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THE ROLE OF CONSORTIA IN MICROBIALLY INFLUENCED CORROSION

Nicholas J.E. Dowling, Marc W. Mittelman, David C. White

Institute for Applied Microbiology, University of Tennessee, Blg. 1, Suite 300, 10515 Research Drive, Knoxville, TN. 37932.

"I am the undertow
Washing tides of power
Battering the pillars
Under your things of high law.

I am a sleepless
Slowfaring eater,
Maker of rust and rot
In your bastioned fastenings
Caissons deep.
I am the Law
Older than you
And your builder proud.

I am deaf
In all days
Whether you
Say 'Yes' or 'No'

I am the crumbler: tomorrow." Carl Sandburg, 1916.

In Mixed Cultures in Biotechnology. (J. G. Zeikus ed) McGraw Hill, N.Y.

I. Introduction

Microbially influenced corrosion (MIC) is not a recently recognized problem (Houghton et al., 1988; Tiller, 1986; Miller, 1981). Despite adequate descriptions of microbial corrosion early in this centuary, little progress has been made in the elucidation of mechanisms and reasonable solutions to the problem. One notable exception was an article by Von Wolzogen Kuhr and Van der Vlugt, who in 1934 proposed a hypothesis for "cathodic depolarization" where certain sulfate-reducing bacteria increased the corrosion rate by accelerating the reductive, cathodic reactions (the consumption of hydrogen generated). Cathodically produced hydrogen is oxidized by these organisms and establishes a link between their well-being and the corrosion reaction itself. Other processes cannot be disregarded. Sulfate-reducing bacteria require the presence and activity of other organisms to exist in aerobic environments. Some of the characterized 10 or 11 genera of sulfate-reducing bacteria (Widdel, 1988) do not exist exclusively as autotrophs but require organic substrates which are available from other organisms within biofilm communities. Large, complex

molecules such as proteins and polysaccharides are commonly available and do not accumulate in biofilms for the same reasons as are applicable in sedimentary systems (Laanbroek and Veldkamp, 1982). Indeed it is reasonable to assume that sediments have similar substrate dynamics to those of biofilms.

Partial degradation by primary degrading organisms channel monomeric substrates to fermentative bacteria which in turn provide short chain acids and alcohols to terminal oxidizing bacteria. The latter, including the sulfate-reducing bacteria tend to be obligately anaerobic and are situated close to the solid substratum. This model, which has been described at length by Hamilton (1985), appears to fit well with the significant deterioration of metal structures observed in the environment. Clearly, the different physiological groups within the biofilm operate in concert. The obligately anaerobic bacteria, for example, are never found in biofilms without other organisms which scavange oxygen. Under some conditions, new metal surfaces (produced by mechanical scouring for example) have a large oxygen requirement to maintain the surface oxide layer. This, however is a short term event and seems unlikely to be sustained long enough to provide the anaerobic environment required by obligate anaerobes. Thus other bacteria are required to not only provide the substrates required by sulfate-reducing bacteria, but also scavange the oxygen which prevents growth of these organisms. In this way, we see that interaction is imperative for some classes of microorganisms. The "closer" relationship of syntrophic organisms with their hydrogenophilic counterparts (Wolin, 1982) in biofilms not been examined.

From a practical standpoint, there appears to be no simple solution to the problem of MIC beyond materials selection, mechanical cleaning and low level biocide use. Few materials are truly impervious to attack in biological systems (Kuhn and Rae, 1988) and all structural materials are considered to have a finite life in service. Studies are often instituted only when a catastrophic shortening of this service life is demonstrated and are specific in nature and limited in scope. Even in the event of a putative link between MIC and the observed failure, subsequent studies are likely to focus on short term solutions. Perhaps the single biggest flaw of research into this topic has been the study of the corrosion properties of individual organisms in axenic cultures. While the relationship of a single microorganism in a biofilm to the substratum is important, there can be no reasonable extrapolation of material performance under environmental stress until the substratum is challanged with the <u>in situ</u> fouling community.

Engineering materials of most kinds are usually composed of complex arrangements, whether ordered by crystalline structure such as metals or aggregates such as concrete. Many of these materials have flaws which are exploitable either by virtue of their surface

structure or composition. This chapter sets out to explore the deleterious effect of different microorganisms in consortium on these diverse materials from problematic and mechanistic points of view.

II. ECONOMIC IMPORTANCE OF MIC

The economic effects of microbially influenced corrosion (MIC) are as diverse and far-reaching as are the consortia responsible. Perhaps the best studied of these activities has been the corrosion of underground iron gas and water pipelines by sulfate-reducing bacteria. Corrosion of these mild steel and cast-iron pipe systems is classically viewed as depending upon the availability of oxygen. At the end of the nineteenth century however, it was noted that serious forms of pitting-type corrosion were associated with extremely anoxic soils. Apparently another type of corrosion process was involved. Von Wolzogen Kuhr and van der Vlugt (1934) coined the term "graphitization" to describe the action of sulfate-reducing bacteria (obligately anaerobic organisms) on cast iron. Furthermore they proposed that a hydrogenase enzyme system previously described by Stephenson and Strickland (1931) was responsible for the "cathodic depolarization" of the metal and this resulted in accelerated corrosion.

It is clear that SRB are found in corrosive soil environments in association with a broad range of physiologically distinct microorganisms. Gaylarde and Johnston (1986) described accelerated corrosion of mild steel when cultures of D. vulgaris were grown with V. anguillarum. Their results demonstrated the importance of conducting coculture experiments and explain, in part, the relatively low corrosion rates obtained with laboratory SRB monocultures compared with those observed in the environment. Since many estimates of environmental MIC rates are based upon monoculture experiments, it seems likely that most projections of the economic impact of anaerobic corrosion on buried pipelines are over conservative.

A number of workers have attempted to place a dollar figure on the economic effects of underground pipeline corrosion. In one of the first studies of MIC effects on pipeline corrosion, Greathouse and Wessel (1954) described U.S. economic losses in excess of 100 million dollars per year. Allred et at (1959) found that SRB were responsible for more than 77% of the corrosion of oil-well underground pipelines. Postgate (1984) has noted that despite the "obvious economic importance of anaerobic corrosion, useful estimates of its economic cost are difficult to make."

Biological fouling of heat exchanger surfaces, cooling tower structures, and attendant distribution systems accounts for over 4.5 billion dollars in economic losses in the non-communist world. Losses to U.S. refinery heat exchangers amount to greater than 1.4

billion dollars per year (Knudsen, 1981). Purkiss (1971) has quoted losses in British industrial systems exceeding 300 million pounds per year in 1971 pounds. It is often difficult to separate the results of fouling phenomena such as mechanical blockages, heat transfer resistance, and corrosion. Aerobic, slime-forming consortia create differential oxygen cells as well as heat transfer resistance and decreased pipeline capacity. Open cooling towers are particularly susceptible to this type of multi-faceted fouling problem. Eukaryotes such as algae and diatoms can produce significant slime deposits in addition to acidic metabolic by-products. Within an aerobic layer composed of such slime-forming isolates as Bacillus subtilis, B. cereus, Flavobacterium spp., Aerobacter spp., and Pseudomonas spp., conditions become ideal for the growth of SRB. Iron oxidizing bacteria such as Sphaerotilus and Crenotrix spp. produce ferric hydroxide, a potent initiator of pitting corrosion in addition to forming differential oxygen cells (Purkiss, 1971).

The fouling and corrosion of jet fuel storage tanks by microbial consortia--principally, fungal contaminants--creates tremendous problems for military and civilian aircraft operations. Severe structural damage resulting from biogenic organic acid attack was commonplace in the 1950's and 1960's, prior to the introduction of biocides and improved measures to prevent water intrusion into the fuel systems. Cabral (1980) analyzed samples of kerosene A1-type jet fuel from aircraft fuel systems in Argentina; 65% of those tested were found to be contaminated with Cladosporium resinae, a ubiquitous, filamentous fungi. While a number of other fungi were isolated, most notably Penicillium spp. only C.resinae was capable of utilizing the fuel as a sole carbon source. Various consortia were tested on a a kerosene medium; however, only those mixtures containing C.resinae were able to utilize the kerosene as a sole carbon source. Schiapparelli and Meybaum (1980) have identified dodecanoic acid (a kerosene breakdown product) as a major organic acid involved in the corrosion of aluminum fuel tanks. Other mechanisms of these aluminum fuel tanks include the differential oxygen cells created by bacteria as result of oxygen uptake in localized areas (Hill, 1984).

Estimates of costs associated with abiological and biological corrosion in the pulp and paper industry have ranged from five to nine dollars per ton of product or up to 20% of maintenance costs including capital expenditure (Davies, 1984; Safade, 1988). Approximately 50% of these costs are associated with the Kraft process alone. Tatnall (1981) described severe microbial corrosion in a paper mill closed water ("white water") system. He found that corroded areas were covered by aerobic slime consisting of Pseudomonas, Aerobacter, Flavobacterium, and Bacillus spp. A brown, gritty deposit containing Desulfovibrio, Desulfotomaculum, and Clostridium spp. was found in association with the aerobic slime layer. Corrosion of paper manufacturing equipment used in the Kraft process by thermophilic Desulfovibrio and Desulfotomaculum spp. has

been described by Grimm et al. (1984).

The growth and activity of Thiobacillus spp. in concrete pipes is an interesting example of consortial involvement in corrosion accelerated by physically separated members. Microbially influenced corrosion of concrete has resulted in catastrophic failures of the upper (aerated) regions of concrete sewage pipes (Sand, 1984). Early investigators attributed the rapid corrosion of sewage transport pipelines to the production of hydrogen sulfide in the sewage followed by a "catalytic oxidation" to sulfuric acid vapor. It was thought that this process was abiological in nature, resulting from a chemical reaction catalyzed at the concrete surface (Thistlethwayte, 1972). Milde et al. (1983) and Parker (1947) have since described a number of species of thiobacilli which are involved in concrete corrosion.

Dissimilatory sulfate-reducing bacteria present in the sewerage and sludge at the bottom of the concrete pipes produce volatile reduced sulfur compounds which dissolve in the thin water film on the upper regions of the pipe. Here the water-film is relatively aerated and thiobacilli can use the sulfide and mercaptans as electron sources. The resulting sulfate and lowered pH leaches out the calcium binder and serious structural loss results. At the initiation of the corrosion process (at neutral pH) facultatively chemolithotrophic thiobacilli such as T. novellus and T. intermedius predominated. As the pH at the concrete surface decreased below 6, T. neopolitanus numbers increased. At pH values below 5, T. thiooxidans predominated, further lowering the pH. It appears that elemental sulfur, a product of abiological sulfide oxidation, can also be used as an energy source for the thiobacilli associated with the Hamburg, FRG concrete pipes (Milde et al., 1983).

Treatment by forced aeration of the sewerage decreased SRB activity and lowering volatile sulfide production. A decrease in the number of thiobacilli and the associated rate of concrete corrosion then resulted (Bielecki and Schremmer, 1987). With the advent of the National Pollution Discharge Elimination Standards (NPDES) in 1972 controlling discharge of pollutants into waterways, the level of heavy metals in sewage has declined significantly. This change, while having salutary benefits for the environment, has resulted in an exacerbation of the MIC problem in concrete sewage systems. By removing metals involved in suppressing bacteria concrete corrosion increased several orders of magnitude. Interestingly, the heavy metals appeared to have been effective against the sulfate-reducing bacteria and not against the thiobacilli (personal communication A. Walker, L.A. Sanitation dept.). Test systems and detection methods for Thiobacilli have been used successfully (Sand et al., 1987; Kerger et al., 1987).

Asphalt, which is the residue from petroleum crude oil distillates, is used as cement in the construction of roads.

Pendrys (1989) has described its degradation by a consortia of seven gram-negative, aerobic bacteria. The organisms belonged to the Pseudomonas, Acinetobacter, Alcaligenes, Flavimonas, and Flavobacterium genera; the consortia was capable of degrading the saturate and naphthene aromatic fractions of an asphalt cement- 20. The primary mechanism for this corrosion process appears to be the production of an emulsifier which is produced by A. calcoaceticus, one of the consortial members. However, consortial members which cannot produce this emulsifier can corrode the asphalt and remain viable by utilizing the water soluble asphalt fraction as a carbon source. Several million tons of asphalt are used annually in the U.S., however there are no figures available for the contribution of MIC to asphalt degradation (Ramamurti et al, 1984).

