A REVIEW OF THE POTENTIAL FOR MICROBIALLY INFLUENCED CORROSION OF HIGH-LEVEL NUCLEAR WASTE CONTAINERS

Prepared for

Nuclear Regulatory Commission
Contract NRC-02-88-005

Prepared by

Center for Nuclear Waste Regulatory Analyses
San Antonio, Texas

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ABSTRACT

The potential for microbially influenced corrosion (MIC) of the candidate and alternate container materials for the proposed Yucca Mountain repository site is examined on the basis of an extensive review of the literature. A brief description of the environmental conditions expected outside the waste packages, in terms of the geology, hydrology, water chemistry, radiation, temperature, and moisture content, is followed by a detailed discussion regarding the characteristics of microbial life in subsurface environments. Energy and nutrient requirements to sustain bacterial colonies are examined in conjunction with the effect of various environmental parameters involved, such as salinity, water availability, temperature, pH, and the presence of electron acceptors. In the extensive discussion on MIC, separate consideration is given to different classes of metallic materials, including mild steels, copper and copper alloys, austenitic stainless steels, nickel and nickel alloys, and titanium and its alloys. It is concluded that all the alloys reviewed, with the possible exception of titanium, may be susceptible to MIC. The lack of relevant information on nickel-base alloys containing chromium as the main alloying element is emphasized. Finally, a set of recommendations is included to address some of the uncertainties confronted in predicting the occurrence of MIC under the environmental conditions anticipated at the proposed Yucca Mountain repository site.

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EXECUTIVE SUMMARY

This report is a review of the literature on microbially influenced corrosion (MIC) of the classes of metallic materials that are considered by the U.S. Department of Energy (DOE) for the construction of high-level waste (HLW) containers for the proposed repository site at Yucca Mountain. These materials include those originally identified in the Site Characterization Plan (SCP) as candidates for the construction of thin-wall containers. These are Fe-Cr-Ni alloys, including types 304L and 316L stainless steels and alloy 825, and copper-based alloys, such as CDA 102 (oxygen-free, high-conductivity copper), CDA 613 (Cu-7Al-3Fe) and CDA 715 (Cu-30Ni). Also included in the review are alternate corrosion resistant alloys, such as alloy C-4 and Titanium Grade 12, that are currently being considered by DOE. MIC of carbon steels is also covered in this review because the waste package design may be modified by the introduction of a double-shell container, in which carbon or low alloy steels may be used as one of the components.

The report has been prepared as an activity of the Integrated Waste Package Experiments (IWPE) research program at the Center for Nuclear Waste Regulatory Analyses (CNWRA). The review was prepared by Dr. Gill Geesey, Department of Microbiology, Montana State University, Bozeman, Montana, to be included as the initial activity in Task 4 of the IWPE program on Microbiologically Influenced Corrosion.

Following a brief introduction in which it is noted that the U.S Nuclear Regulatory Commission (NRC) regulation 10 CFR 60.113 requires waste packages to provide substantially complete containment of the radionuclides for a minimum period of 300 to 1000 years, the main characteristics of the environment outside the waste packages are described for the conditions anticipated at Yucca Mountain. The description includes the consideration of geological, hydrological, and geochemical aspects in order to emphasize that, under the unsaturated conditions present at the repository horizon, it is expected that, with certain emplacement configurations, liquid water may not contact the container surface until at least 10,000 years after the emplacement of HLW. However, it is noted that several scenarios are currently being envisioned that may lead to premature wetting of the container, even during the 300- to 1000-year containment period. Other variables, such as temperature, water chemistry, and radiation are also considered.

In the following section, the ubiquitous nature of many microorganisms is noted, indicating that some of them have survived, and even grown, under extreme temperature conditions or under the high radiation fields encountered in the core of nuclear power reactors. Another important aspect discussed in this section is the fact that microorganisms tend to form biofilms on many types of substrates as a complex assemblage of distinct microbial species attached to the substrate through a matrix of extracellular polymeric substances. The importance of microbial biofilms is emphasized in terms of heterogeneous cell distribution and metabolism-driven chemical gradients at or near metal/solution interfaces which may lead to the occurrence of localized corrosion. The influence of biofilm formation in the modification of the corrosion potential established at a metal/solution interface is noted. This variation may lead to the initiation of localized corrosion if the corrosion potential evolves to values above a certain critical potential for that corrosion process.

The characteristics of microorganisms in subsurface environments are discussed at length. This discussion relies heavily on information obtained in recent years, which reveals that a significant active microbial population exists under such conditions. Relevant information obtained in various DOE sites, such as

Savannah River Plant, Idaho National Engineering Laboratory, Hanford, Rainier Mesa at the Nevada Test Site, and Pajarito Plateau in New Mexico, is summarized as an introduction to a discussion of biotransformations of subsurface microorganisms, including anaerobic biotransformations in the deep subsurface. Although bacteria, as a group of living microorganisms, can function over a wide range of water activity, pH, salinity, temperature, and oxygen concentrations, it is noted that a given species is limited to a substantially narrower range of these parameters for growth. A variety of aerobic and anaerobic microorganisms known to be implicated in MIC are discussed, emphasizing the type of metabolic products that their activity generates, as well as their nutrient requirements. A subsection is dedicated to the examination of the energy requirements to maintain microbial growth under repository conditions. In the examined case of a Swiss repository, it was concluded that the main energy-producing reaction would be the corrosion of the steel container and that the amount of energy available would be a greater limiting factor than the nutrient availability. This preliminary estimation led to the conclusion that microbial action was unlikely to be a significant problem in a HLW repository. However, it is pointed out that the current models are too simplistic to describe the complex microbial processes that may occur on metallic surfaces exposed to repository environments.

MIC of the classes of metallic materials that are currently being considered for the construction of HLW containers are reviewed in detail in Section 5 of the report. However, the lack of relevant information for a particular alloy of interest led to the consideration of related alloys whenever possible. Nickel-based alloys containing chromium as the main alloying element (i.e., alloys 825 and C-4) were a particular problem because published information, even for related alloys, was extremely limited.

In the introduction to MIC of the various alloys systems analyzed, it is noted the MIC manifests itself primarily as localized corrosion in the form of pitting. It is emphasized that, during biofilm development, discrete microcolonies of distinct bacteria are found as a heterogeneous and patchy distribution on the metal surface, occluding some areas and leaving others fully exposed to the bulk aqueous phase. This uneven accessibility of the reducible species present in solution to the metal surface (i.e., oxygen under aerobic conditions) leads to the formation of electrochemical cells characterized by the physical separation of cathodic and anodic areas. The depletion of the cathodic reactant (i.e., oxygen) in the occluded area under the biofilm enhances the anodic dissolution of this area, promoting acidification as a result of the hydrolysis of the metal cations, increasing the concentration of aggressive anions (i.e., chloride) and fostering the oxidative dissolution of detrimental species from the metal (i.e., sulfur species from MnS inclusions). In turn, these physicochemical processes tend to increase even more the rate of localized anodic dissolution. The localized environments in these occluded areas are far more complex in the case of MIC than those present under abiotic conditions in crevices or beneath deposits. This complexity is the result of the multiple biochemical reactions associated with the generation of metabolic products through bacterial activities. For these reasons, it is emphasized that inclusion of appropriate controls to account for all other abiotic corrosion reactions is a necessary requirement for the experimental demonstration of microbial involvement in a given corrosion process.

Although important, an aspect that is not discussed in this review is the role of electrochemical potential on MIC. As clearly presented in the introduction of Section 5, and noted in the preceding paragraph, MIC of metallic materials is an electrochemical corrosion process mediated by the presence of specific microorganisms attached to a metal/solution interface through the formation of a biofilm that leads to the physical separation of anodic and cathodic areas. Although more complex in terms of the chemical composition of the local environments and its physical configuration, the anodic occluded areas are essentially similar to the crevices or under-deposit areas found in abiotic localized corrosion processes such as crevice corrosion. It is also known that in certain cases of MIC, as discussed for sulfate-reducing

bacteria (SRB) in the review, the modification of the local environment by metabolic products (i.e., reduced sulfur species) arising from bacterial activity may decrease the critical potential for the initiation of localized corrosion. From the point of view of predicting long-term resistance to MIC, it is extremely important to determine if a threshold potential also exists in localized corrosion processes influenced by microorganisms. If such threshold potential exists and it is related to the repassivation potential for crevice corrosion, its application as a bounding parameter for performance assessment should be more fully explored. The existence of a repassivation potential for crevice corrosion is well documented in the case of carbon steels, Fe-Cr-Ni-Mo alloys and titanium alloys exposed to abiotic chloride-containing waters.

The MIC of mild steels is discussed in great detail because steels, including low-alloy steels and cast iron, are currently being considered by DOE as suitable materials of construction for the outer shell of a double-shell container that represents the most recent conceptual design for the waste package. In this context, it is fortunate that extensive literature exists on MIC of steels in soil under aerobic and anaerobic conditions. The roles of anaerobic SRB, aerobic sulfur-oxidizing bacteria, and iron-oxidizing and iron-reducing bacteria in the corrosion of mild steel are discussed with special consideration of environmental factors, such as salinity, temperature, pH, and water potential, as well as mechanistic interpretation of the different steps in the electrochemical corrosion process. The potential for stress corrosion cracking and hydrogen embrittlement of steels in the presence of certain microorganisms that generate molecular hydrogen as a by-product of their metabolism is also evaluated. The section on steels is completed with a review of some publications dealing with studies conducted in Switzerland and the United Kingdom on microbial corrosion of steel containers.

Another section in the report considers MIC of copper and its alloys. Most of the information is confined to localized corrosion in the form of pitting of copper water lines in many institutional buildings in Europe as promoted by certain aerobic bacteria, and the corrosion of copper alloys in natural seawater. The roles of different bacteria are discussed, showing that significant increases (i.e., >10 times) in corrosion rates of several copper alloys were found in the presence of certain bacteria (i.e., *Pseudomonas*). The involvement of SRB in the localized corrosion of copper and copper-alloys is also discussed. The formation of complex species by interaction between Cu^{+}, Cu^{2+}, and ligands found in the biofilms (i.e., exopolymers) is indicated to reveal the particular effect that microorganisms may have in the corrosion of copper in groundwater. Also discussed is the specific interaction of gases (i.e., hydrogen sulfide, ammonia), formed by microbial action at a certain distance from the waste package, with a copper container that may lead to accelerated corrosion.

The section on MIC of austenitic stainless steels is based on many recent studies that were prompted by the rash of failures due to localized corrosion observed in auxiliary systems of nuclear power plants. Pitting corrosion is the dominant failure mode, with the specific characteristic that the attack observed in a large number of piping systems is confined to the welds and almost absent in the base metal. Corrosion occurs in the duplex microstructure of the weld. In many cases, it occurred preferentially in the austenitic phase. In some instances, however, the ferritic phase was found to be affected as well as the heat-affected zone away from the fusion line. Several species of bacteria were found to be implicated in this specific type of corrosion of stainless steels, including some SRB.

The MIC of nickel and nickel-base alloys is extensively examined. Unfortunately, almost no information was found for alloys 825 and C-4, which are Ni-Cr-Fe-Mo alloys with a relatively high chromium content among other alloying elements. In separate studies, however, pitting and crevice corrosion of alloy 825 were observed in lakewater and natural seawater, respectively. Bacteria were found to be involved in both

cases. Most of the information available in the literature refers to commercial nickel and alloy 400. Although these materials are not being considered for HLW applications, they were included in the review to complete a description of the behavior of nickel-based alloys.

Finally, the apparent resistance of titanium and its alloys to MIC is noted. Titanium is prone to fouling in natural seawater, but it appears to be extremely resistant to localized corrosion in particularly severe microbial environments, as concluded by comparing the effects of these environments on other corrosion-resistant alloys. Nevertheless, it is suggested that further investigation under the specific conditions at Yucca Mountain (i.e., presence of fluoride ions) is warranted.

The last chapter of this report presents a series of general and specific recommendations. With the understanding that no studies on MIC of the candidate container materials have been conducted in an unsaturated subsurface soil or rock environment, this chapter suggests that all the materials considered may be susceptible to MIC, with the possible exception of titanium and its alloys. However, it is emphasized that all the alloys would be resistant to microbiologically mediated processes if the temperature at the waste package surface is above 100 °C and the moisture content remains below 60 percent over the 300- to 1000-year containment period. On the other hand, it is concluded that sufficient nutrients and microbial life are likely to be present in the near field to promote MIC if the temperature of the waste package drops below 100 °C and the moisture content rises above 60 percent for any period of time. Specific recommendations are included if the latter conditions become dominant, even in the presence of radiation fields, high salinity, or low pH, because these factors are not considered limiting factors for the occurrence of MIC. The specific recommendations comprise development and studies in the following areas: (i) monitoring techniques for MIC, (ii) nutrient availability in the near field, (iii) bioenergetics of container corrosion, and (iv) production of gaseous by-products by microorganisms. It is recommended that site-specific studies that will determine the tolerance of microbes to repository conditions be performed. An interdisciplinary approach is recommended. Additionally, programmatic considerations should be used to determine which of the issues and topics raised in this report in terms of long-term repository performance should be addressed by NRC and which should be brought to the attention of DOE for further evaluation.

Gustavo Cragnolino

1 INTRODUCTION

Commercial nuclear power facilities have been operating since the 1960s using pellets of UO_2 clad in Zircaloy tubes as fuel rods. Once it is removed from a reactor, the spent fuel must be stored and finally disposed of because it is radiologically harmful to people and the environment. The half lives of some daughter products associated with the spent fuel, such as ^{90}Sr and ^{137}Cs, are on the order of tens of years. A minimum of ten half lives are necessary to reduce the radioactivity to a safe and more easily manageable level. This number of half lives equates to a minimum period of 300 to 1000 years. Spent fuels are presently being stored in temporary fuel storage pools at reactor sites and, in a few cases, under dry storage conditions. However, the strategy in the United States has always been that disposal of the spent fuel would eventually occur at a government-controlled repository. This approach imposes the condition of containment of the waste during at least a 300- to 1000-year period. Specifically, the waste packages containing the spent fuel must provide a barrier over this period of time so that radioactive material does not come in contact with liquid or gas in the surrounding subsurface repository environment and become dispersed into the groundwater or other mobile phases. The same is valid for waste packages containing high-level waste (HLW) from defense reprocessing operations vitrified as borosilicate glass.

The long timescale for measurable change is the most difficult parameter to evaluate in the study of radioactive waste disposal (Rosevear, 1991). Four general types of processes must be considered in the development of the technology for ensuring long-term repository integrity and safety: physical, chemical, biological, and catastrophic events. These processes will not only act on the repository as independent entities, but in synergistic ways, as well. The challenge then is to identify and prioritize the significant processes and events that are likely to occur during the decay period (Manaktala and Interrante, 1990). Research and testing may be necessary to assess the significance of some processes for which there is insufficient information to make accurate long-term predictions.

Research in the areas of site selection and waste package design construction has been carried out in the United States over the past two decades. To date, the effects of chemical, physical, and radiation fields on the planned technology have been considered, but the biological processes that could influence waste package integrity have not been examined at the same level. This document was developed to evaluate the potential biological processes that may compromise the integrity of the repository barrier. Specifically, it focuses on the potential for microbially influenced corrosion (MIC) of the metals and related alloys being considered as candidates for the construction of HLW containers.

2 SITE CHARACTERIZATION

2.1 WASTE PACKAGE CONFIGURATION

A proposed waste package configuration has the spent fuel in a dehydrated state packaged in containers fabricated of a metal or metal alloy. The HLW inside the container also may be in the form of borosilicate glass. Although there is no approved design for the waste package, the initial concept was to have a container inserted in a borehole with a steel liner, leaving a small air space between the outer surface of the container and the liner. A concrete plug will seal the borehole from the tunnel system used to transport the spent fuel below ground level. Currently, a double-shell container is being considered, probably emplaced horizontally and covered with backfill material.

2.2 CANDIDATE CONTAINMENT MATERIALS

Several types of pure metals and alloys are being considered by the U.S. Department of Energy (DOE) for the construction of HLW containers: stainless steels, copper and copper-nickel alloys, nickel-base alloys, and titanium and titanium alloys (McCright, 1990; McCright et al., 1991). In addition, an outer liner of carbon steel has been proposed for certain designs for added containment to the waste package (Doering, 1993). Within these classes of metals and their alloys, alloy 825 is being considered as a prime candidate among the nickel-base alloys. Alloy C-4 is also being considered among the nickel alloys, and Ti Grade 12 is the prime candidate of the titanium-based materials (McCright et al., 1991). Table 2-1 lists the composition of these candidate materials. While an effort was made to describe the susceptibility of the candidate metals to MIC, information on related alloys is presented where there is a lack of information on the particular alloy under consideration.

2.3 ENVIRONMENT OUTSIDE THE WASTE PACKAGE

2.3.1 Geology

Yucca Mountain, in the southwestern edge of the Nevada Test Site, is the proposed location for the HLW repository. Thick deposits of unsaturated ash-flow tuffs overlying Paleozoic carbonate deposits predominate at Yucca Mountain (Glassley, 1986). The repository is planned to be located approximately 350 meters or more below the land surface and 225 m above the present static water table in the densely welded, devitrified, rhyolitic tuff of the Topopah Spring Member (Klavetter and Peters, 1986). The rock phase of the environment is described as being uniform in geochemistry with varying degrees of welded and devitrified material composed of quartz latite and rhyolite. The host rock contains many mineral oxides including silicon oxide (78-79 percent), aluminum oxide (12 percent), iron oxide (1 percent), phosphorous oxide (0.01 percent), and magnesium oxide (0.05 percent). These rocks, like most other mineral deposits, contain inorganic nutrients required for microbiological metabolism, for example, nitrogen, sulfur, phosphorous, iron, and manganese. Although the formation in which the repository has been proposed is thought to be seismically stable, an aftershock of M = 5.5 from the Landers earthquake in southern California was recorded in 1992 at Yucca Mountain (Monastersky, 1993).

The near-field repository environment will contain crushed tuff as backfilled material, but it may also contain bentonite and possibly soil. This material assemblage will have higher porosity than the surrounding undisturbed rock. The higher porosity will provide microorganisms that are present with greater access to oxygen in this area (Harris, 1960). As an example, the composition of a bentonite

Table 2-1. Nominal chemical compositions of current DOE candidate and selected alternate container alloys

Alloy	UNS No.	Composition, Wt %						
		C, max	Cr	Cu	Fe	Mo	Ni	Others
304L SS	S30403	0.03	19.0	—	Balance	—	10.0	Mn: 2.0 max S: 0.03 max P: 0.045 max
316L SS	S31603	0.03	17.0	—	Balance	2.5	12.0	Mn: 2.0 max S: 0.03 max P: 0.045 max
Alloy 825	NO8825	0.05	21.5	2.0	29.0	3.0	42.0	Ti: 1.0 S: 0.03 max Mn: 1.0 max
CDA 102	C10200	—	—	99.95 min.	—	—	—	—
CDA 715	C71500	—	—	Balance	0.7	—	31.0	—
CDA 613	C61300	—	—	Balance	2.5	—	—	Al: 6.8 Sn: 0.35
Alloy C-4	NO6455	0.01	16.0	—	3 max.	15.5	Bal.	Ti: 0.7 max
Ti Grade 12	R53400	0.08	—	—	0.30	0.30	0.75	Ti: Balance
AISI 1020 carbon steel (liner)	G10200	0.18-0.23	—	—	Bal.	—	—	Mn: 0.3-0.6 P: 0.04 max S: 0.05 max

Table 2-2. Mineralogy of MX-80 bentonite (from McKinley et al., 1985)

Mineral	% by weight
montmorillonite	75
quartz	15
mica	<1
feldspar	5-8
carbonate	1.4
kaolinite	<1
pyrite	0.3
other minerals	2
organic carbon	0.4

backfill proposed for use in a Swiss HLW repository is presented in Table 2-2.

2.3.2 Hydrology

After emplacement of the volcanic rocks approximately 11 million years ago, interactions of the rocks with an aqueous fluid phase occurred. However, since that time, the formation at the repository horizon has been under unsaturated conditions. Montazer and Wilson (1984) reported the absence of perennial streams at Yucca Mountain. Thus, recharge occurs sporadically through rainfall or snowmelt. Fracture-dominated flow along preferential pathways is considered to be the only credible mechanism capable of bringing liquid water to the entombed waste packages (Buscheck and Nitao, 1992). Montazer and Wilson (1984) determined that the mean porosity of the matrix was 14 percent and that the matrix material had a mean water saturation of 65 percent. Buscheck and Nitao (1991) modeled water infiltration in Yucca Mountain using recharge fluxes of 0, 0.045, and 0.132 mm/yr and obtained repository horizon saturations of 68, 85, and 95 percent, respectively. Water access to the environment at or near the canister surface will be limited to the small amount supplied by downward infiltration from the overlying unsaturated media (Russell et al., 1983).

2.3.3 Water Chemistry

The water chemistry of the area has been assessed in several ways and compared with the ground water from the Nevada Test Site and the nearby Amorgosa Desert area and Oasis Valley (Kerrisk, 1987). Carbon dating of the Yucca Mountain water indicates that it is 10,000 to 40,000 years old. Interstitial, fracture, and tunnel water from the area north of Yucca Mountain at Rainier Mesa have been analyzed. A complete report on the groundwater in areas close to the proposed repository has been published by Kerrisk (1987).

Water from well J-13 in the Topopah Spring Member located east of Yucca Mountain has been proposed to closely approximate repository pore fluid chemistry (Glassley, 1986). A description of the water chemistry is given in Table 2-3. Analysis revealed dissolved oxygen concentrations that ranged from below detection (<0.1 mg/L) to levels as high as 6 mg/L. For the low and intermediate waste repository in the United Kingdom, it was estimated that dissolved oxygen in the groundwater as well as radiolytically generated oxidizing chemical species and air trapped in the field around the repository would all contribute to an oxidizing environment around the canister immediately after it is put in the repository (Marsh et al., 1983).

Both negative and positive redox potentials (E_h) were recorded. Most water measured had E_h values that ranged from 200 to 400 mV_{SHE}, indicating the existence of mildly oxidizing conditions. Some

Table 2-3. Average composition of interstitial, fracture, and tunnel water from unsaturated zone of Rainier Mesa and well J-13 (all values are in mmoles/L except pH) (from Glassley, 1986)

Chemical Species	Rainier Mesa			
	Interstitial	Fracture	Tunnel	Well J-13
Na^+	1.73	1.53	2.30	1.96
K^+	0.18	0.12	0.11	0.14
Ca^{2+}	0.27	0.21	0.08	0.29
Mg^{2+}	0.10	0.06	0.01	0.07
HCO_3^-	1.14	1.61	2.25	2.34*
SO_4^{2-}	0.43	0.15	0.10	0.19
Cl^-	0.75	0.24	0.18	0.18
SiO_2	0.97	0.98	0.73	1.07
pH	7.8	7.5	7.0	6.9

* Measured alkalinity

water samples exhibited negative E_h values, and sulfide was detectable. The pH of the formation ranges from 6.9 to 7.8. Water-mineral reactions stabilize the pH of the water. The predominant cation was sodium while carbonate species were the predominant anions. Based on comparisons with other groundwaters in the United States, the nitrate concentrations (0.05-0.2 mmol/L) were not unusual (Kerrisk, 1987). Nitrate concentration decreases with age of the water. Biological nitrate reduction processes are thought to be responsible for this phenomenon.

Precipitation is considered the primary source of chloride and sulfate in water around Yucca Mountain. Most of the tuffaceous waters at Yucca Mountain and vicinity have low chloride (0.16-0.3 mmol/L) and low sulfate (0.17-0.32 mmol/L) concentrations. Both chloride and sulfur are present in rocks at Yucca Mountain. Oxidizing conditions and carbonate and fluoride complexes are believed to have the largest impact on solubility and speciation of inorganic ions (Kerrisk, 1987).

The native organic carbon concentrations within the formation range from 0.15 to 0.55 mg/L (Kerrisk, 1987). Although the organic carbon concentrations in the formation are expected to be low, an estimated 15 million gallons of drilling fluids were released into the Topopah Spring block a short distance from the anticipated repository site (West, 1988). Lateral movement of these drilling fluids may potentially reach the proposed repository. In addition to the drilling fluids now in the formation, many more gallons are expected to be introduced directly to the site during construction, along with lesser amounts of diesel fuels, hydraulic fluids, and gaseous exhausts (Hersman, 1987).

Water chemistry can affect the stability and composition of contacting minerals, especially under varying thermal loading profiles. Water chemistry also determines what microbial populations will be active and the extent of activity. The report of Kerrisk (1987) addresses the water flow paths, water chemistry, and water fluxes but does not address the impact of microorganisms on water chemistry and vice versa. Microorganisms are likely to obtain many of their nutrient needs from dissolved chemical species in the groundwater. The chemistry of the groundwater at Yucca Mountain is expected to be similar to that of Rainier Mesa (Kerrisk, 1987). The forms of nitrogen, sulfur, phosphorus, and carbon at Rainier Mesa are those utilized by most microorganisms in the subsurface. The important unanswered question is what is the rate of introduction of these nutrients to the repository near-field environment?

2.3.4 Radiation

Neutron and gamma radiation generated within the package are expected to penetrate the package material and interact with the environment (Glassley, 1986). The initial gamma radiation dose rate at the outer surface of the container has been estimated to be 5×10^2 Gy/h (5×10^4 rads/h) for the nominal burnup 10-yr-aged spent fuel and 55 Gy/h (5.5×10^3 rads/h) for defense high-level reprocessed waste (U.S. DOE, 1988). The neutron dose rate is expected to be about 1×10^4 neutrons/cm^2/s for the spent fuel and negligible for the defense waste. Less than 3 percent of the total thermal energy will be deposited in the surrounding rock as gamma radiation. By the time rehydration of the rock occurs within 1 meter of the container, the gamma radiation flux will have decayed to levels three orders of magnitude below initial levels. Hydrogen gas might be generated during radiolysis of moisture present during the early part of the decay process (Manaktala, 1990). Alternative designs currently considered include a thick double-shell container. In this case, almost complete shielding of γ-radiation is anticipated at the outer surface.

2.3.5 Temperature

The temperature of the formation presently ranges from 25-40 °C. However, thermal loading of the repository site is expected to occur as a result of thermal energy generated by the radioactive waste. No definitive conceptual and detailed designs of the waste package currently exist; however, the strategy currently being considered by DOE is based in maintaining a high thermal loading on the package and the near-field area to avoid the presence of liquid water beyond the containment period (Russell et al., 1983). Temperatures as high as 230 °C will be generated at the surface of the packaging material after 9 years (Glassley, 1986). Manaktala (1990) indicates that the temperature at the canister surface will range from 150-260 °C for the first 200 years and plateau at slightly above 100 °C over the next 1000 years. If the canister contains vitrified waste, the temperature is expected to be in the range 90-140 °C for the first 200 years then plateau at less than 60 °C after 1000 years (Manaktala, 1990). The rock temperature 1 meter from the borehole wall is expected to peak 10-20 years after package emplacement, reaching 190 °C (Glassley, 1986). Thermal loading is then expected to be followed by a period of cooling over hundreds of years. However, thermal loading may vary since much of the fuel that will be placed in the repository will have been aged as a result of storage in temporary storage facilities under submerged conditions (Ramspott, 1992). Other factors that may contribute to reduced thermal loading include the actual spacing of the waste packages within the repository and the rates of thermal energy dissipation. A complete assessment of the effects of thermal loading and radiation on rock properties and rock-water interactions has been published by Glassley (1986). However, the conceptual approach to thermal loading is still under discussion.

