acids, 3 and 2 times respectively and in the least corrosive three acids 4 and 3 times respectively. It becomes apparent, therefore, that there is indeed no correlation between acid strengths and corrosion rates. At first sight this seemed surprising. There are many instances in the literature where corrosion is shown to increase with a decrease in pH. For example corrosion characteristics of a metal may be obtained from Pourbaix[160] diagrams. These are a series of diagrams with pH and potential as co-ordinates with curves representing various chemical and electrochemical equilibria which exist between the metal and the liquid. Such curves serve to indicate the conditions under which immunity, corrosion and passivation may be expected. However, such work has compared an increase in strength of the same acid.

This experiment has compared the corrosivity of different acids. Their concentrations were kept constant which naturally led to their strengths being different. The experiment was set up in this way because the relative importance of the acids as produced in a mixture by C. resinae was to be examined. Previous work had measured overall strength of the mixture by pH and had analysed the relative concentrations of acids within that mixture. Since individual strengths were not known, and could not be predicted in such a complex mixture, it was decided to use known concentrations for the comparison.

It was envisaged that the experiment would show that the stronger organic acids would cause the most corrosion. It would appear that the hydrogen ion concentration, under the

conditions used, was not of major importance. This can be confirmed by examining succinic and itaconic acid, both dicarboxylic acids, which happened to have identical pH values of 3.8 in sea water and 5.2 in Turner's solution (under more standard conditions one would have expected itaconic acid, pKa 3.82, to be stronger than succinic acid, pKa 4.21). Under the experimental conditions, Table 7.3 showed that there was no similarity in corrosive effect between the two acids. Succinic acid was one of the most corrosive acids six out of eight times and was never one of the least corrosive acids. Itaconic acid was only one of the most corrosive one out of eight times and was one of the least corrosive acids three out of eight times. Overall succinic acid was much more corrosive than itaconic acid in sea and Turner's systems with each metal. Thus, initial pH did not influence their corrosivity.

As hydrogen ion concentration has been shown to be relatively unimportant one must consider the anion. An acid in solution dissociates into a cation and an anion, for example:-

$$RCH_3COOH \rightleftharpoons RCH_3\,COO^- + H^+$$

The physical and chemical properties of the anion may be the factor determining corrosivity. For example citric acid with its three possible anionic sites may be expected to complex with metal ions to a greater degree and/or at a faster rate than succinic, a dicarboxylic acid, or pyruvic a monocarboxylic acid. In utilising the metal ions the anions would drive the anodic corrosion reaction. However, this complex may need to be soluble to prevent precipitation on the metal surface from stifling the reaction. The anions may also play an indirect role in the corrosion process. By complexing with various cations in the solution they would increase the relative availability of other anions in that solution e.g. OH^-, CI^-, PO_4^-. If such a metal/anion complex is more soluble or more easily formed than the complex in the non-acidified environment then the corrosion rate may increase. Thus, there are several ways in which the anion formed from the dissociation of

acids may play a role in corrosion.

7.1.3 Conclusion

The acids behaved in either a corrosive or a protective manner depending on both the metal and the aqueous environment. Only succinic acid showed any degree of consistent corrosivity. It was concluded that corrosivity was not determined by initial pH but rather by the physical and chemical properties of the anion formed from the acid dissociation.

As indicated by the mild steel/sea water system a limiting factor of the experiment was the relatively small volume with respect to the size of the corroding metal coupon. Both a larger aqueous volume and shorter exposure times may have yielded more conclusive results. In addition, an experiment using various concentrations of acids to achieve the same pH may have determined the relative importance of the various anions. A more rapid method than weight loss would have been the use of the polarisation resistance technique. Using electrochemical measurements, de Meybaum and de Schiapparelli[45] determined that the pH decrease caused by proliferation of C. resinae did not influence the pitting corrosion of aluminium alloys. They concluded (on rather dubious evidence) that it could be determined by the anionic concentration of the metabolites. They adjusted the pH of a culture filtrate from 4.8 to 8.0 using NH_4OH. In such a complex mixture, indiscriminate addition of alkali would completely alter the speciation[195]. Before the true effect of anionic concentration can be determined more controlled conditions are required.

7.2 The Effect of Metals on the Growth, Acid Production and Enzyme Activities of C. resinae

7.2.1 Introduction

The aim of these experiments was to investigate the effect of stainless steel, mild steel, cupronickel and aluminium coupons and their constituent metal ions on the growth of C. resinae. Any effect on the growth, acid production or associated enzyme activity of the organism is likely to alter the susceptibility of a particular metal to microbial corrosion. As discussed in Chapters 4 and 5 corroding metal coupons did affect the growth of C. resinae in Turner's solution and modified sea water. In order to determine the concentrations at which metals stimulated or inhibited growth, C. resinae was incubated in Turner's solution containing the major components of each alloy.

The effect of the corroding metal coupons on acid production had been difficult to assess in previous experiments both because in the well buffered Turner's solution pH was fairly stable and also the acidity may have been modified by the corrosion products themselves in sea water. Thus direct analysis of citric acid in media culture filtrates exposed to metal coupons was proposed. Citric acid was chosen as a marker acid as it was consistantly detected in relatively high quantities in the presence of stainless steel or no metal as described in Chapter 4.

An effect of metals on growth and acid production would imply a modification of metabolic and therefore enzyme activity. The activity of extracellular enzymes involved in hydrocarbon utilisation may play a role in microbial corrosion processes. For example the oxygenation of an alkane by a mono oxygenase or a mixed function oxidase may be catalysed in the presence of metals (Cu or Fe) or another reducing agent[163].

$$RCH_2CH_3 + O_2 + \text{reduced coenzyme} \xrightarrow{\text{mono oxygenase}} RCH_2CH_2OH + H_2O + \text{oxidised coenzyme}$$

The metal is oxidised therefore aiding the anodic corrosion reaction.

A preliminary investigation of five well studied enzymes was carried out using growth inhibiting concentrations of metal ions.

7.2.2 Results and Discussion

7.2.2.1 The Effect of Differing Metal Concentrations on the Growth of C. resinae in Turner's Solution plus Dieso

The results in Table 7.5 indicate that at concentrations of about 40 μg ml^{-1} Ni and Al had virtually no effect on growth whereas Fe and Cu enhanced it. At about 400 μg ml^{-1} Ni and Al inhibited growth though Fe and Cu still enhanced it. At around 4000 μg ml^{-1} all metals were inhibitory to growth.