The corrosion of containers for high-level radioactive wastes has implications for all of mankind. The materials of construction and design of these containers in geological disposal sites must be sufficient to survive for at least 1000 years (Day et al, 1985). Marsh and Taylor (1988) have considered the theoretical contribution of microbial consortia to the corrosion of these containers. Based upon environmental conditions within the repository, the authors surmised that only SRB (of the catagories examined) would be capable of growth in the progressively anaerobic environment. Employing calculations which assume lactate and/or acetate as sole carbon sources, a "worst case" loss of ca. 13 mm mild steel during the 1000 year lifespan was predicted in addition to the 216 mm lost as a result of "abiological" localized corrosion. Unfortunately, the influence of biofilms as mediators of corrosion was ignored in these calculations as was the potential autotrophic growth of SRB on H_2 and CO_2. In order to form long term projections on the behaviour of such materials some estimation must be made for the water activity of surrounding rock formations. The activity of many microorganisms are severely curtailed in dessicated environments. Most problematic is projection of local weather trends 1000 years in the future and the effect of the spent fuel thermal output for the first 500 years which will tend to draw water to the repository. Obvious measures such as siting these repositories away from gypsum formations (sulfate sources) must also be considered.

III. GENERAL MICROBIOLOGICAL CONSIDERATIONS

MIC testing

Currently, there are no standards for microbiological testing of metallic, ceramic, vitreous or other engineering materials. The American Society for Testing and Materials has yet to define specific microorganisms that may be used in a laboratory test to characterize specific materials similar to the regimes used for textiles and wood resistance, for example (Onions, 1975). This is probably due to the fact that microbiological attack of such materials by definition is slow and nonuniform.

Another interesting requirement is that a "standard test system" must be uniformly applicable. For MIC this will present a problem since the corrosion products of some alloys are toxic. A good example of this are the brasses and copper/nickel alloys where the adhesion of organisms is controlled by the alloying distribution (Chamberlain and Garner, 1988). Any MIC test system will have to address these not insignificant points and as yet no such system has been proposed.

Isolates of specific bacteria that are obtained from a corrosion site and reintroduced to the same alloy in the laboratory often demonstrate little attack. From this we conclude that either microorganisms were isolated that were not responsible for the field corrosion situation or that the isolates are indeed responsible but did not perform the same way in the laboratory. This problem is directly influenced by Winogradsky's (1949) observations on the enrichment of "zymogenous" organisms in batch culture. These observations demonstrate that isolates obtained by such batch cultural methods have a high probability of not being the dominant contributors to a turnover (or effect) in biofilms or sediments. Furthermore if "environmentally relevant" organisms are in fact isolated and used as a test system in batch culture, their performance will undoubtedly be very different in such an arrangement from that experienced in the environment. Dowling et al. (1988) have showed that batch culture is very different from continuous flow situations: the worst corrosion rate associated with carbon steel C1020 was demonstrated to be one in which continuous flow was interrupted after a few days with a period of batch (stagnant) culture. This also appears to be the industrial experience: systems left in batch (stagnant) culture that are untreated tend to be very susceptible to MIC (Pollock, 1989).

Modification of the in situ environment

Considerable time and effort has gone into investigations of the formation of the initial biofilm. Baier (1981) has shown that "conditioning films" are laid down which consist of polymeric macromolecules which chemically adhere very quickly to the "new" surface presented. This is followed by recruitment to the surface of bacteria, perhaps predominantly Pseudomonas spp. or other similar organisms which produce copious quantities of extracellular glycocalyx (Costerton, 1985). The actual attachment of a single bacterium to an uncolonized site appears to follow a specific sequence of events: firstly, a reversible phase of electrostatic attachment followed by an irreversible phase omediated by extracellular polymer production (Marshall, 1976; McCoy, 1987; Haydon, 1961). At some undetermined time these oxygen scavanging organisms are joined by facultative and obligately anaerobic bacteria among which are the sulfate-reducing bacteria.

The problem is that research has only recently produced detailed

information on the nature of adhesion and corrosion and that relationship is far from clear. An example of the disarray in this topic can be illustrated by reading the proceedings of two symposia on the subject edited by Dexter (1986) and Licina (1988). For example, it is entirely possible to have a pipe that is heavily fouled, but after the mixture of detritis and biofilm is removed, no corrosion is observed. In practice, many different environments present themselves which vary in temperature, salinity and pH, etc. and therefore, generalizations about MIC fit badly when conceived as a single set of circumstances. For example, the thickness of the electrical double layer is very dependant upon the salinity. In microbiological terms, temperature and the availability of carbon and energy are probably the most important concerns in MIC. Seasonable variability in MIC attack may follow not just temperature but also the effects on heterotrophic organisms of algal blooms in source water which supplies piping systems. If such a correlation exists, then a lake with high agricultural "runoff" of nitrates and phosphates and the associated algal bloom may well contribute to the deterioration of metal superstructures in contact with that water. An interesting note is that chemical corrosion of ferrous alloys is generally impeded in the presence of nitrate and phosphate.

The nature of a "mature biofilm" can vary considerably. Biofilms have been described as stratified layers (Hamilton, 1985) subject to planar diffusion of oxidized and reduced molecular species to and from the metal substratum (Figure 1). For the purposes of this discussion, planar diffusion describes those mass transport processes which operate perpendicular to the surface. In the lower, reduced layer sulfate-reducing bacteria occupy an anaerobic niche where low molecular weight acids may be oxidized at the expense of sulfate. The product, sulfide, may diffuse away or precipitate with the metal close to the substratum. In the upper layers, oxygen is scavanged by aerobic organisms which force fermentative processes in lower anoxic strata. The fermentative products of this region are fed to the sulfate-reducing bacteria in the reduced zone. This description is quite reasonable for waters with high organic loading such as in the pulp and paper industry (Safade, 1988; Morgan and May, 1970; Cloete and Gray, 1985).

Alternatively, in environments where the organic loading is not so high such as an alpine-type freshwater lake, the biofilms may be discontinuous (figure 2) and form pockets of reduced/anoxic areas in oxic plains (Lappin-Scott and Costerton, 1989). As with the stratified model, the discontinuous biofilm model requires the interaction of aerobic, facultatively anaerobic and obligately anaerobic bacteria. In this latter model however, there are distinct areas where corrosion may be facilitated by differential oxygen cells (NACE, 1970). This occurs when adjacent areas of a metal surface are exposed to different oxygen concentrations and redox potentials. The areas under reduced oxygen tension will tend

to recruit the anodic (dissolutive) processes while the areas of high oxygen tension will recruit the cathodic (reductive) processes. Thus corrosion will tend to occur at sites of maximum bacterial consumption of oxygen (the anoxic zones) and be of a distinctly "localized" nature.

Corrosion activity as a measure of consortial production

There are few studies attempting to correlate bacterial activity with corrosion rate. Partly, this is because in the complex slime layers that occur on metal substrata there is no pure "biofilm" per se, but a mixture of bacterial mass, extracellular polymer, and corrosion/deposition products. In this situation with a very complicated series of corrosion reactions, the contribution of a single species is difficult to ascertain. The Aberdeen group have used $^{35}SO4$ reduction to gain some idea of the rates of sulfate-reduction present in biofilms (Maxwell and Hamilton, 1986; Hamilton et al., 1988). The method involves placing a metal coupon complete with an intact biofilm into a wide-bore Hungate roll-tube. The tube is purged with nitrogen and a reduced mineral salts medium added. Radiolabelled $^{35}SO4$ is introduced for a contact time of approximately five hours (Figure 3). Subsequently, the mineral medium is acidified and the volatilized sulfide captured on zinc acetate-soaked filter paper. The filter paper may then be retrieved for scintillation counting in a suitable phosphor cocktail. This assay was developed from a similar one suitable for the study of sediment SO_4-reduction rates (Rosser and Hamilton, 1983).

While the correlation of corrosion rate (by weight loss) with sulfate-reduction activity is hampered by the acidification of the mineral salts, useful information can still be obtained. This is particularly evident if low grade alloys are abandoned in favour of stainless steels where the corrosion products (pyrite formation) do not interfere with volatilization of the sulfide. In such circumstances however, weight loss must give way to electrochemical monitoring of the corrosion rate since weight loss is relatively insensitive.

Radiolabel studies to determine aerobic incorporation rates into biofilms have also been conducted (Geesey et al., 1986) on inert substrates. In this case glass cover slips were placed into cultures where the bacteria were encouraged to adhere. The slips were recovered, washed to remove loosely attached cells then exposed to ^{3}H-glucose for varying periods of time. The results showed that adherant cells were significantly more "active" than planktonic cells. This kind of arrangement is certainly amenable to work in the corrosion field.

The significance of the data as obtained above needs some qualification. A large part of the biomass associated with such films are not obligately anaerobic. Neither can they be considered aerobic. Both measures of estimating heterotrophic "production"

have serious flaws. In reality the organisms interact together and not as subsets to catabolize incoming substrate and in so doing create mass transfer discontinuities (Rittmann 1982). These discontinuities must also take into account "pockets" of organisms which are subject to radial diffusion (ie. not just perpendicular to the surface). In this way it is easy to see that MIC can be a dynamic process where the corrosion rate can oscillate as a function of the type of biofilm which is itself affected by the versatility of the member species and the incoming substrate.

IV. INTERACTIONS AMONGST CONSORTIAL MEMBERS

It has been a widely held contention amongst corrosion specialists that corrosion appears to be worse when a wide variety of microorganisms are present. Certainly, isolations from fresh corrosion tubercles have yielded a wide variety of isolates that fall into a diverse number of physiological types (Franklin et al., 1989). Recent experiments have documented the enhancement of corrosion processes by consortia of bacteria in controlled experimental systems. Experiments utilizing a sterilizable, flow-through test system after that reported by Franklin et al., (1989), have shown that the corrosion of mild steel measured non-destructively by electrochemical impedance spectroscopy for a consortium concentrated from corrosion tubercles in a freshwater system with a trace of sulfate was 7.5 times faster than the sterile control (White et al., 1989). Monocultures of aerobic heterotrophic bacteria, resulted in corrosion rates which were 3 times faster than the sterile control. A biculture of any of several facultative heterotrophic bacteria and a sulfate-reducing bacterium resulted in a corrosion rate which was 4.2 times faster than that of the control (White et al., 1989). Electrochemical impedance spectroscopy (EIS), a method of deriving corrosion information from low amplitude sinusoidal voltage oscillations, has proved to be quite valuable in MIC studies due to its relatively non-destructive properties (Dowling et al., 1988). EIS data showed that the mechanism of corrosion for bicultures and environmental consortia is quite different from that with monocultures and sterile chemical corrosion.

The presence of fermentative bacteria that produce acetate stimulate the growth of the sulfate-reducing bacterium Desulfobacter sp. (Dowling et al., 1988). The presence of acid producing heterotrophs in aerobic seawater systems also stimulates corrosion by these sulfate-reducing bacteria on coupons of stainless steel. Presumably the is occurs both by creating the metabolic niche and supplying the nutritional substrate (White et al., 1990). With the judicious utilization of ^{13}C and gas chromatographic/mass spectrometric analysis (GC/MS), it should be possible to demonstrate the transfer of carbon between the heterotrophic bacteria and the obligate anaerobe by following the incorporation into the phospholipid ester-linked fatty acid 10-methyl palmitic acid which is characteristic of this

sulfate-reducing bacterium (Dowling et al., 1986). In this system, the consortium could be fed low levels of a ^{13}C labeled substrate such as glutamate that is not utilized by the anaerobe and the coupon containing the consortia extracted for GCMS analysis for the "heavy" fatty acid biomarkers.

Pope et al.(1984) has described corrosion by local reduction of the pH by organic acid production. While it can be established that the pH in propagating pits may be very low (less that 2.0), it seems unlikely that only acetic acid (pKa 4.75), a very important and common fermentation product, could be the cause. Coupled with other organisms which utilize this short chain acid at the expense of oxidizing other electron acceptors (perhaps ferric ions) corrosion rates are faster than observed with just the fermentative bacteria (Gaylarde and Johnston, 1982). Further support against the specific acetate-mediated corrosion hypothesis is given by chemical corrosion studies: supplementation of mineral salts medium with acetic acid achieves only a low increase in corrosion rate which does not compare to the high rates observed with microorganisms.