McKinley et al. (1985) indicated that the bentonite backfill in the near field of the waste canisters at the Swiss HLW repository will achieve a maximum temperature of 140 °C, but that this temperature will be of relatively short duration (< 10 years) and that the outer areas of the bentonite backfill will always be less than 100 °C.

2.3.6 Moisture Content

The heat generated by the radioactive decay of materials inside the containers initially will keep their exterior surfaces dry. Thermal loading models predict drying of the near-field rock by evaporation of the vadose water in the rock matrix and the flow of water vapor through fractures to cooler regions, where it will condense (Buscheck and Nitao, 1992). Eventually, formation water will approach and condense on the container surface as the temperature inside the container decreases. Because it provides a solid matrix with a suction potential, any material packing used would assure that water arriving at the edge of the borehole wall eventually will contact the container (Ramspott, 1992). With an airgap in an unsaturated site, it is postulated that a single package could remain uncontacted by liquid water from the borehole wall for up to 10,000 years. However, this postulation is currently being challenged, and several scenarios are envisioned that may lead to premature wetting of the container surface.

2.4 ENVIRONMENT INSIDE CONTAINERS

High-level waste in a geological disposal site will have a lower organic carbon content and higher thermal output than intermediate- or low-level waste (Rosevear, 1991). The waste will not be uniform in radioactivity. Some wastes will be reprocessed defense spent fuels, vitrified as borosilicate glass HLW. Although the exact configuration or material composition of the interior of the waste package is not presently known, it will likely be in contact with the inside metal surface of the container. There will be spent-fuel assemblages that have been at a number of locations prior to emplacement in the waste package at the repository. Most of the aged fuel will have been stored beneath water. Upon transfer to the HLW repository, the waste will become dehydrated so that the environment inside the waste package can be considered dry, providing the intended thermal loading is maintained. Different alternatives are being evaluated at the present time, including the use of a multipurpose container for storage, transportation, and disposal. These uncertainties preclude a more detailed analysis of the internal environment.

3 BIOLOGICAL PROCESSES

3.1 THE UBIQUITOUS NATURE OF MICROORGANISMS

Bacteria and other microorganisms are the most ubiquitous life forms on Earth. As man has developed new technologies to probe the most extreme reaches of the planet, he has been met by the diversity of viable microbial species waiting to be discovered. Not 100 years ago, scientists doubted the existence of life in the deep sea; today we value the decomposition processes that marine bacteria carry out in the water column and on the seafloor, even at great depth. Less than 15 years ago, scientists were unaware of the microbiologically mediated chemosynthesis that occurs along the seafloor spreading zones and the thermophilic bacteria in the hydrothermal plumes that survive temperatures of greater than 350 °C (Jannasch and Wirsen, 1979; Straube et al., 1990). Today thermophilic bacterial enzymes are commercially exploited in new biotechnological processes such as the Polymerase Chain Reaction (Mullis, 1990). Scientists were surprised to find bacteria resistant to long-term, high-level radiation growing in the damaged core of the failed nuclear reactor at Three Mile Island (Booth, 1987). In this instance, microbial growth interfered with remote-handling equipment during the defueling of the reactor. Many types of aerobic and anaerobic microorganisms have been associated with and isolated from nuclear reactor systems at commercial power plants (Miller et al., 1988). Microorganisms have also been isolated from defense spent fuel storage pools[1]. Thus, long-term exposure to high-level radiation in nutrient-depleted environments does not appear to inhibit microbial growth and activity.

Microorganisms have further surprised the scientific community in recent years by revealing their viability and diversity in deep subterranean formations that have been buried for millions of years and presumably isolated from nutrients for much of that time. Just how much activity these microbes must sustain in order to remain viable over this period of time remains to be established. Microbes are now considered to be present in virtually every natural environment, no matter how extreme (Rosevear, 1991). Thus, microbial activities must be considered when developing long-term disposal strategies for HLW at the Yucca Mountain repository.

The ubiquitous distribution of microorganisms is due to their exquisite adaptability to the environment in which they find themselves. Their ability to extract energy from a wide variety of reduced organic and inorganic compounds that exist on Earth, combined with their rapid generation time and mutation rates, have permitted them to survive, if not flourish, in the most remote and seemingly inhospitable habitats known to man. Given the evidence that viable bacteria have survived in the unsaturated subsurface environment over geological time, some low level of cellular maintenance activity must have occurred during this time in order to maintain the integrity of the cell. Thus, we must anticipate that a variety of viable and metabolically active microorganisms will inhabit the environment surrounding the waste packages containing the spent nuclear fuels at the proposed Yucca Mountain, Nevada, underground HLW disposal facility. The question under consideration in this review is whether the microorganisms that are present in this environment at the time of canister burial or that find their way to this environment at a subsequent time will carry out biochemical reactions that promote corrosion of the containment material to the extent that this barrier is breached within a 300- to 1000-year period.

[1] Personal Communication from J. Wolfram, March 1993

3.2 THE BIOFILM MODE OF GROWTH

Microorganisms may be found in one of two modes of existence: a swarmer or planktonic mode in which individual cells are dispersed in the bulk aqueous medium or a sessile or aggregated mode in which cells exist as a biofilm adhering to a substratum. In most surfaces exposed to aqueous environments, particularly those with a high surface area to volume ratio, the vast majority of cells may be found in a biofilm at any point in time. Planktonic cells are formed when biofilm cells slough into the adjacent bulk aqueous phase.

The soil environment, like any porous medium, exhibits an extremely high surface area to volume ratio. Thus, the vast majority of microorganisms found here may be expected to form biofilms over the surfaces of the soil particles, in the pore spaces between particles, and on other surfaces buried in the soil horizon.

In nature, biofilms are composed of complex assemblages of physiologically distinct microbial species that interact to maximize their survival in that environment (Figure 3-1). A matrix of extracellular polymeric substances (EPS) excreted by these bacteria anchor them to the substratum and to each other, trap essential nutrients derived from either the substratum with which they are associated or the overlying bulk aqueous phase, and protect the cells from harmful chemicals in the environment. Within the biofilm, the bacterial cells are buffered from various environmental assaults, such as fluctuating pH, toxic metals, biocides, or antibiotics. The EPS matrix also allows accumulation of metabolic products in the biofilm at or near the substratum (Geesey, 1982).

The biofilm mode of growth promotes a patchy distribution of cells over the substratum. Cells initially present in the bulk aqueous phase attach to surfaces in contact with the bulk phase in a random manner unless there are physical features, such as crevices or other surface discontinuities, that promote colonization (Figure 3-1). As the cells replicate on the surface and produce EPS, discrete microcolonies are formed over localized areas of the substratum. The biofilm may accumulate more rapidly in some areas than in others and their specific metabolic activities may change the chemistry to varying degrees at the surface of the substratum. This chemical fluctuation may change the electrochemical potential established across the surface/solution interface. It is these two phenomena—heterogeneous cell distribution and metabolism-driven chemical gradients at or near the substratum surface—that implicate microbial biofilms in localized corrosion processes. Both the French and United States research programs on low-level radioactive waste suggest that biofilms on the inorganic matrix are an important factor in the distribution of the microorganisms in the radioactive waste (Rosevear, 1991).

Biofilm Development

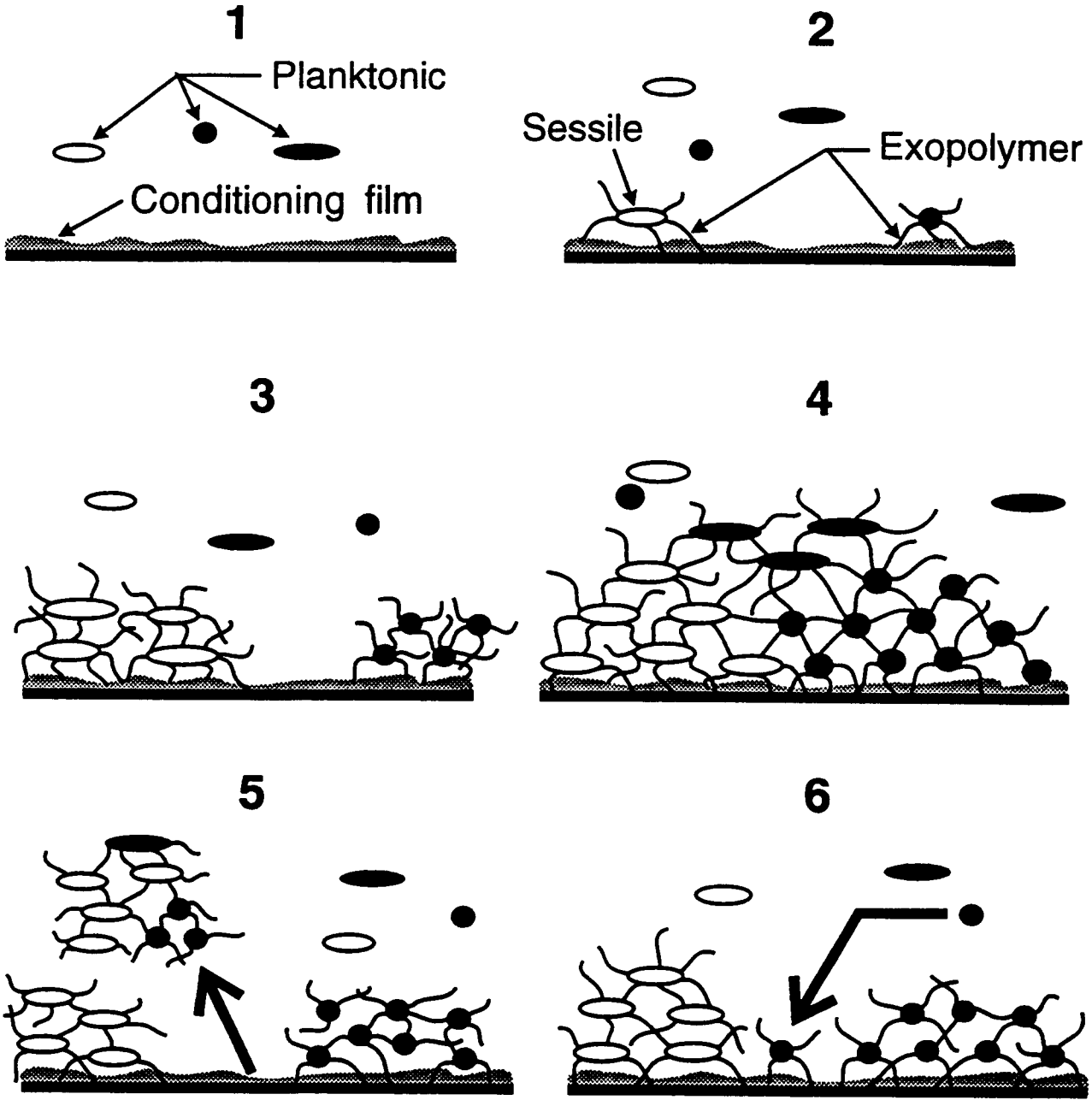

Figure 3-1. Schematic showing successive stages in biofilm development

4 CHARACTERISTICS OF MICROORGANISMS IN SUBSURFACE ENVIRONMENTS

4.1 INTRODUCTION

As mentioned previously, the HLW repository housing the waste packages will be located at a depth of approximately 350 m below the surface of Yucca Mountain and 213-427 m above the static water table. This environment is termed the deep subsurface vadose (unsaturated) zone. Historically, there has been little information on the microbiology of deep subsurface terrestrial environments. Lack of suitable aseptic sampling equipment and techniques, as well as the high cost and difficulties associated with drilling, have contributed to the dearth of microbiological data in this remote environment (McNabb and Mallard, 1984). While isolated work on the microflora of sulfur and oil deposits provided some evidence of life in the deep subsurface (McNabb and Dunlop, 1975), it was thought to be restricted to only those zones that were rich in nutrients.

Beginning in the early 1980s, renewed interest in the subsurface environment led to a number of microbiological studies that indicated higher densities of microorganisms than previously realized. White et al. (1983) found biochemical evidence that bacterial populations equivalent to 107 bacteria per gram sediment existed at a depth of 410 meters in the Bacatunna clay formation near Pensacola, Florida. Weirich and Schweisfurth (1985) demonstrated the presence of heterotrophic bacteria in the vicinity of a coal deposit at 405 meters in a sandstone formation near Kaiserslautern, West Germany. The plain sediments of Maryland were shown by Chapelle et al. (1987) to contain up to 10^6 bacteria per gram down to a depth of 182 meters. Sinclair and Ghiorse (1989) found 10^6 bacteria in valley aquifer sediment down to 86 meters in Kansas. Three types of microorganisms were found in rock samples taken from boreholes drilled into granite in the northern part of Scotland in Case Nest (West, 1990). The types obtained were aerobic and anaerobic heterotrophs, sulfate-reducing bacteria (SRB), and sulfur- and iron-oxidizing bacteria. Another group, which precipitated but did not oxidize metals directly, was also recovered. Thus, significant numbers of viable microbes exist in subsurface horizons.

Many of the microorganisms in the subsurface are metabolically active. King and Parker (1988) used a water-soluble tetrazolium salt to demonstrate dehydrogenase activity in ground water collected from various sites in Virginia. Those populations recovered from more nutrient-rich coastal plain wells contained 20.6 percent active cells ($5\text{-}7 \times 10^4$ cells/mL), whereas those recovered from more nutritionally deprived groundwaters contained 17.6 percent active cells ($5\text{-}11 \times 10^4$ cells/mL). Unfortunately, the sampling depths were not identified in the report. The results indicate, however, that a significant active microbial population exists in the subsurface sediment environment.

While there is evidence of an active microbial population in subsurface environments, the cells appear to be exposed to suboptimum conditions. Thorn and Ventullo (1988) determined the growth rates of subsurface microbial communities. They estimated production rates of $5\text{-}10 \times 10^5$ cells/g of sediment per day. These rates are 2 to 1000-fold lower than growth rates measured in surface soils. On the basis of these results, they suggested that subsurface bacterial communities are nutritionally stressed. Other evidence that the microorganisms are stressed in the subsurface comes from morphological characterization of the cells. Microorganisms recovered from groundwater were morphologically distinct from those recovered from surface soils. Bengtsson (1989) found that cells were generally less than 0.5 μm in diameter in groundwater but increased in size with the addition of carbon and phosphorus. Thus, restricted access to nutrients appears to limit microbial growth in some subsurface environments.

4.2 CHARACTERIZATION OF SPECIFIC SITES

4.2.1 Savannah River Plant Site

In 1984, the DOE initiated the Deep Subsurface Science Program to characterize the microbial populations and their activity at the Savannah River Plant (SRP) near Aiken, South Carolina. The subsurface environment from which the microorganisms were recovered was a porous sand in a water saturated aquifer. The work was undertaken primarily to examine the fate of deep subsurface aquifers with respect to contamination by pollutants and the activity of microorganisms. To date, this study has been the most extensive characterization of the microbiology of a deep subsurface environment. While the geology and hydrology of the SRP differ significantly from that of the Yucca Mountain site, the unanticipated numbers, diversity, and activity of the microorganisms isolated from the SRP provide some insight on what might be expected at Yucca Mountain.

The SRP site is a 768 km^2 restricted-access facility that rests on the Aiken Plateau, a comparatively flat surface that slopes southeastward. The Plateau is dissected by several tributaries of the Savannah River. The surface at the site is underlain by about 400 meters of unconsolidated sands, clayey sands, and sandy clays of Tertiary and Cretaceous age. These sands and clays overlie a basement of dense metamorphic rock, igneous intrusives, or consolidated sedimentary rock. An overview of the geology and hydrology at the SRP is given by Sargent and Fliermans (1989).

The types and variety of microorganisms at the SRP site have recently been described. Sinclair and Ghiorse (1989) were able to isolate bacteria, protozoa, algae, and fungi from samples taken at depths of over 250 meters. Furthermore, they found that the number of viable bacteria in some of the deepest sediment samples equaled those in the surface soil at the site. While conducting a study on heterotrophic bacteria, Balkwill (1989) recovered up to 10^8 microbes per gram sediment from depths to at least 265 meters, and found that the density of organisms showed no decline in numbers with increasing depth (0-265 m). If active, such a large number of microorganisms would likely exert a marked effect on groundwater chemistry (Wilson et al., 1983).

Subsurface samples from the SRP site yielded a diversity of physiological types of bacteria. Fredrickson et al. (1991a) isolated and cultured a total of 198 morphologically distinct colony types recovered from 365 to 467 meters below the surface. Francis et al. (1989) were able to recover denitrifying microorganisms, while Fredrickson et al. (1989) isolated nitrogen-fixing bacteria, and sulfur- and H_2-oxidizing lithotrophs. Anaerobic activity including sulfate reduction was detected by Madsen and Bollag (1989), and Jones et al. (1989) recovered both sulfate reducers and methanogens throughout the depth profile. SRB densities varied inversely with clay content of the samples. SRB distribution did not correlate with groundwater sulfate concentration. No sulfide was detected in pore waters from any of the subsurface samples. Methanogen densities were below the detection limit of the Most Probable Number (MPN) assay (0.3 cells/g soil). Nevertheless, methane was detected in samples of soil recovered from depths ranging from 60-150 m. It was estimated that methanogens ranged from 3-10 cells/g soil in surface samples to 1 cell/270 g or more subsurface soil. Coliform bacteria were detected in most subsurface samples, ranging in abundance from undetectable to 10^4 cells/g soil. Coliforms were represented by *Enterobacter*, *Citrobacter*, and *Serratia* sp. Coliforms are facultative anaerobes and can excrete various organic acids into the surrounding environment under either aerobic or anaerobic conditions (Krieg, 1984). The most common genera that the isolates fell into included *Pseudomonas*, *Acinetobacter*, and

Agrobacterium (Balkwill et al., 1989). The results of the investigations at the SRP site demonstrated the existence of an abundant, diverse, and active microbial community.

4.2.2 Idaho National Engineering Laboratory Site

The microbiological work of most general relevance to an assessment of microbial impact on the containment of nuclear wastes at the repository planned at Yucca Mountain is that conducted in unsaturated, deep subsurface zones. Colwell (1989) characterized the microbiology of unsaturated subsurface soil samples obtained from the Radioactive Waste Management Complex in the semiarid high desert in south central Idaho. This site has an aquifer at 177 meters below the surface that is overlaid by alternating layers of fractured basalts and thin sediment beds consisting of clayey silt. The region receives 20 cm of mean annual precipitation. Samples taken at about 70 meters (from the basalt-sediment interface and from within the sediment zone) contained cell densities that averaged 5×10^5 cells/g of dry soil compared to approximately 10^6 bacteria per gram of soil from the surface. Both culturable and total numbers of bacteria decreased with depth. A total of 32 morphologically distinct aerobic bacteria were cultured from the 70-meter interbed. Most isolates were gram positive (84 percent) and strict aerobes (79 percent).

The subsurface chemistry at the sampling site was characterized by a pH of 7.9, organic carbon content of 0.05-0.17 percent, a water content of 25.7 percent, and a gas phase similar to air; in one case, the carbon dioxide concentration was 0.16 percent. The author indicated that bacteria with aerobic metabolism are the most active microbes in the unsaturated subsurface environment, except in microzones of low oxygen concentration. The presence and localized distribution of these bacteria in the subsurface soil of an arid environment were suggested to be the result of colonization via draining of water from surface soils through fractures in the basalt.

The types of microorganisms isolated by Colwell in unsaturated basaltic soils were clearly different from those isolated by others in saturated, sandy soils. Several studies have identified environmental parameters that seemed to control the abundance and distribution of microorganisms in the subsurface environment. Phelps et al. (1989) suggested that initial data from the SRP site point to moisture and sediment texture as the principal determinants of the abundance and activity of the microbes found during the study. Areas in which clay was shown to predominate (>20 percent) consistently yielded lower numbers and diversity of organisms than did predominantly sandy sediments (Fliermans and Balkwill, 1989). Sinclair and Ghiorse (1989) also concluded that sediment texture was the best predictor of microbial abundance and activity; however, pH and the presence of metallic ions also correlated well with microbial numbers. Interestingly, organic carbon concentration was not shown by these authors to correlate with microbial abundance.

4.2.3 Hanford Site

The microbiology of formations exhibiting different properties was assessed at the Hanford Site in the state of Washington (Brockman et al., 1992). Microbial abundance and activities were related to water content and total organic content of the soils. Water content was highest in the Upper Ringsgold layer (19-36 percent) and lowest in highly developed paleosols (11 percent). Total organic carbon was highest in the highly developed paleosols (0.27 percent) and lowest in the Upper Ringsgold (0.09-0.05 percent). Total bacterial densities, enumerated by acridine orange direct count, were approximately one order of magnitude greater than the number of respiring cells, based on 2-(p-iodophenyl)-3-

(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT)-formazan reduction, in the highly developed paleosols and 1.5 to 2.5 greater in the Upper Ringsgold layer. Most of the metabolically active microbes were nonculturable under the growth conditions employed.

Microbial activities were evaluated by radiolabeled substrate uptake and conversion to radioactive carbon dioxide (Brockman et al., 1992). First order mineralization rate constants were determined for acetate but not specified. The constants from layers with native moisture content of 19 percent were statistically less than those from layers exposed to artificial recharge (36 percent moisture content). It was concluded that a more extensive and responsive microbial community was present in the highly developed, total organic carbon-rich paleosol than in the weakly developed paleosols. It was proposed that microorganisms in the deep vadose zones may originate from:

(i) entrainment during deposition
(ii) colonization during soil development
(iii) transport to the zones during artificial or geologic moisture recharge events
(iv) combination of processes described above

Future research on deep subsurface microbial ecology should focus on the manner in which bacteria have found their way to these locations.

4.2.4 Nevada Test Site, Rainier Mesa

Recent information has been obtained in a subsurface environment that resembles that of the proposed repository at Yucca Mountain. Viable microorganisms were recovered from ashfall tuff in a mined tunnel system 350-450 m below the surface but hundreds of meters above the regional water table of Rainier Mesa at the Nevada Test Site (Amy et al., 1992). Microbial counts ranged from 10^2 to 10^4 cells/g dry rock sample. Water flowing from faults in a perched groundwater lens contained 10^2 cells/mL. Many of the bacteria were small (< 1 μm) when viewed in the rock matrix and remained small even when cultured. Dwarfism is a well-documented strategy for survival of microbes in extreme environments, and small cells are capable of penetrating geological formations (Lappin-Scott and Costerton, 1990).

Large mucoid colonies dominated the isolates recovered on R2A Agar (Amy et al., 1992). The majority (89 percent) of the isolates were gram negative bacteria. Only a small percentage of the isolated bacteria were amenable to identification by conventional identification systems. The genus *Pseudomonas* was the most commonly encountered. Gram positive bacteria of the genera *Bacillus*, *Pasteurella* and *Micrococcus* were present in lower abundance. The isolate profiles demonstrated that groundwater isolates were considerably different from the endolithic isolates. A large number of the subsurface isolates exhibited arginine hydrolase and urease activity. These reactions liberate carbon dioxide and ammonia into the surrounding environment and alter the pH. No fungi were recovered from the subsurface rock. The similarity of Rainier Mesa and Yucca Mountain subsurface environments and the recovery of an abundant and diverse microbial population from these and other deep subsurface habitats indicate that the proposed repository environment will have a comparable native microflora.

4.2.5 Pajarito Plateau, New Mexico

The microbiology of a variety of deep vadose zone nonpaleosol materials from nonwelded and welded tuff in New Mexico was characterized by Hersman et al. (1988). These material were not influenced by artificial recharge. Culturable heterotrophic bacterial densities ranged from undetectable to 7×10^2 colony-forming units/g dry material. It was suggested that deep vadose zone microbial populations have evolved nutrient uptake systems that enable slow, steady uptake of organic carbon at matrix water potentials lower than uptake systems studied to date. Alternatively microbes could enter a resting state with little or no metabolism of exogenously supplied nutrients or very slow metabolism of endogenous reserves at low matrix water potentials. Bursts of nutrient uptake and metabolism may have occurred during episodes of moisture intrusion. The data from deep subsurface vadose zones suggest that total organic carbon is an important control on the culturability, activity, and diversity of microbes in this environment. The fact that small increases in water potential stimulate microbial growth and activity suggests that water availability also controls microbial processes here.

4.3 BIOTRANSFORMATION OF SUBSURFACE MICROORGANISMS

It is relatively easy to show that microorganisms are present in an environment; however, it is much more difficult to illustrate how metabolically active these cells may be *in situ*. Ghiorse and Wilson (1988) summarized the types of microorganisms recovered from the subsurface and their activities between 1977 and 1987. Although the highest numbers of microorganisms were recovered from saturated aquifers, samples from most vadose zone and clayey aquitard sediments also harbored viable microbes. Rates of biodegradation of a wide variety of common metabolites (carbon sources) were described as being instantaneous relative to the residence time of the water. This rapid biodegradation suggests that unless the microbes can utilize nutrients in the surrounding rock matrix, their activities are limited by those nutrients transported by groundwater.

The densities and activities of the bacteria in the subsurface SRP site are similar to or greater than those found in the top 3 meters of the soil surface (Fliermans and Balkwill, 1989). One-third of the 1500 isolates recovered from the SRP site metabolized all of the carbon sources listed in Table 4-1, and most of the remaining isolates assimilated more than half the tested compounds (Fliermans and Balkwill, 1989). Fredickson et al. (1991a) identified the organic compounds that were most commonly metabolized by the subsurface bacteria from the SRP site. These compounds were Tween 40 (the palmitic ester of sorbitol) and beta-hydroxybutyrate. Organic acids such as acetate and succinate were also commonly metabolized by subsurface bacteria. Complex polymers such as glycogen and dextrin were also commonly metabolized, as were the simple sugars glucose, fructose, and mannitol. One bacterial isolate utilized toluene, naphthalene, dibenzothiophene, salicylate, benzoate, p-cresol, and xylene as a sole carbon source under microaerophilic (40-80 μmoles/L oxygen) conditions (Fredrickson et al., 1991b). In general, the different types of microorganisms found in the subsurface environment, when taken as a whole, will metabolize a wide variety of carbon sources.

Most of the bacteria recovered from deep subsurface samples at the SRP site were oxidative with only a small percentage exhibiting fermentative metabolism (Balkwill et al., 1989). Hicks and Fredrickson (1989) demonstrated oxidation of acetate, phenol, and 4-methoxybenzoate to CO_2 in most samples. Many samples mineralized 40-60 percent of the 10 μg/L carbon amendments within a 21-day period at 24 °C. Mineralization was positively correlated with colony-forming units. Large microbial populations and extensive biodegradation were associated with sandy sediments and were absent or nearly

Table 4-1. Common carbon sources metabolized by bacterial isolates recovered from the deep subsurface at the Savannah River Plant site (from Fliermans and Balkwill, 1989)

arginine	arabinose
urea	ornithine
sorbitol	glucose
amygdalin	sucrose
lysine	sodium citrate
tryptophane	inositol
rhamnose	melibiose

absent in formations containing relatively high amounts of clay. Aerobic metabolic potential did not decrease with depth. Although dissolved organic carbon (DOC) content of subsurface samples varied between 0.9 and 7.9 μg/mL, there did not appear to be any significant correlation between DOC, depth and metabolic potential.