Such results conform to the observations made in Chapter 4. Mild steel coupons (Fe) enhanced growth whereas aluminium coupons (Al) inhibited it. Cupronickel coupons had little effect indicating that enhancement by Cu counteracted inhibition by Ni. This was found to be the case when both Cu and Ni were presented to C. resinae (results not shown). Possibly the Ni concentration was reduced by its adsorption onto hydrous copper oxides.

An additional experiment, where C. resinae was grown in either Turner's solution or modified sea water and undecane with the addition of 1000 ppm metal gave the following results:- After 2 weeks static growth at 30°C had reached factor 3 in the no metal control; Mn and Zn stimulated it to factor 5; Ni totally inhibited it and Fe, Mo, Cu, Mg and Al inhibited it to Factor 1. After 8 weeks, compared to a no metal control at Factor 5, Ni was totally inhibitive, Mo and Al inhibited growth to Factors 1 and 3 respectively and the other metals had no effect. Again Ni and Al were found to be especially inhibitive. Molybdenum is a component (0.3%) of stainless steel but previous experiments have not shown the latter to be toxic.

A recent review by Duxbury[53] has noted that although the majority of literature quotes concentrations in ppm that a more useful parameter from a biochemical point of view would be molar values. The range of atomic weights throughout the heavy metals is quite large thus a value in ppm of each metal may give rise to a quite different

molar concentration. For example, 100 ppm of Mo, Fe, Al and Mg are equal to 1.0, 1.8, 3.7 and 4.1 mM though 100 ppm of Cn Fe, Mn, Ni, Cu and Zn are very similar, that is between 1.5 and 1.8 mM.

Table 7.5

Growth of C. resinae when Exposed to Fe, Cu, Ni and Al ions in Increasing Concentrations

Metal	$\mu g\ ml^{-1}$	Weeks 1	2	4	6	8
		Growth Factor				
Control	-	0	1	1	2	2
Control	-	0	0	1	1	2
Fe	43	1	1	2	3	4
Fe	430	0	1	2	3	3
Fe	4304	0	0	0	0	0
Cu	41	2	2	3	4	4
Cu	410	1	2	3	4	4
Cu	4100	0	0	0	0	0
Ni	41	0	1	1	1	1
Ni	412	0	0	0	1	1
Ni	4123	0	0	0	0	0
Al	45	0	1	1	2	2
Al	450	0	0	0	1	1
Al	4500	0	0	0	0	0

Key: Growth Factor - Refer to Table 2.2.

7.2.2.2 The Effect of Metal Coupons on Extracellular Citric Acid Production by C. resinae in Turner's Solution and Modified Sea Water with Undecane as the sole Carbon Source

The results are shown in Table 7.6. No citric acid was detected in the uninoculated controls.

Turner's Solution

The metal coupons had no effect on citric acid production after two weeks growth except for mild steel which doubled the amount of acid produced and stimulated growth. After 4 weeks aluminium stimulated citric acid production though it did not appear to alter the amount of growth. With the exception of mild steel systems the concentration of citric acid more than doubled between 2 and 4 weeks followed by a greater increase in all flasks between 4 and 6 weeks. Although most growth was observed in the presence of mild steel the concentration of citric acid was least after 6 weeks. Presumably a stage of growth had been reached where utilisation of citric acid was preferable to that of undecane. The pH was reduced further, indicating that alternative acids were produced. The presence of aluminium stimulated citric acid production above that of the no metal control after 4 and 6 weeks though growth did not appear to be greater. By the eighth week of growth citric acid concentration had fallen dramatically to a similar level in all flasks though the pH had also fallen which was indicative of production of alternative acids. Monitoring to 14 weeks showed the concentration to be further reduced to about 52 $\mu g\ ml^{-1}$ in the presence and absence of metals (results not presented). In summary, citric acid was produced during the first six weeks of growth when it was utilised and/or converted to an alternative acid.

The experiment reported in Chapter 4 showed higher concentrations of citric acid, as measured by GLC, in the presence of stainless steel after 4 + 9 weeks than those found in this experiment.

Table 7.6

Growth, pH and Concentration of Citric Acid in Cultures of C. resinae Grown in Turner's Solution and Modified Sea Water in the Presence of Metals

Weeks	2			4			6			8		
System	G	pH	µg ml^{-1} Acid	G	pH	µg ml^{-1} Acid	G	pH	µg ml^{-1} Acid	G	pH	µg ml^{-1} Acid
TURNER'S SOLUTION												
No metal	2	5.9	56	3	5.7	153	4	5.5	509	5	4.9	117
Mild steel	3	5.9	141	4	5.7	150	5	5.4	421	5	5.2	118
Stainless steel	2	5.9	58	3	5.7	137	4	5.5	442	5	4.9	117
Cupronickel	2	5.9	60	3	5.7	164	4	5.5	479	5	5.1	112
Aluminium	2	5.9	62	3	5.7	202	4	5.4	540	5	5.2	112
MODIFIED SEA WATER												
No metal	2	6.2	29	2	5.2	26	3	4.7	20	3	4.7	18
Mild steel	0	6.8	1	0	7.0	4	0	7.1	1	1	7.3	1
Stainless steel	2	6.5	30	2	5.2	27	3	4.8	21	3	4.8	19
Cupronickel	0	7.8	8	0	8.0	6	0	7.9	1	1	8.0	1
Aluminium	0	7.7	4	0	8.0	5	0	8.0	2	1	8.1	1

Key: G = Growth; Acid = Citric Acid

Modified Sea Water

In the no metal control and in the presence of stainless steel, growth, pH fall and citric acid concentration were similar over the eight week period though the amount of growth and subsequent acid production were very much lower than in Turner's solution. The pH fall indicated that significant amounts of acid were produced especially after 6 weeks, though evidently not due to citric acid. Chapter 4 reported an average concentration of 58 $\mu g\ ml^{-1}$ of citric acid in the absence of metal again higher than in this experiment.

Although visible growth was not apparent in the presence of mild steel, cupronickel and aluminium a low concentration of citric acid was detected after 2, 4 and 6 weeks. The growth observed after 8 weeks did not increase the concentration of citric acid.

Stimulation of citric acid production by mild steel and by aluminium may result in their enhanced corrosion in Turner's solution. In the case of aluminium results in the previous section and in Chapter 6 conform to this suggestion, i.e. citric acid was found to be moderately corrosive to aluminium increasing its corrosion rate 2.5 times and adherent C. resinae continuously increased the corrosion rate.

It was illustrated that citric acid production varied with time and was independent of pH. It was evident that other acidic products were formed and if their production were selectively stimulated by the various metals then those metals may inadvertantly "seal their own fate".

7.2.2.3 Estimation of Selected Enzyme Activities from Cell Extracts Prepared from C. resinae Grown in the Presence of Various Metals

The results from the method described in Section 2.4.4.2 are presented in Table 7.7.

