UCRL-CR-125059
S/C-B310596

Microbiologically Influenced Corrosion Final Report for Fiscal Year 1995

Denny A. Jones
Penny J. Amy

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MICROBIOLOGICALLY INFLUENCED CORROSION
FINAL REPORT FOR FISCAL YEAR 1995
Yucca Mountain Project
JUNE 6, 1996

Denny A. Jones,
Chemical and Metallurgical Engineering
University of Nevada, Reno

Penny J. Amy,
Biological Sciences
University of Nevada, Las Vegas

Microbiologically influenced corrosion (MIC) is a serious concern when considering measures to guard against long-term corrosion of waste package containers at Yucca Mountain [1]. Harmful organisms are known to be present in the host tuff rock at the repository site. Probably more important, most critical organisms with the necessary nutrients for growth will be unavoidably imported into the repository environment during construction. During long term operation of the repository, water accumulations and temperatures below 100°C will almost surely evolve to activate unpredictable and aggressive MIC.

An experimental program has been initiated to gain a better fundamental understanding of MIC in repository environments. Some engineering objectives will be achieved during the investigation: a reproducible apparatus and procedure for electrochemical monitoring of MIC will be developed; the most aggressive combinations of bacteria will be determined, and the MIC resistance of various candidate alloys for the multipurpose container (MPC) will be measured.

A collaboration has been established between Dr. Denny Jones, Professor of Metallurgical Engineering at the University of Nevada and Professor Penny Amy, Professor of Biology at UNLV. The work is being conducted primarily in the microbiology laboratories at UNLV where the biological preparations and procedures can be most conveniently conducted, with electrochemical corrosion measurements performed under supervision of Dr. Jones at UNLV.

Technical Background

Microbiological organisms (bacteria) grow by charge (electron) transfer between reduction/oxidation (redox) reactions within the bacterial cell. The similarity to electrochemical charge transfer reactions during aqueous corrosion of metals is remarkable. Mutual interactions between electrochemical corrosion and bacterial cell growth are suspected but not well understood. For example, imposed cathodic potentials stimulate bacterial growth, and microbiologically influenced corrosion (MIC) concentrates at welds where local galvanic currents are present.

Growth and metabolism in microbiological organisms (bacteria) require energy and carbon [2]. Energy is derived from electron transfer in the bacterial cells. Certain cell components facilitate exchange of electrons between reduction (electron acceptor) and oxidation (electron donor) reactions. The resulting overall reactions are normally very slow at ambient or near ambient temperatures, but various bacteria have developed genetic schemes to enhance electron transfers and utilize the liberated reaction energy for cell growth.

Carbon is an essential part of cell structure and chemistry. It is most often derived from oxidation of reduced organic compounds (the electron donor) or is absorbed (fixed) from CO_2 in the environment.

Specific bacterial strains have developed to operate on one or a few electron transfer reactions. For example, *thiobacillus ferrooxidans* enhances the ferrous/ferric oxidation reaction,

in an aerobic environment with dissolved oxygen, which is readily reduced by

The liberated Fe^{3+} forms relatively insoluble ferric hydroxide and oxyhydroxides which deposit on a steel surface where there is a plentiful supply of corrosion product Fe^{2+} . Incorporation of these hydroxides into the surface exopolymer (slime) layer excreted by the bacteria forms tubercular deposits under which aggressive anaerobic bacteria can thrive.

The best known of the anaerobic bacteria are the sulfate reducing bacteria (SRB), *desulfovibrio desulfuricans*, for example. They enhance the reduction of sulfate to sulfide,

in the absence of oxygen. The distinctive odor of H_2S is often detected when the tubercles are broken. The acidity from H_2S enhances corrosion and formation of Fe^{2+} which feeds aerobic bacteria in the outer deposit layers. Both *thiobacillus* and *desulfovibrio* have the remarkable ability to thrive in acidic solutions. Some species of *thiobacillus* also derive energy from reoxidation of H_2S back to sulfate.