Another common hypothesis is the effect of the differential oxygen cell mentioned earlier. This is an established mechanism in chemical corrosion. Oxygen-depleted areas tend to recruit the anodic processes. Since a large number of microorganisms respire at the expense of oxygen the probability is that such microbes will easily enter crevices, create local anoxic conditions and thereby promote corrosion. Further complicating this study has been the interaction of obligate anerobic organisms which often produce significantly corrosive end products. Thus not only is there an oxygen differential but also a reduced redox potential at the anodic site.

A significant flaw associated with almost all field studies has been the failure to identify sources of nutrient flux. It is axiomatic that microorganisms require carbon and energy. In grossly polluted systems such as busy, enclosed harbours, the biofilms generated are very thick and diverse in community structure (Desmukh et al., 1988) and the resulting MIC is quite extensive and rapid. It seems reasonable to assume that the problem would be mitigated by a simple reduction in the pollution level. Desmukh et al. (1988) demonstrated the high levels of corrosion associated with the presence of waste effluent. Sulfate-reducing bacteria were specifically implicated by interaction with other organisms which provided primary breakdown of the suspended waste. Unfortunately, while such experimentation on marine corrosion has shown that the corrosiveness of certain waters vary from place to place is influenced by pollution loading, attempts to isolate MIC as the sole cause have not been successful.

V. MECHANISMS

Some simple mechanisms have already been discussed for various types of MIC (organic acid production, differential oxygen cells etc.). While it is clear that they have an important bearing on MIC, they cannot be the sole cause. Other mechanisms put forth include the "cathodic depolarization hypothesis" where hydrogen generated in the reductive processes is rapidly removed to low partial pressures by hydrogenophilic bacteria. This has been shown for methanogenic (Daniels et al., 1987) as well as sulfate-reducing bacteria (Pankhania et al., 1986) and a range of others (Mara and Williams, 1971). The presence of "hydrogenase" enzymes have been taken as indicating potential for cathodic depolarization. Although organisms with hydrogenase enzymes may be responsible for higher corrosion rates than those without (Booth and Tiller, 1968), there has been few attempts to separate the "effect" of the enzyme from other potentially corrosive and possibly never-described activities. Microorganisms are involved in a great many processes which may affect the corrosion rate besides a hydrogenase-linked cathodic depolarization hypothesis. Supporting the depolarization hypothesis has been electrochemical data which describes cathodic polarization curve movements and current direction (Ringas and Robinson, 1987; Daumas et al., 1988). Only recently has the technology been available to rigorously examine this phenomenon at the molecular level.

Hydrogen is available not only from corrosion reactions but also from certain fermentative bacteria. No estimations have been conducted to quantitate these different sources of hydrogen in biofilms and assess their effect on the corrosion rate. Further complicating this problem are sulfate-reducing bacteria which contain more than one hydrogenase. These enzymes can be engaged in either import or export functions (Lupton et al., 1984). Only a few studies have been performed where the interactions of sulfate-reducing bacteria with fermentative organisms have been examined with respect to the corrosion rate of their metal substratum. Gaylarde and Johnston (1982) showed that a coculture of Vibrio anguillarum and Desulfovibrio vulgaris accelerated the corrosion rate of a mild (carbon) steel over that exhibited by either of those organisms in pure culture. This study was carried out using a reduced medium and lactate. No mechanistic information was provided however. Dowling et al. (1988) subsequently showed that a nominally aerobic coculture arrangement with Vibrio natriegens and two sulfate-reducing bacteria substantially changed the impedance diagram associated with the overall corrosion process as compared to pure culture of vibrio alone. This study was different from the first in that no substrate was specifically incorporated into the medium for the sulfate-reducing bacteria and anaerobiosis was provided by the activity of the vibrio alone. Electrochemical impedance analysis (figure 4) indicated that an unspecified colloidal corrosion product associated with a low frequency impedance capacitive loop may have been removed in the presence of

the sulfate-reducing bacteria. These data together indicate that not only can sulfate-reducing bacteria accelerate the corrosion process in coculture with another organism but that the corrosion processes promoted by each organism are distinct.

Hydrogen sulfide, whether of chemical or biological origin provides aggressive anions in solution (Assefpour-Dezfuly and Ferguson, 1988). The generation of these anions by sulfate reducing bacteria contributes to the overall corrosion process. This effect has been separated from any other corrosive activity that these bacteria might possess in two ways: 1) Examination of the corrosion rates associated with non-hydrogenase sulfate-reducing bacteria (no cathodic depolarization), and 2) corrosion rates associated with hydrogenase-containing bacteria with benzyl viologen (no sulfide production) replacing sulfate as the electron acceptor (Booth and Tiller, 1968).

Another mechanism of metal corrosion associated with microorganisms is hydrogen embrittlement (Ford and Mitchell, 1989). This is a process whereby microorganisms release diatomic hydrogen as a result of fermentation processes which diffuses into the solid metal and concentrates in a particular area. This hydrogen coalesces to form a bubble which presses the metal out in a blister. While hydrogen embrittlement is not immediately a corrosion problem, there is the potential for stress corrosion cracking associated with blister formation. Catastrophic failure can occur without warning. While embrittlement is a well-known phenomenon the contribution due to microorganisms is undetermined.

A serious but unquantified aspect of microbial corrosion has been the scavanging of chloride anions from service water systems and other raw water conduits. In order for corrosion to occur electroneutrality must be observed at the anodic sites (Lin et al., 1981). Thus some anions must diffuse to the surface in order to account for the positive charges generated in the anodic process. Biofilms tend to recruit metal ions by the extracellular polysaccharide acting as an exchange resin (Rendleman, 1978). These charges must be balanced by anions obtained from solution. The degree to which the polysaccharide retains aggressive anions such as chloride and affects the corrosion rate by this method is not understood. Jolley et al. (1988) have used Auger electron spectroscopy and X-ray photoelectron spectroscopy to examine the biocorrosion of copper by Pseudomonas atlantica polysaccharide. Figure 5 shows the Auger electron lines where bacteria and various organic materials and culture supernatant contributed to the dissolution of copper. This information shows that the extracellular polymer acts not just as a support matrix for the bacteria but also behaves as a trap for ions which promote corrosion.

VI. THE EFFECTS OF SURFACE PERTURBATIONS ON MIC COMMUNITIES

Weld effects

Elements such as iron, copper, zinc, and aluminium all have extensive use as engineering materials. The more desirable properties however, such as strength, elasticity and corrosion resistance generally improve with the incorporation of specific alloying elements. Alloys, unfortunately, are subject to types of corrosion not found in uniform, single element materials. An example is "intergranular attack". This is a type of corrosion whereby adjacent grains of slightly differing composition form a galvanic couple. These discontinuities produce anodic and cathodic segregation and hence localized corrosion. In ordinary circumstances, stainless steels are manufactured to produce an even distribution of elements. Localized heat treatments such as welding cause a discontinuity in this arrangement. The grain size, elemental composition, and crystal structure all vary across a weld. Such areas are heavily attacked. Whether microorganisms are recruited to these sites (by magnetic or electric fields, for example) or organisms present merely exert a greater corrosive effect has not been established. While huge conglomerations of bacteria arranged in tubercules have been observed with many types of welds, their role in the actual deterioration of the weld is not understood (Borenstein, 1988a, Tatnall, 1981; Kobrin, 1976).

Heat Affected Zone (HAZ).

Welds can be divided into three areas: the base metal, which is unaffected by the weld adjacent to it; the weld metal which contained the liquified pool of metal behind the heat strike (a mixture of the rod and base metal); and the heat affected zone (HAZ), an area where dislocations occur due to the proximity of the heat strike but where no rod material is incorporated.

While the weld metal itself can be attacked, the heat affected zone is an area that may be preferentially attacked. Figure 6 depicts a weld failure where the primary region of attack is the heat affected zone immediately adjecent to the fusion line. MIC in such cases is characterized by tiny perforations in the pipe wall which lead to very large subsurface cavities (Borenstein, 1988b). Figure 7 shows a 304L stainless steel weld. A large pit was found immediately under the tubercule. So-called "weeping" welds are those where several of these subsurface cavities have joined at a single point and provided a continuous path through the wall of the pipe. While it is certain that these cavities are anaerobic, no knowledge of the microbiology of the interior of these regions has been obtained due in part to the kinetic heating produced when slicing with a saw (no cooling jets may be used which might contaminate the site). Dowling et al. (1989) have shown that MIC attack of AISI 316/E308 stainless steel weldments in seawater initiates in the heat affected zone. Rather interestingly, if the

weld is autogenous (ie. heat strike only - no weld metal) then corrosion initiates in the weld zone at places where slag accumulates. Slag is composed of mangenese and silicon globules which are produced in the welding process.

Weld metal

The weld metal is a mixture of the melted composition from the rod or welding wire, and the base metal. Depending upon the criteria required for that weld, the rod and base metal may or may not be identical. In certain stainless steel welds; the overriding importance will be for resistance to "hot-cracking" and not corrosion resistance. Thus, these welds are manufactured without regard to their susceptibility to MIC (Borenstein, 1988c). A duplex structure is characteristic of 300 series stainless steel welds. In this type of arrangement, two crystal structures, ferrite and austenite, are produced by differential cooling-solidification rates after the heat strike has passed, where previously the base metal was fully austenitic. MIC attack of such welds appears to selectively remove either one phase or the other, but not both. Figure 8 depicts the skeletal remains of the ferrite after removal of the surrounding austenite in the pit. Borenstein (1989) found that the content of ferrite in a weld apparently had no effect on MIC susceptibility although attack of these welds apparently required a duplex structure. Tubercule formation was never observed on solution annealed (heat treated to produce single phase austenitic) welds whereas similar, untreated duplex welds were attacked. Thus it seems that MIC attack requires the presence of such dislocations. Examination of welded materials other than stainless steels tends to confirm that elemental segregation is an important factor in weld failures (Little et al., 1988).

The microbiology of stainless steel tubercules is complicated. Sulfate-reducing bacteria are frequently found associated with pseudomonads, Bacillus and Alcaligenes species. Whereas the distribution of organisms does not appear to change much, the total number of individuals varies a great deal. Dexter (1988) described a tubercule that had a structured arrangement with obligately anaerobic organisms on the interior and aerobic, iron-precipitating bacteria on the exterior. Extrapolation from the microbiological composition of a tubercule to that of the subsurface cavities, however, seems unreasonable.

Metal composition

The surface chemistry of the metal substratum exerts a significant effect on adhesion and, therefore, corrosion. An excellent example of this was work by Chamberlain and Garner (1988), which involved exposure of 90/10 copper-nickel alloys to varying amounts of iron in the sea. The alloy containing no iron corroded rapidly and demonstrated little bacterial adhesion. It appears that the rapid (chemical) dissolution of toxic metals may therefore have been

involved in the prevention of colonization similar to an antifouling paint which releases copper. Other studies have shown that copper-nickel alloys are particularly affected when voracious, oxygen-consuming bacteria are present (Schriffrin and De Sanchez, 1985). The same authors also described a significant increase in corrosion rate when mixed cultures of Pseudomonas spp. Micrococcus spp. and Corynebacterium spp. were introduced into the test system. The deterioration of copper and copper-based alloys have been described as associated with the formation of exopolymers (Geesey et al., 1986) which would tend to support the data of Schiffrin and De Sanchez (1985). The NACE (1970) basic corrosion course lists sulfate-reducing bacteria as significantly corrosive to brasses (copper-zinc alloys) due to the presence of aggressive HS- anions.

A large number of commonly used engineering alloys contain elements that are considered toxic. Stainless steels, for example, contain high levels of nickel, chromium, and molybdenum. Corrosion of these alloys poses the interesting question of why biofilm organisms are apparently immune to the consequent production of toxic cations. An ESCA survey, however, showed that significant quantities of these metals do not accumulate in the biofilm (Dowling et al., 1989). One possible explanation is that these cations are not retained by the bacterial extracellular polymer and diffuse rapidly away. The case for copper has been discussed above. Nickel-based alloys (where nickel is present in the highest quantity of any element) have also been implicated as susceptible to MIC. Stoecker (1984) discussed a case-study where a Monel tube (72% nickel) was severely attacked by selective leaching of the nickel, leaving a copper sponge. Unfortunately, follow-up microbiology is rarely conducted to determine elementary conditions such as total direct counts, viable counts, nickel or copper resistance of the isolates, numbers of anaerobes etc.