The concentrations of metals used were those thought to be partially inhibitory to growth of C. resinae.

Table 7.7

To Show the Effect of Fe, Cu, Ni and Al on the Specific Activities (n moles produced/min/mg protein) of five Enzymes

Metal µg ml^{-1}	Protein mg ml^{-1}	Acid Phosphatase	Alkaline Phosphatase	Malate Dehydrogenase	Catalase	Cytochrome c oxidase
No metal	1.8	3.3	15.9	790.6	1000	31.8
Fe (1000)	1.3	35.0	13.3	523.4	761	20.9
Cu (1000)	0.7	4.1	0.8	42.9	0	8.0
Ni (412)	0.8	0	2.5	194.6	269	20.5
Al (450)	1.8	45.1	8.1	105.4	282	5.2

Of the enzymes examined acid phosphatase, a lysosomal marker, was the only one to show an increase in specific activity in the presence of metals (an exception being with Ni). Iron and aluminium particularly increased the activity and copper had little effect.

The other four enzymes examined all showed a decrease in specific activity in the presence of metals. Generally speaking iron was least inhibitory and copper most inhibitory to production of the enzymes. Previous experiments however, have not shown copper to be inhibitory to growth.

The resistance of C. resinae mycelia to breakage dictated the use of a fairly harsh method to prepare cell free extracts. Improved technique using enzymic breakage of cell walls would improve the experiment, however, it was shown that metals did affect enzyme activities. Further examination of malate dehydrogenase, found in the mitrochondrial matrix, and other enzymes involved in acid conversions may prove useful. Section 7.1 of this Chapter showed that of the various acids produced by C. resinae all those examined were corrosive to different extents. Particular metal alloys may not suffer from acid related corrosion if their components are capable of altering the activity of enzymes which produce especially corrosive acids, for example those of the tricarboxylic acid cycle.

Section 7.1 showed that succinic acid was particularly corrosive towards cupronickel and aluminium. If for example Cu, Ni or Al inhibited the enzyme succinyl - CoA synthetase or stimulated the enzyme succinate dehydrogenase the build-up of succinic acid by C. resinae may be prevented thus reducing the effects of microbial corrosion. Thus a metals' susceptibility to corrosion by a particular microorganism may be dictated by it's effect on that microorganism. Hence the mechanism of corrosion may differ for each organism on each metal. Thus alloys may be chosen which are intrinsically resistant to microorganisms known to be a potential problem.

7.2.3 Conclusion

This section has shown that the growth, acid production and enzyme activities were altered in the presence of metals. Such

modifications may well account for the relative resistance of some metals to microbial corrosion whilst others corrode rapidly. For example the release of inhibitive or toxic ions may protect a metal whereas the release of stimulatory ions may enhance it's further corrosion. Also the ability of a pure or mixed culture to tolerate such ions would determine the relative corrosivity of different microorganisms. In addition the environment in which the corroding metal and living organism are subject to will play a major part in the effective metal concentration and the organisms response to it. Thus, there are many interacting factors in the effect a metal has on an organism and care must be taken in interpretation of results.

7.3 Growth, Adherence and Corrosive Effects of a Drop of C. resinae Spore Suspension on Stainless Steel, Mild Steel, Cupronickel and Aluminium

7.3.1 Introduction

Chapter 4 illustrated that corrosion was particularly severe below adherent growth. The aim of this experiment was to further investigate such corrosion with special regard to the adherent substance. Preliminary work by Engel and Swatek[57] showed that different metals could stimulate or inhibit slime formation of several fuel utilising bacteria and modify it's adherent properties. For example, 10 ppm of Fe, Mg and Zn followed by Al, Mn and Mo promoted slime formation whereas Cu and Ni did not. Metal alloys were found to promote faster slime production than metal ions in solution with aluminium promoting particularly adherent material.

7.3.2 Results and Discussion

After six weeks there was no growth in either Turner's or modified sea water drops on mild steel and cupronickel. Excepting those in Turner's solution on mild steel, the spores were shown to be non-viable. The toxicity of mild steel and cupronickel corrosion products was surprising in view of the prolific growth of C. resinae

in fuel systems, especially mild steel fuel tanks. Germination may only take place away from the metal surface and/or by protection from other microorganisms.

Modified sea water supported poor growth on aluminium and stainless steel. After six weeks, growth was concentrated at the circumference with small isolated colonies in the centre of the drop. Plate 7.1 gives an example of growth on stainless steel in modified sea water.

After one week growth was well defined in Turner's solution on both stainless steel and aluminium. A heavy band of green coloured growth was apparent at the circumference. Growth radiated outwards by 1.5 mm onto the metal surface in a band of green followed by white hyphae. After three weeks the band was ~ 3 mm wide and a brown colouration had developed (Plate 7.2). After six weeks the band had extended to ~ 4 mm, the growth rate slowing as the available mineral solution was depleted. Before dehydrating the centre of the drop had isolated colonies throughout with a developing hyphal membrane over its surface. This had the effect of stabilising the drop at the metal surface as opposed to the uninoculated drops which had a tendency to move if they were disturbed. Thus, in a ship's fuel system the stabilisation of potentially corrosive condensation or splashed water droplets may be an indirect mechanism of corrosion by C. resinae.

Tarnishing was apparent below the uninoculated drops especially at the circumference. Corrosion associated with drops on stainless steel and aluminium was examined before and after adherent growth was removed. Stainless steel was little affected and there was no corrosion below the drop itself. A band of corrosion in the form of small pits and tarnish was apparent radiating from the circumference. Plate 7.3 illustrates this band of corrosion and Plate 7.4 shows growth of C. resinae on the surface of stainless steel. Aluminium corroded more readily especially with Turner's solution which supported more growth. A pattern of corrosion emerged whereby the area below the drop was generally corroded, the circumference of the drop was more corroded and the area radiating from this was pitted and suffered a 'channelling' effect, Plate 7.5. The channels are believed

Plate 7.1 (~ x 3.6)

Growth of C. resinae Spore Suspension in a Drop of Modified Sea Water after Six Weeks