The metabolic abilities and nutrient requirements of groundwater microorganisms vary greatly within an aquifer. In samples from an uncontaminated shallow, saturated, sandy aquifer near Lula, Oklahoma, Swindoll et al. (1988) found that the aerobic mineralization to CO_2 of a variety of organic compounds added at trace amounts (100 ng/g sediment) in some cases exhibited a lag period of from less than 10 days to as much as 60 days. Addition of inorganic nutrients, vitamins, and amino acids reduced the lag period. In the case of phenol degradation, no acclimation period was required for biodegradation. Well water from this aquifer contained, in mg/L: inorganic orthophosphate, 0.12; nitrogen as ammonia, <0.05; and nitrogen as nitrate plus nitrite, 0.8. Recent studies have shown that bacteria are the principal CO_2-producing agents in the subsurface environment of the Atlantic Coastal Plain (Chapelle, 1990). Organic matter is oxidized to carbon dioxide with the reduction of electron acceptors, such as O_2, ferric iron, and sulfate. In another study, however, McMahon and Chapelle (1990) indicated that bacterial CO_2 accounts for less than 1 percent of the carbon input to the Cretaceous Black Creek aquifer of South Carolina. In this case, there appears to be a lack of terminal electron acceptors in the groundwater in this environment. The balance of the CO_2 production appears to have been derived from the oxidation of organic carbon using sulfate that exists in the confining beds of the matrix rock. Thus, microorganisms may have the capacity to extract essential inorganic nutrients from the solid mineral matrix phase.

Major portions of the metabolizable organic carbon in the subsurface material may be converted to storage products such as poly-B-hydroxyalkanoate or extracellular polysaccharides. Microbial populations in the confined Lula aquifer site in Oklahoma contained almost 10 times as much uronic acid-containing exopolysaccharide and polyhydroxyalkanoic acid storage products than those microbial populations just below the root zone (Smith et al., 1986). The groundwater at the Lula site contained 3.5 mg/L of nitrate and 0.12 mg/L of orthophosphate, and nondetectable organic carbon. However, the soil particles likely contained sufficient organic carbon to support exopolysaccharide-producing bacteria (Ghiorse and Wilson, 1988).

Starved bacteria recovered from the deep subsurface appear to be adapted to degrade organic compounds present in low concentrations. A pure culture of a strain of *Pseudomonas cepacia*, recovered from a depth of 203 m, when starved for 60-80 days, degraded quinoline at steady state (39 μmoles/L) more efficiently than cells starved for only 2 days (Truex et al., 1992). The results suggest that bacteria indigenous to oligotrophic (low nutrient) subsurface environments (as simulated by long-term starvation in the laboratory) may initiate more complete degradation of a substrate than may be indicated by laboratory experiments with recently cultured, actively metabolizing cells.

4.4 ANAEROBIC BIOTRANSFORMATIONS IN THE SUBSURFACE ENVIRONMENT

There is a growing data base on anaerobic microbial processes in the subsurface environment. Anaerobic metabolism of organic compounds such as glucose or acetate is usually slower and never faster than aerobic metabolism of these substrates (Phelps et al., 1989). Suflita and Miller (1985) described a methanogenic site and a sulfate-reducing site in a shallow aquifer next to a municipal landfill. A gas detector placed down a borehole leading to the top of the water table detected active methane production at the methanogenic site. No methane activity was noted in the sulfate-reducing site at which the aquifer material was colored black, contained less than 2 ppm oxygen, and emitted a hydrocarbon and sulfide odor. The belief that anaerobic biotransformations are important in the subsurface environment is supported by the rapid rate at which organic contaminants are removed from sites where alternate electron acceptors (nitrate, ferric iron, sulfate, carbonate, etc.) are used by the microorganisms when O_2 is not available (Ghiorse and Wilson, 1988). For example, anaerobic degradation of xylenes can be supported by denitrification (reduction of nitrate to molecular nitrogen or nitrogen oxides). Organic contaminants such as chlorinated phenols and benzoates can also serve as electron acceptors (Gibson and Suflita, 1986). Thus, there is a every reason to believe that microorganisms can metabolize common and toxic compounds in anaerobic subsurface environments. The redox potential of the environment determines which alternate electron acceptors are used. As the E_h decreases, the sequence with decreasing energy yield is $MnO_2 > NO_3^{2-} > Fe^{3+} > SO_4^{2-} > CO_3^{2-}$ (Stumm and Morgan, 1981).

Oxidized forms of iron (Fe^{3+}) and manganese (MnO_2) are believed to be important electron acceptors for anaerobic respiration in soil microorganisms (Lovley, 1991). Indeed, the metabolism of iron- and manganese-reducing microorganisms is believed to be responsible for most of the oxidized forms of these elements in sedimentary environments. Most of the oxidation of organic matter coupled to iron reduction in Precambrian sedimentary environments was probably the result of the activity of dissimilatory iron-reducing microbes that could completely oxidize organic compounds to carbon dioxide, with ferric iron as the sole electron acceptor. Iron is an abundant, if not the most abundant, electron acceptor for organic matter oxidation in soils. With Fe^{3+} as electron acceptor, various species of *Thiobacillus* and *Sulfolobus* can oxidize elemental sulfur to sulfuric acid. Likewise, a species of *Pseudomonas* and *Shewanella putrefaciens* can use Fe^{3+} as an electron acceptor to oxidize hydrogen gas produced by various fermentative microorganisms to protons, thus reducing the pH of the environment. Respiratory ferric-reducing bacteria are capable of enzymatically catalyzing the complete oxidation of naturally occurring organic compounds to carbon dioxide (Lovley, 1991). The ferric-reducing bacteria recovered from pristine deep aquifers in which iron was being reduced were capable of reducing Fe^{3+} present in the subsurface sediments deposited approximately 80 million years ago (Lovley et al., 1990).

The contamination of groundwater with organic compounds frequently leads to the development of anaerobic conditions. With the onset of anaerobiosis, Fe^{3+} is the most abundant potential electron acceptor for most subsurface environments. Sulfate reduction and methane production are generally inhibited in soils in which organic matter oxidation is being coupled to iron reduction. The reduced forms of iron and manganese generated by the metabolic activities described above can be spontaneously oxidized to insoluble oxides of these elements when they come in contact with oxygen-containing groundwater. Thus, Fe^{3+} and MnO_2 are again available to precipitate as electron acceptors for anaerobic microbial biotransformations.

Anaerobic microbial dissolution of several crystalline, water-insoluble forms of metal oxides commonly associated with the waste from energy production has been demonstrated. An anaerobic nitrogen-fixing *Clostridium* sp. with an acetic, butyric, and lactic acid fermentation pattern, isolated from coal-cleaning waste, solubilized Fe_2O_3 and MnO_2 by direct enzymatic reduction; CuO was solubilized by indirect action due to the production of metabolites and the lowering of pH (Francis and Dodge, 1988). Extracellular heat-labile components of the cell-free spent medium solubilized a significant amount of iron oxide. Direct contact of the cells with the oxide resulted in complete dissolution of the oxide. Lesser amounts of MnO_2 were solubilized by the cell-free metabolites of these bacteria. Ferrous and manganous oxides appeared to be used as sinks for excess electrons generated from glucose fermentation. Cr_2O_3 and NiO were not solubilized by direct or indirect action. Significant amounts of solubilized copper were immobilized by the bacterial biomass, and the addition of Cu^{2+} inhibited the growth of the bacterium. These observations further support the evidence that subsurface microbes can modify, solubilize, and transform soil minerals in their environment. These same transformations can be directed to the metals or metallic alloys proposed for waste package construction.

4.4.1 Anaerobic Biotransformations in the Deep Subsurface

Although the regions sampled at the SRP site were not predominantly anaerobic, the potential for anaerobic mineralization of exogenous and endogenous carbon was present in almost all samples recovered ranging from the surface to depths of 300 m (Jones et al., 1989). Anaerobic degradation of aromatic hydrocarbons appeared to be restricted to some geological strata by the absence of acetoclastic (acetic acid utilizing) methanogens. Acetate accumulation and methane production in slurries without added carbon indicated that inorganic nutrients or other environmental factors limited microbial activities in some areas of the deep subsurface. Many unamended soil slurries accumulated acetate and produced methane (Jones et al., 1989). The data indicated that some of the acetate and methane were derived from carbon reserves not measured by DOC determinations. It was suggested that some of the metabolizable carbon in these environments exists in the form of particulate matter.

Amendment of subsurface soils with various organic carbon sources resulted in stimulation of microbial activity in some samples and not in others. Slurries of formation material were prepared as 20-50 percent weight/volume in sulfate-free mineral salts medium, amended with various carbon sources, sealed in bottles, and incubated in the dark at 25 °C for 11 months (Jones et al., 1989). Disappearance of the amended carbon substrates and appearance of methane in the head space were then determined. Lactate and formate were metabolized in most samples. The time required for degradation of 20 ppm of amended lactate to nondetectable levels ranged from 38-240 days, depending on the sample. No methane formation was observed during degradation of either lactate or formate. Methane accumulation occurred 1-3 months after lactate or formate concentrations had dropped below detection limits. Metabolism of amended acetate displayed one of four patterns, depending on the sample. In one pattern observed in surface samples from saturated sandy strata, acetogenesis occurred during early stages of incubation, followed by disappearance of accumulated acetate, accompanied by methane production. The stoichiometry of acetate consumption and methane production was determined to be 1.6 ± 0.55 μmoles methane produced per μmole acetate consumed. It was estimated that 63 percent of the methane produced was derived from acetate that was either added experimentally or produced by fermentation of other carbon sources present. Samples from other strata exhibited either slow accumulation of acetate or loss of acetate over time with no methane accumulation. In other studies on samples collected from the SRP site, Phelps et al. (1989) were unable to demonstrate methane production. Likewise, no methanogenesis was detected at any depth in any core from the SRP site by Madsen and Bollag (1989).

Anaerobic degradation of benzoate was strictly associated with strata capable of methane production (Jones et al., 1989). Benzoate persisted in nonmethanogenic samples from all boreholes. Anaerobic degradation of benzoate was accompanied by a transient accumulation of acetate. Benzoate and acetate disappearance occurred during the 2- to 5-month period of methane accumulation. Phenol biodegradation was not detected in any samples, although methane production occurred in many of the phenol-amended samples. Under conditions of low E_h and the presence of organic and inorganic nutrients, the principles of carbon metabolism were found to be similar to those of other environments. It was concluded that the distribution of microorganisms and carbon reserves in subsurface soils is very heterogeneous. The investigators stress the need to examine larger and more representative samples when evaluating microbial numbers and activities in deep subsurface environments.

Sulfate-reduction activity was absent in approximately 80 percent of the samples examined in one core and completely absent from all samples from the only other core collected from the SRP site (Madsen and Bollag, 1989). In those samples in which sulfate reduction was detected, electron donor or acceptor were not limiting at some depths, electron donor was limiting at other depths, and both electron donor and acceptor were limiting at yet other depths. No correlations were presented between sulfate reduction and other features of the geology or hydrology of the site. No rates were presented for any of the reactions.

Microbiological sulfate reduction was responsible for mineralization of significant amounts of reduced carbon in oil field waters of the Shor-Su in Russia (Ivanov, 1990). Almost 2000 tons of biogenic hydrogen sulfide have been formed in 4 years in the groundwater of the Shor-Su sulfur deposits. Cultures of SRB have been grown on extracts from the sulfur deposits of the Cis-Carpathian region. Sulfate-reducing bacteria have been found in 90 percent of the water-bearing sulfur ores. When the deposit lacked water, it did not contain any microflora.

Dissimilatory nitrate reduction (denitrification or the conversion of nitrate to N_2) was demonstrated in subsurface samples from the SRP site by Francis et al. (1989). Denitrification was observed in unamended samples that received only prereduced deionized water at almost all depths from all sediments sampled. Denitrification rates in unamended samples in the deepest depths evaluated (289 m) ranged from 0 to 1.5 nmoles N_2O produced/g dry sediment). The addition of nitrate, but not carbon, stimulated denitrification in most samples, indicating that nitrate and not carbon is limiting this reaction in this environment. Denitrification rates at the greatest depths sampled that were amended with nitrate (300 nmol) ranged from 1 to 85 nmol/g dry sediment. There was no correlation between nitrate concentration in pore waters and denitrification activities in unamended samples, suggesting that denitrification may not occur at significant rates in the subsurface zones sampled.

Fe^{3+} reducers are also active in the deep subsurface. In a zone of high levels of dissolved Fe^{2+} in the Middendorf aquifer at the SRP site, there was a direct correlation between the accumulation of dissolved Fe^{2+} along the groundwater flow path and the accumulation of dissolved inorganic carbon (Chapelle and Lovley, 1992). Carbon isotope determinations suggested that the inorganic carbon was derived from a local organic carbon source. Iron-reducing organisms were recovered that could oxidize acetate to carbon dioxide, using the Fe(III) oxides present in the deep subsurface as electron acceptors.

The results indicate that, although the SRP deep subsurface site was oxidizing, microniches occurred that permitted anaerobic biotransformations. One overall conclusion of the SRP site study was that more research needs to be carried out to characterize the geochemistry, mineralogy, and hydrology

of the formations under study in order to better understand and predict the occurrence of specific types of organisms and their activities.

4.4.2 Summary of Subsurface Microbiology

The body of information describing the microbiology of deep subsurface environments is still limited to only a few sites, none of which has been exhaustively characterized. However, some conclusions can be drawn based on the information that has been made available over the past 10 years. It is likely that a diversity of microorganisms will be present in the formation in which the HLW repository is proposed at Yucca Mountain. Only a small fraction of the total numbers and physiological types of microorganisms present in subsurface environments has been cultured, and even fewer identified. Those present in the subsurface repository environment can be expected to exhibit a wide range of metabolic capabilities both aerobic and anaerobic respiration, with their energy needs met by either chemoheterotrophy or chemolithotrophy.

It is difficult to predict biological activities based on measured carbon turnover in low-nutrient environments. Carbon can be directed to various metabolic pathways, each with different by-products, depending on the types of nutrients available and the prevailing environmental conditions. If the subsurface microbial population is demonstrated to be active, there is a high likelihood that such activity has had significant impacts on the geology and movement of nutrients at depth. It is also likely that some *in situ* microbial activities such as sulfate reduction and iron reduction will affect the integrity of the radioactive waste barrier over the long term.

It is reasonable, therefore, to conclude that we can not rule out the presence of a particular microorganism simply because it is several hundred meters below the soil surface under unsaturated conditions. Predictions of the types and activities of subsurface microorganisms should be based on the physical and chemical parameters of the particular site. In other words, if the site provides a niche for microorganisms having a certain type of activity, then we should expect to find some type of organism at that site, with that activity. The key question now is whether specific corrosive activities are likely to proceed at rates that will compromise container integrity over a 300- to 1000-year period.

4.4.3 Environmental Parameters Affecting Subsurface Microbial Processes

The physical and chemical properties of the repository environment at Yucca Mountain will, to a large extent, control the microbial activities in the vicinity of the canisters. As a group, bacteria function over a wide range of temperature, pH, water activity, and nutrient and oxygen concentrations. Any one species, however, is limited to a substantially narrower range of these parameters for growth. The range widens when we refer to survival rather than growth. Balkwill (1989) indicated that vegetative cells rather than spores were the predominant form in the deep subsurface. Like marine bacteria in the ocean, these cells survive in low-nutrient environments with a very low maintenance energy and can respond rapidly when nutrients do appear. Survival of a particular physiological type or species in the microbial world requires that only one viable cell be maintained out of the many millions of cells generated during the short period that environmental conditions permit such activity.

4.4.3.1 Ionizing Radiation

Gamma radiation is believed to be the primary form of ionizing radiation that escapes the HLW package barrier. The destructive action of ionizing radiation results from the physical cleavage of chemical bonds of biomolecules, especially of nucleic acids (e.g., DNA). However, microbial tolerance to radiation can vary significantly. One of the more tolerant bacteria known is *Micrococcus radiodurans*, first detected by Anderson et al. (1956) in canned meat that had been exposed to several mrad of gamma irradiation. This organism has been shown to withstand doses as high as 500 krad without showing any significant inactivation (Mosely and Laser, 1965). Numerous other bacteria, such as *Escherichia, Salmonella*, and *Pseudomonas* sp., have been shown to possess enhanced resistance to irradiation (Nasim and James, 1978). Francis et al. (1980) were able to recover sulfate reducers, denitrifiers, and methanogens as well as *Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp., and *Clostridium* sp. from trench leachates containing beta emitters such as ^{14}C, ^{3}H, ^{60}Co, ^{90}Sr, $^{134,137}Cs$, and alpha emitters such as $^{238,239,240}Pu$. Growth of mixed culture trench water bacteria in the laboratory was not affected at total radioactivity levels of 2.6×10^2 and 2.7×10^3 pCi per mL. In a separate study, Wildung and Garland (1982) were able to culture bacteria, actinomycetes, and fungi in a medium containing up to 180 μg/g ^{239}Pu. Thus, microbes have evolved mechanisms to repair radiation-damaged molecules essential for their survival. Unfortunately, none of the studies cited above reported dosage of radiation received by the microbes.

Incidental information on survival of microorganisms in high radiation fields is derived from observations in operating nuclear facilities. Microorganisms have been found in reactor cooling water that experienced a flux of 5×10^{14} neutrons/cm$^2 \cdot$s (Mergeay et al., 1984). Microorganisms were found growing in real radioactive waste under radiation doses of 10 Gy/h, utilizing the small amounts of nutrients derived from hydraulic fluid that leaked into the system (Booth, 1987). The questions that need to be answered are: (i) What is the gamma radiation flux at the container surface and near-field environment? and (ii) Can the indigenous and introduced bacteria in this environment survive, and, if so, can they carry out activities in the radiation field that are corrosive to the container material?

4.4.3.2 Salinity

Salt often crystallizes imperfectly, trapping brines and microbes in pockets within growing crystals. Methane and hydrogen sulfide have been detected in brine inclusions, indicating active microbial activity under these extreme conditions (Javor, 1989). There have been brine depositions in sulfur deposits of Kosha-Haur, Central Volga and some oil deposits in Central Asia (Ivanov, 1990). Sulfate-reducing bacteria are consistently isolated from these saline waters. Sulfate-reducing bacteria tolerate up to 30 percent salinity with an optimum range of 0-20 percent. A *Desulfovibrio* sp. capable of growth in 19 percent salt solution was isolated but not characterized physiologically (Javor, 1989). Methanogens tolerate up to 30 percent with an optimum range of 0-25 percent. Enrichment studies showed that methanogens and SRB did not compete for carbon substrates in hypersaline environments as they do in marine sediments. Most halophilic methanogens isolated to date require methylamines or methanol rather than hydrogen and carbon dioxide or acetate for growth (Javor, 1989). Thus, the SRB use carbon substrates that are present in hypersaline habitats other than methylamine and methanol. The lack of competition suggests that both methanogenesis and sulfate reduction can occur simultaneously.

Hypersaline soils contain halophilic *Pseudomonas*, *Bacillus*, *Acinetobacter*, and gram-positive cocci. Most halophilic bacteria are chemoheterotrophs, aerobic, or facultatively anaerobic by nitrate reduction or fermentation. They convert amino acids or sugars to glycerol, pyruvate, or other organic

acids. Most halophiles are mesophilic with temperature optima near 35-37 °C. Their temperature maxima, however, may be as high as 50-55 °C, which is more typical of thermophilic bacteria. Some could withstand a temperature of 100 °C for 20 min. Sulfur-oxidizing bacteria tolerate salinities up to 30 percent with an optimum range of 0-12 percent.

4.4.3.3 Water Availability

Water availability may be expressed as water activity (a_w), as water potential, or as matrix potential in the case of porous media (Brown, 1990). Water activity is often used to describe the water content of solutions or porous media. Values range from $a_w = 0$ to $a_w = 1.0$. Although water potential is a form of free energy, it is normally expressed in pressure units (MPa) as a result of the relationship between chemical potential and fugacity, or between chemical potential and partial pressure by assuming ideal gas behavior of the vapor phase. As a_w decreases from a value of 1, water or osmotic potential becomes more negative. Lowering the water potential effectively slows biochemical reaction rates. Matrix potential is of importance to microbes in the soil in that it affects access of microbes to liquid water as well as the diffusion of nutrient solutes and gases. Low soil water content will minimize transport of nutrients to microbes and thus reduce their ability to grow and be active but not necessarily reduce viability.

Microbial growth is possible over a range of water activities from 0.60 to 0.998 (Mossel and Ingram, 1955). Halophilic bacteria have been observed to grow in environments in which the $a_w = 0.75$. The tolerance limit for growth of most gram-negative bacteria is $a_w = 0.95$ (−7.1 MPa); for *Bacillus* and some other gram-positive cocci, the tolerance limit is $a_w = 0.90$ (−14.5 MPa) (Brown, 1990). Xerophilic (low water potential loving) fungi exhibit a growth optimum at a_w of 0.90 or above.

Fungi are considered to be the most desiccation-resistant of soil microbes. Cells of *Xeromyces bisporus* remain active down to $a_w = 0.60$ (Schlegel and Jannasch (1987). Few bacteria are known to remain active at a_w values below 0.9.

Xerotolerant microbes are defined as those able to tolerate low water potentials. An *Acinetobacter* sp. isolated from a depth of 324 m in Middendorf clay layer at SRP site maintained viability under starvation conditions in sterilized aquifer material even when subjected to sever desiccation (−22 MPa) (Kieft et al., 1990).

Given that the moisture content of the Yucca Mountain repository site is 65 percent (a_w of approximately 0.65), significant bacterial activity, including that of halophilic species, is not anticipated in this environment. Fungal activity can be anticipated at this moisture level, however.

While cell activity may be arrested at water activities below 0.60, certain microbes are capable of survival in a dormant state. Bacteria are able to form special resistant structures such as endospores and cysts to survive periods of desiccation. These structures can preserve viability for many years as has been shown by Sneath (1962), who cultured bacteria from soil associated with the roots of plants stored at Kew Gardens since 1640. Under certain conditions, even vegetative cells are capable of surviving long periods of desiccation. Cells of the bacterial genus *Arthrobacter* were shown by Boylen (1973) to persist in desiccated soil samples for over 6 months.

Low water activity stimulates exopolysaccharide production in *Pseudomonas aeruginosa* (DeVault et al., 1990). The acidic polysaccharides elaborated by soil microorganisms retain large

quantities of water under desiccating conditions (Roberson and Firestone, 1992). The fact that acidic exopolysaccharides were associated with mucoid colonies of bacteria isolated from the deep subsurface suggests that these bacteria are adapted to this dehydrating environment. Cells entrapped in polysaccharide gels retained viability for more than 3 years at 28 °C when maintained in an environment with a water activity of less than 0.069 (Mugnier and Jung, 1985). High molecular weight nutrients promoted survival under desiccating conditions. Survival of vegetative cells also depends on the type of soil, the velocity of the drying process, and the local water activity.

The critical range for suppressing bacterial movement in most soils is likely from −0.05 to −0.5 MPa, except for filamentous organisms. In relatively dry, nonsaline soils, the dominant microbes are the fungi and actinomycetes. Viability under chronic solute stress requires accumulation of a "compatible solute" inside the cells to a sufficient concentration to ensure that intracellular osmotic potential is below external water potential (Brown, 1990). A compatible solute is one that can vary widely in concentration inside the cell without disturbing other intracellular processes. Betaine is an example of a compatible solute used by procaryotes.

Evidence to date suggests that moisture content of a soil is a determinant of subsurface microbial densities and activity. Generally, sediments with high water content exhibit greater water permeability than sediments with low water content. Permeability influences the amount of nutrients available to the microbes. The greater the permeability of the sediment to nutrient-containing water, the greater the flux of dissolved nutrients to the bacteria. Kieft et al. (1990) showed that samples collected from material exhibiting water potentials ranging from −2.7 to −2.1 MPa had very low numbers of culturable bacteria, low microbial biomass based on adenosine triphosphate (ATP) analysis, and low microbial activities based on respiration measurements. Water-saturated subsurface sands exhibited three to four orders of magnitude higher densities of culturable microorganisms and microbial activity than the dense clay zones, which had low permeability (Phelps et al., 1989). DNA replication and phospholipid fatty acid synthesis (two measures of bacterial growth) occurred at higher rates in samples saturated with water. Samples containing greater than 20 percent clay gave the lowest activities or, in some cases, none at all. In a crude evaluation of the importance of water content on microbial processes, Phelps et al. (1989) determined that sand with a water content of less than 70 percent saturation exhibited no significant difference in viable cells or fatty acid synthesis rates than sand with greater than 70 percent water saturation.

The water content of sediments also influences the types of microbial activities that will occur. In the presence of comparable organic carbon loads, saturated sediments become anaerobic more easily than unsaturated sediments due to respiratory activities of the microorganisms present. The higher the organic content, the greater the likelihood of establishing anaerobic conditions at any particular sediment water activity.

4.4.3.4 Temperature

The range of temperatures over which bacterial activity can take place is broad. Microorganisms as a group have adapted to survive in virtually every habitat on earth, regardless of temperature. Baross and Deming (1983) were able to isolate an obligate anaerobic bacterium capable of growth and activity in boiling water at 0.1 MPa pressure. This organism was isolated from a sulfide-encrusted deep-sea hydrothermal rift community in which the temperature at depth was in excess of 250 °C. This extreme tolerance to temperature is believed to be associated only with organisms at elevated pressures where water can still be found in its liquid form. Once temperatures become sufficiently elevated to allow boiling, most bacteria in the environment cease to remain active. Nevertheless, there have been reports

of some bacteria being able to grow in super-heated hot springs at 101 °C (Brock, 1978). Stetter et al. (1986) described an obligately autotrophic bacterium (*Pyrodictium occultum*) that was found in geothermally heated sea floor area close to a volcano. *Pyrodictium occultum* converted hydrogen gas and elemental sulfur to H_2S, exhibiting an optimum growth temperature of 105 °C and a maximum growth temperature of 110 °C. Another bacterium (*Acidothermus infernus*) that has a similar metabolism exhibited an optimum growth temperature of 88 °C and a maximum growth temperature of 95 °C. Stetter et al. (1986) suggest that life at 250 °C as reported by Baross and Deming (1983) is not possible. They also suggest that, although the upper limits of temperature at which life can exist is still unclear, it is likely in the range of 110-150 °C.

At sub-boiling temperatures, a wide range of bacteria are capable of growth and survival. Sulfide amendments to hot springs often stimulate acetate metabolism by microbes that are presumably heterotrophic sulfur oxidizers (Brock, 1978). Optimum sulfide concentration was found to be 13 μg/mL. The temperature optimum for metabolism of all organic compounds added to the hot springs was 80-90 °C. The temperature optimum for bacterial colonization of surfaces submerged in the hot springs was 90-92 °C. Sulfide becomes deposited on surfaces around the bacteria. Elemental sulfur deposits also appear in filamentous bacteria that associate with surfaces submerged in the hot springs. None of the microorganisms have been cultured in the laboratory.

Many of the chemolithotrophs, such as sulfur- and iron-oxidizing bacteria, along with many of the SRB are thermophilic, growing optimally at temperatures of 45 to 80 °C. Strains of *Thiobacillus ferrooxidans* generally have an optimum growth temperature ranging from 30 to 35 °C (Norris, 1990). Reduction in environmental pH extends the upper temperature limit for growth. This bacterium can continue to oxidize iron without growth at temperatures up to 37 °C, and one strain has been shown to oxidize iron at 40 °C (Ehrlich, 1981). The thermophilic, chemoautotrophic bacterium, *Sulfolobus* oxidizes iron at 80-85 °C but not at 90 °C.

West (1990) determined that of the isolates representing different physiological classes of bacteria from subsurface soils, the SRB were best able to survive elevated temperatures. Species of *Desulfovibrio* grow at temperatures ranging from 10 to 40 °C. *Desulfotomaculum* sp. grow over a temperature range of 30 to 70 °C. Thus, many metal-corroding bacteria are active at temperatures well above ambient temperatures.