Cathodic protection

It is often uneconomical to use high grade alloys to perform low-risk tasks that are not "safety-related". To this end, many construction companies select materials that are more susceptible to corrosion but are less expensive and have better mechanical or welding capabilities for example. To offset the increased corrosion, interim measures such as painting with anticorrosion/antifouling coatings and cathodic protection may be employed. The latter is quite a common procedure in which the anodic sites associated with the corrosion process segregate to a separate electrode either by impressed current (use of an industrial potentiostat) or via sacrificial anodes (made of zinc or magnesium, for example). Further details may be obtained from McKenzie (1987).

The cathodic (reductive) reactions segregate to the "protected" structure and locally the pH increases due to production of hydroxyl ions. Two major effects spring from this that have the

potential to affect and be affected by many different types of organisms: 1) deposition of a calcareous shell due to the elevated pH, and 2) production of cathodic hydrogen. These effects include elevating the numbers of hydrogenophilic bacteria (such as sulfate-reducing bacteria) surrounding the protected structure (Guezennec et al., 1988) and removal of the calcareous shell (Edyvean et al., 1988). These processes do not, however, constitute immediate corrosion but rather economic loss due to the increased current required to maintain the displaced potential (Fiksdal and Guezennec, 1988). Serious corrosion is not appreciated until accidental disruption of the cathodic protection (whether by complete dissolution of the sacrificial anode or interrupted power to the potentiostat) occurs. In this event, the "cloud" of organisms (including sulfate-reducing bacteria), which have been encouraged by the evolution of cathodic hydrogen, will be available to accelerate corrosion. Microorganisms of different physiological types are therefore likely to have a synergistic effect on the deterioration of cathodic protection effectiveness. Factors influencing the effectiveness and efficiency of cathodic protection include the water activity in soils, salinity in water systems, and organic loading.

VII. DISSECTING MIC COMMUNITY STRUCTURE.

Problems with classical methods

The ability of microbiologists to adequately characterize physiologically distinct members of MIC communities has often been limited by the available cultural techniques. Isolation and enumeration of the unusual bacteria which comprise MIC communities is frustrated by the diverse growth requirements of consortial members. Mittelman and Geesey (1987) and Pope (1986) have described classical cultural techniques for corrosion consortia. Unfortunately, media for enumeration of the sulfate-reducing, sulfur-oxidizing, and slime-forming consortial members are unacceptably selective. The presence of "viable, non-culturable" organisms in extreme environments and biocide treated industrial systems adds a further complication to classical cultural techniques.

Lipid analyses

In order that the deficiencies associated with cultural methods may be addressed, techniques are now being employed which utilize signature biomarkers, rather than the presence of viable cells, as indicators of community structure and biomass. Vestal and White (1989) have reviewed the use of lipid analyses to evaluate the biomass, community structure, metabolic status, and activity of consortial communities. These techniques exploit the ubiquitous nature and relatively short half-life of membrane-associated phospholipid fatty acids (PLFA). Since these compounds are present

in all cells and they are turned over rapidly in the environment (White, 1979), their presence is an indicator of viable biota.

Analysis of specific PLFA patterns provides a means by which physiologically distinct groups of bacteria may be identified and enumerated. With the exception of archaebacteria, which contain ether-linked rather than ester-linked fatty acids, these lipid-based methods can be used to assess the eukaryotic and prokaryotic biotic structure at a given point in time. Dowling et al (1988) utilized PLFA analysis to characterize the biofilm community structure of corroded carbon steel coupon. The analysis showed the presence of large numbers of facultative anaerobes, with smaller numbers of sulfate-reducing bacteria present in the corroded areas.

An assessment of community activity, an important determinant of corrosion potential, can be made using a measure of substrate incorporation into cellular lipids (White et al, 1977). ^{14}C-acetate, which can be readily metabolized by most eubacteria and fungi, is quickly incorporated into lipids. Following incubation with the labeled acetate, biofilms can be extracted and the amount and rate of lipid synthesis estimated. Franklin et al (1989) used this uptake procedure to evaluate the effects of oxidizing biocides on biofilm activity and corrosion rates of mild steel coupons.

Molecular methods

The techniques of molecular biology provide new tools to examine the distribution and community structure of microbial consortia in biofilms. The techniques that have been developed for quantitative recovery of DNA and ribosomal RNA from sediments should work for the biofilms involved in corrosion. It is particularly difficult to recover nucleic acids from soils or sediments rich in clay and the nucleic acids recovered are typically fragmented. Presumably these problems would be decreased in the analysis of corrosion biofilms. This emerging technology is enormously powerful as oligonucleotides made with sequences of more than 10-20 bases provide a specificity that is virtually absolute. Once the sequence is known and the appropriate fragment defined, the synthetic DNA probe can be made in substantial quantities. This probe is then tagged (most often with 32-P) by nick-translation. The environmental samples are treated with detergents to lyse the bacteria and the nucleic acids recovered and purified. Once the nucleic acid is recovered from the environment, it is placed in appropiate ionic strength buffers and heated to denature (form single strands). The single strands are then allowed to anneal (hybridize) with the probe and the un-hybridized single stranded nucleic acid, and excess probe removed by various techniques. The degree of homology can be controlled by the hybridization conditions. Under proper conditions it is possible to detect single gene copies if enzymatic amplification techniques, such as polymerase chain reaction, are utilized (Ogram and Sayler, 1988).

Selection of the appropriate DNA probe is dependent on the gene of interest, augmented by the DNA sequence information available for that gene. With access to gene libraries it is possible to select for a wide variety of genes. Genes that are associated with certain groups of bacteria can be selected if the distribution of the enzyme is known. As more of the sequences of genetic determinants of specific processes become known, the probes can be modified or mixed probes utilized. The probe defines the presence of the specific gene, not the enzyme of the metabolic activity. It has been established that certain genes can be detected outside of bacteria adsorbed to the sediment (Ogram and Sayler, 1988). Even with these provisos, the technique is powerful and can give insight into the enzyme distribution in a community. Problems may exist in referring the presence of the gene to the presence of a specific bacteria. Some genes are widely distributed; for example, the APS-reductase enzyme which activates sulfate and is essential to all the sulfate-reducing bacteria also was found to occur in sulfur oxidizing bacteria as well (Postgate, 1984). DNA probes are particularly useful in the detection of genetically engineered microbes in the environment where the specificity of the probe can be controlled. The use of DNA gene probes has been reviewed (Jain et al. 1988).

An alternative or complementary method that allows detection of the ribosomal RNA (rRNA) with probes has some additional properties. The rRNA are remarkably conserved molecules that are involved in protein synthetic systems common to all life. Initial work concentrated on the 5S rRNA which consists of about 120 nucleotides. This short sequence was sufficently invariate that it showed a paucity of independently varying nucleotide positions. The 16S rRNA (~1600 nucleotides) was ideal for phylogenetic comparisons and with the advent of DNA cloning and sequencing methods, provides exciting possiblilities. There are invariate sequences universal to all life, sequences common to the major kingdoms--eukaryotes (plants, animals and microeukaryotes) and the prokayotes (eubacteria and archaebacteria). There are sequences which can be used to define closely phylogenetically related groups (which may not be functionally obvious such as the plant mitochondrial rRNA, the methane oxidizing bacteria and the plant tumor inducing Agrobacterium) and individual species or strains. The facts that there are 10^4 copies of the rRNA in each cell and the analytical systems can detect 50 or so molecules, and that the probe nucleotides can penetrate the intact cells of bacteria in environmental samples provides a system in which appropiately labeled probes can be used to identify specific bacteria or groups of bacteria in biofilms (DeLong et al., 1989).

The disadvantages of the rRNA probe technology are that for maximum effectiveness the sequence of the 16SRNA must be known. This means the organism must be culturable and the sequencing performed. Determination of the sequence is an artful procedure

not the least of which comes in defining the best positioning (placement of "skipped" bases) to use in phylogenetic matching. There is a constant problem of the almost ubiquitous RNAase contamination which can easily ruin the experiments. None-theless, the application of nucleic acid probe technology to environmental systems is a powerful means to defining the distribution of microbes in samples and their community structure.

Immunofluorescent techniques

When fluorescent probes are applied to biofilms, the localization of specific cells can be readily determined. This exciting new technology is reviewed by Olsen et al.(1986). An elegant use of this technology in examining the effects of a commonly used antibiotic on the community structure in the bovine rumen has been reported (Stahl et al. 1988). Pope (1986) has reviewed techniques for detecting members of MIC communities. Included in these discussions is a summary of immunofluorescent methods for detecting sulfate-reducing and sulfur-oxidizing bacteria in tubercles. As with other immunochemical techniques, however, the use of antibody probes for the detection of specific consortial members is somewhat limited by the ability to produce the probes and target them to specific environmental isolates. Despite their current technological limitations, these emerging biochemical, molecular and immunological techniques could provide great insight into the activities of specific organisms in the corrosion consortium.

VIII. TREATMENT CONSIDERATIONS.

The literature is replete with descriptions of biocide and/or physical treatments for the control of biofouling and MIC activities. Unfortunately, the majority of these studies are performed with monocultures and are designed to evaluate treatment efficacy with respect to planktonic populations. The phenomenon of "bacterial regrowth" in municipal water systems is an example of the failings of classical treatment evaluations (LeChevalier, 1988). Biofouling and corrosion treatments which fail to control adherent populations cannot succeed in eliminating or controlling deleterious microbial activities. All too often, failures in control measures are attributed to "biocide resistent" microbial populations. Indeed, this apparent resistance is sometimes used as a rationale for regular changes in the type(s) of biocides employed in the treatment regime. In reality, "biocide resistance" can nearly always be attributed to the presence of adherent biofilms (Costerton and Lashen, 1984). Resistance to commonly employed biocides such as sodium hypochlorite, in the classical sense of antibiotic resistance, has not been shown. The multitude of extra- and intra- cellular target sites and relatively high concentrations utilized in industrial systems effectively precludes

such resistance mechanisms.

Franklin et al (1989) examined the effects of sodium hypochlorite and sodium hypochlorite/sodium bromide biocide combinations on a consortia of MIC putative bacteria. The test system employed mild steel coupons in a flowing freshwater medium maintained at pH 8.5. An inoculum of bacteria obtained from a tubercle containing actively respiring bacteria was used in the biocide challenge assays. The consortium, which was found to consist of a spore-forming Bacillus sp.; an iron-reducer (Fe^{+++}); an iron precipitating, slime-forming, strict aerobe; and an acetic acid producing, facultative anaerobe; and a lactate-utilizing, sulfate- reducing bacterium. Both the corrosion rate as measured by electrochemical impedance spectroscopy and the metabolic activity of the community decreased following addition of 16 mg l^{-1} sodium hypochlorite/sodium bromide (Figure 9). A 3-4 order-of-magnitude decrease in each of the community members was noted following the biocide dosage. However, within 24 h following cessation of biocide dosing, the community had recovered to its approximate pre-biocide structure and number. The intrinsic biocide demand associated with adherent biofilms and their associated corrosion products can account for the failure of apparently high concentrations of oxidizing biocides to completely inactivate biofilm bacteria (Ruseska et al, 1982).

The corrosion products which result from MIC activities can interact with a number of commonly employed biocide treatments. For example, the oxidizing biocides, such as sodium hypochlorite, can oxidize ferrous iron, manganese, and hydrogen sulfide. The resulting deposits often lead to deleterious deposits within cooling towers and other industrial water systems (Trulear and Wiatr, 1988). Other, non-oxidizing biocides, such as acrolein, are rapidly inactivated by hydrogen sulfide. While differential sensitivity to biocide application has not been reported for MIC consortial bacteria, Ridgway (1987) has shown that fast-growing Mycobacteria spp. are significantly more resistant to inactivation by chlorine than are other aerobic bacteria present in consortial biofilms on wastewater treatment reverse osmosis membrane surfaces.