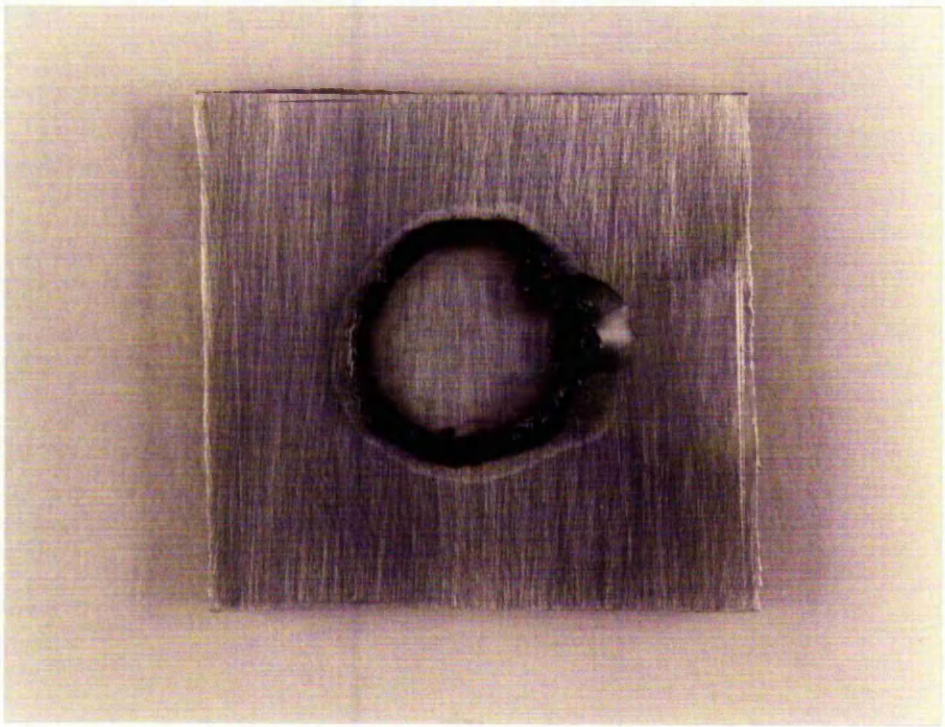
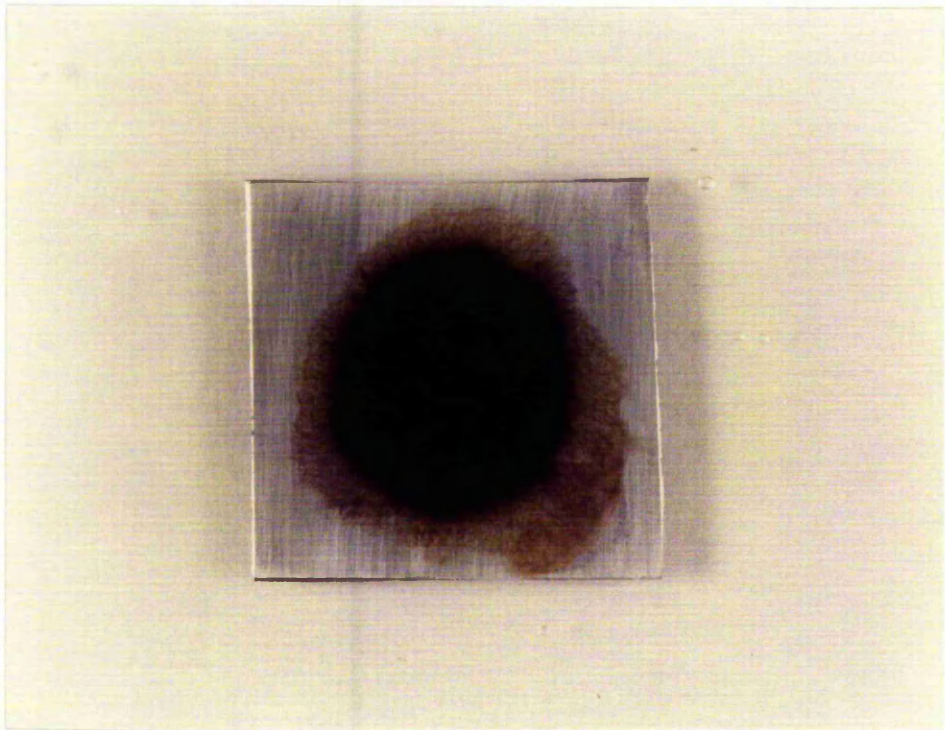


Plate 7. 2 (~ x 2.8)

Growth of C. resinae Spore Suspension in a Drop of Turner's Solution after Three Weeks



to be due to hyphal growth on the metal surface and are shown in Plate 7.6.

It was surprising that hyphal channels were not observed on stainless steel considering its known susceptibility to crevice corrosion. Their formation on aluminium may have been due to several factors namely:

1) production of corrosive metabolites.
2) formation of an oxygen concentration cell.
3) nature of mycelial development.

These will be considered individually.

Firstly, the effect of corrosive metabolites would be worse around the radiating hyphae because, contrary to the situation in the drop, less water (if any) is available to dilute them. As the hyphae grow into the fuel they may carry a surrounding film of mineral solution though internal transport would be their main method of getting water. There is no direct evidence as to where secretion occurs along hyphae; it seems reasonable to suppose, however, that this is largely or wholly concentrated in the region towards the hyphal tip where compartmental protoplasm contributes to apical extension and where metabolism may be expected to be highest[72]. Thus as the hyphae were seen to grow outwards from the drop onto the metal surface they would leave a trail of extracellular metabolites behind them. This occurred below non-polar n-undecane, indicating that the necessary electrolyte for corrosion was provided by these secretions.

The ability of C. resinae to modify the local environment of stainless steel thus affecting its corrosion rate was examined by forming a drop of spore suspension in modified sea water around the tip of a micro pH electrode. The results (Figure 7.1) indicate that between the second and eleventh day of the experiment the pH fell from 6.5 to 2.7. Thus, germination and growth of C. resinae in modified sea water gave rise to an acidic environment in a short period of time. It is reasonable to suppose that due to lack of dilution the acidity below the radiating hyphae would always be greater than that

Plate 7.3 (x 140)

Tarnishing Below the Growth at the Circumference in a Drop of C. resinae Spore Suspension on Stainless Steel

Plate 7.4 (x 140)

Growth of C. resinae, Stained with Ruthenium Red on Stainless Steel

Plate 7.5 (x 80)

Corrosion of Aluminium by a Drop of Spore Suspension of *C. resinae* in Turner's Solution