Eucaryotic organisms such as fungi are not considered to be capable of growth above 60 °C. Most of the microorganisms isolated from the SRP site were mesophilic, growing best at approximately 23 °C, the *in situ* temperature of the soil (Fliermans and Balkwill, 1989). No studies were found that determined the cardinal temperatures (minimum, optimum, and maximum) of the bacterial isolates recovered from the deep subsurface environments. Heat-resistant bacterial spores were not predominant in the subsurface samples, however.

Some bacteria are capable of surviving at elevated temperatures by becoming dormant, either through the process of sporulation or desiccation. The limits to survival at elevated temperature are not well established for many soil organisms. At decreased water activities, many bacteria can survive indefinitely at temperatures above 100 °C. Most bacterial activity, however, is restricted to temperatures below 100 °C at atmospheric pressure. The temperature at the exterior surface of the HLW containment package to a distance 1 meter into the surrounding subsurface environment at Yucca Mountain is expected to remain above 100 °C over the 1000-year period. Thus, while many bacteria may survive the high temperatures in the immediate vicinity of the HLW containment package, they are not likely to be active.

However, bacterial activity should be anticipated immediately outside this envelope. Should the thermal loading of the waste package not maintain a temperature greater than 100 °C at the package surface and surrounding near-field environment, then bacterial growth and activity should be anticipated at this site.

The lower temperature limits for bacterial activity are determined by the freezing point of water. The deep subsurface conditions at Yucca Mountain are not expected to approach the freezing point of water. Thus, bacterial activity will not be limited at the minimum temperatures experienced at the repository.

4.4.3.5 Hydrogen Ion Concentration

Very high or very low concentrations of hydrogen ions are normally toxic to most organisms. The known limits to growth and activity of microorganisms due to a high concentration of hydrogen ions are around pH 1.0 reported for a few bacteria and fungi (Souza et al., 1974). Among the obligate extreme acidophiles are chemoautotrophic bacteria *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*. The optimum pH for growth of *T. ferrooxidans* ranges from 3.0-3.6 but they tolerate pH ranging from 1.4-6.0 (Ehrlich, 1981). Both these bacteria are capable of oxidizing hydrogen sulfide or other reduced forms of sulfur to sulfuric acid. Other bacteria such as *Thiobacillus acidophilus* can grow either autotrophically on reduced sulfur or heterotrophically on reduced organic carbon at pH 2.5-3.0. These bacteria can oxidize sulfide minerals in groundwater aquifer systems, producing local acidification, and *T. ferrooxidans* is capable of oxidizing ferrous iron to ferric iron. Numerous other microorganisms also participate in the oxidation of sulfur compounds to sulfuric acid in their immediate environment.

While specific strains of organisms are not generally active over a wide pH range, a succession of species may occur when the pH changes significantly. *Thiobacillus thioparus* may initially oxidize reduced forms of available sulfur in concrete or minerals to elemental sulfur at basic or neutral pH. As the pH drops to below 5, *Thiobacillus concretivorus* and *Thiobacillus ferrooxidans* oxidize the elemental sulfur to sulfuric acid, which causes the pH to drop even further. Thus, *Thiobacillus thiooxidans* continues to grow and to produce more sulfuric acid until the pH drops to 1-2.

The lower limit of hydrogen ion concentration for growth and activity of microorganisms is believed to be around pH 11, which has been reported for several algae, fungi, and bacteria (Souza et al., 1974). The pH at Yucca Mountain ranges from neutral to slightly basic (Glassley, 1986), a range that coincides with that favored by most microorganisms isolated from subsurface soils.

Microorganisms growing in colonies on surfaces of soil particles can excrete acidic or basic metabolites that lead to large local changes in pH. Those nearby species that were previously inactive at the original pH may find the new conditions more conducive for their growth and activity.

4.4.3.6 Electron Acceptors and Redox Potential

As mentioned previously, the redox potential controls the availability of electron acceptors used for microbial respiration. Under oxidizing conditions, oxygen acts as the preferred electron acceptor by many microorganisms due to the high energy yield. Many microbes can utilize more than one electron acceptor, depending on E_h. For example, some soil Pseudomonas can utilize NO_3^{2-} in place of O_2 under conditions of reduced E_h. Some microorganisms are poisoned by oxygen and require a reducing environment (negative range of E_h values) in order to maintain activity. Sulfate-reducing bacteria, methanogens, acetogens, denitrifying bacteria, and fermentative bacteria and fungi are metabolically

active in those environments in which molecular oxygen is either in very reduced concentrations or absent. Although the subsurface of Yucca Mountain contains some oxygen and is considered to be an aerobic environment (mildly oxidizing conditions), localized areas and low permeability zones will become depleted of oxygen, creating anaerobic, reducing conditions. This process will occur especially under conditions of water saturation in which there is an adequate supply of electron donors for microbial energy production. The recovery of anaerobic bacteria and the detection of anaerobic reactions such as sulfate reduction in deep subsurface samples indicate that both anaerobic and aerobic conditions exist in this environment and that the alternate electron acceptors are present and available to the microorganisms in this environment.

4.4.3.7 Nutrient Availability

Microorganisms have specific requirements for those compounds that supply their cellular carbon, nitrogen, phosphorus, and sulfur. The specific compounds vary with the type of microorganisms. The availability of these compounds often limits microbial activity and growth. Some microorganisms (chemoautotrophs) in the deep subsurface are capable of deriving their carbon requirement through the fixation of carbon dioxide and their energy from reduced inorganic compounds such as hydrogen sulfide, elemental sulfur, and ferrous iron. Many methanogens, sulfur oxidizers, and ammonia oxidizers utilize carbon dioxide rather than organic carbon for their carbon needs. *Thiobacillus* and *Sulfolobus* sp. are chemoautotrophs, using carbon dioxide as their source of cell carbon, reduced forms of sulfur as energy source for growth, and oxygen as their terminal electron acceptor. This type of metabolism requires an abundant source of reduced sulfur to produce a significant amount of biomass. Pyrite ore serves as a source of reduced sulfur for these microbes. Hydrogen sulfide or metal sulfides produced by sulfate-reducing bacteria can also serve as a source of reduced sulfur for these chemoautotrophs. Sources of sulfate at Yucca Mountain repository include: groundwater, formation minerals, and concrete that may be used to seal off the waste packages from the transportation tunnels. The carbon dioxide needed by chemoautotrophic bacteria is thought to be derived from chemoheterotrophic microbial respiration and fermentation reactions in surface layers. The carbon dioxide is then transported to depth by percolation of water at the surface (Chapelle, 1990).

Some types of subsurface chemoautotrophic microorganisms can grow in the absence of oxygen. Nitrate is anticipated in the groundwater at this site. Under anaerobic conditions over a pH range of 6-8.4 (similar to the pH range predicted for the Yucca Mountain repository site), *Thiobacillus denitrificans* can, through the process of denitrification, oxidize sulfide, elemental sulfur, sulfite, and thiosulfate to sulfuric acid using nitrate as the terminal electron acceptor (Ehrlich, 1981).

The predominant microorganisms recovered from deep subsurface sediments are chemoheterotrophic bacteria that utilize organic carbon compounds as a source of cell carbon and energy. Most of the oxygen-respiring aerobes and nitrate- and sulfate-respiring anaerobes are chemoheterotrophic bacteria. They obtain their nitrogen, phosphorus, and sulfur requirements from inorganic or organic compounds that contain these elements.

The most important habitats for denitrification are those in which aerobic-anaerobic interfaces occur. Nitrate from oxidation of nitrogenous compounds by autotrophic and heterotrophic microbes in an aerobic zone diffuses to a nearby anaerobic zone where it is quickly reduced by denitrifiers in the presence of metabolizable organic carbon. Two-thirds of the surface sediment samples recovered from depths ranging from the surface to 289 m at the SRP displayed denitrification when amended with only prereduced deionized water (Francis et al., 1989). Nitrate supplementation to sediment samples stimulated

denitrification in all cases. Denitrification in the unsaturated zone sediments was limited by a lack of available organic carbon based on comparison of rates in the presence and absence of added succinate. This result led the authors to suggest that either the energy source or nitrate were limiting in the environment from which the samples were taken. The extent of denitrification decreased with depth. Although the nature of the organic compounds, the source of nitrogen and the characteristics of the denitrifiers in the subsurface environment are not fully known, the available data indicates that denitrifiers are present in the deep subsurface environment and that under appropriate conditions, they can be active. Nitrate is expected to be present in the minerals and water at the Yucca Mountain subsurface site proposed for radioactive waste disposal.

Dissimilatory SRB can be found in terrestrial, freshwater, and marine environments. SRB use sulfate as the terminal electron acceptor in anaerobic respiration. Some genera grow on a narrow range of organic electron donors (e.g., lactate), releasing acetate as a metabolic product. Other genera are able to metabolize a wider range of organic electron donors (e.g., formate, butyrate, and propionate) and produce carbon dioxide as an end product. Still other genera of SRB utilize long-chain fatty acids and some aromatic compounds (Imhoff-Stuckle and Pfennig, 1983). One species of SRB can utilize methane as an energy source (Ehrlich, 1981). The species about which most is known is *Desulfovibrio desulfuricans*.

Deep subsurface environments vary with respect to nutrient concentrations. The deep subsurface at the SRP site, the Idaho National Engineering Laboratory, and the Nevada Test Site contained detectable amounts of organic carbon and inorganic nitrogen and phosphorous. The low-permeability clayey layers, in contrast, were low in these nutrients. In some cases, amendments of these nutrients stimulated *in situ* microbial activities, whereas in other samples, amendments produced no detectable change in rates of microbial processes (Swindoll et al., 1988). The fact that there appeared to be no correlation between total organic carbon (TOC) and microbial cell densities in the many samples collected suggests that organic carbon concentration did not control the activities of deep subsurface microbes as it does in many aquatic environments (Jones et al., 1989). The presence of reserves of fermentable carbon in some samples from the SRP site suggests that metabolic activities in some subsurface zones may not necessarily be limited by carbon. However, organic carbon limitation was noted for several sulfate-reducing zones at the SRP site (Madsen and Bollag, 1989). Identification of the limiting nutrients for the different physiological groups of microorganisms present in the Yucca Mountain repository and the flux of these nutrients to the repository environment are areas of research that needs to be developed. Answers to these questions will help determine the potential rates of microbial activities in the environment immediately outside the thermal envelope surrounding the waste package.

At Yucca Mountain, the activity of man will undoubtedly result in the introduction of nutrients in the vicinity of the repository. Drilling activities will likely introduce diesel fuels, hydraulic fluids, and drilling fluids that can serve as nutrients to the microorganisms present (West, 1988). This increased nutrient load is expected to stimulate microbial growth and activity at the repository (Hersman, 1987).

McKinley et al. (1985) estimated that the bentonite backfill in a Swiss HLW repository is expected to provide a major source of nutrients to the microbial populations. The composition of this backfill is presented in Table 2-2. Although direct determinations have been made with respect to nitrogen and phosphorus, estimates of 400 and 1000 ppm, respectively, have been used for modeling purposes. Philp et al. (1984) isolated a large number of different microbial groups in a similar material (Fuller's Earth) but found them to be limited for carbon and possibly phosphorus. Sulfide in the pyritic fraction of bentonite may be microbiologically oxidized to sulfuric acid. Other minerals associated with the

bentonite in Table 2-2 may be microbiologically modified in such a way that structure and permeability of the backfill material are altered.

No detailed experimental work on the nutritional or energetic value of the backfill material to microorganisms is known. This is an important area that requires more research.

Models have been developed by Swiss researchers to evaluate the microbial population sizes and activities in the near field of a HLW repository based on simple assumptions about nutrients and energy sources (McKinley et al., 1985). Nutrient sources in the near field include: the waste matrix, bentonite minerals, trapped air in bentonite, and groundwater. Available organic carbon was identified as the main limiting factor for microbial growth and activity. Organic carbon was assumed to be derived from the bentonite backfill and the inflowing groundwater (McKinley et al., 1985). The organic carbon inventory in the backfill around each canister is 3.5×10^5 g/canister, which corresponds to 1.5×10^4 moles carbon, 3.5×10^3 moles nitrogen, 3.4×10^2 moles phosphorus, and 1.1×10^2 moles sulfur. Based on a water supply of 0.7 L per canister per year, the annual supply of organic carbon in groundwater amounts to 5.6×10^{-4} g/canister, which can be similarly apportioned to 2.3×10^{-5} moles carbon, 5.6×10^{-6} moles nitrogen, 5.4×10^{-7} moles phosphorus, and 1.8×10^{-7} moles sulfur. Integration of these inputs over a 1000-year period reveals that the bentonite backfill is the most important source of these nutrients. Similar determinations need to be made for nutrient input to the Yucca Mountain repository.

4.4.4 Energy Requirements of Microorganisms in the Repository Environment

Although there appear to be no laboratory studies that have studied the energetics of the microbial community expected to be in the repository environment, estimations have been made on populations in less extreme environments. For bacteria that utilize exogeneously supplied organic carbon as a source of cell carbon, it has been determined that an energy input of 64 kJ/g dry weight cell mass is required (McKinley et al., 1985). This value must be increased for chemoautotrophic bacteria such as *Thiobacillus*, which need additional energy to fix CO_2 into cell carbon. The efficiency of the reactions involving the conversion of carbon dioxide to organic cell carbon is not very good since 90 mol of Fe^{2+} are required to assimilate 1 mol of carbon from CO_2. There is presently a lack of knowledge of the energy required to maintain a cell in the viable state.

The major process that supplied energy for microbial activity at a Swiss HLW repository was identified as the oxidation of the steel canister (McKinley et al., 1985). This process can be mediated by either biological or chemical iron oxidation mechanisms. The anoxic corrosion process can be represented as:

$$Fe + nH_2O \rightarrow FeO_n + nH_2 \qquad (4\text{–}1)$$

followed by

$$FeO_n + mH_2O \rightarrow FeO_{(m+n)}H_{2(m-x)} + xH_2 \qquad (4\text{–}2)$$

with varying m, n and x.

Assuming that the stable oxidation product is magnetite (Fe_3O_4), the net reaction can be expressed as:

$$3Fe_{(s)} + 4H_2O_{(l)} \rightarrow Fe_3O_{4(s)} + 4H_{2(g)} \quad (4\text{–}3)$$

The net free energy change in this reaction at standard temperature and pressure (STP) is calculated to be -64 kJ/mol of magnetite. If the moles of the iron in the canister material (1×10^5 mol) are divided by 3, and the resulting value multiplied by -64 kJ, an estimate of the free energy for microbial activity may be estimated.

Other reactions that were considered to be important sources of energy for microbial activity include:

(i) iron corrosion by oxygen in the trapped air space adjacent to the waste package represented by:

$$3Fe + H_2O + 3/2\ O_2 \rightarrow Fe_3O_4 + H_2 \quad (4\text{–}4)$$

where the magnetite is the stable oxide at higher temperatures, otherwise, ferric hydrate [$Fe_5(HO)_8 \cdot 4H_2O$] or goethite [α–FeOOH] is formed. The free energy of the reaction is about -775 kJ/mol Fe_3O_4 or -258 kJ/mol of Fe oxidized. If one estimates that there are 3.8×10^2 moles of O_2 trapped in the air space surrounding the canister, the maximum free energy from this source is about 2×10^8 J per canister (McKinley et al., 1985).

(ii) radiolysis of water to hydrogen peroxide with subsequent reaction with magnetite. This energy-yielding reaction could occur after the barrier of the canister wall is breeched (McKinley et al., 1985). This reaction would provide an estimated free energy of 120 J per canister.

Combining these energy-yielding reactions, corrosion of the canister is estimated to yield 2.5×10^3 J/yr. This energy was estimated to yield a maximum initial bacterial population of 2×10^{18} cells followed by a maximum steady-state production rate of 2.6×10^{13} cells/yr.

McKinley et al. (1985) estimated that for the Swiss repository, there is 3.5×10^5 g organic nutrients available in the canister environment. Conversion of this potential energy source to microbial biomass requires about 2.2×10^{10} J, which is about an order of magnitude greater than the energy produced by anoxic corrosion of the entire canister. It was concluded, therefore, that energy is likely to be more limiting than available nutrients.

4.4.5 Microbial Influence on the Integrity of Radioactive Waste Repositories: Near-field Effects

In view of the fact that high-level radioactive waste material has been stored for up to 30 years in a hydrated environment at ambient temperatures and that microbes have been isolated from these materials, the racks that support the material, and the surrounding fluid[2], most national research

[2] Personal Communication from J. Wolfram, March 1993

programs recognize that microbes cannot be excluded from temporary spent nuclear fuel storage facilities and are now assessing the safety issues related to their presence in these as well as long-term disposal repositories (Rosevear, 1991). The United Kingdom and Switzerland have undertaken the most extensive research in this area (Rosevear, 1991). The United States approach to radioactive waste research has been much more fragmented than the main European initiatives.

In the United Kingdom, the Nirex Safety Assessment Research Program considered the effect of microbial action on low and intermediate-level radioactive waste, the repository in which the waste is disposed, and the backfill environment. The program has concentrated on the deterioration of the cement that will be used to seal off the repository (Colasanti et al., 1989). High alkalinity and low-nutrient conditions are the two environmental factors that have received the most attention (Rosevear, 1991). A computer simulation of microbial action in a low- and intermediate-level waste repository has been developed. The results of the simulation indicated that microbial action will persist inside the repository for up to 400 years after closure (Colasanti et al., 1989). Another conclusion drawn by the research is that a bacterial biofilm may develop on the surface of the cement and reduce the buffering capacity of the cement, allowing a reduction in pH from highly alkaline (pH of 11-12) to values more conducive to waste degradation.

The biotechnology group at Cadarache, Commissariat a L'Energie Atomique (CEA), has also evaluated microbiology of concrete degradation in the study of radioactive waste disposal in France. Under aerobic conditions, cultures of the fungus *Aspergillus niger* and the bacterium *Pseudomonas aeruginosa* produce organic acids such as malic and gluconic, while sulfur-oxidizing bacteria produce sulfuric acid close to the concrete surface (Perfettini et al., 1991). This production of acids caused decalcification, which resulted in structural changes and lowering of pH.

In Switzerland, the HLW waste material is more radioactive and has lower organic carbon content than the low-level waste (LLW) studied by Nirex in the United Kingdom. McKinley et al. (1985) identified canister corrosion, alteration of gross pore-water chemistry, and physical disruption of the bentonite buffer as major microbial processes in the near field. According to McKinley et al. (1985), it is likely that both introduced and resident microbes will colonize the near-field HLW repository environment even at times when ambient temperature and radiation fields are relatively high. Based on a simple quantitative model, microbial growth in the near field is limited by the rate of supply of chemical energy derived from the corrosion of the canister. The quantification of canister corrosion was presented in the previous section.

The main effects of microbial processes on near-field groundwater chemistry is expected to be alteration of E_h and pH (McKinley et al., 1985). Anaerobic conditions are expected to predominate over most of the near-field Swiss HLW repository environment during the period of radioactive waste decay (McKinley et al., 1985). Redox disequilibria is not able to be quantitatively evaluated at current levels of sophistication of geochemical modeling. Decreases in pH would be expected to occur in the presence of actively respiring (CO_2-producing) microbial populations, populations of sulfur-oxidizing bacteria, and organic acid-producing bacteria. Increase in groundwater pH would be expected to occur in the presence of active populations of ammonia-producing bacteria. Local changes in pH at the container surface are anticipated as a result of the electrochemical corrosion reactions promoted by either abiotic or biotic reactions. Current equilibrium thermodynamic models are not sensitive to nonequilibrium microbial reactions that alter groundwater. The nonequilibrium conditions that have been noted in low-temperature groundwaters can not be accounted for by these models (Lindberg and Runnells, 1984). The actual changes in groundwater chemistry caused by microbiological processes cannot be predicted at the present

time due to the lack of knowledge of the rates of transport of nutrients to the repository environment and the types of indigenous and introduced microorganisms that will be present following waste disposal.

Disruption of the bentonite buffer is only anticipated if very large amounts of microbial biomass were distributed throughout the backfill (McKinley et al., 1985). On the basis of the calculations presented in the previous section, the estimated 30 mg dry weight microbial biomass per year is insufficient to promote significant changes in buffering properties.

An additional process mediated by microorganisms in the near field is plugging of pore space in the bentonite backfill and surrounding rock matrix by microbial biofilms. Microorganisms in nutrient-limited soil environments are, for the most part, attached to particle surfaces (Colasanti et al., 1989). As they replicate on these surfaces, they tend to elaborate exopolysaccharides that fill in the pore space between the particles. This phenomenon decreases porosity of the matrix material and reduces transport of nutrients to down-field sites (i.e., the container surface). This phenomenon has been demonstrated in the formation face of water injection wells in oil-producing strata (Davis and Updegraff, 1954) and in laboratory simulations based on core flood experiments (Geesey et al., 1987b). The overall effect is to retard nutrient and water transport to the container surface.

Models have been developed to simulate major changes in the repository environment. Modeling calculations led to the conclusion that microbial action was unlikely to be a significant problem in a HLW repository (McKinley et al., 1985). However, the current model is not capable of describing the complex microbial processes that occur (Arter et al., 1991). More powerful models are being developed that are intended to predict localized heterogeneous reactions on metal surfaces and in porous media at the Center for Interfacial Microbial Process Engineering at Montana State University.

5 MICROBIALLY INFLUENCED CORROSION OF METALS

5.1 INTRODUCTION

Microbially influenced corrosion manifests itself primarily as pitting corrosion. Pitting is a localized corrosion involving a small area of the surface that may lead to through-wall perforations and leaks. Pitting corrosion must be distinguished from generalized corrosion. Monitoring techniques developed for generalized corrosion, such as weight loss measurements, are not particularly useful in evaluating pitting corrosion initiated by microorganisms.

It is difficult to relate a failure to MIC due to the multiple reactions that have occurred at the surface since the material was put into service. The interdependence of various corrosion processes makes it difficult with existing technology to distinguish between cause and effect (Clayton, 1987). Corrosion of metals covered by microbial biofilms involves both biological and abiotic reactions and is further influenced by the nature of the physical properties of the substratum.

Microbes probably exert their primary influence at the stage of pit initiation. Discontinuous bacterial biofilms that develop on metal surfaces create crevices or occluded volumes. This reaction has been attributed to metallurgical, biological, or chemical heterogeneities within the biofilm on the metal surface. Metallurgical heterogeneity includes weldments and inclusions.

Biological heterogeneity has been attributed to patchiness of bacterial colonization and biofilm formation on the metal surface, and the formation of discrete microcolonies of physiologically distinct bacterial types distributed nonrandomly over the surface. During growth and metabolism on the metal surface, the bacteria occlude some areas of the surface while exposing other areas to the bulk aqueous phase. This variation leads to the formation of differential aeration cells. Bacterial metabolism in the microcolonies results in the excretion and accumulation of different types and amounts of chemicals. The matrix polymers of the biofilm form a diffusion barrier and promote the establishment of chemical gradients across the surface. Hydrogen ion, metal ion, and oxygen gradients are formed. Thus, all microorganisms capable of colonizing metal surfaces have the potential to promote the establishment of electrochemical cells on a metal surface, mainly characterized by the physical separation of anodic and cathodic areas.

In aerobic environments, the areas under the deposits (biofilm) become depleted in oxygen and the local environment becomes acidic, causing the metal to become electrochemically active and dissolves anodically. The area of the metal surrounding the deposit is the cathode where oxygen is reduced, and the solution in that area shifts to a more alkaline pH. The initial driving force for corrosion is the oxygen concentration cell established between the occluded anodic area and the area of the surface free of deposit. The depletion of oxygen is followed by depassivation of the occluded area either due to acidification, increase in the chloride concentration or formation of reduced sulfur compounds (i.e., from MnS inclusions in steels).

Pit initiation does not necessarily result in through-wall perforation. Changing conditions at the surface will frequently lead to passivation of a pit. When evaluating the threat of MIC, the frequency with which pits form over a surface must be combined with the likelihood of any one pit extending itself completely through the material. In most instances, the researcher is interested in determining the probability of <u>one</u> through-wall perforation occurring somewhere over a relatively large surface area.

Reasonable predictions of this event require that the studies be conducted on a total surface area at least as large as that over which the prediction is being applied. In practice, the surface area over which the prediction is based is orders of magnitude less than that for which it is applied. In these instances, there is only a slight probability that the small test area (coupon) would show representative pitting. Proper use of extreme value statistics can be used to overcome this problem (Ellor et al., 1986; Gehring et al., 1983).

Demonstration of microbial involvement in a corrosion reaction requires the inclusion of proper controls that account for all other abiotic corrosion reactions. To date, only a few studies have included these critical controls (Little et al., 1986; Bremer and Geesey, 1991).

5.2 MICROBIALLY INFLUENCED CORROSION OF MILD STEEL

The most comprehensive data on the localized corrosion of mild steel buried in soils, regardless of the mechanism of attack, was obtained by Romanoff (1957). He conducted 30 year field testing in soils to obtain the mathematical expression:

$$P = 3.19\ t^{0.485} \qquad (5\text{-}1)$$

to determine P, the maximum pit depth, in mm over time, t, in years. Using this expression, he predicted that a maximum penetration of 91 mm would occur over a 1000-year period. The author cautioned that there was significant variability in the experimental results. He also cautioned that localized corrosion rates on small sample areas of coupons may not provide the highest rates that might be experienced over an area the size of a canister. He suggested applying the statistical theory of extreme values developed by Gumbel (1954) and applied to pitting by Eldredge (1957) and Aziz (1956). Marsh et al. (1983) plotted the maximum penetration depths versus cumulative frequency and showed that the local corrosion depths have an exponential distribution. It was concluded that standard corrosion tests are inadequate to assess localized corrosion of candidate metals. There is usually a discrepancy between envisaged service life and realistic laboratory test periods.

The role of microorganisms in the corrosion of iron and steel has been the subject of numerous articles, and a number of excellent reviews have been published, including those by Miller and Tiller (1970) and Ford and Mitchell (1990b).

Recognition that microbes play a role in the corrosion of metals buried in the ground or immersed in water occurred as early as the late 19th century. Gains (1910) was one of the first to suggest that iron bacteria and sulfur bacteria might be responsible for the corrosion of ferrous metals buried in soil. Von Wolzogen Kuhr and Ven der Vulgt (1934) were among the first to attempt to provide a mechanism to explain the corrosion of ferrous metals in soil due to the action of dissimilatory SRB.

SRB are considered to be the most important mediators of anaerobic corrosion of steel. Fifty percent of the failures of underground pipe are thought to be due to microbial action (Booth, 1964). Dissimilatory SRB most commonly associated with corrosion of steel are distributed into the genera *Desulfovibrio, Desulfotomaculum, Desulfobulbus, Desulfobacter, Desulfobacterium, Desulfococcus, Desulfosarcina,* and *Desulfuromonas*. Most laboratory studies on MIC employ these genera when evaluating the influence of SRB. The genus *Desulfotomaculum* is often isolated from deep subsurface oil

production fluids. The thermophilic species *Desulfotomaculum nigrificans* is likely to promote corrosion of mild steel that comes in contact with the produced fluids from oil reservoirs.

Other important groups of bacteria associated with corrosion of steel are the chemoautotrophic iron- and sulfur-oxidizing bacteria. Organisms such as *Thiobacillus thiooxidans* and *Sulfolobus* sp. have the ability to oxidize reduced forms of sulfur to meet their energy needs with the subsequent production of sulfuric acid. The sulfuric acid destabilizes the protective iron oxide films that form on carbon steel (Miller and Tiller, 1970). Acidification also facilitates corrosion by shifting the corrosion potential in a more noble direction.