Clearly, there is justification for improving evaluation methodologies. It is apparent that the presence of consortial corrosion communities within adherent biofilms is the primary limiting factor in treatment efficacy.

IX. SUMMARY

1. MIC attack is usually associated with consortial activities. One caveat to this is that a single species within a community may exert a disproportionate effect. The corrosion of aluminum fuel tanks by C.resinae provides an example of such an effect.

2. Complicated consortia are involved in promoting corrosion in particular areas depending upon materials and their condition (eg. welds). The relationship of these organisms to each other and to their substratum is not well understood.

3. Most significant economic effects of corrosion which have been identified as microbiological in origin is found to be a direct result of consortial, rather than monospecies, activities.

4. Novel methods for dissection of MIC consortia and their activities are currently available but have yet to be extensively used. These include non-destructive electrochemical techniques, signature biomarker analyses, and molecular genetic techniques.

5. Treatment and prevention maintenance strategies must consider the influence of consortial biofilms on their efficacy. Evaluations of treatment efficacies should utilize biofilm communities. Treatments which do not consider their influence are, at best, ineffective. At their worst, they can often exacerbate preexisting corrosion conditions.

6. The development and use of new surface-specific biocides, MIC-resistant alloys (containing bactericidal elements perhaps), self draining pipe systems with constant flow (no stagnant areas) and limiting zones where detritis may collect, will be more important in the design of efficient plant operations in the future.

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REFERENCES

Allred, R. C., J. D. Sudbury, and D. C. Olson: Corrosion is controlled by bactericide treatment. World Oil 149:111-112, 1959.

Assefpour, M. and W.G. Ferguson: Effect of low concentrations of hydrogen sulfide in seawater on fatigue crack growth in a CMn structural steel. Corrosion, 44 (7), 443-449, 1988.

Baier, R.: Initial events in microbial film formation. In: Marine Biodeterioration: An interdisciplinary study. Proceedings of the symposium on marine biodeterioration. Uniformed Services University of Health Sciences. 20-23, April, 1981. Naval Institute Press, Annapolis, Maryland, 1981.

Bielecki, R. and H. Schremmer: Leichtweiss-Institut fur Wasserbau der Technishen Universitat Braunschweig. Mitteilungen Heft 94/1987. ISSN 0343-1223, 1987.

Booth, G.H., and A.K. Tiller: Cathodic characteristics of mild steel in suspensions of sulphate-reducing bacteria. Corrosion Science 3, 583-600, 1968.

Borenstein, S.W.: Microbiologically influenced corrosion failures of austenitic stainless steel welds. Materials Performance, August pp. 62-66, 1988.

Borenstein, S.W.: Guidelines for destructive examination of potential MIC-related failures. EPRI publication # ER-6345. Microbial corrosion: 1988 workshop proceedings. Ed. G.J.Licina, 1988b.

Borenstein, S.W.: Microbiologically influenced corrosion failure analyses. Materials Performance. March, pp. 51-54, 1988c.

Borenstein, S.W.: Influence of welding variables on microbiologically influenced corrosion of austenitic stainless steel weldments. MS thesis, University of Tennessee, Knoxville, 1989.

Cabral, D.: Corrosion by microrganisms of jet aircraft integral fuel tanks. Int. Biodeterion. Bull. 16(1): 23-27, 1980.

Chamberlain, A.H.L and B. J. Garner: The influence of iron content on the biofouling resistance of 90/10 copper-nickel alloys. Biofouling, 1(1), 79-96, 1988.

Cloete, T.E. and F. Gray: Microbiological Control in paper mills. Paper Southern Africa 51 (4), 26, 1985.

Costerton, J.W.: The role of bacterial exopolysaccharides in

nature and disease. Developments in Industrial Microbiology Vol. 26, 249-261, 1985.

Costerton, J. W. and E. S. Lashen: Influence of biofilm on efficacy of biocides on corrosion-causing bacteria. Materials Performance 23(2), 13-17, 1984.

Daniels, L., N. Belay, B.S. Rajagopal and P. Weimer: Bacterial methanogenesis and growth from CO^2 with elemental iron as the sole source of electrons. Science 237, 509-511, 1987

Daumas, S., Y. Massiani and J. Crousier: Microbiological battery induced by sulphate-reducing bacteria. Corrosion Science 28(11), 1041-1050, 1988.

Davies, R. D.:Corrosion problems in the pulp and paper industry. Paper Southern Africa 4(4):18-27, 1984.

Day, D.H., A.E. Hughes, and G.P. Marsh: The management of radioactive wastes reports on progress in physics. 48:101-169, 1985.

DeLong, E. F., G. S. Wickham, and N. R. Pace: Phylogenetic stains: Ribosomal RNA-based probes for the identification of single cells. Science 243, 1360-1363, 1989.

Deshmukh, M.B., R.B. Srivastava and A.A. Karande: Corrosion of mild steel and copper by microorganisms in polluted seawater. In: Marine Biodeterioration: Advanced techniques applicable to the Indian Ocean. pp. 725-737. Eds. M.-F. Thomson, R. Sarojini, and R. Nagabhushanam. Oxford & IBH Publishing Co., New Delhi, Bombay, 1988.

Dexter, S.C.: Role of microfouling organisms in marine corrosion. Plenary lecture: 7th International Congress on Marine corrosion and fouling. Valenica, Spain. November, 1988.

Dexter, S.: Biologically Induced Corrosion. Proceedings of the conference on Biologically Induced Corrosion, June 10-12, 1985, Gaithersburg, MD. National Association of Corrosion Engineers (NACE), Houston,TX, 1986.

Dowling, N.J.E, J. Guezennec, M.L. Lemoine, A. Tunlid, D.C. White: Analysis of carbon steels affected by bacteria using electrochemical impedance and direct current techniques. Corrosion 44 (12), 869-874, 1988.

Dowling, N.J.E., M.J. Franklin, D.C. White, C.H. Lee and C. Lundin: The effect of microbiologically influenced corrosion on stainless steel weldments in seawater. Page No. 187. NACE '89.

New Orleans, 1989.

Dowling, N.J.E., F. Widdel and D. C. White: Phospholipid ester-linked fatty acid biomarkers of aceate-oxidizing, sulphate-reducing, and other sulfide-forming bacteria. J. General Microbiology. 132:1815-1825, 1986.

Edyvean, R.G.L, C.J. Hutchinson, and N.J. Silk: Observations on the cathodic protection of steel in biologically active seawater. 7th International congress for marine corrosion and fouling. Valencia, Spain, 1988.

Fiksdal L. and J. Guezennec: Increased current demand required by cathodic protection of carbon steel in the presence of Vibrio natriegens. 7th International Congress for marine corrosion and fouling. Valencia, Spain, 1988.

Ford, T.E. and R. Mitchell: Hydrogen embrittlemant: a microbiological perspective. Corrosion 89. Page No. 189. NACE, New Orleans, 1989.

Franklin, M.J., D.E. Nivens, M.W. Mittelman, A.A. Vass, R.F. Jack, N.J.E. Dowling, R.P. Mackowski, S.L. Duncan, D.B. Ringleberg and D.C. White: An analogue MIC system with specific bacterial consortia, to test effectiveness of materials selection and countermeasures. Corrosion 89. Page No. 513. NACE proceedings of conference. New Orleans, 1989.

Gaylarde, C. C. and J. M. Johnston: Anaerobic metal corrosion in cultures of bacteria from estuarine sediments. pp. 137-143. In Dexter, S.C. (ed.), Biologically induced corrosion. National Association of Corrosion Engineers, Houston, TX, 1986.

Gaylarde, C.C. and J.M. Johnston: The effect of Vibrio anguillarum on the anaerobic corrosion of mild steel by Desulfovibrio vulgaris. Internat. Biodeterioration Bull. 18 (4), 111-116, 1982.

Geesey, G.G., S.D. Salas and M.W. Mittelman: Effects of environmental conditions on the sessile existance of an estuarine sediment bacterium. GERBAM: Deuxieme Colloque International de Bacteriologie marine- CNRS, Brest. 1-5 octobre 1984. IFREMER, Proceedings of conference 3, 243-247, 1986.

Geesey, G.G., M.W. Mittelman, T. Iwaoka, P.R. Griffiths: Role of bacterial exopolymers in the deterioration of metallic copper surfaces. Materials Performance, February. 37-40, 1986.

Greathouse, G.A. and C. J. Wessel: Deterioration of materials. Reinhold, New York, 1954.

Grimm, D.T., C.G. Hollis, and S.T. Threlkeld: The nature of sulfate-reducing bacteria isolated from a high temperature Kraft liner board machine. Proceedings of the Technical Association of the Pulp & Paper Industry, pp 111-113, 1984.

Guezennec, J., M. Therene, N. Dowling, and D.C. White: Influence de la protection cathodique sur la croissance des bacteries sulfato-reductrices et sur la corrosion d'aciers doux dans les sediments marins. 7th International Congress on Marine Corrosion and Fouling. Valencia, Spain, 1988.

Hamilton, W.A.: Sulphate-reducing bacteria and anaerobic corrosion. Ann. Rev. Microbiol. 39, 195-217, 1985.

Hamilton, W.A., A.N. Moosaavi, N. Pirrie: Mechanism of anaerobic microbial corrosion in the marine environment. Paper submitted to European Federation of Corrosion First Workshop on Microbial Corrosion at Sintra, Portugal 7th-9th March, 1988.

Haydon, D.A.: The surface charge of cells and some other small particles as indicated by electrophoresis. Biochimica et Biophysica Acta 50, 450-457, 1961.

Hill, H.C.: Biodegradation of petroleum products. pp. 582-617. In Atlas, R.M. (ed.) Petroleum Microbiology, MacMillan, New York, 1984.

Houghton, D.R., R.N. Smith, and H.O.W. Eggins: Biodeterioration. Proceedings of Conference of the Seventh International biodeterioration symposium, Cambridge, UK 6-11 September 1987, 1988.

Jain, R. K., R.S. Burlage, and G. S. Sayler: Methods for detecting recombinant DNA in the environment. CRC Critical Reviews in Biotechnology 8, 33-84, 1988.

Jolley, J. G., G. G. Geesey, M.R. Hankins, R. B. Wright, and P.L. Wichlacz: Auger electron spectroscopy and X-ray photoelectron spectroscopy of the biocorrosion of copper by gum arabic, bacterial culture supernatant and Pseudomonas atlantica exopolymer. Sur. Inter. Analy. 11, 371-376, 1988.

Kerger, B.D., P.D. Nichols, C.A. Antworth, W. Sand, E. Bock, J.C. Cox, T.A. Langworthy and D. C. White: Signature fatty acids in the polar lipids of acid producing Thiobaccilli: methoxy, cyclopropyl, alpha-hydroxy-cyclopropyl and branched and normal monoenoic fatty acids. FEMS Microbial Ecology, 38, 67-77, 1987.

Knudsen, J.G.: Fouling of heat transfer surfaces. pp. 57-82. In P.J. Marto and R.H. Nunn (ed.), Power condenser heat transfer technology, Hemispher Publishing Co., New York, 1981.

Kobrin, G.:Corrosion by microbiological organisms in natural waters. Materials Performance 15, (7), 38, 1976.

Kuhn A.T. and T. Rae: Aqueous corrosion of Ni-Cr alloys in biological environments and implications for their biocompatability. British Corrosion J. 23 (4), 259-266, 1988.

Laanbroek, H.J. and H. Veldkamp: Microbial interactions in sediment communities. Phil. Trans. R. Soc. Lond. B, 297, 533-550, 1982.

Lappin-Scott, H.M., and J.W. Costerton: Bacterial Biofilms and Surface fouling. Biofouling 4, 323-342, 1989.

LeChevallier, M.W., C.D. Cawthon, and R.G. Lee: Inactivation of biofilm bacteria. Appl. Environ. Microbiol. 54, 2492-2499, 1988.

Licina, G. Microbial Corrosion: 1988 Workshop Proceedings. Electric Power Research Institute (EPRI) publication ER-6345. October 18, 1988, Charlotte, NC, 1988.

Lin, L.F., C.Y Chao, and D.D. MacDonald: A point defect model for anodic passive films. II. Chemical breakdown and pit initiation. J.Electrochemical Soc. 128 (6), 1194-1198, 1981.