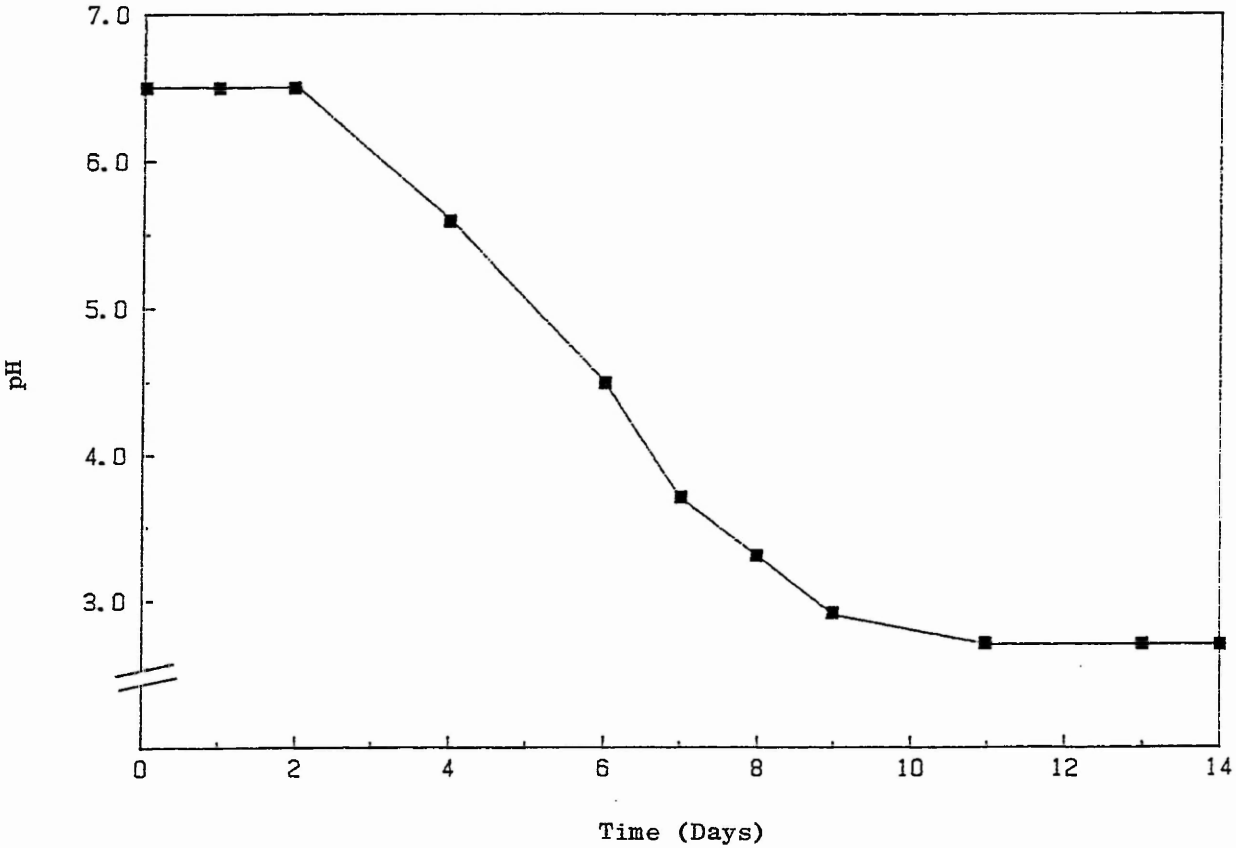
The top of the picture shows the undecane phase with hyphae radiating into it. The circumference is shown as a dark pitted band.

Plate 7.6 (x 130 (x 5))

Hyphal Channels on Aluminium

Figure 7.1

To show the Fall in pH of a Drop of C. resinae Spore Suspension in Modified Sea Water on Stainless Steel Surrounded by n-undecane



of the environment. Stabilisation of the drop and adhesion of radiating hyphae together with the excretion of acidic metabolites may be considered to be corrosion mechanisms of C. resinae.

Secondly, differential oxygen concentrations are well established as causing pitting corrosion. It is reasonable to suppose that the areas below the hyphal film and the water drop itself were oxygen depleted. Technical difficulties prevented the monitoring of oxygen levels in the drop. At the mycelial edge, however, there probably existed a steep oxygen gradient where the outer area was well supplied with oxygen from undecane and the inner area was depleted by metabolic activity.

Thirdly, the nature of mycelial development, particularly the method of adhesion, may be important in forming hyphal channels. Uptake of ruthenium red by a sheath around the hyphae showed the adherent material to be composed of an anionic polymer with 'polysaccharide-like' characteristics[63] (Plate 7.7). It was interesting to note that the polymer was mainly found on the hyphae radiating from the drop. The mass of older or less actively growing hyphae at the edge and in the centre of the drop may have stopped producing or modified the polysaccharide to a water soluble form. Generally speaking extracellular polysaccharides of microorganisms vary with age and conditions[52].

Finally, the extracellular material in bringing the hyphae in close contact with the metal surface (Plate 7.8) may serve to allow sequestration of metal ions. A non-specific uptake is likely because of the polyanionic nature of the adherent material. Alternatively, it might allow active sequestration by organic chelating agents in order to satisfy the specific requirements of the cell. In either case the removal of metal ions would provide a continuously depolarised corroding surface. The sequestration of aluminium, manganese or magnesium ions may account for the hyphal channels seen below adherent growth on aluminium coupons. The alloy examined had a high concentration of Mg (4.9%) and active uptake of such a metal might be expected in view of it's role in the assembly and stability of the plasma membrane[12]. The accumulation of metal ions by C. resinae is discussed in the next section.

Plate 7.7 (x 140)

Hyphae of C. resinae Stained with Ruthenium Red

The stain was taken up by a sheath around the hyphae.