The bacteria *Thiobacillus ferrooxidans* and *Sulfolobus* oxidize ferrous iron to ferric iron to obtain energy for growth. The oxidation of passivating or protective ferrous iron films promotes oxidation of the metallic iron in steels (Miller and Tiller, 1970).

Another group of iron-oxidizing bacteria often associated with corrosion of iron, carbon steel, and stainless steels is the iron-precipitating bacteria. This group includes the genera *Gallionella, Leptothrix, Crenothrix, Clonothrix*, and *Lieskeella* (Ehrlich, 1981). These bacteria appear in tubercles or deposits, under which various type of corrosion can occur. These organisms promote the oxidation of ferrous iron to ferric iron under slightly reducing conditions at pH greater than 5. The ferric hydroxides precipitate around the outside of the cell. It is believed that at least some of the iron oxidation that occurs in the presence of these microbes is the result of biochemical reactions, such as ammonia production and excretion, that raise the pH of the solution (Ehrlich, 1981).

Iron-reducing bacteria also appear to be important in the corrosion of mild steel. Some species of *Pseudomonas* have been reported to reduce ferric iron to ferrous iron (Obuekwe et al., 1981a; Obuekwe et al., 1981b; Obuekwe et al., 1981c; Sorensen, 1982). These organisms have been recovered from oil production facilities as well as various freshwater and marine environments. They appear to utilize ferrous iron as an alternative electron acceptor when oxygen becomes depleted in their environment. They are chemoheterotrophs in that they utilize short-chain organic acid such as lactate as a carbon source.

Other aerobic and facultatively anaerobic organisms that have been implicated in the corrosion of ferrous metals through their ability to produce and excrete organic acids include: *Escherichia coli, Bacillus megaterium, Serratia marcescens*, and *Salmonella typhimurium* (Umbreit, 1976). Other investigators (Booth and Wormwell, 1962) report that the hydrogenase enzyme synthesized by some of these organisms promotes corrosion of steel by removing hydrogen from the metal surface, causing cathodic depolarization.

Methanogenic bacteria have been implicated in the corrosion of iron and steel in anaerobic environments (Daniel et al., 1987; Boopathy and Daniel, 1991). *Methanococcus thermolithotrophicus* and *Methanosarcina bakeri* normally use molecular hydrogen and carbon dioxide to produce methane, but can use elemental iron in mild steel as a source of electrons to produce energy for the reduction of carbon dioxide to cell material and methane. As mentioned previously, methanogens have been isolated from deep subsurface environments.

5.2.1 Factors Influencing MIC of Mild Steel

The factors controlling MIC of mild steel include temperature, pressure, pH, E_h, oxygen concentration, salinity, water activity, and nutrient concentration. The influence of oxygen concentration on MIC of mild steel is the factor that has received the most attention due to its control over SRB activity and growth.

The availability of oxygen determines in large part the types of microorganisms and their metabolic activities and products. In the absence of oxygen and at negative redox potentials, corrosive microbial reactions such as sulfate reduction, ferric iron reduction, production of organic acids by facultative anaerobes, and methanogenesis will be favored. In the presence of oxygen at positive redox potentials, oxygen-respiring chemoheterotrophic bacteria utilize a wide variety of reduced organic carbon compounds and convert them to products that serve as electron donors to SRB and facultatively anaerobic bacteria.

The production of sulfuric acid by *Thiobacillus* and the oxidation of ferrous iron is also favored in the presence of oxygen. Microbially influenced pitting corrosion can thus occur over a wide range of oxygen concentrations (i.e., from zero to saturation).

5.2.1.1 MIC of Mild Steel Under Anaerobic Conditions

SRB have been proposed to participate in the MIC of steel through the following alternative mechanisms.

(i) Stimulation of the cathodic reaction by removal of hydrogen by SRB

Some species of SRB and methanogenic bacteria produce the enzyme hydrogenase (Daniel et al., 1987). When these bacteria grow as a biofilm on the steel surface, the enzyme can oxidize the molecular hydrogen generated at the cathodic site on the metal surface to facilitate cathodic depolarization. Removal of the hydrogen will stimulate the overall corrosion process (Bryant et al., 1991).

There are two consecutive steps for the hydrogen electrode reaction in the absence of H_2S. A primary step is:

$$M + H^+ + e^- \rightarrow M\text{-}H_{ads} \qquad (5\text{–}2)$$

coupled with the molecular recombination desorption step

$$M\text{-}H_{ads} + M\text{-}H_{ads} \rightarrow H_2 + 2M \qquad (5\text{–}3)$$

or with the electrochemical desorption step

$$M\text{-}H_{ads} + H^+ + e^- \rightarrow H_2 + M \qquad (5\text{–}4)$$

Where combination of adsorbed H atoms to produce H_2 gas is considered to be the rate-controlling step, bacteria effectively increase the hydrogen evolution rate and accordingly increase the corrosion rate.

(ii) Stimulation of the cathodic and anodic reactions by dissolved hydrogen sulfide

Hydrogen sulfide produced by SRB is known to be corrosive towards ferrous materials because it can accelerate both anodic and cathodic reactions. When H_2S is present, there is an increase in proton discharge rate (Eq. 5-2), but the hydrogen evolution rate is suppressed (Berkowitz and Horowitz, 1982). Stimulation of the cathodic reaction by bacterially produced hydrogen sulfide has been suggested by Costello (1974). The rate of cathodic reduction of hydrogen sulfide is limited by the rate of diffusion of H_2S to the cathodic site.

Stimulation of the anodic reaction by microbially produced hydrogen sulfide has been suggested by Wanklyn and Spruit (1952), but is considered to be important only at the initial stages of the corrosion process due to the eventual formation of a semiprotective sulfide film over the metal surface.

(iii) Stimulation of the cathodic reaction by SRB indirectly by the formation of iron sulfides

For corrosion to occur by this mechanism, iron sulfides must have access to the bare surface of the steel. This access is achieved when SRB grow at the base of biofilms formed on the steel surface. These bacteria produce hydrogen sulfide during respiration. This hydrogen sulfide is trapped in the film long enough to react with the iron at the base of the biofilm to form iron sulfide and a galvanic cell. Once a galvanic cell is established, mild steel behaves as an anode and proton discharge and electron transfer occur on and through the iron sulfide. Iron sulfides increase the rates of corrosion by decreasing hydrogen overpotential and absorbing the cathodically produced hydrogen.

For surface-adherent sulfides, there is evidence that corrosivity is minimized with pyrite (FeS_2) (Tayler, 1978; Tapping et al., 1983). The more perfect crystal lattice of pyrite reduces the diffusion of the cations and electrons through the film (Greco and Sardisco, 1969). This reduced diffusion increases polarization and reduced corrosion rates.

Different types of iron sulfides exert different effects on mild steel. In abiotic systems, all suspended iron sulfides are excellent anodic and cathodic depolarizing agents. In biotic systems where the iron sulfide is produced and retained in an adherent biofilm, the initial corrosion rate was attributed to iron sulfide-induced anodic and cathodic depolarization (Lee and Characklis, 1993). The initial anodic depolarization is due to a change in mechanism related to the sulfide-free system. Further polarization studies showed only increased cathodic current accompanied by little change in anodic behavior (Lee and Characklis, 1991). The increased cathodic current was thought to be due to increased accumulation of loosely adherent iron sulfide with consequent increase in cathode surface area for increased hydrogen evolution reaction on the iron sulfide. Loose accumulation of sulfide particles at the metal surface is not a barrier to ionic transport of ferrous ions and results in little change in anodic polarization curves.

In summary, the type of iron sulfide formed on the steel surface significantly influences the corrosion rate. Corrosion rate is at a minimum when an adherent sulfide film forms on the steel surface. The low corrosion rate observed under these conditions is due to polarization of both the anodic and cathodic reactions. On the other hand, the acceleration of corrosion in the presence of loosely adherent iron sulfides is due to cathodic depolarization, apparently the result of an accelerated hydrogen evolution rate.

5.2.1.2 MIC of Mild Steel Under Fluctuating Aerobic and Anaerobic Conditions

Fluctuating oxygen concentration influences the types of sulfur products formed at the steel surface that initially appeared as hydrogen sulfide released from the SRB (Hamilton, 1990). In the absence of oxygen, protective iron sulfides such as mackinawite (FeS_{1-x}), greigite (Fe_3S_4), and smythite (Fe_3S_{4-x}) are formed. If oxygen is introduced to the system, the formation of pyrite (FeS_2) and elemental sulfur are favored. In addition, the introduction of oxygen favors the anodic dissolution of iron in the steel in the form of ferrous ions. These ions react with oxygen in solution to form ferric hydroxides. Hydrolysis reactions promote the formation of hydrated ferric oxide, $Fe_2O_3 \cdot x\ H_2O$, and ferric oxyhydroxide, FeOOH, which react with the hydrogen sulfide generated by the SRB when oxygen is absent to form elemental sulfur and iron sulfide (Nielsen et al., submitted for publication). Oxygen will also react directly with iron sulfide to produce elemental sulfur and iron oxide. Both molecular oxygen and elemental sulfur can depolarize the cathode by reacting with electrons to form hydroxyl ions or hydrogen sulfide, respectively.

Deposition of iron sulfide on a steel surface results in the formation of a galvanic cell. FeS is cathodic to the underlying iron in the steel, causing the iron to dissolve at the anode and react with the oxygen and hydroxyl ions present at the cathode.

Finally, transient introduction of oxygen to the system provides sulfide-producing bacteria with additional electron donors. As mentioned previously, SRB utilize a rather narrow range of reduced organic compounds. Aerobic and facultatively anaerobic heterotrophs, on the other hand, can utilize a wide range of reduced organic compounds present in the system and convert them to lactate, acetate, butyrate, and propionate, all of which serve as electron donors for the SRB. Thus, SRB activity during periods of anaerobiosis is stimulated by the accumulation of electron donors in the biofilm provided by the aerobic and facultatively anaerobic bacteria during periods of oxygen availability.

5.2.1.3 Corrosion of Mild Steel by Iron-Reducing Bacteria

Bacterial iron reduction under anaerobic conditions can modify the protective oxide film that forms over a mild steel surface under aerobic conditions to promote anodic depolarization (Obuekwe et al., 1981b). The cathodic reaction is not affected by bacterial iron reduction.

5.2.1.4 MIC of Mild Steel Under Aerobic Conditions

Acid Production

The corrosion rate of mild steel is independent of pH over the range of 4-10 in aerated water at room temperature (Uhlig, 1971). Once the pH drops below 4, however, the corrosion current will increase since the hydrogen exchange current at the cathode and iron exchange current at the anode are both proportional to the square of the hydrogen ion concentration. *Thiobacillus* sp. can reduce the pH

to 1. Other heterotrophic bacteria can release organic acids that can reduce the pH to below 4. When these reactions are carried out in biofilm populations, the matrix polymers restrict the diffusion of the acids and keep them in close proximity to the metal substratum. Consequently, acid-producing bacteria enhance corrosion of iron and steel through both anodic and cathodic depolarization.

Mechanism of Corrosion Enhanced by Iron-Oxidizing Bacteria

Iron-oxidizing bacteria do not directly catalyze the corrosion of steel. Rather, they convert ferrous iron to ferric iron, which reacts with oxygen in the system to form ferric oxide and oxyhydroxides, which are insoluble in water and precipitate. The precipitated iron likely reacts with the organic matrix polymers of the biofilm to form a shell or tubercle over the source of ferrous ions generated from the base metal. The role of iron-oxidizing bacteria in the corrosion of iron and steel is based on the following: (i) primarily through the formation of differential aeration cells by the growth of iron bacteria over limited regions of the iron surface, (ii) a mechanical strengthening of the tubercle caused by the felt-like structure of the sheaths of iron bacteria, and (iii) a decrease in oxygen availability at the surface due to occlusion by the tubercle and oxygen-respiring bacteria. This decrease in oxygen causes the potential at the anode to decrease and the potential difference between the anode (under the tubercle) and the cathode (outside the tubercle) to increase, with the result of an increase in corrosion current (Olsen and Szybalski, 1949).

Range of Environmental Variables Over Which Steel-Corroding Bacteria Are Active

Temperature

The temperature range over which microbial corrosion of mild steel occurs is quite broad. The SRB *Desulfotomaculum* grows over a temperature range of 30-70 °C (Ehrlich, 1981). Iron-oxidizing bacteria such as *Sulfolobus* sp. are active at temperatures of 80-85 °C but not at 90 °C (Ehrlich, 1981). While most strains are mesophilic with an optimum temperature of 15-20 °C, some strains of *T. ferrooxidans* oxidize iron without growth at 37 °C, and one strain reportedly is active at 40 °C. Brock (1978) described a pink bacteria growing on the surface of glass slides after 2 days immersed in boiling water. Generation times ranging from 2.5-3 hours were observed at 91 °C.

pH

The pH range over which steel-corroding bacteria are active is also very broad. *T. ferrooxidans* exhibits optimal iron oxidation at pH 3-3.6, optimal growth at pH 2.5-5.8, and tolerates pH over the range 1.4-6.0 (Ehrlich, 1981). *Sulfolobus* sp. function over the pH range 0.9-5.8.

Salinity and Water Potential

Salinities of up to 3.5 percent are typical in estuarine and marine waters. Corrosion of mild steel submerged in waters of salinities ranging from 0-3.5 percent have, in some cases, been accelerated by bacteria. Little work has been done on MIC of mild steel under low water potentials.

5.2.2 Influence of Microorganisms on Hydrogen Embrittlement and Stress Corrosion of Mild Steel

Walch and Mitchell (1986) have proposed a role for microorganisms in hydrogen embrittlement of steel. Many bacteria produce molecular hydrogen as a by-product of fermentative metabolism, which may be dissociated into atomic hydrogen and adsorbed into the steel by dissociative chemisorption. Other bacteria produce hydrogen ions along with organic or mineral acids, which may be reduced at cathodic sites to atomic hydrogen. SRB produce hydrogen sulfide, which impedes formation of molecular hydrogen. The buildup of atomic hydrogen at the surface of the steel promotes absorption into the metal. Ford and Mitchell (1989, 1990a) reported that when high-strength steel was exposed to hydrogen-producing bacteria, longitudinal cracks appeared on the upper surface of the steel. Some initiation of cracking was apparent on control steel, but to a much lesser degree than steel exposed to the bacterium. Possible mechanisms of the involvement of the organisms in the cracking process proposed by the authors were similar to those for hydrogen embrittlement.

5.2.3 Physical and Chemical Conditions for Corrosion of Mild Steel

The physical and chemical factors that influence corrosion of steel in soil has been extensively investigated by Booth et al. (1967a, 1967b). Mild steel would not passivate in natural environments and was shown to experience serious deterioration under certain physical, chemical, and biological conditions. Factors that are important in controlling corrosivity of mild steel in soil include: (i) soil resistivity, (ii) redox potential, (iii) water content, (iv) pH, (v) soluble iron, (vi) hydrogen uptake, and (vii) biological activity. Booth et al. suggested that aggressive soils were characterized by a mean soil resistivity less than 2000 ohms cm or a mean redox potential more negative than 400 mV_{SHE}. If water content was greater than 20 percent, the soil was regarded as aggressive, and if below this figure, nonaggressive. Alternating moisture content with alternating aerobic and anaerobic conditions is also likely to accelerate the rate of corrosion of mild steel. Soils with a mean soluble iron content greater than 120 $\mu g/g$ are considered to be aggressive. The concentration of soluble iron is important in governing the nature of any sulfide film formed on the steel surface and can modify the overall rate of corrosion.

5.2.3.1 Anion Concentrations

The abundance and ratio of anions in solution also influence the corrosion of mild steel. The most aggressive ions in groundwater are likely to be Cl^- and SO_4^{2-}, while the main passivating ions are CO_3^{2-} and HCO_3^- (Marsh et al., 1983). Carbon steel was found to passivate only under comparatively alkaline conditions. At 6.1 ppm bicarbonate concentrations, the metal did not passivate between pH 6-11. Local passive breakdown potential decreased with increasing chloride concentration. Sulfate ion concentration effects were similar to chloride ion.

5.2.3.2 High Salinity/Low Water Content

Ions of varying concentrations are commonly encountered where corrosion reactions are occurring. Pitting, crevice corrosion, and underground pipe corrosion, particularly those connected with heavy brines, are examples of types of corrosion dependent on elevated concentrations of specific ions. At high ionic strengths, the activity of water falls below 1; while the effect of ion concentration is often taken into account in corrosion reaction rates, the influence of decreased water activity is rarely considered (Smart and Bockris, 1992). Nevertheless, water is known to be one of the reacting species that

governs electrochemical mechanisms of corrosion. McCafferty and Hackerman (1972) found that water activity had an insignificant effect on the electrochemical behavior of iron. Smart and Bockris (1992) investigated the electrochemical kinetics of iron in solutions of $ZnCl_2$ or $ZnCl_2$ plus $CaCl_2$ up to 24 M. Linear relationships were observed for log $i_{o,Fe}$, log $i_{o,H+}$ and log i_{corr} with the estimated water activities a_{H2O} in these solutions. All decreased linearly with a_{H2O} between 0.1 and 1.0. Thus, the rate of iron corrosion decreased linearly with water activity under these conditions if one assumes that a_{H+} remains constant over the range of water activities studied. Log i_{corr} decreased from -4.5 Acm^2 at a_{H2O} of 1.0 to approximately -5.5 Acm^2 at a_{H2O} of 0.1.

Thus, the rate of iron dissolution described below should decrease with decreasing H_2O in solution, according to the following equations:

$$Fe + H_2O \rightleftharpoons FeOH + H^+ + e^- \quad (5\text{-}5)$$

$$FeOH + Cl^- \rightleftharpoons FeCl + OH^- \quad (5\text{-}6)$$

$$FeCl \rightleftharpoons Fe^{2+} + Cl^- + e^- \quad (5\text{-}7)$$

When Cl^- concentration is greater than 0.2 M and the electrodes are active, the breakdown of passive films on iron does not occur and the interface is saturated with Cl^-. Thus, pitting from Cl^- does not occur. The results are in agreement with those predicted from electrochemical theory for the influence of water activity.

5.2.4 Container Corrosion Studies on Mild Steel and Summary

Marsh et al. (1983) estimated that a canister made of carbon steel subject to chemically induced localized corrosion would have to be 91 mm in thickness in order to prevent leakage of canister contents into the surrounding environment. The author indicated, though, that these estimates were made without consideration of corrosion reactions mediated by microorganisms.

The repository corrosion research program in the United Kindgom has included studies on microbially mediated corrosion of steel (Philip and Taylor, 1987). The program has concentrated on gas and acid production caused by microbial degradation of the organic and inorganic components of the waste (Pugh et al., 1989). A mathematical model that simulated microbial processes in the repository environment was developed. The model simulates gas production through metal corrosion (Colasanti et al., 1989). The worst microbial case was encompassed by the limits established for chemical degradation and corrosion. The current research is focusing on the manner in which microorganisms form biofilms and survive high pH.

The main microbiological concern related to intermediate- and low-level waste in Sweden was the corrosion of steel packages (Rosevear, 1991). No details or references have been obtained on any research performed in this regard.

Carbon steel has been tested as a candidate canister material in the Swiss research program, as well. Laboratory vessels were filled with synthetic groundwater simulating that around a Swiss repository and some were inoculated with microbes, including SRB, iron-oxidizing bacteria, sulfur-oxidizing

bacteria, and alkalinophilic bacteria and incubated for up to 18 months in the presence of cement and iron coupons. During this time, the pH increased to 11.5-12, E_h decreased to $-100\ mV_{SHE}$, and there was evidence of cement degradation. The final population of microorganisms was the same regardless of the type of inoculation and appeared to be capable of surviving alkaline conditions (West et al., 1992). Steel coupons all exhibited signs of corrosion. Although weight loss measurements indicated that the overall corrosion rates were low for aerobic conditions, detailed metallographic observations revealed that the corrosion was localized in deep crevice pits. Some coupons had been penetrated to 50 percent of their thickness by the localized corrosion. This study suggests that the coupons could be breeched two orders of magnitude faster than indicated by the average corrosion rate. Corrosion products appeared to be ferric oxyhydroxide and magnetite. Formation of the latter may produce localized microenvironments with a lower pH. This type of corrosion was considered to be a microbial influence (West et al., 1992).

Microbial attack of mild steel occurs in both soil and water. SRB appear to promote the corrosion reaction by several proposed but as yet unconfirmed mechanisms. If conditions for microbial activity develop at the surface of the mild steel liner, these activities could either accelerate abiotic corrosion reactions or promote MIC under anaerobic conditions. Further work needs to be done to estimate the rate of localized corrosion under repository environmental conditions. Such data could be used to determine the metal thickness required to eliminate the possibility of a single through-wall perforation over the 300- to 1000-year waste disposal time.

5.3 MICROBIALLY INFLUENCED CORROSION OF COPPER AND COPPER ALLOYS

5.3.1 Corrosion and Fouling of Copper

Pure copper is a metal known for its good ductility, heat and electrical conductivity, and anti-fouling properties (Mendenhall, 1977). It is commonly chosen for industrial applications because of one or more of these properties combined with its relative affordability. Environments to avoid in prudent applications include those containing oxidizing acids (e.g., nitric acid), nonoxidizing acids (e.g., carbonic acid) in aerated solutions, hot concentrated sulfuric acid (aerated or nonaerated), agents that complex with copper ions, notably ammonia or amines, oxidizing heavy metal salts, such as $FeCl_3$ and $Fe(SO_4)_3$, and hydrogen sulfide, as well as some other sulfur compounds (Uhlig, 1963; Leidheiser, 1971).

Pure copper is protected by a layer of cuprous oxide, which forms immediately upon exposure to oxygen or aerated aqueous environments. This oxide film is adherent to the substratum unless subjected to high water flow rates. Flow-assisted corrosion is caused by anodic dissolution enhanced by mass transfer of the dissolution products across the diffusion layer (Syrett, 1976). Furthermore, this oxide film is deficient in cations, and thus not completely insulating. Therefore, cathodic reduction can occur as electrons are transported across it.

Pure copper is one the most fouling-resistant materials known, a property conferred by the toxicity of copper ions to organisms. Historically, the anti-fouling properties of copper have led to its use in marine environments. However, many microbes are at least somewhat resistant or tolerant of copper ions. A classic example is the bacterium *Thiobacillus thiooxidans*, which can tolerate a 2-percent aqueous solution of copper in the form of cupric ions (Pope et al., 1984). Some fungi have been reported to tolerate saturated solutions of $CuSO_4$ at pH 0.1 (Starkey and Waksman, 1943). Many strains of microbes that are normally sensitive to copper become tolerant upon exposure to sublethal concentrations of the

metal (Trevors and Cotter, 1990). Thus, selection of copper or copper alloys for their toxic effects on microorganisms leads to the selection of copper-tolerant populations as the fouling community.

5.3.2 Characteristics of Copper Corrosion in Freshwater Involving Microorganisms

Two unusual types of pitting corrosion have been observed that are thought to involve microbial biofilms (Angell et al., 1990). One type is characterized by numerous pits beneath a common basic copper sulfate crust with a randomly perforated oxide membrane across the pits. This type of pitting has been reported in copper water lines of certain institutional buildings in southwest Scotland. These buildings use surface waters that contain relatively high concentrations of dissolved organic compounds, suspended particulates, including microorganisms, and low buffering capacity (pH 7.4-9.3). The other type of pitting believed to involve microorganisms occurs in cold, warm, and hot water systems and exhibits features of both Type 1 and Type 2 pitting (Figures 5-1 and 5-2) (Fischer et al., 1992). The pits are hemispherical and contain soft crystalline cuprous oxide with varying amounts of cuprous chloride under a cuprous oxide membrane. The oxide on the surface between the pits is largely cupric. The mounds above the pits are principally basic copper sulfate, often with a deposit of powdery cupric oxide around the periphery and on parts of the deposit itself.

Microbiological involvement was indicated by the presence of a patchy, slimy biofilm on the surface of the piping that stained positive with periodic acid-Schiffs base (PAS) reagent or Gentiana-violet, indicating the presence of polysaccharide (Fischer et al., 1992; Angell et al., 1990). This unusual type of pitting has been referred to as "Type 1 1/2" pitting and has been reported in copper water lines of institutional buildings in Saudi Arabia, Germany, Scotland, and more recently in southwest England (Figure 5-2). A description of the corrosion products from copper tubing collected from the various institutional facilities has been reviewed (Angell et al., 1990; Fischer et al., 1990; Fischer et al., 1992).

With one exception, cases in which these unusual types of pitting occurred were from soft water supplies in which the water temperature was less than 60 °C and the water remained stagnant for periods of time. The waters in which the unusual type of pitting corrosion have been observed have total hardness of between 25 to 40 mg/L $CaCO_3$, alkalinity (carbonate hardness) between 10 and 20 mg/L $CaCO_3$, chloride between 15 and 20 mg/L, and sulfate between 10 and 30 mg/L. In most cases, the sulfate content is approximately twice the carbonate content. Since the water is so poorly buffered, pH is quite variable in water associated with this type of pitting. A more complete description of water characteristics in which the unusual types of pitting corrosion have been observed is presented by Fischer et al. (1992) and by Wagner et al. (1992).

Aerobic bacterial species have been cultured and isolated from biofilms removed from corroded areas of copper pipes recovered from various institutional buildings. *Methylobacterium* sp., *Achromobacterium* sp. and *Pseudomonas* sp. were recovered from all samples (Chamberlain and Angell, 1990). Some SRB were found in some biofilms recovered from corroded copper pipe.