Little, B., P. Wagner, and J. Jacobus: The impact of sulfate-reducing bacteria on welded copper-nickel seawater piping systems. Materials Performance. 27 (8), 57-61, 1988.

Lupton, F.S., R. Conrad, J.G. Zeikus: Physiological function of hydrogen metabolism during growth of sulfidogenic bacteria on organic substrates. J. Bacterio. 159 (3), 843-849, 1984.

Mara, D.D., and D.J.A. Williams: Polarization studies of pure Fe in the presence of hydrogenase-positive microbes- I. Non-photosynthetic bacteria. Corrosion Science 11, 895-900, 1971.

Marsh, G.P. and K.J. Taylor: An assessment of carbon steel containers for radioactive waste disposal. Corrosion Science, 28, 289-320, 1988.

Marshall, K.C.: Interfaces in Microbial Ecology. Harvard University Press, Cambridge, London, 1976.

Maxwell, S, and W.A. Hamilton: Modified radiorespirometric assay for determining the sulfate reduction activity of biofilms on metal surfaces. J. Microbiol. Meth. 5, 83-91, 1986.

McCoy, W.: Fouling biofilm formation. In: Biological Fouling of Industrial water systems: a problem solving approach. Eds.

Mittelman, M.W. and Geesey, G.G. Water Micro Associates, CA, pp. 24-25, 1987.

McKenzie, S.G.: Techniques for monitoring corrosion of steel in concrete, Corrosion Prevention and Cantrol, February, 11-17, 1987.

Milde, K., W. Sand, W. Bock: Thiobacilli of corroded concrete walls of the Hamburg sewer system. J. General Microbiol. 129, 1227-1333, 1983.

Miller, J.D.A., E. R. Schiapparelli and D.E. Meybaum: Metals In: Microbial Biodeterioration. Ed. Rose, A.H., 1981. Academic Press, London, New York, 1981.

Mittelman, M.W. and G.G. Geesey: Biological fouling of industrial water systems: a problem solving approach. Water Micro Assoc., San Diego, CA, pp. 316-339, 1987.

Morgan, F.L. and O. W. May: Scale control in White-water systems. TAPPI, 53 (11), 2096-2098, 1970.

NACE: NACE Basic Corrosion Course. National Association of Corrosion Engineers, Houston, Texas, 1970.

Ogram, A.V. and G.S. Sayler: The use of gene probes in the rapid analysis of natural microbial communities. J. Indust. Microbiol. 3, 281-292, 1988.

Olsen, G.J., D.J. Lane, S.J. Giovanni, N.R. Pace and D.A. Stahl: Microbial ecology and evolution: a ribosomal approach. Ann. Rev. Microbiol. 40, 337-365, 1986.

Onions, A.: Organisms for Biodeterioration Testing- moulds and fungi. In: Microbial Aspects of the Biodeterioration of materials. Eds. Lovelock D.W., and Gilobert R.J. Academic Press, London, New York, 1975.

Pankanhania,I.P., A. N. Moosaavi, W.A. Hamilton: Utilization of cathodic hydrogen by Desulfovibrio vulgaris (Hildenborough). J.General Microbiology, 132, 3357-3365, 1986.

Parker, C.D.:Species of sulphur bacteria associated with the corrosion of concrete. Nature 159, 439, 1947.

Pendrys, J.P.: Biodegradation of asphalt cement-20 by aerobic bacteria. Appl. Environ. Microbiol. 55:1357-1362, 1989.

Pollock, W.I.: Hydrotest water can cause MIC pitting. Materials Performance. 28 (7), 68, 1989.

Pope, D.H., D.J. Duquette, A.H. Johannes and P.C. Wayner: Microbiologically influenced corrosion of industrial alloys. Materials Performance, April, 1984.

Pope, D.H.: Discussion of methods for the detection of microorganisms involved in microbiologically influenced corrosion. In Dexter, S.C. (ed.) Biologically induced corrosion. NACE Publications, Houston, TX, pp 275-283, 1986.

Postgate, J.R.: The sulphate-reducing bacteria. Cambridge University Press, London, 1984.

Purkiss, B.E.: Corrosion in industrial situations by mixed microbial floras. pp. 107-128. In Miller, J.D.A. (ed.), Microbial aspects of metallurgy. Medical and Technical Publishing Co., Aylesbury, England, 1971.

Ramamurti, K., G.P. Jayaprakash, and C.F. Crumpton: Microbial biodegradation of asphalt and related hydrocarbons--a literature review. pp. 1-44. In Report no. FHWA-KS-84/1 Interim report. Division of Operations,, Bureau of Material and Research, Kansas Department of Transportation, Topeka, KS, 1984.

Ratledge, C. and S. G. Wilkinson: Microbial Lipids. Volume 1. Academic Press, New York, N. Y. 1988.

Rendleman, J.M.: Metal-polysaccharide complexes- Part I. Fundamental Chemistry 3, 47-79, 1978.

Ridgway, H.F.: Microbial fouling of reverse osmosis membranes: genesis and control. pp 138-193. In Mittelman, M.W. and Geesey, G.G. (eds.) Biological fouling of industrial water systems: a problem solving approach. Water Micro Associates, San Diego, CA, 1987.

Ringas, C. and F.P.A. Robinson: Corrosion of stainless steel by sulfate-reducing bacteria- electrochemical techniques. Corrosion 44 (6), 386-396, 1987.

Rittman, B.E.: Comparative performance of biofilm reactor types. Biotech.Bioeng. 24, 1341-1370, 1982.

Rosser, H.R. and W.A. Hamilton: Simple assay for accurate determination of [35_S] sulfate reduction. Appl. and Environ. Microbiol. 45 (6), 1956-1959, 1983.

Ruseska, I., J. Robbins, J.W. Costerton and E.S. Lashen: Biocide testing against corrosion-causing oilfield bacteria helps control plugging. Oil and Gas J. 80, 253-264, 1982.

Safade, T.L.: Tackling the slime problem in a paper-mill. Paper Technology and Industry. 25(6), 280-285, 1988.

Sand, W. and E. Bock: Concrete corrosion in the Hamburg sewer system. Environ. Technol. Lett. 5, 517-528, 1984.

Schiapparelli, E.R. and B.R. Meybaum: The role of dodecanoic acid in the microbiological corrosion of jet aircraft integral fuel tanks. Int. Biodet. Bull. 16 (3), 61-66, 1980.

Schiffrin, D.J. and S. R. De Sanchez: The effects of pollutants and bacterial microfouling on the corrosion of copper- base alloys in seawater. Corrosion 41 (1), 31-38, 1985.

Stahl, D.A., B. Flesher, H.R. Mansfeld, L. Montgomery: Use of phylogenetically based hybridization probes for studies of ruminal microbial ecology. Appl. Environ. Microbiol. 54, 1079-1084, 1988.

Steocker, J.G.: Guide for the investigation of microbiologically induced corrosion. Materials Performance. 23,(8), 48-55, 1984.

Stephensen, M. and L. H. Stickland: Hydrogenase. II. The reduction of sulfate-to-sulfide by molecular hydrogen. Biochem. J. 25, 215-220, 1931.

Tatnall, R.: Case histories: Bacteria induced corrosion. Materials Performance 20, (9), 32, 1981.

Thistlethwayte, D.K.B.: Control of sulfides in sewerage systems. Butherworth, Melbourne, 1972.

Tiller, A.K.: A review of the European research effort on microbial corrosion between 1959 and 1984. In: Biologiocally induced corrosion. Ed. S.Dexter. National Association of Corrosion Engineers, Houston, Texas, 1986.

Trulear, M.G. and C.L. Wiatr: Recent advances in halogen based biocontrol. Proceedings of the National Association of Corrosion Engineers Annual Meeting, March 21-25, St. Louis, MO. Paper no. 19, 1988.

Vestal, J.R. and D.C. White: Lipid analysis in microbial ecology. Bioscience 39, 535-541, 1989.

Von Wolzogen Kuhr, C.A.V. and L. S. Van der Vlugt: Water (The Hague), 18 (16), 147, 1934.

White D.C., R.F. Jack, N.J.E. Dowling, M.J. Franklin, D.E. Nivens, S. Brooks, M.W. Mittelman, A.A. Vass, and H.W. Isaacs: Microbially influenced corrosion of carbon steels. Submitted to Corrosion/90, National Association of Corrosion Enginners, Las Vagas, NV, 1990.

White, D.C., R.J. Bobbie, S.J. Morrison, D.K. Oosterhof, C.W. Taylor and D.A. Meeter: Determination of microbial activity of estuarine detritus by relative rates of lipid biosynthesis. Limnol. and Oceanogr. 22, 1089-1099, 1977.

White, D. C.: Validation of quantitative analysis for microbial biomass, community structure, and metabolic activity. Adv. Limnol., 31, 1-18, 1988.

Widdel, F.: Microbiology and ecology of sulfate- and sulfur- reducing bacteria. In: Biology of anaerobic microorganisms. Ed. A.J.B. Zehnder. Wiley-Interscience, New York, Chichester, 1988.

Winogradsky, S.: Microbiologie du Sol. Oeuvres Completes. Mason, Paris, 1949.

Wolin, M.J.:Hydrogen transfer in microbial communities in biotechnology. In: Microbial Interactions and communities. Volume 1. Eds. Bull, A.T. and Slater, J.H. Academic press, London, New York, 1982.

Figure 1: A stratified biofilm in high organic-loading waters.

Figure 2: A "non-uniform" biofilm in low organic-loading waters.

Figure 3: Radiolabel method to survey sulfate-reduction rates on metal coupons exposed to seawater. By kind permission of the authors: S. Maxwell and W.A Hamilton (1986)

Figure 4: Electrochemical impedance spectra (Z'-real impedance Vs. Z"-imaginary impedance) which shows the inception of a low frequency capacitive loop (right side of the diagram) associated with the corrosion of carbon steel. This was only observed when mild steel coupons were in sterile conditions or in contact with Vibrio natriegens alone (closed circles). A coculture of the vibrio and two sulfate-reducing bacteria (open circles) apparently removed the low frequency capacitive loop. Note that the occurrance of a secondary loop indicates diffusion through a particular corrosion product which was apparently removed by the sulfate-reducing bacteria (after Dowling et al. 1988).

Figure 5: Auger electron spectral lines associated with various combinations of bacteria and biopolymers in contact with copper surfaces (Jolley et al.,1988).

Figure 6: Typical structure of a MIC-mediated weld failure of the heat affected zone (HAZ). Note that a tubercule covers a pin-hole entrance to series of caverns within the heat affected zone. Attack can also occur in the weld metal, apparently without HAZ deterioration.

Figure 7: Tubercular mound covering a 304L weld in the bottom of a 304L pipe that had been exposed to untreated "freshwater". Note that these mounds may appear within a two week exposure period. By kind permission of Susan W. Borenstein.

Figure 8: Residual ferrite stringers observed from a AISI 316L/E308 resulting from microbially influenced corrosion. The austenite phase has been selectively removed leaving the chromium rich ferrite behind. This residual crystal structure has been only been observed with MIC and ferric chloride attack. Photograph courtesy of Rebecca Wood, Singleton Laboratories.

Figure 9: The effect of chlorine biocide treatment on corrosion rate (as measured by reciprocal polarization resistance) and metabolic activity (^{14}C-acetate incorporation into phospholipid fatty acids).

A. (▲) HOCl, inoculated; (△) HOCL, sterile; (◍) Non-treated, incoculated; (○) Nontreated, sterile.

B. (○) Non-treated, inoculated; (◭) HOCl, treated, inoculated.

Fig 1

FIGURE 1.