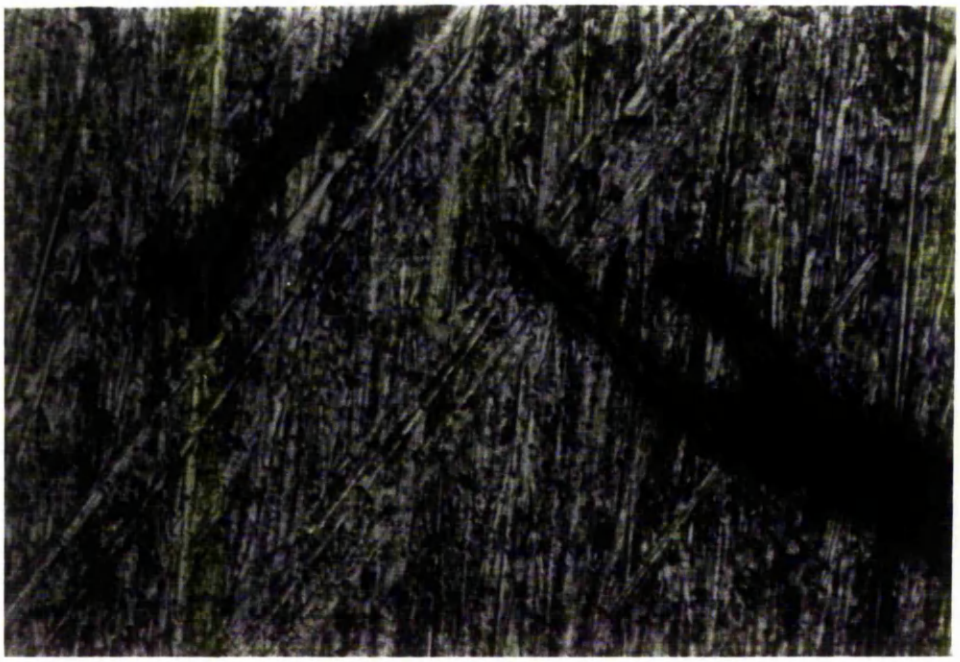
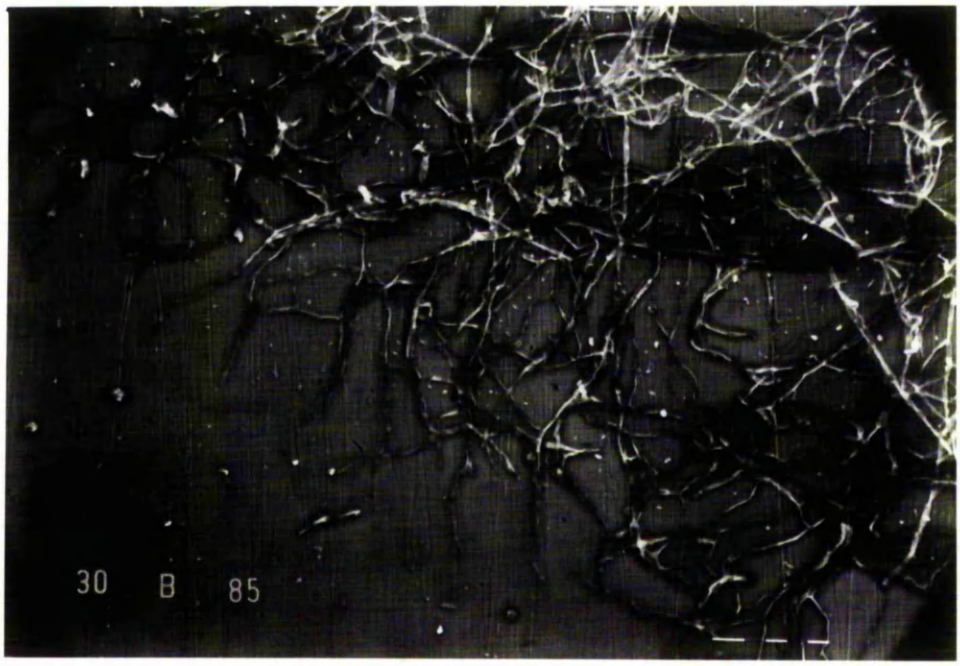


Plate 7.8 (x 300)

Electron Micrograph Showing C. resinae Adhering to Stainless Steel



The extraction and characterisation of the polysaccharide and associated enzymes might be a first step in the manipulation of the adhesive properties. For example, the fungal polymerase may be presented with a compound that mimics it's normal substrate thus occupying the enzymes active site but preventing the formation of a polysaccharide fibre. However, its feasibility in a ship's fuel system where large volumes and mixed populations prevail is unlikely.

The adhesion of microorganisms in fuel systems contaminated with water will affect the corrosion rate of the underlying metal. Adhesion is a complex process which can be influenced by the chemical and physical nature of the substratum, the composition and temperature of the bulk and local aqueous phases, local hydrodynamic conditions, and the nature and history of the organisms themselves[52]. Study of these processes is complicated by the ability of the organisms to adapt to a changed environment and, in a mixed culture, for the relative populations to change with time and changing conditions. However, the improved understanding of the ways in which adhesion of organisms depends upon these variables gives good reason to expect that improved methods for control of biofilm formation, and thus of the corrosion which often accompanies it, will be achieved.

7.4 The Sequestration of Metal Ions by C. resinae

7.4.1 Introduction

Previous experiments have illustrated that corrosion by C. resinae was particularly severe below adherent growth. Several suggestions were put forward to account for such a phenomenon one of which was the sequestration of metal ions from the surface thereby providing a continuously depolarised corroding material. It is well known that certain metals, e.g. Ca, K, Mo, Fe and Mg are concentrated from the environment or actively sequestered by organic chelating agents such as the exochelins to satisfy the specific requirements of metal/enzymes and/or structural components[12]. In addition much non-specific uptake is likely to occur at the potential anionic sites

in the cell wall (e.g. teichoic phosphodiester, free carboxyl groups of peptidoglycan, sugar hydroxyl groups of both wall polymers and amide groups of peptide chains) and by extracellular polysaccharide material. The previous section showed the existence of an adherent polyanionic sheath around C. resinae and the aim of this experiment was to investigate the uptake of metal ions from a solution containing the organism. The metals chosen for study were components of metal structures in ships' fuel systems. Factors studied were age of culture, exposure time and concentration of metal challenging the culture.

7.4.2 Results and Discussion

After challenging the culture (12 mg dry weight) with a concentration of metal for a specified period of time the amount of metal adhered to the extracellular layers (and removable by EDTA) was compared to that which had been taken up intracellularly or was strongly adhered to C. resinae.

The first experiment examined uptake of 100 µg metal by cultures of different ages. The intra- and extracellular % uptake is shown in Figure 7.2 and the total uptake per mg C. resinae is shown in Table 7.8. The % uptake of the different metals varied from a total of 6 to 42%, the former value representing the poor sequestration of zinc and magnesium which on average amounted to only 0.6 µg metal per mg C. resinae.

Some caution in interpretation of the apparently low magnesium uptake was necessary. That is, had concentrations been expressed in terms of molarity rather than as µg ml^{-1}, the experimental load of 10 µg ml^{-1} would have 0.41 mM for Mg and 0.15 to 0.19 for the other metals. Thus the load for magnesium was at least twice as great in terms of molarity and uptake calculations would have demonstrated a greater uptake than has been illustrated.