Microbes that can colonize the surface of pure copper are abundant in natural waters. They have been successfully isolated by placing coupons in fresh tap water for several months. Under controlled laboratory conditions at 20 °C, one of these isolated strains was shown to enhance the corrosion of copper significantly compared to a sterile control (Bremer and Geesey, 1991). The MIC was initiated by terminating flow in the bulk aqueous phase (0.36 mL/h) to quiescent conditions. Once initiated, the corrosion reaction proceeded quite rapidly compared to the sterile control. The strain involved was a

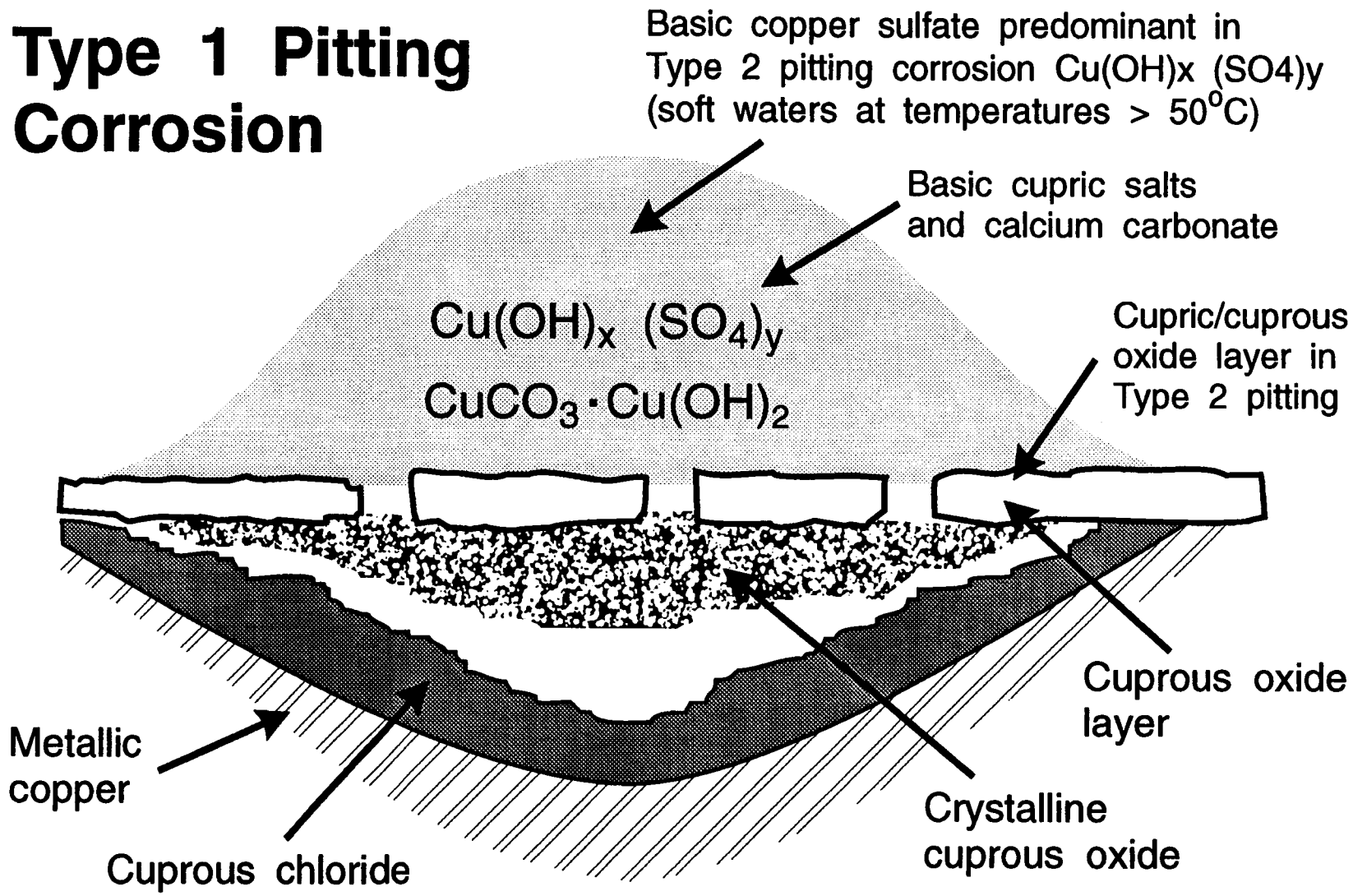

Figure 5-1. Schematic of the morphology and the various processes associated with Type 1 pitting corrosion of copper in stagnant, hard waters. In the same figure, variations corresponding to Type 2 pitting corrosion are also indicated.

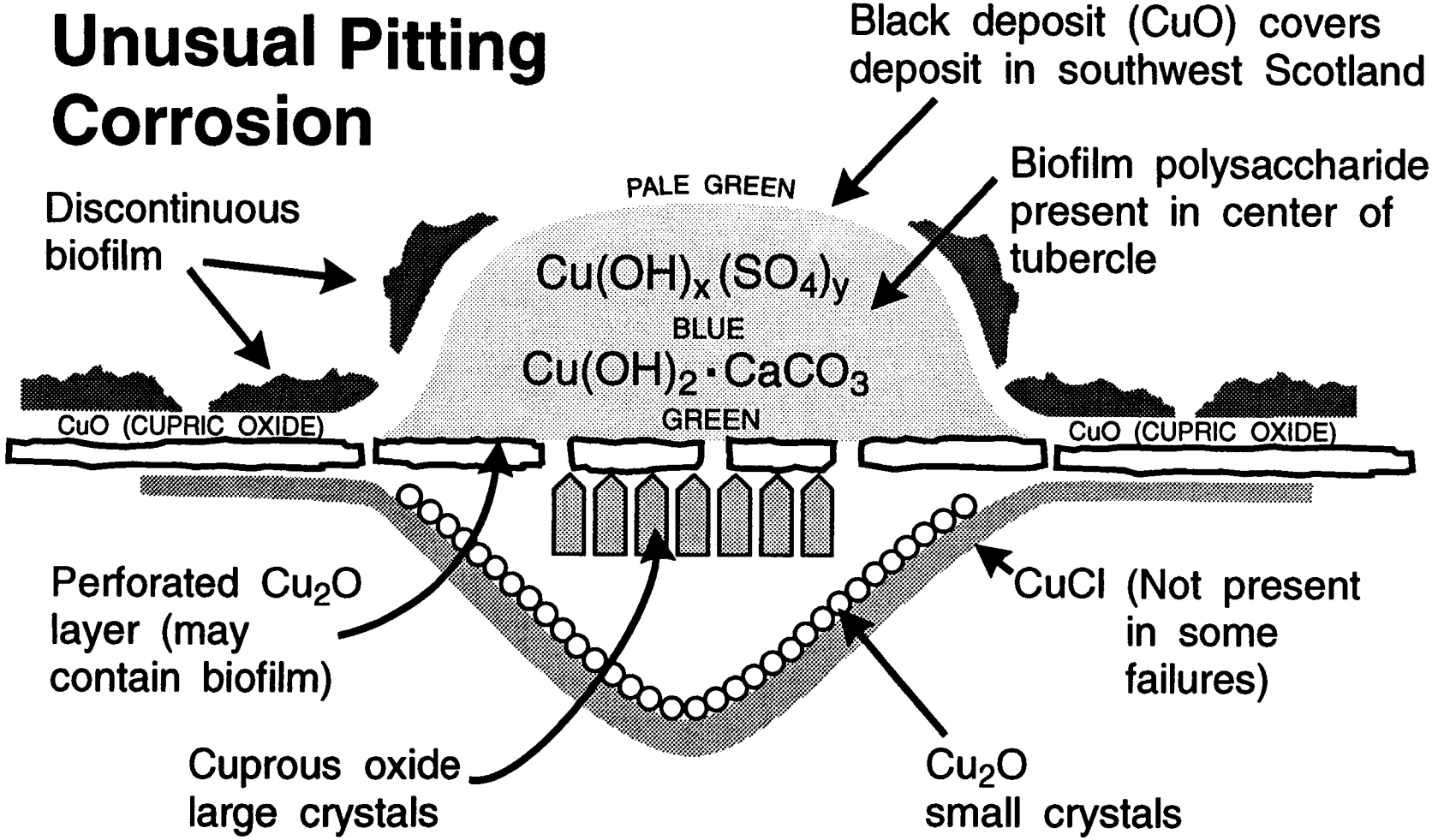

Figure 5-2. Schematic illustrating the morphology of the unusual pitting corrosion of copper observed in the presence of bacteria

facultative anaerobe not yet identified. In a completely deaerated aqueous environment, *Desulfovibrio desulfuricans* has been shown to increase the corrosion rate of copper to 140 μm/yr [5.5 mils per year (mpy)], compared to a negligible rate for a sterile control (Schick, 1990). The authors did not indicate whether attack was generalized or localized, perhaps because the monitoring techniques (potential versus time curves, linear polarization curves, potentiodynamic polarization curves and weight loss measurements) are not particularly well-suited to distinguish between these types of corrosion processes.

5.3.3 Factors Contributing to Microbially Influenced Pitting Corrosion of Pure Copper in Freshwater

5.3.3.1 Temperature

Substandard water maintenance was thought to contribute to the corrosion problems. The institutional buildings experiencing pitting corrosion had a lower temperature in their hot water systems than those without corrosion. Practical experience has shown that MIC has not been a problem in water systems maintained above 60 °C. Studies by Walker et al. (1991) showed that the hot water in the buildings that experienced the pitting corrosion was not maintained at a sufficiently high temperature to inhibit microbial growth. Most of the time the temperature was below 50 °C, which enabled the bacteria to proliferate. A two-stage continuous culture model was used to mimic the corrosive environment of one of the buildings (Walker et al., 1991). Using a microbial inoculum from the corroded copper tubing, these authors showed that the biofilm could be established at temperatures up to 55 °C. However, at temperatures above 55 °C, the biofilm was greatly reduced. The fact that pitting corrosion and biofilm accumulation were much more pronounced at temperatures below 55 °C than above that temperature suggests the involvement of a biological rather than a nonbiological reaction. It was concluded that the temperature of the hot water supply has a significant role in the control of MIC in these institutional buildings (Walker et al., 1991).

5.3.3.2 Water Chemistry

Although the quality of the supply water was considered to be good, the level of assimilable organic carbon (AOC) was found to be higher than in other areas where corrosion problems were absent. The water in buildings that did not maintain their hot water above 50 °C exhibited higher AOC, presumably due to sloughing of biofilm from the walls of the tubing. In this respect, the growth of biofilm microorganisms on the walls of the piping can lead to the degradation of water quality. Besides serving as nutrients for the bacteria, some types of organic carbon (i.e., humic substances in the supply water) are strong chelators of copper and counteract the bactericidal properties of copper ions in solution. Aluminum-chelating phenolic material was recovered from the biofilm/corrosion deposits (Angell and Chamberlain, 1989). Dissolved oxygen concentration in the water of buildings experiencing corrosion dropped to 0.1 mg/l during the 11-hour period that building activities were minimal. This drop was believed to be due to the respiratory activities of the biofilm bacteria since the planktonic bacterial densities were not high enough to account for the oxygen demand. The establishment of anaerobic conditions was thought to promote the growth of SRB that were found in the biofilm and may have contributed to the corrosion reactions.

5.3.4 MIC of Pure Copper in Marine Environments

Marine bacteria, isolated from a copper-containing protective coating, have been shown to enhance the corrosion of copper. An exopolymer-producing marine bacterium promoted a five-fold increase in corrosion rate of copper in an artificial seawater-glutamate solution (2.7 $\mu A/cm^2$) over sterile glutamate-containing artificial seawater (0.53 $\mu A/cm^2$) during a 37-day experiment (Wagner et al., 1991). The formation of a Cu^{2+}/Cu^{+} couple or accumulation of acidic polysaccharide was proposed to accelerate the cathodic reaction under these circumstances.

A marine isolate identified as a *Pseudomonas* species (B-3) was shown to be capable of reducing Cu^{2+} and oxidizing Cu^{+} under specific conditions. By using a dual copper electrode setup connected by a zero-resistance ammeter, workers demonstrated that the microbial slime may be instrumental in depolarizing the cathodic reaction when Cu^{2+} ions are added to the cathodic compartment (Gerchakov et al., 1985). Of the 45 bacterial strains isolated from brass surfaces exposed to pristine Gulf Stream water, 43 were found to corrode copper (Gerchakov et al., 1978), indicating that MIC-associated bacteria are abundant in marine environments.

5.3.5 Corrosion of Copper Alloys

Copper alloys, though more prone to fouling than pure copper, are generally more resistant to corrosion. The procedure of alloying copper with zinc to produce brass was followed by the addition of tin and aluminum for applications involving exposure to high-velocity seawater (e.g., heat condenser tubes). Finally, nickel was alloyed with copper to produce the copper-nickels. It was found that the addition of a small amount of iron improved the corrosion resistance in seawater still further in these cupronickel alloys. The protective film formed on copper alloys can have a highly variable chemical composition, depending upon the alloy and the environment (Parvizi et al., 1988). However, in general, it does appear that corrosion of copper alloys is under cathodic control (Hack et al., 1986).

Copper alloys are generally susceptible to corrosion in aqueous environments containing small quantities (< 10 ppm) of ammonia or amines, especially in the presence of a depolarizer (e.g., oxygen). Homogeneous alloys such as copper-nickels are more resistant in this respect. Brass is subject to dezincification in aqueous environments containing chloride and oxygen. This attack on brass is exacerbated by high temperature and the existence of crevices. Intergranular attack occurs with the grain boundary being anodic to the grain. Gases that promote corrosion under nonsaturated conditions (i.e., moist air) are sulfur dioxide, carbon dioxide, and oxides of nitrogen.

The presence of NaCl (0.51 M) favors the dissolution of copper in 70/30 Cu/Ni alloy (Videla et al., 1989). The formation of a $Cu_2(OH)_3Cl$ deposit hinders further dissolution of the copper.

Water containing sulfides is very aggressive towards copper and its alloys (Manaktala, 1990). The addition of 1 mM Na_2S to solutions containing 70/30 Cu/Ni increases the current density (Videla et al., 1989). CuS or Cu_2S is formed under these conditions and promotes formation of a very unstable copper oxide layer that has defects and poor protective properties. Cu_2S is more cathodic than the cuprous oxide film formed in sulfide-free water. As little as 0.1 mg/L sulfide in the water can accelerate attack on copper alloys. The maximum pit depths of 0.5 mm in 15 days can be expected in alloys exposed to sulfide (Manaktala, 1990). Sulfide generated by SRB is very aggressive towards copper and its alloys (Manaktala, 1990).

5.3.6 MIC of Copper Alloys in Marine Environments

Studies of MIC of copper alloys in marine environments have been performed. Surface seawater is a chemically complex solution and varies, depending on location. The presence of minor components can have a large influence on the corrosion processes (Parvizi et al., 1988). Surface seawater in which most corrosion studies have been performed has a large buffering capacity with a typical pH between 8.1 and 8.3, 3.5 percent salinity, 1.9 percent Cl^-, 7-8 ppm dissolved oxygen, and 900 ppm sulfur as sulfate. Sulfide and free chlorine are not generally detectable in open water but are present in sewage effluent that is discharged into marine waters. Sulfide is often detected in microbial biofilms containing SRB.

In marine environments, the nonprotective scale that overlays the protective cuprous oxide layer is typically paratacamite (or atacamite) [$Cu_2(OH)_3Cl$] or malachite [$CuCO_3 \cdot Cu(OH)_2$] (Pollard et al., 1989). If sulfide is present at greater than 10^{-2} M, sulfur-containing minerals (e.g., chalcocite, Cu_2S) will be present. Even when the sulfide concentration is below detectable limits, the scale will contain Cu_2S when SRB are present. Unusual sulfur-containing minerals [digenite (Cu_9S_5), spionkopite ($Cu_{39}S_{28}$), and djurleite ($Cu_{31}S_{16}$)] appear as corrosion products in the presence of SRB. These minerals are not detected in the case of abiotic corrosion (McNeil et al., 1991). Some of the minerals (djurleite) are more adherent than the common minerals found in corrosion deposits. Within a year, pits can form under these surface deposits, which are rich in chloride and sulfur and contain microbes including SRB (Little et al., 1991).

5.3.6.1 Short-Term Experiments on MIC of Copper Alloys

Exposure of cupronickel (90/10) to natural seawater for 24 h causes its electrochemical behavior (polarization curves) to be distinctly different from that in a 3.4 percent NaCl solution, presumably with a low bacterial concentration (Castle et al., 1983). X-ray photoelectron spectroscopy (XPS) of the surface after removal from the seawater revealed the presence of organic material on the 90/10 surface. Polarization curves under deaerated conditions supported the interpretation that adsorption of organic material at the surface caused cathodic depolarization.

After one day of exposure to artificial seawater (pH 7.5) inoculated with two isolates from polluted seawater (either *Pseudomonas* sp or *Vibrio alginolicus* in separate experiments), the surface of 70/30 Cu-Ni was colonized, and signs of corrosion under the colonies were observed using scanning electron microscopy (SEM) (Gomez de Saravia et al., 1989). Oscillations in the corrosion potential were pronounced over a 7-day period as compared to a sterile control and mimicked the electrochemical behavior of *in situ* samples (placed directly in Mar del Plata Harbor, Argentina). The scatter in the corrosion potential was believed to result from repeated sloughing of portions of the corrosion/slime layer that had formed on the metal. In support of this interpretation, SEM revealed that after 6 days of exposure, bacteria had colonized in strata sandwiched between layers of corrosion products.

Introduction of *V. alginolyticus* to culture medium displaced the corrosion potential in the active direction from 0.2 to 0.4 V_{SCE} over a 4-day period (Videla et al., 1989). Mixed cultures of *V. alginolyticus* and an SRB caused the corrosion potential to shift from -0.2 to -0.7 V_{SCE} over a 7-day period.

Coupons of 90/10 and 70/30 Cu-Ni, aluminum brass, and aluminum bronze were exposed to different types of bacteria in flowing seawater (20 °C) that had been previously filter sterilized (Schiffrin

Table 5-1. Corrosion rates of various copper alloys in the presence and absence of bacteria after a 10-day exposure period (from Schiffrin and de Sanchez, 1985)

Material	Corrosion Rate, mg/cm^2		
	Sterile Seawater	Seawater + Mixed Bacteria	Seawater + Pseudomonas
90/10 (C70600)	0.5	1.5	12
70/30 (C71500)	0.2	0.5	—
Al-brass	1.3	2.2	—
Al-bronze	0.7	2.5	15

and de Sanchez, 1985). Inocula consisted of a *Pseudomonas*, a *Micrococcus*, and a *Corynebacterium* isolated from the inside of condenser tubes. Coupons were exposed for 10 days to both mixed and pure cultures of these bacteria. Weight loss of the coupons indicated an approximately twenty-fold increase in corrosion rate for the 90/10 alloy and aluminum bronze exposed to the *Pseudomonas* culture compared to the sterile control (Table 5-1).

5.3.6.2 Medium-Term Studies on MIC of Copper Alloys

Copper alloy CuNi30Fe, after exposure to flowing (0.2-0.3 m/s) seawater from the intake channel of a thermal power plant (pH 7.57-7.68, 9.7-20.4 °C, O_2 1.37-2.36 mL/L) for 4 weeks, was heavily fouled with filamentous biological material (Mele et al., 1989). SEM examination revealed bacteria and diatoms entrapped between corrosion products. Bacteria isolated from the fouling layer were determined to be gram-positive and oxidase-negative *Coccobacillum* and *Vibrio alginolyticus*.

Fouling deposits formed in seawater environments were monitored electrochemically for extended periods of time in several studies. Open-circuit potentials on 90/10 Cu-Ni in fresh seawater (9 °C) collected from a 60-m depth off the coast of Norway were monitored for 100 days. The electrochemical data suggested that an active fouling layer was not formed when the temperature was held above 30 °C (Holthe et al., 1989). An unspecified copper alloy, when exposed to seawater used for the cooling water for the Hsin-Da power plant (Taiwan), was heavily fouled after 92 days (Chen et al., 1988). The underlying metal appeared shiny after marine organisms and scale were peeled off. Pure copper and 90/10 Cu-Ni were exposed to seawater from the Thames estuary (United Kingdom) for 27 weeks (Blunn, 1984). Samples examined by transmission electron microscopy (TEM) revealed bacteria associated with corrosion products on both metals. Corrosion products and bacteria formed alternating layers on pure copper. On 90/10 Cu-Ni, bacteria were embedded throughout the film of corrosion products, but most were concentrated in the upper and lower layers.

Pure copper, 90/10 Cu-Ni, and 70/30 Cu-Ni coupons were exposed for 4 months to mixed undefined cultures of facultative aerobic bacteria and SRB isolated from various marine environments (McNeil et al., 1991). The experiment was performed in culture media under anaerobic conditions. Corrosion products on pure copper were nonadherent, while those on the other alloys (90/10 and

70/30 Cu-Ni) bound more tenaciously to the surface. Mineral analysis by x-ray diffraction determinations revealed that deposits on samples exposed to bacterial cultures contained copper-sulfur compounds not usually found in deposits formed under abiotic conditions. These minerals may be considered fingerprints for MIC of these copper alloys.

5.3.6.3 Long-Term Studies of MIC of Copper Alloys

Long-term utility of copper alloys has been established in relatively corrosive marine environments over a wide temperature range (< 0 to 150 °C). Parvizi et al. (1988) found that the average service life of 90/10 Cu-Ni condenser tubes was 30 years. Microorganisms have been associated with pitting corrosion of copper alloys exposed to seawater. Severe pits were found under surface deposits on sections of a 90/10 Cu-Ni (CDA706) seawater piping system. The pits were observed after 12 to 18 months of construction and 1 year of service (Little et al., 1991). Bacterial populations enriched in SRB were found under deposits within the pits. Nickel had been preferentially removed from pits, leaving copper-rich spongy deposits. The role of the microorganisms associated with the deposits was not elucidated.

The effects of heterogeneities in the metal structure have been the subject of several studies. Welds of 90/10 Cu-Ni (CDA706) exposed for 1 year to seawater from the mouth of the Pascagoula River, Michigan (9-30 °C, pH 7.4-8.2, 4.3-20 percent Cl^-, O_2 4.3-10.5 mg/L), were fouled more heavily by SRB than the smooth surfaces on the base metal (Little et al., 1989). Weld filler material was 70/30 Cu-Ni (CDA710). Welds were of various types: bead, socket, and bolt. Both aerobic bacteria and SRB were present in the seawater and in biofilms. Pits were observed downstream from the fouled welds. Exposure of 90/10 Cu-Ni (CDA706) piling sheathing to seawater at Wrightsville Beach, North Carolina, for a 2-year period resulted in profuse hard shell biofouling, which was concentrated on the bead welds (Melton, 1987). Weight loss measurements on the anodes that were used on cathodically protected piling indicated a consumption rate of 0.38 kg/yr.

Microbiological involvement in crevice corrosion of 70/30 Cu-Ni (C71500) was evaluated with the use of biocides. Coupons were exposed to flowing (2.4 or 3.4 m/s) or quiescent natural seawater containing chlorine residuals of 0, 1, and 90 ppm for periods of 6 months to a year (Klein et al., 1991). Artificial crevices were created by a series of annular sleeves and O-rings. Maximum corrosion resistance was achieved in the presence of 1 ppm chlorine, and the C71500 alloy performed better than a high nickel alloy Monel 400, Ni-35Cu (N04400). Superficial pitting was found on the C71500 alloy after a 6-month exposure to quiescent conditions and after 1-year of exposure to flowing conditions. Pitting was more pronounced in unchlorinated water than in water containing 1 ppm residual chlorine.

5.3.7 Summary of Fouling and Corrosion of Copper Alloys

At ambient temperatures (9 to 20 °C), fouling and MIC of copper alloys progress in the following manner: Organic matter in the bulk aqueous phase adsorbs to copper alloys (and all other substrata, for that matter) within minutes of submersion, forming a conditioning film that changes the electrochemical behavior of the alloy (Castle et al., 1983). In a matter of days, bacteria colonize the surface, and the metal begins to reveal signs of corrosion underneath microcolonies (Gomez de Saravia et al., 1989). Colonization by a particular bacterium for a period of a week can enhance the corrosion rate by as much as twenty-fold (Schiffrin and de Sanchez, 1985). In a matter of weeks, available microbes

will be incorporated into lamella of corrosion products (Mele et al., 1989), which form a scale over the alloy.

Modification of the liquid/metal interface may be induced by film-forming bacteria through: (i) generation of diffusion barriers, (ii) change in the structural characteristics of the corrosion products under bacterial colonies, and (iii) production of aggressive metabolites that reduce the protective characteristics of the oxide layer.

The discussed results, while not an exhaustive review of the literature on MIC of copper and copper alloys, are representative of the types of experiments performed to date. Long-term experiments tend to be conducted in the field, while short-term experiments tend to be performed in the laboratory. Short-term experiments yield similar results regardless of the environment under study. Early events such as formation of an organic conditioning film and the colonization of the substratum by pioneering bacterial species do not exhibit wide variations in different environments. Long-term *in situ* studies tend to produce results specific to that particular environment as the duration of the experiment increases. Macrofouling in the marine environment exerts effects on the underlying substratum that are different from a mature fouling community associated with surfaces submerged in freshwater systems. Extrapolation of biological activities and corrosion rates determined in the marine environment to the deep subsurface repository environment of Yucca Mountain should therefore be done with caution.

Based on the fouling of pure copper observed in freshwater and seawater environments, it is likely that copper alloys will also foul and corrode in soil environments. In one study of severe corrosion of underground brass pipes (humid, clay soil), SRB were found associated with the black sulfide-containing corrosion products (Alanis et al., 1985). Thus, SRB appear to be widely associated with fouling and corrosion of copper alloys.

5.3.8 Proposed Mechanisms of MIC of Copper and Its Alloys

Pitting appears to be the most important type of corrosion experienced by copper and its alloys in the presence of microorganisms. All copper-based alloys being considered as candidates for the HLW canister are susceptible to pitting. Microbially influenced pitting is one form of copper corrosion for which mechanisms and kinetics are poorly understood.

5.3.8.1 Role of Microbial Exopolysaccharides in Localized MIC of Pure Copper

Studies by Fischer et al. (1989) and Paradies et al. (1992) indicated that polysaccharides and oligopeptides were associated with corrosive biofilms. Exopolysaccharides isolated from biofilm bacteria have been shown to destabilize copper thin films. When a copper-coated internal reflection-sampling element (IRE) was exposed to a 1-percent solution of a crude acidic exopolymer from an unidentified sediment bacterium, the copper film corroded immediately (Geesey et al., 1987a). A similar phenomenon was observed when the copper-coated IRE was exposed to a suspension of other acidic polysaccharides such as alginic acid and gum arabic (Jolley et al., 1989a). The results suggest that metallic copper is sensitive to a wide range of acidic polysaccharides, including those produced by some biofilm-forming bacteria that colonize copper tubes.

Results from several studies suggest that the metallic copper is oxidized by acidic polysaccharides. X-ray photoelectron spectroscopy has shown that some of the copper deposited on the

IRE after exposure to gum arabic and alginic acid was oxidized to Cu^{2+}, that copper was exposed to other bacterial polysaccharides was oxidized to Cu^{+}, and that copper exposed to yet other types of bacterial exopolysaccharides did not undergo oxidation and remained as Cu^{0} (Jolley et al., 1988; 1989b). These results are consistent with a corrosion mechanism based on a copper concentration cell formed by the excretion of exopolysaccharides with different affinities for copper ions by different bacterial species within a biofilm (Geesey, 1991; Geesey et al., 1986).

Paradies et al. (1990) presented evidence that the imidazole or L-histidine groups of the oligopeptide chains in biofilms participate in complex formation with Cu^{+}. These authors also have detected a Cu^{3+} complex in corrosive biofilms that was formed through a reaction with peroxide. A corrosion mechanism involving these complexes has been proposed.

The chemistry, and possibly the structure of exopolysaccharides of copper-corroding bacteria, is influenced by the presence of copper in the surrounding environment. Bremer and Geesey (1993) demonstrated that the copper-complexing carboxyl groups contributed by uronic acid subunits are significantly more abundant in exopolysaccharides produced by cells exposed to copper than those produced in environments with little or no copper present. The exopolymer produced in the presence of copper bound approximately 16 percent of its weight in copper. It is likely that some microorganisms produce large amounts of copper-complexing exopolymers to protect themselves from the toxic effects that Cu^{2+} has on essential reactions inside the cell. These polymers accumulate in a heterogeneous manner over the surface during biofilm growth. This heterogeneity may promote localized dissolution of metallic copper, which takes the form of pitting. Whether pitting propensity is simply related to the quantity of exopolymer produced or a reflection of subtle differences in exopolymer chemistry or structure remains to be determined. Further research will be required to gain a more complete understanding of the role of specific bacterial exopolymers in pitting corrosion of copper. Nevertheless, significant progress has been made in demonstrating the participation of microbial biofilms in this phenomenon.

Although evidence strongly supports the hypothesis that microbes are responsible for accelerating corrosion processes in the cases that were reported, the mechanisms involved have not been clearly delineated.