A STRATIFIED BIOFILM IN HIGH-ORGANIC-LOADING WATERS

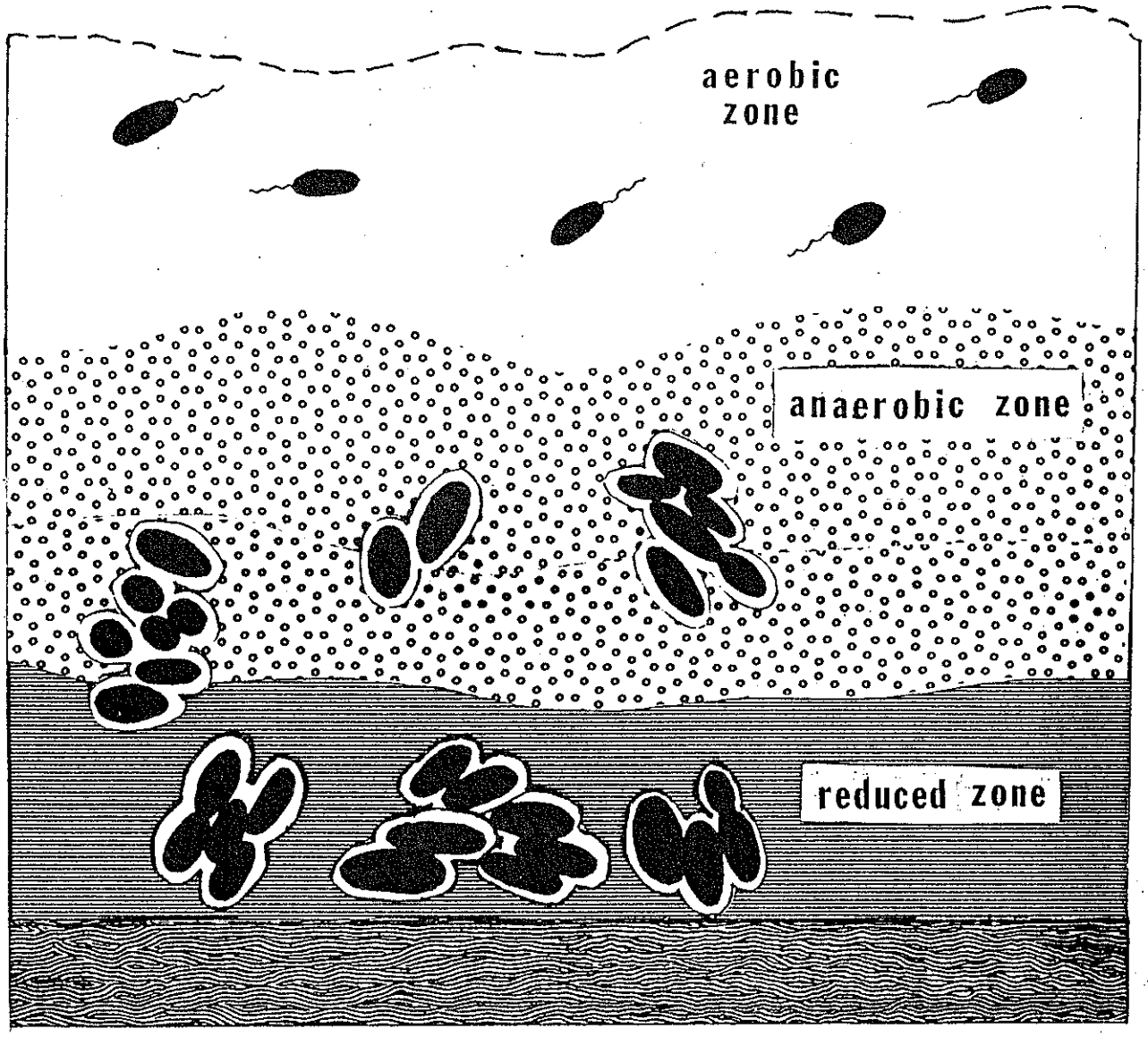


FIGURE 2. (fig 2)

A "NON-UNIFORM" BIOFILM IN

LOW-ORGANIC-LOADING WATERS

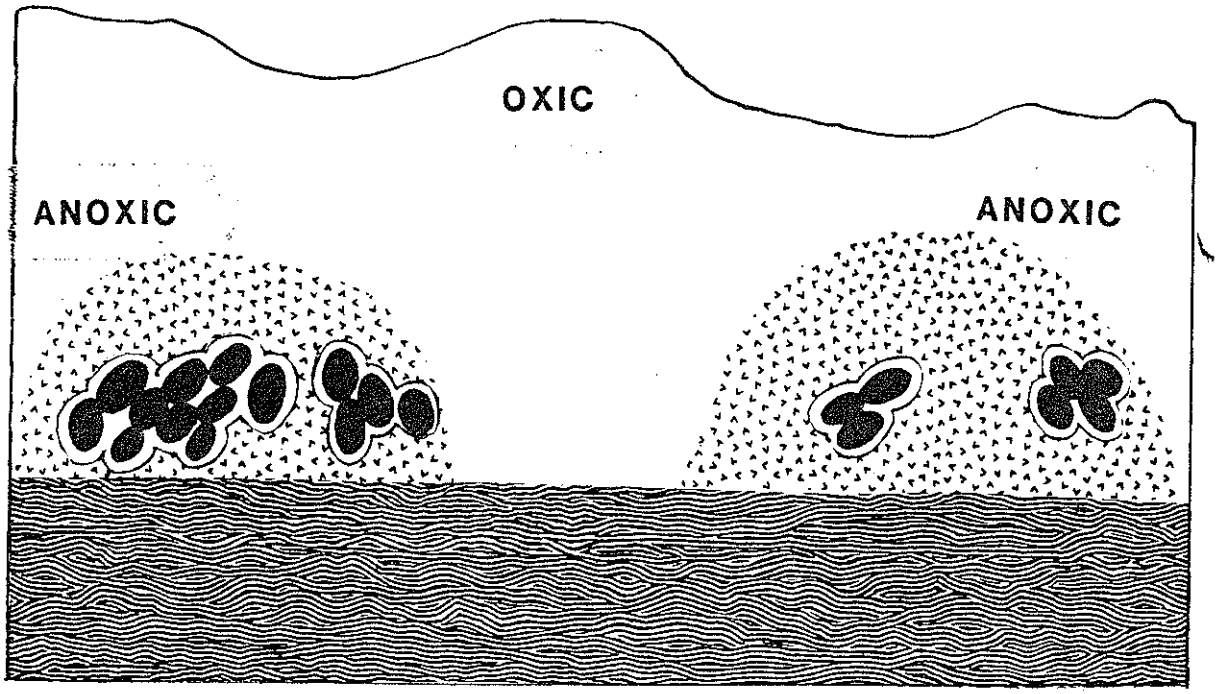
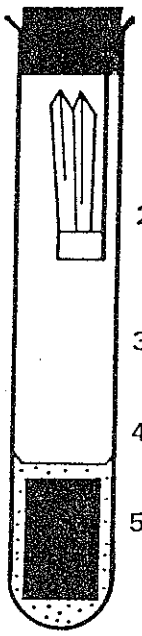


Fig 3



1. Metal coupon placed into 4 mls of anaerobic, filtered, sterile sea water containing 10 μCi [^{35}S–sulphate]
2. Bung seated securely and 0.5mls of oxygen-free 2N zinc acetate immediately injected onto the filter paper wick
3. Metal coupon incubated as desired temperature for optimum incubation time (in this case 5 hrs)
4. 0.5 mls of oxygen-free 6N hydrochloric acid injected past the wick into the solution
5. Acid volatile sulphides, including any [$H_2{}^{35}S$] formed, trapped during a 2hr equilibration period at 35 C, 100 osc. min^{-1} in a shaking water bath

(All manipulations carried out under oxygen-free nitrogen)

fig 4

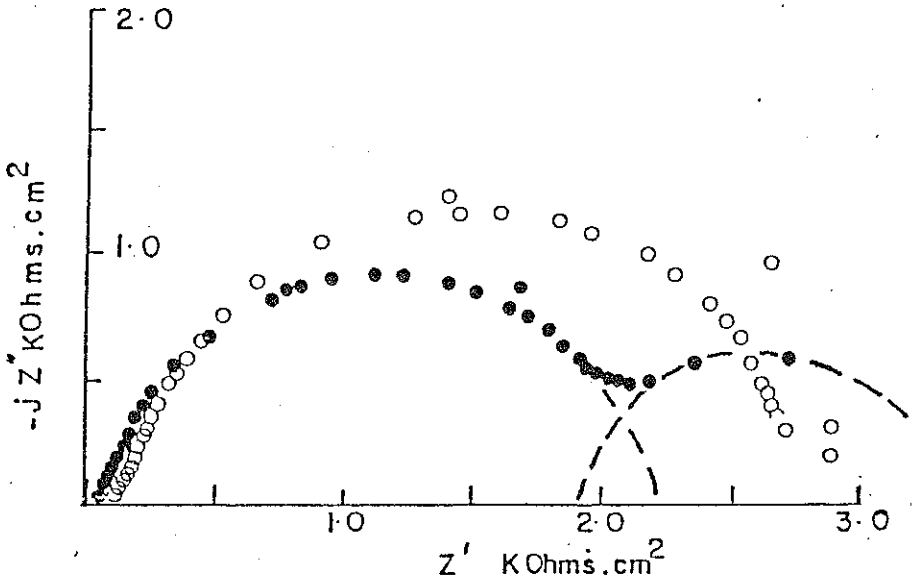
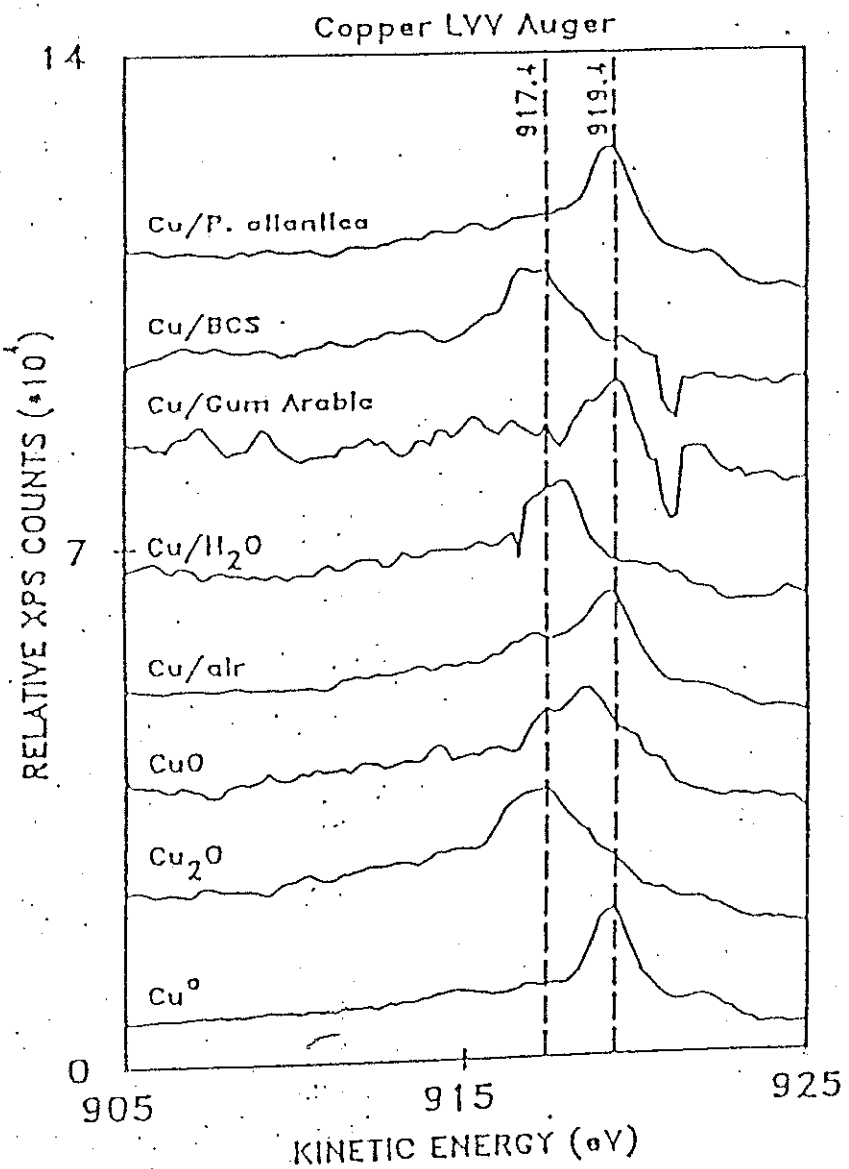


fig 5.