Of the manganese sequestered most of it was found to be intracellular, the amount increasing up to 18 days when 14.4% of the challenge was intracellular and a further 4.4% extracellular. This represented a total of 1.6 µg Mn per mg C. resinae. Uptake of iron

Figure 7.2

To show the Intra- and Extracellular % Uptake of Various Metal Ions by C. resinae

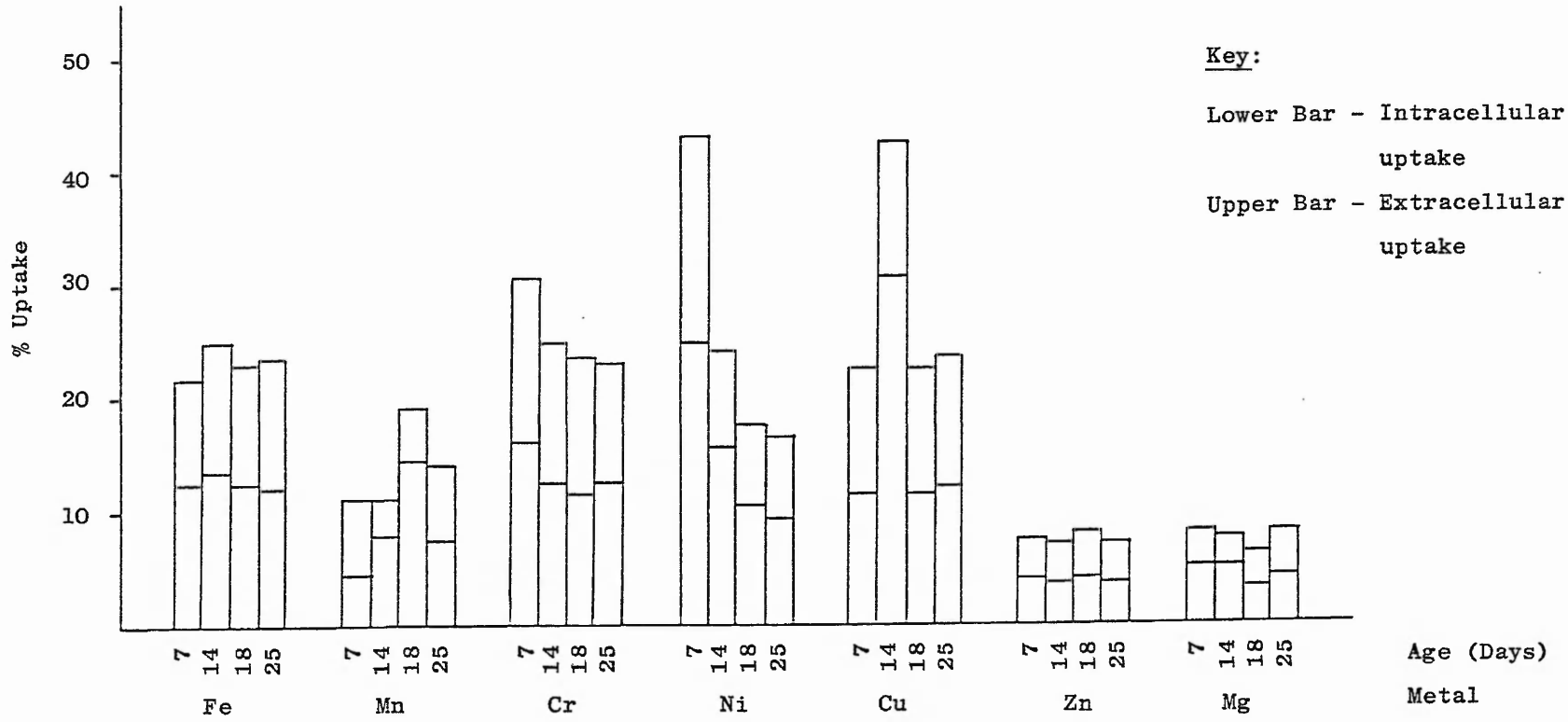


Table 7.8

To Show the µg Metal Uptake per mg (Dry Weight) C. resinae of Age 7, 14, 18 and 25 Days

	µg Metal Uptake per mg (Dry Weight) C. resinae						
Days	Fe	Mn	Cr	Ni	Cu	Zn	Mg
7	1.8	0.9	2.6	3.4	1.9	0.6	0.7
14	2.1	0.9	2.1	2.0	3.5	0.6	0.6
18	1.9	1.6	2.0	1.4	1.9	0.7	0.5
25	2.0	1.2	1.9	1.4	2.0	0.6	0.7

Table 7.9

To Show the µg Metal Uptake per mg (Dry Weight) C. resinae Challenged with a 20,100 and 500 µg Load

Load Total	µg Metal Uptake per mg (Dry Weight) C. resinae						
µg	Fe	Mn	Cr	Ni	Cu	Zn	Mg
20	0.4	1.3	0.3	0.4	0.5	0.1	0.2
100	2.3	1.6	1.7	1.1	1.8	0.5	0.5
500	11.4	15.1	9.0	5.5	8.3	1.8	3.9

varied slightly from 21 - 25% (2.1 μg mg^{-1}) the intra- and extracellular levels being approximately the same. Chromium uptake was greater with 7 day old C. resinae than with the older cultures, a maximum uptake of 31% was recorded (2.55 μg mg^{-1}) of which slightly more was intracellular. Sequestration of nickel decreased with increasing age of C. resinae, large differences being observed up to 18 days. The majority of the metal was found to be intracellular, 25% compared to 16% of the challenge after 7 days. Uptake of copper was about 23% with the exception of the 14 day culture which more than doubled its intracellular uptake bringing the total uptake to 42% or 3.5 μg Cu per mg hyphae. The combined high sequestration of nickel and copper is more than likely a mechanism of corrosion by C. resinae of cupronickel.

In summary, it was evident that in many cases metal uptake varied with age of culture. An increased uptake was generally due to intracellular or strongly bound metal which is indicative of an active mechanism and/or a requirement for the metal. Excepting nickel (at 7 days) the extracellular uptake of any one metal remained fairly constant suggesting that between 7 and 25 days the polyanionic sheath surrounding C. resinae either did not alter or any change in either it's nature or quantity cancelled the other out. There was however much variability between the extracellular uptake of different metals suggesting the occurrence of sites with particular affinities.

The second experiment examined the effect of incubation time between the metal and C. resinae. For an incubation time of 50, 100 and 200 minutes there was no difference in uptake of Cu, Ni or Zn indicating that C. resinae was fully saturated within 50 minutes. The metals Fe, Mn and Mg were sequestered in lower quantities both extra- and intracellularly after 200 minutes than after 50 and 100 minutes. This may indicate the presence of a protective mechanism in C. resinae whereby metals are taken up non-specifically and then toxic concentrations eliminated from the cell. Chromium was the only metal to be sequestered in a greater quantity (extracellularly only) after 100 and 200 minutes than after 50 minutes.