Based on the information presented previously, it appears that a combination of factors contribute to the unusual forms of pitting corrosion of copper tube in institutional buildings. These factors include the use of soft water with low pH, high suspended solids, and assimilable organic carbon content; the long-term or periodic stagnation of water in the pipeworks, which produces widely fluctuating oxygen concentrations; and the use of water temperatures that promote rapid growth and activity of naturally-occurring bacteria that form biofilms on the pipe wall. It was felt that removal of the humic substances from the water, increasing the water temperature to 55-60 °C, and a general cleanup of the system was needed to control the corrosion problem. Unfortunately, once this type of corrosion is allowed to start, it is difficult to control.

5.3.8.2 Mechanisms of Corrosion of Copper Alloys in Seawater

Enhanced corrosion of copper alloys has been attributed to cyclic sloughing of a deposit consisting of bacterial slime mixed with corrosion products (Gomez de Saravia et al., 1989). In the case of a deposit containing sulfide generated by SRB, it has been proposed that the most corrosive condition for copper alloys is exposure to alternating oxic and anoxic seawater. The cuprous oxide layer formed

in the presence of oxygenated seawater is partially replaced by a Cu_2S porous black scale produced under anoxic conditions in the presence of sulfide-producing SRB and Cu^+ anodic reaction product (Little et al., 1989). The cathodic reduction of H^+ ions occurs at the boundary of the oxide-type and sulfide-containing layers, increasing the concentration of OH^- ions that react with anodically produced Cu^+ ions. Most of the Cu^+ ions migrate through the Cu_2S film, react with dissolved sulfide, and increase the thickness of the film. Corrosion under these conditions is under cathodic control. When the system becomes aerobic again, the sulfide film transforms to an oxide film again, resulting in a change in volume that weakens the bonds between the thick black sulfide film and the oxide-type scale, causing the deposit to spall (Little et al., 1989). From electrochemical polarization measurements, it was concluded that as little as 1 ppm HS^- promotes the electrocatalytic reduction of oxygen, accelerating the dissolution rate without affecting the anodic reaction (Schiffrin and de Sanchez, 1985). In a soil environment, sulfide has been shown to increase the corrosion current of brass by a factor of 40 (Alanis et al., 1985).

5.3.9 Extrapolation of Copper Corrosion in Marine and Freshwater Systems to the Yucca Mountain Repository Site

Copper and copper-based alloys have never been corrosion tested under conditions that may promote bacterial activity in an environment similar to that proposed for the Yucca Mountain HLW repository. Based on what is known about MIC of copper and copper alloys, the following reactions are proposed at the wall of a container constructed of these materials when water and microbes are present.

Episodic rainfall and flooding of the surface soils at Yucca Mountain are hypothesized to cause percolation of water down through fissures to the deep subsurface repository site. Under such conditions, the water would drip at a slow rate onto a site on the outer surface of the container, which is exposed to the backfill matrix material and subsurface atmosphere. It is proposed that small amounts of liquid water as droplets or film on the canister surface cause the most severe corrosion (Manaktala, 1990). The oxygen-containing gas phase in contact with the outer container surface equilibrates with water vapor. Microbes transported with the water, introduced during waste package disposal or present in the surrounding soil before repository construction, colonize the wetted surface using nutrients transported with the water or present in the surrounding matrix rock. The bacteria replicate on the surface of the container at the wetted site and form a local biofilm deposit. Local attack of the substratum will commence and continue until the influx of water subsides. As the water evaporates due to thermal loading from inside the container, the bacteria will produce acidic exopolysaccharides to maximize retention of the remaining water (Govan and Harris, 1986). Advancement of desiccating conditions will inactivate the microorganisms in the biofilm and preserve them until the next episode of water intrusion, leaving behind a small surface deposit of corrosion products mixed with exopolymeric material and viable cells.

Thermal loading will also promote evaporation of water droplets on the container surface, concentrating aggressive anions such as sulfate and chloride from the water and soil matrix as well as aggressive microbial by-products such as sulfide and ammonia in the biofilm matrix to further accelerate pitting corrosion. Both ammonia and amines are known to enhance stress corrosion of copper alloys, especially in oxygenated environments (Uhlig, 1963). The presence of small concentrations of ammonia (10 ppm) is expected to increase the anodic current. Ammonia liberation is anticipated by the indigenous microflora in subsurface environments similar to the environment at the Yucca Mountain repository (Amy et al., 1992). High concentrations of chloride will overshadow the ammonia effect, however (Schiffrin and de Sanchez, 1985). Other microbe-produced substances that may promote corrosion of copper and copper alloys are CO_2, organic acids, and metabolites that act as depolarizers, mercaptans, and disulfides

(Pope et al., 1985). Low pH prevents copper-based alloys from developing protective films, leading to high rates of corrosion (Manaktala, 1990). Carbonic acid formed during the evolution of CO_2 production by various bacteria also prevents formation of protective films on copper.

If this cycle is repeated with each 100-year flood, the corrosion deposit, bacterial biofilm, and underlying pit would grow. Episodic rehydration of acidic exopolymer deposited on the container surface in earlier times can stimulate corrosion in the absence of active or viable microorganisms by complexing copper and other metal ions and decreasing local pH (Ford et al., 1987; Geesey et al., 1986). Rates of localized corrosion under deposits containing biotic material can be as much as twenty-fold higher than rates observed in the absence of biotic material. The impact of water droplets on the deposit will eventually cause portions to spall and expose bare areas of the metal, which are highly susceptible to corrosion in the presence of water or water vapor.

If the surrounding soil or bentonite backfill is in direct contact with the container, intruding water will likely coalesce in voids between the surface of the container and adjoining soil particles. This condition will also promote microbial growth and activity on the container surface. Alanis et al. (1985) predicted that under these conditions, SRB-influenced localized corrosion may be greatly enhanced (forty-fold higher).

Microbial growth and activity in the repository environment remote from the waste package surface have a higher likelihood than at the container surface. Thermal loading and radioactive flux will decrease rapidly with distance from the waste package, allowing microorganisms in this zone greater access to water and nutrients than at the container surface. Aggressive by-products of microbial metabolism that are volatile (hydrogen sulfide, ammonia), produced at locations remote from the container surface, may migrate to the surface through the gas phase and promote corrosion without intimate contact with the microbes that produce these products. Excessive growth of microorganisms in the near-field environment, on the other hand, could lead to formation plugging and effectively isolate the container from intruding water entering the near-field or aggressive microbial products produced there.

To summarize, it appears that copper and copper alloys may be subjected to rapid, microbially-influenced, through-wall pitting corrosion if water, nutrients, and certain microorganisms gain access to the container surface or near-field repository environment. It is not possible to assess at this time the relative importance in affecting MIC of the container of those microbial processes occurring at the container surface versus those in the near-field environment. Either scenario may promote failure of containers constructed of this class of candidate materials during the 300- to 1000-year containment period.

5.4 MICROBIALLY INFLUENCED CORROSION OF STAINLESS STEELS

Stainless steels have been used in many applications requiring corrosion resistance for many years, and their metallurgical properties are well characterized. Because stainless steels are thought to be highly resistant to corrosion, they have become the material of choice for piping where environmental conditions are aggressive to mild steel and other metals. This condition includes power generating industries, chemical plants, paper mills, and wastewater systems. However, stainless steels are vulnerable to corrosion in those systems in which the water is stagnant or the flow is low or intermittent (Licina, 1988; Wolfram et al., 1988; Kearns and Borenstein, 1991).

Austenitic stainless steels are commonly used because their high ductility and tensile strength make them ideal candidates for tubing and piping fabrication. The austenitic steels comprise AISI 300 series stainless steels, in which the most common alloys are types 304, 304L, 316, and 316L. Table 2-1 gives the composition of types 304L and 316L, which are two of the candidate container materials selected by the DOE for disposal of high-level nuclear waste.

The high chromium content (as high as 20 percent) reduces general corrosion by forming a very stable passive film on the surface. Under normal conditions, an adherent chromium oxide layer forms on the surface of these alloys (Lula, 1986). This passive film constitutes a barrier layer, decreasing the cationic transport into solution that occurs during the corrosion process (Duquette and Ricker, 1986; Mesarwi and Ignatiev, 1990).

If the protective chromium oxide layer is breeched by mechanical abrasion or halide attack, corrosion can occur at an accelerated rate if repassivation does not occur (Ibars et al., 1992). Breeching of the layer may occur more readily at the grain boundaries, where the passive layer is already thin. Alteration of the protective properties of the passive layer can occur by welding and heating. These areas appear to be more susceptible to pitting corrosion in certain natural, stagnant waters. Microorganisms have been suggested to participate in or promote this type of corrosion (Kearns and Bornstein, 1991).

Although stainless steels are more resistant to corrosion than carbon steels, they are not immune to some types of corrosion, particularly pitting corrosion associated with microbial biofilms. Tatnall (1981) suggests that iron-oxidizing bacteria such as *Gallionella* tend to concentrate iron, manganese, and chlorides, with the result that their deposits are rich in ferric and manganic chlorides. These acidic, oxidizing chloride salts cause generalized corrosion of carbon steel when they are dissolved in solution. On stainless steels, they produce rapid pitting that penetrates walls of piping and other structures within weeks at ambient temperature. The pits appear as pin holes at the surface, which expand into cavities in the bulk metal.

Stainless steels are susceptible to pitting corrosion in the presence of SRB or their metabolites (Moreno et al., 1992). SRB may have an anodic or cathodic effect, depending on the specific conditions at a surface. SRB are generally considered to be the major culprits of localized pitting corrosion, which is the most common type of corrosion to occur on stainless steels (Hamilton, 1985; Ibars et al., 1992; Duquette and Ricker, 1986; Scott et al., 1991; Iverson, 1987). SRB generally produce open pitting or gouging on stainless steel, whereas along the edges of gasket joints, SRB produce shallow crevice corrosion (Tatnall, 1981). The production of sulfides such as HS^- and H_2S in localized high concentrations in chloride-containing media causes the breakdown potential to move toward more negative values (Newman et al., 1982).

Pitting of 304 SS in freshwater environments has been proposed to occur indirectly through reactions catalyzed by SRB-containing biofilms (Newman et al., 1991). H_2S, produced by SRB respiration, reacts with sulfate ions to produce thiosulfate ions. Pitting occurs even in the absence of chloride ions, so long as the ratio $[SO_4^{2-}/S_2O_3^{2-}]$ ranges from approximately 10-30. Pitting has been observed at thiosulfate concentrations as low as 1 ppm (10^{-5} M). The aggressiveness of thiosulfate as compared to sulfide is due to its anionic nature: it is attracted into pits along with Cl^- and SO_4^{2-} by electromigration, whereas sulfide is uncharged at the acidic pH encountered inside pits (Newman et al., 1989). In environments containing SRB, the rapid failures experienced by stainless steels must involve a locally anaerobic environment, with maintenance of an oxidizing potential through access of oxygen to remote areas. Pitting corrosion of stainless steels in a uniformly anaerobic environment is not likely,

as the potential would be lower than any value at which corrosion has been observed. It is incorrect to assume that a lowering of the potential in itself increases the likelihood of corrosion. In stainless steels, there is no active loop in neutral solution, and acidification at the anode can only be achieved if there is a remote cathode other than hydrogen evolution. SRB-induced pitting of stainless steels is proposed to be an H_2S-catalyzed dissolution process in a locally anaerobic microenvironment, driven by remote reduction of oxygen or another cathodic reductant (Newman et al., 1991).

5.4.1 Microbiological Reactions at the Metal Surface

When bacteria colonize a surface, a patchy biofilm develops (Geesey et al., 1992a). The respiratory activities of the bacteria limit oxygen access to the underlying metal surface and promote establishment of an oxygen concentration cell on the surface. The influence of this microbial process on corrosion of the underlying metal has been discussed previously.

Once a biofilm on a stainless steel substratum has been established, it has the ability to trap and entrain certain species of chemicals that may be particularly aggressive towards stainless steel (Videla and Characklis, 1992). Chloride is one such species that has been shown to be of particular importance (Lula, 1986). Chloride concentrations have been found to be surprisingly high within an established tubercle, even when the chloride concentration in the surrounding medium is quite low (Borenstein, 1991). Chloride ions have the ability to permeate the passive film, thus initiating or enhancing the electrochemical reactions that are responsible for localized corrosion (Fan and Bard, 1989). In addition, chloride can initiate stress corrosion cracking that affects many alloys and has been characterized as transgranular in austenitic stainless steel (Lula, 1986). Costerton and Geesey (1986) have proposed the formation of a corrosion cell in which there are distinctively localized anodic and cathodic sites due to microbial activity concentrated at the surface.

EPS produced by the biofilm-forming bacteria prevent the corrosive metabolites from diffusing across the surface or into the bulk aqueous phase, thereby accumulating at localized sites near the substratum surface. Beech et al. (1991) and Nivens et al. (1986) showed that the SRB *Desulfovibrio desulfuricans* produced exopolymer during colonization of stainless steel.

The EPS produced by many bacteria found in aquatic and terrestrial environments, including the deep subsurface, contain uronic acid subunits. These polyanionic polysaccharides could produce an acidic environment at specific locations on the surface or create metal concentration cells on the stainless steel surface as a result of differential binding of metal ions as has been shown in the case of copper ions (Jolley et al., 1989a; Geesey et al., 1992b). Slime-forming pseudomonads have been associated with some cases of stainless steel pitting (Pope et al., 1984). Species of bacteria that have been associated with slime-related stainless steel corrosion include: *Clostridium, Flavobacterium, Bacillus, Desulfovibrio,* and *Desulfotomaculum* sp. The consortium of physiologically-distinct bacteria that develop within a biofilm create strong chemical concentration gradients across the surface[3].

Bacteria may contribute to anodic dissolution by production of acidic substances, such as CO_2, or organic acids such acetate, butyrate, and other short-chain fatty acids. The production of these substances decreases the local pH by increasing the proton concentration (Hamilton, 1985; Dowling et al., 1992; Iverson, 1987). Biofilms of acetic acid-producing bacteria grown in aerated artificial seawater

[3] Personal Communication from Z. Lewandowski, March 1993

destabilizes or dissolves the protective calcareous film that forms on stainless steels during cathodic polarization (Little et al., 1987). The presence of the bacterial biofilm led to an increase in current density when the surface was polarized to -900 mV_{SCE}. Cathodic depolarization has often been cited as the mechanism of MIC to explain an undefined change in electrochemical response of a system containing microbes (Little et al., 1987).

Bacteria can colonize those areas of the surface at which the protective oxide layer has been breeched by mechanical or halide attack, produce a slime layer composed of cells and matrix exopolysaccharide, and prevent the penetration of oxygen to the surface and subsequent passivation (Pope et al., 1984). The growth of SRB in oxygen-depleted regions of a respiring microbial biofilm also prevents passivation of the protective oxide layer where breeches have occurred.

For reasons that are not entirely understood, MIC seems to be more likely to occur at or near a weld than at any other area on a stainless steel surface (Borenstein, 1991; Licina, 1988; Ibars et al., 1992). Welding of some austenitic stainless steel leads to a duplex microstructure because the filler metal is chosen to promote the formation of delta ferrite. Under oxidizing acidic conditions the austenitic matrix is preferentially attacked (Ibars et al., 1992). Similar preferential attack of the austenitic phase has been observed in type 316L weld metal (Licina, 1988). However, there are cases in which the delta ferrite is preferentially removed and the austenite is unattacked. As a rule, the duplex condition seems to be more susceptible to MIC than the austenitic condition alone, particularly in the proximity of the weld fusion line. The heat-affected zone (HAZ) has also been shown to be susceptible to MIC (Licina, 1988; Ibars et al., 1992). This susceptibility is due to the fact that chromium carbide precipitation at the austenite-grain boundary results in the formation of chromium-depleted zones, which will be preferentially affected by MIC. In an attempt to combat this attack, a variety of different weld metals have been tried. So far all have shown susceptibility to MIC, and are more susceptible to MIC than the base metal; however, little investigation has been done to find the reasons of this increased susceptibility (Borenstein, 1991; Ibars et al., 1992).

Hydrogen embrittlement is another means by which MIC can cause material failure of stainless steel (Ford and Mitchell, 1989, 1990a). Hydrogen embrittlement involves atomic hydrogen entering the stainless steel lattice, causing loss of ductility and tensile strength, as a result of crack propagation. Any bacterium that produces an acid byproduct may be a potential problem, as well as SRB, which produce sulfides that prevent the transformation of atomic hydrogen to molecular hydrogen. Some SRB can consume hydrogen, thus cathodically depolarizing the stainless steel. The overall outcome of hydrogen embrittlement may be determined by an abiotic/biotic competition between the abiotic metal and the biotic hydrogen- producing and consuming bacteria. Since the metal surface characteristics determine the number of adsorption sites for the atomic hydrogen, biofilm composition remains as the controlling variable for hydrogen adsorption. Despite the fact that hydrogen can be adsorbed in austenitic stainless steel, diffusion rates are less than for ferritic or martensitic steels; hence, austenitic stainless steels are less prone to hydrogen embrittlement than other steels.

Because stainless steel is mainly used as a piping material in contact with water or other aqueous media, all studies on the MIC on stainless steels have been performed in aqueous environments. Little is known about its susceptibility to MIC in unsaturated environments in which water and nutrients may be limited and contact with a gas phase of unknown composition for extended periods of time may significantly influence the redox chemistry on the metal surface.

Periodic or intermittent wetting of parts of a container constructed of stainless steel is likely to lead to microbiologically influenced reactions similar to those described in the section on copper and its alloys. The corrosion frequently observed after hydrotesting of pipelines suggests that alternating saturated and unsaturated conditions may be the most corrosive situation. Pipes that are filled with water for pressure testing and then drained leave water droplets that contain bacteria on the surface of the metal. These bacteria initiate corrosive reactions that fix the anodic and cathodic sites on the metal so that, when water is reintroduced to the system, focused attack resumes.

Based on observations of MIC resulting from hydrotesting, periodic wetting of the HLW container surface may be anticipated to produce similar results. Microbes associated with the container surface during its deployment in the repository or those bacteria transported to the container surface during periodic water intrusion would form a patchy biofilm where water and nutrients contacted the surface. During dehydration, hygroscopic EPS is synthesized by the attached bacteria to trap water and concentrate ions. The polymeric matrix and associated bacteria would remain on the surface during periods of dehydration in an inactive state until water penetrated the repository environment at some later time. Renewal of water and nutrients at the container surface would revive the bacteria and allow them to continue their intermittent localized attack on the surface. Research should be conducted to determine the validity of the scenario described above.

5.4.2 Microbiological Reactions Distant from the Metal Surface

Microbial reactions outside the stainless steel containment material may have significant effects on the corrosion resistance and stability of the stainless steel. Although the temperature of the container surface and near field will initially be too high for microbial activity and growth, the temperature of the adjacent field will be low enough to permit water and nutrients to condense and microbial processes to occur. Thermophilic SRB could grow in these areas and convert sulfate in the soil to a corrosive gas such as hydrogen sulfide, which could diffuse through the pore space in the backfill or surrounding soil to the stainless steel surface (Pankhania, 1988). Microorganisms such as SRB, which have a slower growth rate when compared to most aerobes (Voordouw et al., 1992; Okabe and Characklis, 1992), may be able to function successfully under conditions of low nutrient input.

5.4.3 Summary

Heat-affected zones and weldments on containers constructed of stainless steel would be the areas most sensitive to microbial attack. Grain boundaries would be the next most vulnerable site of attack by microbes. Both forms of attack would be localized and would lead to pitting corrosion for which there is currently no good means of measuring for long-term predictions. Hydrogen embrittlement and stress corrosion cracking could be other types of corrosion failures that could occur on stainless steel containers either through direct growth of microbes on the metal surface or by diffusion of hydrogen gas from areas distal to the containers where lower temperatures permit microbial growth and activity. However, austenitic stainless steels are less prone to hydrogen embrittlement than ferritic or martensitic steels.

5.5 MICROBIALLY INFLUENCED CORROSION OF NICKEL ALLOYS

Nickel is generally regarded as a corrosion-resistant material. Alloys containing more than 40 percent nickel tend to show the passivity associated with pure nickel. Turbulent conditions do not seem to impair the passive layer formed on these nickel alloys, but high-nickel alloys are prone to pitting

corrosion (Little et al., 1990). Nickel-copper alloys have a marked tendency for pit initiation in chloride-containing environments in which the passive film can be disturbed. Corrosion of pure nickel is essentially of an intergranular type (Cid and Petit, 1983). The passive layer on nickel has a characteristic duplex structure with an inner NiO layer that increases linearly with the potential and a constant $Ni(OH)_2$ overlay (Strehblow, 1991). The inner NiO layer is the barrier, which grows to a maximum thickness of 3.5 nm in 1 M NaOH. In 0.5 M sulfuric acid (pH 0.3), the increase of the oxide thickness is much less pronounced. It starts to form at around 0.7 V_{SHE}, increasing in thickness to a maximum of 2.5 nm at 1.5 V_{SHE}, and then decreases to undetectable thickness in the transpassive range.

Pitting of nickel has been shown to develop preferentially at grain boundaries and imperfections in the surface (Tokuda and Ives, 1971). Under stagnant conditions, chlorides can penetrate the passive film at breech points and cause pitting attack. Sulfides can modify the oxide layer as described above for copper or cause breakdown of the oxide film. When sulfur is introduced as an H_2S-H_2 mixture, the sulfur atoms adsorbed to nickel or nickel-iron alloys act as a catalyst for the anodic dissolution reaction and delay passivation of the surface (Marcus and Oudar, 1983). In addition, the adsorption of sulfur to nickel and nickel-iron surfaces causes a two-fold reduction in the double layer capacity and the potential of zero charge is shifted towards more positive values (Marcus and Oudar, 1984). Adsorption of sulfur to the metal also reduces the capacity of the metal to adsorb hydrogen or hydroxyl ions (Marcus and Oudar, 1984). Schumacher (1979) reported that nickel alloys were susceptible to under-deposit corrosion and oxygen concentration cells. On nickel, high-nickel alloys, and cupronickels, SRB are reported to produce conical pits, containing concentric rings or steps (Kobrin, 1976).

Nickel alloys are vulnerable to MIC. Formation of a biofilm on passive alloys such as nickel-chromium and nickel-copper in seawater promotes depolarization of the cathodic reduction of oxygen (Molica et al., 1990). Depolarization produces an increase of the free corrosion potential for alloys in the passive state, which, in turn, may result in increased susceptibility to localized corrosion. The mechanism of this phenomenon is still unclear. Two possibilities have been proposed:

(i) The reduction of pH at the metal surface due to metabolic activities of the biofilm bacteria and entrapment of the acids produced at the metal surface, causing an excursion of the cathodic curve to a more noble potential

(ii) Acceleration of the rate of the oxygen reduction reaction when mass transfer is not limiting.

Thus, unlike very passive metals such as Ti and stainless steels in which the biofilm increases the corrosion potential markedly, nickel and some of its alloys (i.e., those with Cr content less than 15 percent) exhibit increased generalized corrosion in the presence of biofilms which cause only a minor increase in corrosion potential.

5.5.1 MIC of Commercial Nickel

Wagner and Little (1986) used a cell composed of two galvanically coupled compartments, electrochemically connected but biologically separated, to evaluate the electrochemical impact of naturally occurring estuarine microorganism on Nickel 201 (N02201) (Ni 99 percent, Fe 0.4 percent) and Titanium Grade 2 (unalloyed). One electrode was exposed to filtered sterilized water (9 ppt salinity) and the other to unfiltered water containing a natural population of microorganisms. Current measurements between

the two compartments over a 96-h period were collected using a zero resistance ammeter. Nickel and titanium were colonized by rods and filaments. These metals developed stable anodic currents in the 10^{-3} $\mu A/cm^2$ range after 24 h. An anodic current indicates that oxidation has occurred. The anodic currents may be due to respiration, creating differential aeration cells, to secretion of organic acids by the bacteria or to galvanic currents between the substratum and chelated metals. Control studies indicated that differential aeration could not account for the anodic currents observed with nickel or titanium at room temperature. Although surfaces were examined by SEM for bacterial colonization, no statement was made about the type of corrosion that occurred.

The practical significance of bacteria growing in environments with elevated temperatures has not received much attention in terms of corrosive metabolic reactions mediated by thermophilic bacteria. Little et al. (1986) have demonstrated that the thermophilic bacterium, *Thermus aquaticus*, isolated from a failed Nickel 201 brazed joint, promoted corrosion in a solution consisting of a basal salts medium supplemented with 0.025 percent tryptone and yeast extract. Using the dual cell and a zero resistance ammeter described above, they showed that the rate of the corrosion reaction in the cell inoculated with the bacterium did not increase with temperature between 20-80 °C as would be expected for a nonbiologically driven chemical reaction. Rather a maximum rate was observed at 60 °C, suggesting a biological process with maximum activity at this temperature. It was concluded that *Thermus aquaticus* controlled the rate of the corrosion reaction. After 20 h the current increased anodically and stabilized after 80 h at 3.8 $\mu A/cm^2$ generating a corrosion rate of 40.6 $\mu m/yr$ (1.6 mpy). Approximately 75 percent of the surface of the nickel electrode in the inoculated cell was colonized by bacterial biofilm. The electrode surface contained 2×10^6 cells/cm^2 after 6 h incubation based on direct microscopic examination (Little et al., 1984). No current was observed when both cells were sterile. It was concluded that current measurements made in the presence of a mixed population of microbes cannot be predicted by results of previous experiments using identical metal substrata and known pure cultures of a single microorganism.

Depletion of oxygen combined with temperature of 60 °C produced an anodic current of 35 $\mu A/cm^2$ (Little et al., 1986). Respiration of oxygen at elevated temperatures may have contributed to the anodic currents observed in the presence of the thermophilic bacterium. Whereas differential aeration could not account for the anodic currents observed at room temperatures (Wagner and Little, 1986), it was found to be responsible for anodic currents on nickel at elevated (60 °C) temperatures (Little et al., 1986).

Nickel forms a passivating film in slightly alkaline solutions. Acidic metabolites secreted by bacteria either prevent passivation or destroy an existing passivating film. Dilute solutions of isobutyric acid and isovaleric acid at 60 °C impact the corrosion of Ni 201 (Little et al., 1986). Acetic acid at a concentration of 10 mmoles/L caused Ni 201 to become more anodic with a stable anodic current of 0.1 $\mu A/cm^2$ (Wagner and Little, 1986). The thermophilic bacterium produced isobutyric and isovaleric acid at concentrations of 0.033 and 0.035 mmoles/L, respectively. At concentrations 10 times those above, the nickel exhibited anodic currents of 3.0 and 5.0 $\mu A/cm^2$ at a temperature of 60 °C. At this temperature, the acids were effective in accelerating corrosion. They proposed that concentrations of these acids in biofilms in contact with the metal surface could be 10 times greater than those found in the bulk aqueous phase. The ionized forms of the organic acids can form complexes with the metal cations and thereby lower the thermodynamic activity of metal ions to promote metal dissolution.

5.5.2 MIC of Nickel-Copper Alloys

Nickel and 70/30 Ni-Cu alloy are not so passive in seawater as those nickel-base alloys with chromium as the main alloying element (Cubicotti and Licina, 1990). For those nonpassivating metals, the corrosion potential increases by only a few tens of millivolts, even though the biofilm catalyzes the oxygen-reduction reaction. The rates of anodic dissolution were found to increase by two orders of magnitude. There was a 3-mg weight loss for a nickel specimen exposed for 40 days to sterile seawater as compared to a 600-mg weight loss in biologically active seawater. A similar effect was observed for 70/30 Ni-Cu.