Cu $L_3M_{4,5}M_{4,5}$ Auger electron lines.

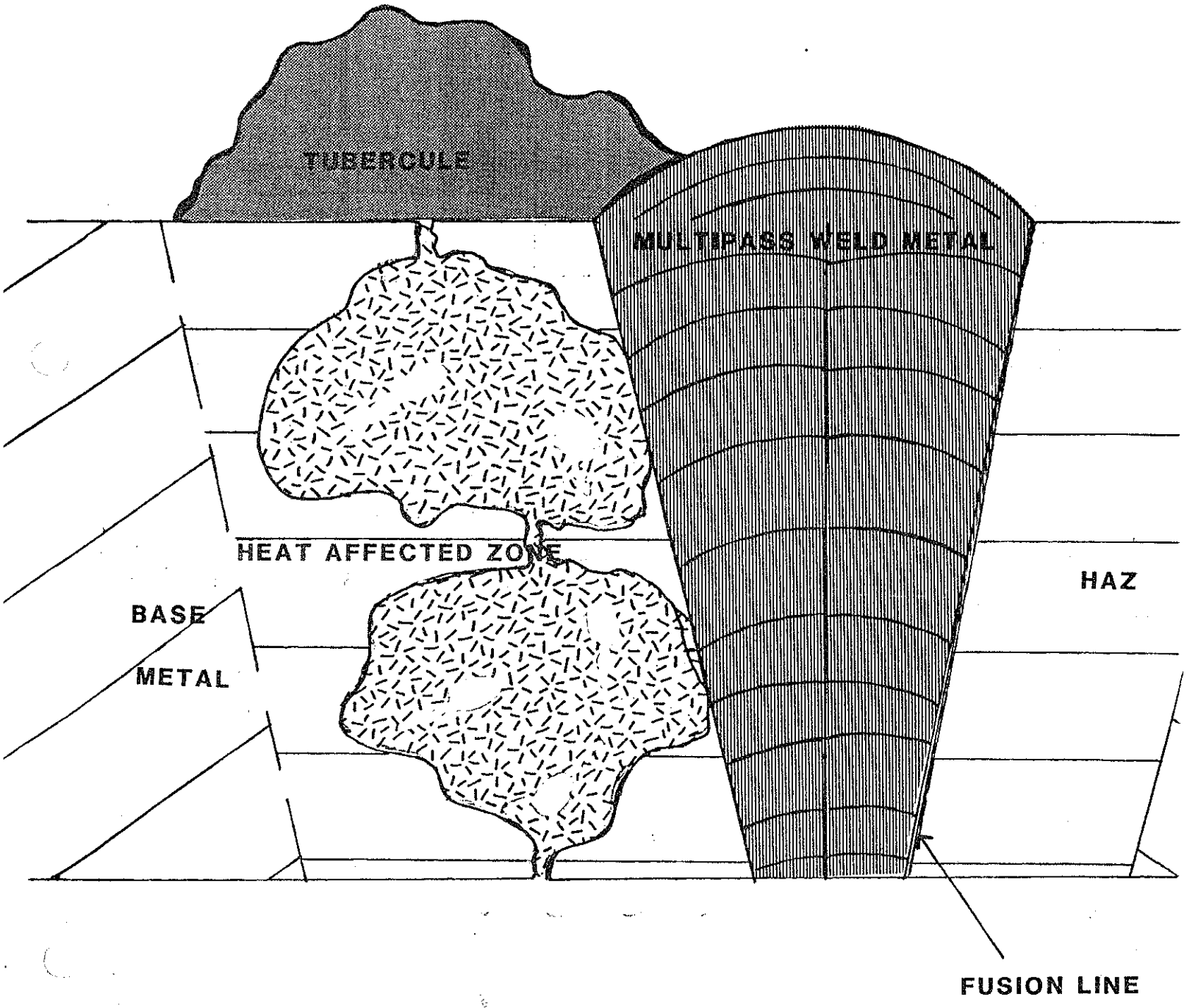
FIGURE 6

Fig 6

TYPICAL ARRANGEMENT OF MIC ATTACK OF A WELD

WHERE A TUBERCULE COVERS A PINHOLE OPENING

TO A SERIES OF CAVERNS IN THE HEAT AFFECTED ZONE



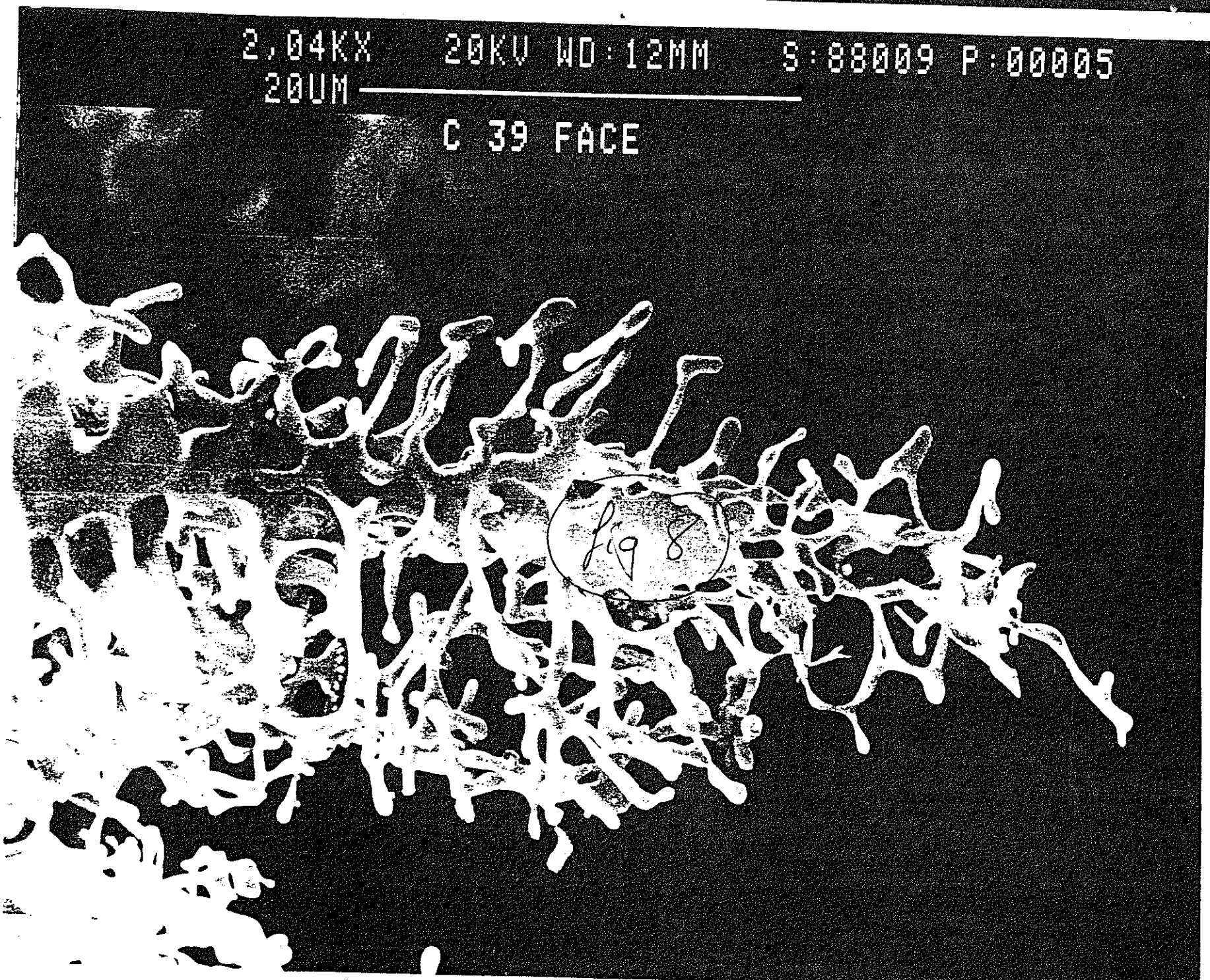
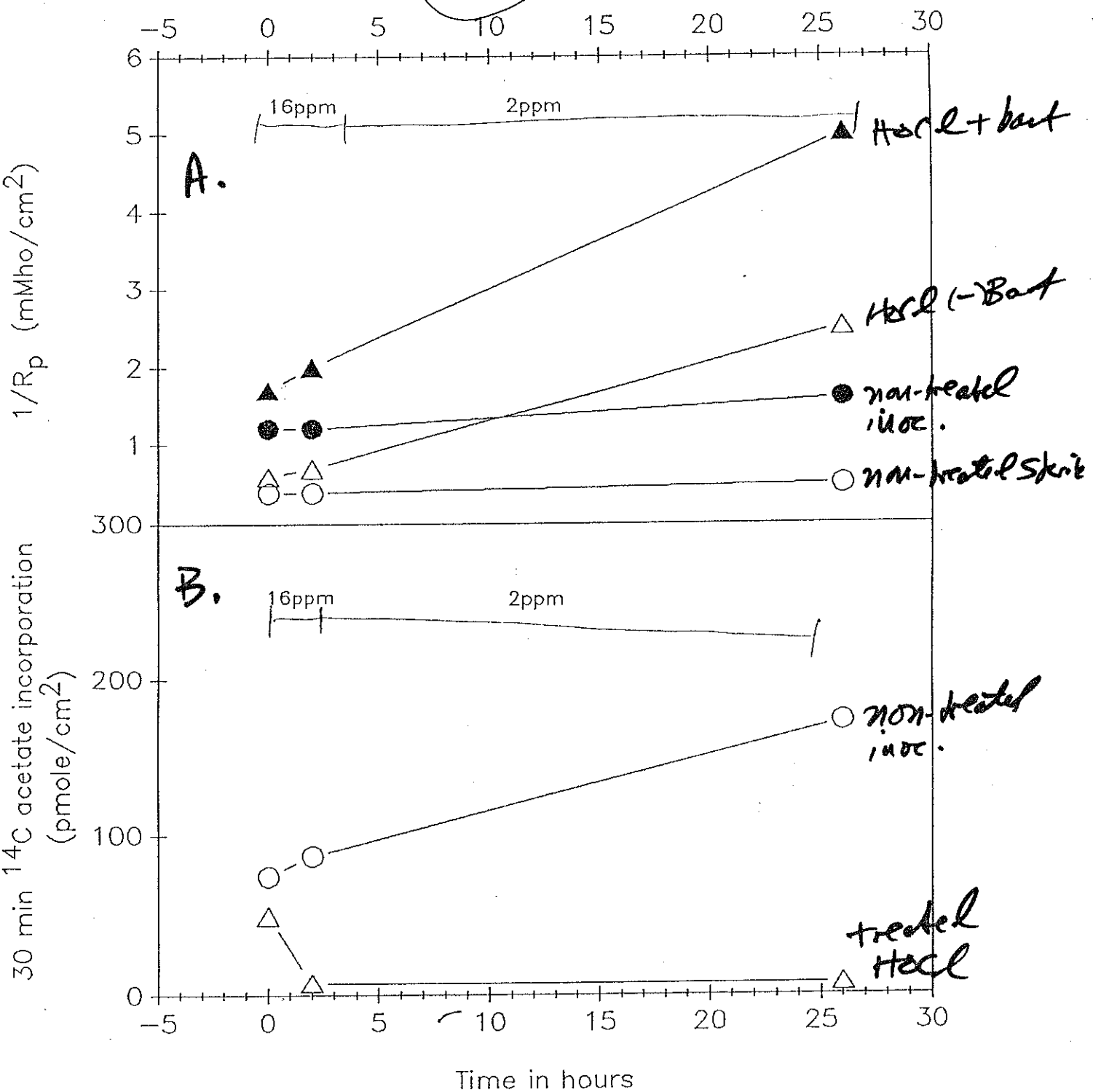

2,04KX 20KV WD:12MM S:88009 P:00005
20UM
C 39 FACE
fig 8

Figure 9


A.
B.
16ppm
2ppm
HCl + Bact
HCl (-) Bact
non-treated intact
non-treated skin
treated HCl
1/Rp (mMho/cm2)
30 min 14C acetate incorporation (pmole/cm2)
Time in hours
-5
0
5
10
15
20
25
30
1
2
3
4
5
6
100
200
300