The third experiment varied the challenge of the metal (20, 100 and 500 μg in 10 ml) to 18 day old C. resinae. It is apparent

from Table 7.9 that as the load increased the uptake increased. Somehow the increasing ionic pressure forced increased uptake. For example, with a 500 µg load of Fe the uptake was 11.4 µg per mg C. resinae which corresponds to a total of 136.8 µg Fe taken up. Thus one might have expected all of the 20 and 100 µg load to have been sequestered. This was evidently not the case, instead the values generally represented an equivalent 'percentage' uptake. That is, for Fe, 24, 27 and 27% of the challenge was taken up. Although this percentage differed the same was true for each metal. The lower affinity for metal at low concentrations may be indicative of non-specific and non-active uptake mechanisms, any uptake being fortuitous.

In conclusion it was evident that the metals Fe, Mn, Cr, Ni, Cu, Zn and Mg were sequestered by C. resinae. It may therefore be hypothesised that C. resinae is capable of corroding metal alloys containing these components by their selective uptake. For example, stainless steel contains 18% Cr, 9% Ni, 1.5% Mn in addition to the main component Fe, all of which were susceptible to sequestration.

CHAPTER 8

FURTHER WORK

The difficulty in identifying fuel contaminants by conventional means has been illustrated and supports the alternative strategy proposed by Hill[90] which uses tests to convey spoilage significance. Fifteen tests would be sufficient to label the isolated organisms and would include tests to clarify the significance of the organism in corrosion, for example nitrite reduction or acid production. In addition, data on distribution of organisms could be derived from examination of samples from various sources. Further work on the development of such numerical profiles and the production of an appropriate 'identikit' would greatly aid the assessment of a contamination problem as a corrosion hazard.

Another area which would benefit from further investigtion is the development of more appropriate growth media for the isolation and culturing of corrosion causing bacteria. For example, commercially available isolation media do not reflect the high osmotic pressure and low water activity exerted in glycol based industrial fluids and fuels. However, the use of the API Method RP-38 is a practical method for detecting the presence of Desulphovibrio spp.[132] and Hill[91] has developed several "sig-tests" which use an indicator medium designed to show some significant spoilage activity for example, gelatin liquefaction, tributyrin hydrolysis, sulphate reduction, petroleum sulphonate reduction. These tests indicate whether the microbial contamination is actually participating in degradation or corrosion. Many more tests could be developed based on this concept to suit particular situations. In addition, the development of rapid techniques to monitor the total number of contaminating organisms may be useful. However, as this Thesis has shown, the most severe corrosion is generally due to adherent organisms such that low numbers in the bulk aqueous phase may not be indicative of a corrosion free area. Conversely high numbers may not be indicative of a corrosion problem. Semi-quantitative dip slides have been developed[64] but these are subject to an incubation of up to several days. The development of enzyme assays (more enzyme generally reflects more microbial flora), measurement of ATP, measurement of radioactive $^{14}CO_2$ produced from a labelled substrate, or measurement of accelerating impedance change of an inoculated growth medium have

all been given consideration[91] though several problems still have to be overcome.

Although the pure culture study provided much data that was relevant to the understanding of microbial corrosion mechanisms of ship related alloys, it was not ideal. The Thesis illustrated that generally speaking both mixed and sludge cultures gave rise to more corrosion than a pure culture of C. resinae. In addition, to the greater degree of corrosion, possibly qualitatively as well as quantitatively, the use of pure cultures denies analysis of genetic exchange between populations that may lead to an altered corrosive activity. For example, by enzymic activity, resistance to bioaccumulation of metals, biocide resistance or degradation.

Analysis of corrosion by mixed cultures would be more appropriate in a continuous rather than a batch culture system especially for long term experiments where infinitely variable and unique environments can be established. Continuous culture would allow more environmentally relevant laboratory models to be used. Booth et al.[2] found sulphate reducing bacteria to be more corrosive in semi-continuous culture than batch culture. Continuous cultures generally show good replicability and show fair correlation with the environment.[24]

Using such a technique, the effect, as regards subsequent corrosion, on a variety of communities, of biocides, alkaline pH's, oxygen scavengers or other changes not conducive to growth may be studied. The vessels themselves could be manufactured from the metal under study or perhaps more simply coupons could be inserted. Such metals may be new resistant alloys, may be painted with a variety of protective coatings or may even be cathodically protected. In this way the microorganisms, the metals and the physicochemical environment could be modified allowing an analysis of the corrosion process under many varying conditions. A chemostat would also allow the use of 'once through' sea water which although subject to differences in composition due to location and seasonal changes is far more representative than recirculated or synthetic preparations. However, they can never totally replace field trials and ideally long term experiments in service conditions should be carried out to monitor

microbial corrosion in ships' fuel systems.

The factors affecting adhesion of microorganisms to surfaces also warrants further study. The Thesis has illustrated that more severe corrosion occurs below adherent growth than in a growth modified environment. A better understanding of the factors which affect adhesion therefore offers the possibility of being able to control or even eliminate such corrosion. The effect of the chemical and physical nature of the surface, cell growth conditions, environmental composition, temperature and fluid velocity on attachment are areas to be investigated. In addition, bacterial enumeration and corrosion rate measurements to monitor early microbial fouling and assessment of it's association with the subsequent growth of macroscopic organisms and corrosion of the metal would provide useful information. Modification of early non-cellular and microbial fouling may well prevent serious corrosion, either in it's own right or by preventing further macroscopic fouling.

Contamination by microorganisms changes the biological, chemical and physical aspects of the environment in which they are growing. Several chemical parameters, e.g. pH, redox, corrosion potential were monitored in this Thesis to determine their usefulness as fouling indicators and potential corrosion hazards. Further study of several more such parameters may give rise to a reliable assessment of the environment such that a hazardous situation may be predicted and subsequent steps taken to eliminate it. Such tests would be most useful if they could be used for 'In-Service' or 'on-line' monitoring. However, no one test can take into account all the variables which affect the rate and type of corrosion and several methods should be considered.[21]

Despite all the microbial corrosion work which has been carried out in the past and the protection techniques which have been developed corrosion incidents still continue to occur, often causing considerable financial loss. The role which microorganisms play is complex and it is frequently difficult to assess their contribution to corrosion incidents. In many instances several corrosion mechanisms, including microbial corrosion, have all contributed more or less equally to the overall corrosion. Moreover, there is often a

considerable time interval between the occurrence of a corrosion failure and its diagnosis and the primary corrosion mechanism may have been masked by secondary reactions. Nevertheless a greater awareness of microbial corrosion by designers and operators would improve the design features, operating procedures and system monitoring which are important if microbial problems are to be contained and controlled.

CHAPTER 9

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