Monel 400, a Ni-Cu alloy (66.5 percent Ni, 31.5 percent Cu, 1.25 percent Fe) experienced localized pitting under a multilayer scale after exposure to estuarine water for 6 months (Little et al., 1990). Surface scales appeared as blisters containing corrosion products and bacterial cells. SRB were typically present in densities of 10^4 cells/cm^2. A black layer within the pits was enriched in sulfur and chlorine. Regions under the biofilm were depleted in nickel and iron and enriched in copper. Differential aeration cells appeared to have formed with low oxygen concentrations under the deposits suitable for SRB activity. The surface area under the deposit became anodic and corroded, while the electrons generated from the dissolution of the metal reacted with oxygen outside the deposit at the cathode. A spongy, copper-rich material accumulated in the base of the pits.

Microorganisms colonizing surfaces excrete exopolymers that bind metal ions selectively. Binding of metal ions by exopolysaccharides of *Deleya marina* followed the sequence Mn > Cu > Ni > Fe (Ford et al., 1987). Reduction of copper by the exopolymers at the metal surface created a copper-nickel galvanic couple for cupronickel alloys (Little et al., 1984).

Monel 400 is fouled to a similar extent by SRB in seawater as Corten A steel (Fe 98 percent, Cu 0.4 percent, Cr 1 percent, C 0.12 percent) (Sanders and Maxwell, 1983). However, it takes SRB much longer (150-288 days) to achieve a stable sulfate reduction rate (20-27 nmol SO_4/coupon/day) on Monel 400 than on the steel. Corrosion rates (8-12 mg/dm^2 • d) were similar, however. Severe deep pitting was observed. Localized anaerobic corrosion is a characteristic of high-nickel alloys.

Specimens of Monel 400 were exposed to Arabian Gulf seawater for up to 4 months and also exposed to cultures of active sulfate-reducing bacteria (Gouda et al., 1993). The Arabian Gulf seawater contains organic carbon concentrations of 8 ppm (Gouda et al., 1993). The active SRB was cultured on a rich nutrient medium, enriched with anaerobic sewage sludge, and then deaerated in an atmosphere of hydrogen and carbon dioxide prior to exposure to the Monel substratum. After 10- to 21-days exposure of the Monel to the active SRB culture in deaerated seawater in the laboratory, a sulfide layer containing a bacterial biofilm had formed on the metal surface. The biofilm contained actively growing cells of *Desulfotomaculum* sp. as well as other possible genera of SRB including *Desulfovibrio*. These bacteria were surrounded by EPS. After 3-weeks exposure to anaerobic laboratory conditions, corrosion was slight. However, when air was introduced for 1 week into the system, localized corrosion was observed. The effect of oxygen on the corrosion behavior of Monel mimics to some extent that observed with mild steel. The localized areas that had been attacked contained a cracked layer that exposed the grain boundaries of the alloy. Intergranular attack by the SRB was observed at this time and became more severe with further incubation. Intergranular corrosion has been observed in the presence of microorganisms in freshwater (Stoecker, 1984) and seawater environments (Schiffrin and de Sanchez, 1985). According to the authors, the response of the Monel to aeration implicated differential aeration

cell as the mechanisms of corrosion: low oxygen concentrations under areas of high biofilm bacterial density (anode) and higher oxygen concentrations at areas of the metal surface containing lower bacterial densities (cathode). The protective surface film at the anodic sites is disturbed and breeched, leading to enhanced localized corrosion. In addition, the sulfide generated by the SRB promotes the breakdown of the oxide films on nickel alloys (Schumacher, 1979).

The authors indicated that both active laboratory cultures of SRB or from naturally occurring microbes in Arabian Gulf seawater produced similar forms of attack on Monel. Monel was reported to be more susceptible to MIC than brass, 70/30 Cu-Ni alloy (CDA715), Sanicro 28, alloy 825, or Ti when exposed to the same environmental conditions. They indicated that failure and leaking of Monel heat exchange tube can take place whenever SRB are present, irrespective of their densities. Through-wall perforations (2 mm thickness) occurred within 3-8 months of the time the tubes were placed in service. The reason that Monel serves as an attractive material for SRB bacterial biofilm development remains to be determined.

5.5.2.1 Effect of Temperature on MIC of Monel

Exposure of Monel 400 to chlorinated Arabian Gulf seawater for 10 days at different temperatures resulted in the development of a few small black nodules, circular in shape (Gouda et al., 1993). These nodules developed from discrete microcolonies of bacteria growing on the Monel surface. After 1 month, the black layer became covered with green deposits. The size and number of nodules were greater at 30 °C than at 19 or 50 °C. The number of nodules increased significantly at all temperatures after 4 months exposure. Two types of corrosion attack were identified: deep localized attack that produced cavities and irregular shaped, shiny, shallow and broad attack that was spread all over the surface at all temperatures studied. Intergranular corrosion attack occurred inside the cavities, very similar in appearance to that produced in the presence of the active SRB culture. The other type of attack resembled the etching attributed to chloride pitting corrosion. Prolonged exposure to the Arabian Gulf seawater under laboratory conditions for up to 4 months resulted in pit propagation and severe intergranular corrosion. In summary, the laboratory seawater studies demonstrated that even though the water was not deaerated and contained 0.2 ppm free residual chlorine, SRB-associated localized corrosion occurred within a 3-month period.

5.5.2.2 Effect of Chlorination on MIC of Monel

Immersion of Monel 400 surfaces in unchlorinated or chlorinated natural Arabian Gulf seawater for 50 days resulted in the appearance of nodular deposits (1 mm dia) on the metal surface similar to those observed in the laboratory immersion tests (Gouda et al., 1993). The deposits were made up of a black layer composed of one or more iron nickel sulfides $(Fe,Ni)_xS_y$ overlayed with a green layer of $CuCl_2 \cdot 3Cu(OH)_2$ and $NiCl_2$. The areas under the deposits displayed severe intergranular attack below which a copper-rich alloy was detected. These results suggest preferential attack of iron and nickel by SRB.

Although deposit density was not reported, the numbers and size of the deposits were reported to be much greater in unchlorinated seawater than in chlorinated seawater. After 50 days exposure to chlorinated and unchlorinated seawater, 1.6×10^2 SRB/cm^2 and 6×10^1 SRB/cm^2 were recovered from the metal surfaces. After 70 days exposure, the SRB cell densities increased to 3.3×10^2 and 5.1×10^3/cm^2. The authors indicated that the biofilm population rather than the planktonic cell population was responsible for the observed corrosion. The authors further indicated that chlorination at

the levels applied (0.5 ppm) in their studies was ineffective in keeping the SRB cell densities in the biofilm under control. No controls were described, however, that considered the role of abiotic reactions in these corrosion studies.

Monel 400, when submerged in quiescent seawater, developed evidence of corrosion in crevices (Klein et al., 1991). Treatment of the seawater with 1 mg/L chlorine per liter of seawater rendered the alloy more resistant to crevice corrosion in the presence of microorganisms. After 180 days exposure, depths of attack were about one-half that observed in unchlorinated natural seawater. In the absence of chlorination, it appeared that biological activity present in natural seawater promotes ennoblement of the corrosion potential and, consequently, pitting of the Monel.

5.5.3 MIC of Nickel Alloys Containing High Levels of Chromium

Soracco and coworkers (1988) reviewed the susceptibility to MIC of various alloys used in the power-generating industry. According to their survey, no cases of MIC of nickel-chromium alloys had yet been documented. However, very few studies of MIC of nickel-chromium alloys had been carried out at that point.

Heat exchange tubing made of alloy 800 (NO8800) (alloy composition: Ni 32 percent, Cr 21 percent, Fe 46 percent, Cu 0.02 percent) suffered from under-deposit pitting corrosion on the lake water (Lake Ontario) side of the tubing (Brennenstuhl et al., 1990). Model heat exchanger test rigs were rapidly colonized by a biofilm of bacteria comprised of aerobic heterotrophs, actinomycetes, pseudomonads, and SRB. Initially, the biofilm was almost completely organic in nature. After maturation, however, inorganic deposits from the surrounding environment accumulated in the film. After 2-months exposure, a calcarious layer eventually covered the film. Microbial biomass contributed approximately 10 percent of the total deposit mass. The exopolymers excreted by the bacteria in the biofilm trapped the inorganic particles, which acted as nuclei for calcium carbonate deposition. The calcareous layer, along with the sediment material, led to the formation of crevices where localized breakdown of the passive oxide layer occurred. Treatment of the tubing with sodium hypochlorite before biofilm accumulation reduced the buildup of a fouling layer. Tube surfaces became fouled, but sloughing occurred before the tubes became fully enveloped.

Electrochemical noise measurements suggested that negligible attack occurred on alloy 800 during initial stages of biofilm buildup in the presence of lake water (Brennenstuhl et al., 1990). Over a period of several months, passivity was still maintained as long as sufficient water was circulated over the tube surface to provide an adequate supply of oxygen to the metal surface. Passivity breakdown occurred after 6 months when flow was interrupted for a short time. One year after the tubing had become heavily fouled, the corrosion current remained high during both normal and low-water flow operation. The environment at the tube surface had become sufficiently aggressive to ensure pit propagation. The authors felt that bacteria had an important role in the pitting process (Brennenstuhl et al., 1990).

Similar studies with alloy 825 (NO8825; Ni 42 percent, Cr 21 percent, Fe 30 percent, Mo 3 percent, Cu 2 percent, Ti 1 percent) and Sanicro 28 (NO8028; Ni 31 percent, Cr 27 percent, Fe 36 percent, Mo 3.5 percent, Cu 0.6 percent) indicated that they experienced pitting corrosion after 3 years (Brennenstuhl et al., 1990). These two alloys contained molybdenum, whereas alloy 800 did not. Pitting was always more prevalent at the end of the tubing receiving the hot fluid. The material extracted from

the pits and bulk deposits formed on alloy 800 was enriched in chromium (63 percent) and calcium (16 percent). Pits may have initiated at TiC inclusions. Examination of the pits suggested crystallographic attack indicating an activation controlled dissolution process. Ca, Fe, and S were enriched in the vicinity of the pits. The Fe/S ratio was as high as 2.4. P and Cl were also enriched but not to the extent of S. Cl^- and SO_4^{2-} concentrations in the lake water were 35 and 25 ppm, respectively. It is believed that oxygen respiration by aerobic bacteria and anaerobes such as SRB assist in the formation of micro galvanic cells, which leads to accelerated attack. Chloride ions are likely to be involved in the breakdown of passivity of the protective oxide film.

The initiation of crevice attack on alloy 825 at 25 °C was more than four times as severe in natural seawater than in synthetic seawater (Dexter et al., 1985). Heating the metal to kill the bacteria recruited to the surface from the natural seawater produced no detectable change in crevice initiation, indicating that once a biofilm has become established, viable bacteria are not required to initiate corrosion.

Based on a survey of the current literature, it appears that there have been no other studies on MIC of nickel alloys with high chromium content. There is a clear need to obtain information on MIC of alternate candidate container alloys, such as alloy C-4.

5.5.4 Summary

In summary, nickel alloys containing copper and iron as alloying elements are susceptible to similar types of microbial attack as copper-nickel alloys. Sulfide, chloride, and acids produced by microorganisms will accumulate in biofilms on the metal surface and, through various proposed mechanisms, initiate or promote localized pitting of the metal. In the case of nickel-copper alloys, attack is focused at the grain boundaries. Nickel alloys are therefore ranked similar to copper alloys in terms of resistance to MIC. Considerable research still needs to be conducted to evaluate the resistance to MIC of nickel-base alloys with a high chromium content in the repository environment because the information and data available on these alloys is very limited.

5.6 MICROBIALLY INFLUENCED CORROSION OF TITANIUM AND ITS ALLOYS

Titanium is a material known for its high strength-to-weight ratio and excellent corrosion resistance (Everhardt, 1954; Abkowitz et al., 1955; Barksdale, 1966). Titanium has low thermal and electrical conductivity. It has good workability, and it is a highly reactive metal. The considerable effort involved in purifying it from its natural mineral form, most commonly an oxide, is responsible for its relatively high cost.

The corrosion resistance of titanium derives from a tenacious oxide layer, primarily TiO_2, which forms rapidly upon exposure to oxygen or aqueous environments. This oxide layer is stable over a wide range of pH values and redox potentials (Schutz, 1991). For this reason, titanium is extremely resistant to both localized corrosion and erosion corrosion. It is also not susceptible to corrosion normally associated with the breech of the protective oxide layer in stainless steels (i.e., stress corrosion) (Everhardt, 1954; Barksdale, 1966), or intergranular corrosion (Schutz, 1991). In addition, a similar stable, imperturbable oxide layer forms over weldments, which appear to be just as passive as the wrought metal. Although, titanium has been developed recently for commercial use as compared to metals

such as copper and copper alloys, there has been ample time (about 45 years) to evaluate long-term corrosion effects. Titanium samples have endured 20 years of service in marine environments without displaying any signs of corrosion (Schutz, 1991).

From the perspective of choosing a material for its resistance to various forms of corrosion, titanium appears to be superior to other candidate metals. Titanium has limited resistance to concentrated (> 10 percent) sulfuric acid. At elevated temperatures (> 100 °C), even a 1 percent solution will cause high corrosion rates. At 100 °C, a 1 percent solution of hydrochloric acid will corrode titanium rapidly. It is extremely susceptible to hydrofluoric acid. In 5 percent sulfuric acid solutions at ambient temperature (30 °C), small amounts of fluoride ion (50 ppm) can increase the corrosion rate three-fold (Barlett et al., 1980). It is not resistant to concentrated, hot solutions of certain organic acids (formic, trichloracetic, or oxalic). Crevice corrosion of titanium in contact with certain polymers in saturated salt solutions (Kobayashi et al., 1980), or at high temperatures (95 to 150 °C) and concentrated chloride concentrations (6-10 percent) has been reported (McKay and Mitton, 1983; Moroishi and Miyuki, 1980). However, it has also been found that titanium is superior to other materials (types 316 and 304 stainless steels, and alloy 825) in aqueous solutions containing Cl^-, Br^-, and thiosulfate ions (Nakayama et al., 1992).

Marsh et al. (1983) found that a titanium-palladium alloy exhibited the least amount of corrosion of the various titanium alloys tested over the temperature range 50-150 °C. Commercially pure titanium resisted corrosion up to 90 °C but experienced severe crevice corrosion at 150 °C. The titanium metals resisted localized attack, exhibiting breakdown potentials and repassivation potentials in excess of 1200 to 800 mV_{SCE} for pure Ti and titanium-palladium, respectively.

5.6.1 Superiority of Titanium Over Other Metals in Resistance to MIC

The literature dealing specifically with microbial influenced corrosion of titanium and its alloys is not extensive. Although titanium fouls rapidly and extensively (Blunn, 1984; Videla et al., 1988; Chen et al., 1988; White and Benson, 1984), at least in marine environments, it does not appear to corrode. After 72 h of exposure to an environmentally isolated gram negative, acid-producing, facultative anaerobic bacteria in aerated culture media, stainless steels (types 304L and 316L) showed signs of localized corrosion when examined by light microscopy and SEM, whereas titanium, under identical conditions, showed no signs of localized corrosion (Zhang et al., 1989). Titanium also exhibited the most stable open-circuit potential during this period. During exposure to estuarine seawater for 96 h, the galvanic currents in a dual cell between filter sterilized and nonfiltered seawater were more stable for titanium than for Nickel 201, 90/10 Cu-Ni, or carbon steel (Wagner and Little, 1986). After 120 days exposure to flowing seawater at Port Hueneme, California (22 L/h, 21-23 °C, pH 8.2, salinity 35 ppt), Titanium Grade 2 showed no signs of localized corrosion. However, neither did samples of 304 SS, 316 SS or A16XSS stainless steel (Cr 20 percent, N1 24 percent, Mo 6 percent, Fe 49 percent) (Mansfeld et al., 1992). The water contained both heterotrophic bacteria and SRB. Exposure of stainless steel alloy SMO254 (Cr 20 percent, Ni 18 percent, Mo 6 percent, Mn 1 percent, Cu 1 percent, Fe 52 percent) to SRB at 55 °C under anaerobic conditions for 167 days produced extensive pitting, which was not observed on Titanium Grade 2 (Little et al., 1993). Titanium exposed to seawater from the Thames estuary (United Kingdom) showed no signs of corrosion after 27 weeks (Blunn, 1984). Both 90/10 Cu-Ni and pure copper surfaces were covered with a scale consisting of interdispersed layers of corrosion products and bacteria during this same period. One year exposure to seawater at Port Hueneme, California, caused no detectable corrosion to titanium (Little et al., 1992).

5.6.2 Susceptibility of Titanium to Severe Microbial Environments

The apparent resistance of titanium to localized corrosion in the presence of different bacteria needs further consideration. One approach is to attempt to formulate worst case scenarios based on knowledge of conditions of enhanced abiotic corrosion and attempt to find biotically induced circumstances that might mimic those conditions. Two papers summarized below describe this type of approach (Little et al., 1992; Little et al., 1993).

One possible scenario for corrosion of titanium is by formation of a crevice having high chloride concentration and low pH surrounded by a large cathode at elevated temperatures (Schutz, 1991). This type of crevice could in principle be created by heterogeneous colonization by SRB, if they could survive elevated temperatures. As mentioned previously, colonization of Titanium Grade 2 by a thermophilic SRB (isolated from a corrosion failure on a ship) at 55 °C for 167 days under anaerobic conditions did not produce any sign of corrosion when examined by environmental SEM combined with energy dispersive x-ray analysis (EDX). The electrochemical measurements (open circuit potential, electrochemical impedance spectroscopy) indicated that the surface remained passive. No weight loss was observed after this time period.

The susceptibility of titanium to concentrated sulfuric acid has been mentioned previously. Exposure of Titanium Grade 2 samples to *Thiobacillus ferrooxidans* (a mesophilic bacterium capable of oxidizing elemental sulfur) for 75 days in culture media adjusted to pH 2.5 by titration with sulfuric acid, produced no signs of localized corrosion at temperatures between 25-40 °C (Little et al., 1992). In a similar experiment, *Sulfolobus acidocaldarius* (an acidophilic, facultative autotroph), also capable of oxidizing elemental sulfur, induced no signs of corrosion in Titanium Grade 2 samples after 84 days in culture media at pH 3.0 at a temperature of 70 °C (Little et al., 1993).

5.6.3 Possibility of Microbial Enhanced Hydrogen Embrittlement of Titanium

Hydrides of titanium have been observed in crevices after immersion for 2000 h in 6 percent NaCl at 120 °C (Moroishi and Miyuki, 1980), which may be an indication of impending hydrogen embrittlement. There exists some evidence that titanium particles may absorb significant amounts of biotically evolved hydrogen at ambient temperatures (37 °C) (Walch and Mitchell, 1986). However, exposure of Titanium Grade 2 weld material (unspecified composition) to either of the hydrogen-producing bacteria *Clostrium acetobutylicum* at 25-40 °C or *Clostrium thermosaccharolyticum* at 70 °C under anaerobic conditions did not produce any evidence of hydride formation on the surface (Little et al., 1992; Little et al., 1993).

5.6.4 Possible Near-Field Effects

It is difficult to imagine probable scenarios resulting in significant microbe-induced far-field effects on titanium containers. As mentioned previously, both formic and oxalic acid can corrode titanium, but only in boiling, concentrated solutions. Both hydrochloric and sulfuric acid solutions must be fairly concentrated (> 1 percent) to produce noticeable corrosion even at 100 °C. Titanium is resistant to ammonium hydroxide, nitric acid, and most organic acids (acetic, lactic, citric, stearic, tartaric) at all concentrations (Everhardt, 1954; Barksdale, 1966). There is some evidence that blood serum proteins (e.g., albumin, fibrinogen) increase corrosion rates of commercially pure titanium at ambient

temperatures (Williams et al., 1988). However, this conclusion has been contradicted by other experimental results (Clark and Williams, 1982).

5.6.5 Titanium as an HLW Container Material

On the basis of available data, it appears that most grades of titanium are exceptionally resistant to MIC. This resistance is most substantiated for Titanium Grade 2. No cases of MIC of titanium or its alloys have yet been documented (Soracco et al., 1988). However, no studies have been identified that deal with titanium corrosion in soils where crevice corrosion may be pronounced and concentrations of acid by-products could reach high levels. In addition, as mentioned previously, small amounts of fluoride ion (< 50 ppm) can enhance corrosion caused by sulfuric acid. Since the tuff at the Yucca Mountain site contains fluoride, the possibility of synergistic action resulting from fouling by sulfur-oxidizing species such as *Thiobacillus ferrooxidans* or *Sulfolobus* sp. and small concentrations of fluoride should be examined.

6 RECOMMENDATIONS

6.1 GENERAL RECOMMENDATIONS

Virtually no studies have been conducted on MIC of the candidate container materials in an unsaturated soil environment. It is clear from the review of the literature that MIC could promote localized attack of mild steel, stainless steels, copper and its alloys, and nickel and its alloys. The exception seems to be titanium alloys, and possibly certain nickel-chromium alloys that have not yet been evaluated at temperatures and water activities that support microbial activity. The possibility that any of these alloys may be considered immune to attack in the repository environment stems from the assumption that, for certain conceptual designs of the repository, the temperature at the waste package surface will be above 100 °C and moisture content will remain below 60 percent over the 300- to 1000-year containment period. Other environmental parameters, such as radiation, nutrient concentration, salinity, and acidic pHs, would not exclude microbial activity. Even if episodic, nutrient-rich surface water infiltrated the repository environment, it will vaporize before contact with the container surface, and any microbes present on the container surface will remain inactive and unable to initiate corrosive reactions.

Conversely, if the repository design is such that the temperature at the waste package surface drops below 100 °C and the moisture content rises above 60 percent for any period of time, sufficient nutrients are likely to be present in the near field to promote the activities of corrosion-causing bacteria. If these activities are localized at the waste package surface, microbially influenced pitting corrosion is likely to lead to accelerated degradation of the integrity of the metallic barrier. This statement is valid for all the candidate container materials, with the exception of titanium and its alloys and perhaps some high-chromium nickel alloys. However, these latter materials have not been tested sufficiently for susceptibility to MIC to draw any definitive conclusions at this time.

On the basis of the above observations, it would be beneficial to achieve temperatures above 100 °C inside the waste package immediately upon introduction of the HLW and for some period thereafter to assure dehydration of the waste, particularly in the use of damaged spent fuel. This procedure will ensure that microorganisms associated with the waste are inactivated before they have the opportunity to interact with and degrade the disposal barrier.

The primary concern with respect to MIC is the microbial activity that will occur a distance from the container surface where the soil temperature is below 100 °C. If microbial activities result in the release of even small amounts of volatile corrosive gases such as hydrogen, hydrogen sulfide, carbon dioxide, or ammonia during starvation metabolism over the 300- to 1000-year period, these gases may permeate the porous backfill material and contact the container surface. The following recommendations focus on this scenario. If, however, thermal loading does not achieve temperatures greater than 100 °C at the outer wall of the container surface, a more extensive set of recommendations should be proposed.

6.2 SPECIFIC RECOMMENDATIONS

Quantitative corrosion monitoring methods that are sensitive to MIC should be developed. Most studies described above lack appropriate controls to discriminate biotic from abiotic processes. New analytical techniques need to be developed and applied to MIC studies conducted under *in situ* environmental conditions. These techniques should not introduce unwanted perturbations into the system

(i.e., applied potentials, etc.), and they should be sensitive enough to monitor very slow rates of localized corrosion.

The following aspects should be addressed in order to evaluate the potential for MIC of container materials.

- Determine whether thermophilic sulfide producers, hydrogen producers, ammonia producers, and carbon dioxide producers are capable of these activities in the repository environment.
- Determine the minimum distance from the container surface at which these microbial activities are likely to occur based on the temperature gradient and the maximum temperature for these activities to occur.
- Determine the porosity of the matrix material around the waste container, the permeability of the various gases through the matrix material, and the volume of the gas phase between the active bacterial zone and the container.
- Determine the partial pressure of the various biogases required to effect corrosion of the waste package surface under *in situ* conditions (i.e., high temperature, radiation, water activity).
- Determine combinations of biogases that are most aggressive toward the canister material.
- Studies should be of long-term (> 10 years) duration and should incorporate the most sensitive methods of gas detection available. The studies should be of sufficiently flexible design to incorporate new, more sensitive detection techniques as they are developed. Rates of production and accumulation of these gases that may be undetectable in short-term studies may be significant over a 300- to 1000-year timescale.
- Determine the flux of the limiting nutrients controlling gas production and microbial growth to the location in the repository at which the gas-producing microbes are active.
- Determine how biofilm formation in the active bacteria zone influences diffusion of the corrosive gases to the waste package wall.
- Determine if the presence of a biofilm shields the waste container from the aggressive volatile metabolites produced by the film-forming microorganisms or if the biofilm prevents the gases from escaping into the soil matrix surrounding the repository.

6.3 SUMMARY OF KEY RESEARCH AREAS THAT NEED TO BE CONSIDERED

The principal areas of recommended research may be summarized as:

- New sensitive monitoring techniques for MIC
- Nutrient availability in the near field

- Bioenergetics of container material corrosion
- Production of volatile corrosive by-products by microorganisms in the repository area
- Site-specific studies on above topics
- Tolerance of microbes to expected repository conditions

Many uncertainties exist that prevent reasonable prediction of the rate at which microbially enhanced corrosion of waste package material will occur over a 300- to 1000-year time frame. These uncertainties need to be addressed through experimental studies that combine microbiology, corrosion science and engineering, geochemistry, and hydrology. Additionally, programmatic considerations should be used to determine which of the issues and topics raised in this report in terms of long-term repository performance should be addressed by NRC and which should be brought to the attention of DOE for further evaluation.

7 ABBREVIATIONS

AOC	assimilable organic carbon
ATP	adenosine triphosphate
a_w	water activity
CEA	Commissariat a L'Energie Atomique
CNWRA	Center for Nuclear Waste Regulatory Analyses
DOC	dissolved organic carbon
DOE	U.S. Department of Energy
EDX	energy dispersive x-ray analysis
E_h	redox potential
EPS	extracellular polymeric substances
HAZ	heat-affected zone
HLW	high-level waste
INT	2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride
IRE	internal reflection-sampling element
IWPE	Integrated Waste Package Experiments
LLW	low-level waste
MIC	microbially influenced corrosion
MPN	Most Probable Number
mpy	mils per year
NRC	U.S Nuclear Regulatory Commission
PAS	periodic acid-Schiffs base
SCE	saturated calomel electrode
SCP	Site Characterization Plan
SEM	scanning electron microscope
SHE	standard hydrogen electrode
SRB	sulfate-reducing bacteria
SRP	Savannah River Plant
STP	standard temperature and pressure
TEM	transmission electron microscopy
TOC	total organic carbon
XPS	x-ray photoelectron spectroscopy

8 GLOSSARY

abiotic	characterized by the absence of life
aerobic	characterized by the use of molecular oxygen as terminal electron acceptor during respiration
anaerobic	characterized by the use of molecules other than molecular oxygen as electron acceptor
autotrophic	capable of using CO_2 as source for cell carbon
biotic	characterized by the existence of life
endogenous	derived from within
exogenous	derived from without
facultative	not obligatory
gram positive	holding purple dye when stained with crystal violet following counterstaining with safranin
gram negative	not holding purple dye when stained with crystal violet following counterstaining with safranin
halophilic	able to grow in high salinity water
heterotrophic	requiring organic compounds as source for cell carbon
lithotrophic	characterized by the use of reduced inorganic compounds for energy source
mesophilic	able to grow at moderate temperatures
oligotrophic	deficient in nutrients
planktonic	passively floating
sessile	attached
thermophilic	able to grow at a high temperature

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