In aerobic environments, dissolved oxygen (2) is the "terminal" electron acceptor (oxidizer). In anaerobic environments, another chemical serves as the oxidizer; i.e. sulfate in (3). The *thiobacillus* bacteria derive energy for cell growth from oxidation of Fe^{2+} and sometimes H_2S or other reduced sulfur compounds. These and other iron oxidizing bacteria often obtain the necessary carbon from fixation of atmospheric oxygen. However, most bacteria, including SRB, oxidize organic carbon, retaining part in the cell structure and liberating the rest as CO_2 . A simplified electrochemical reaction for complete oxidation of glucose, $\text{C}_6\text{H}_{12}\text{O}_6$, is

Reaction (4) releases 686 kcal/mol but is incredibly slow without microbiological mediation. Bacteria degrade glucose into smaller molecules in a number of consecutive oxidation steps with energy supplied from adenosine triphosphate (ATP), an energy-rich molecule present in all bacterial cells. ATP liberates energy and phosphate, P_i , by degradation to adenosine diphosphate (ADP):

The reaction (5) is reversible and ATP will be regenerated by absorbing energy. Thus, ATP initiates the oxidation of organic carbon (e.g. in glucose) by degradation to ADP and is reformed from ADP and phosphate during subsequent steps in the oxidation sequence. Pyruvate, $\text{CH}_3\text{COCO}_2\text{H}$, is an intermediate oxidation product of glucose and many other reduced organic compounds, as well. Pyruvate is amenable to further degradation through the tricarboxylic acid cycle.

The tricarboxylic acid cycle feeds four H-atom (or $H^+ + e^-$) pairs into the oxidative phosphorylation process, a sequence of charge transfer (electrochemical) reactions which move electrons through a series of consecutively more oxidized enzyme and protein molecules to ultimately reduce dissolved oxygen. These electrochemical reactions alternately consume and synthesize ATP as electrons progress down the sequence. The electrochemical half-cell reaction (6) utilizes oxidation/reduction of nicotinamide-adenine dinucleotide (NAD) to transfer charge and hydrogen in many biochemical reactions [3]:

Hydrogen transfer accompanies electron transfer in such reactions, which thus exchange hydrogen between oxidized and reduced states. Oxidative phosphorylation emits several excess ATP molecules which provide essential energy for cell multiplication and growth during reduction of dissolved oxygen.

The complex but experimentally satisfying chemiosmotic mechanism is widely accepted for coupling electron flow and energy generation to cell synthesis of ATP [2]. In this mechanism, protons (H^+) are released at the inner surface of the cell membrane by oxidation (4) of a carrier such as NADH, "pumped" across the cell membrane by the electron-carrying intermediates and ejected at the cell exterior, thereby storing energy in a proton gradient across the membrane. The cell membrane is impervious to simple diffusion of protons back into the cell interior. Instead, adenosine triphosphatase (ATPase) residing in the cell membrane provides a directed path for reentry of protons into the cell interior and utilizes the proton gradient energy to reverse reaction (5) and produce ATP [4].

In the absence of dissolved oxygen, other oxidizers substitute, such as MnO_2 (in soils) and ferric, nitrate, nitrite, fumarate, and sulfate ions. Specific anaerobic bacteria have developed genetically to operate on each of these alternative oxidizers. The SRB have been mentioned previously as anaerobic bacteria that specifically mediate the reduction of sulfate. The same respiration mechanisms operate in anaerobic systems with minor modification. Electrons do not fall as far down the oxidation sequence, less energy and ATP are liberated, and growth rates are significantly slower.

Experimental Program

Professor Penny Amy, UNLV, supervised biological aspects of the program in cooperation with Professor Denny Jones, UNR, who supervised corrosion rate measurements and evaluations. Professor Amy's research group have isolated and identified bacteria from exploratory YM bore-hole rock samples for study in this program. These include exopolysaccharide (biofilm-slime) producers (EPS), iron oxidizers (FEOX), and sulfate reducers (SRB).

After the project started in midsummer 1995, an experimental cell was assembled to culture various bacteria and measure effects on electrochemically measured corrosion rates. The cell consists of a rubber-stoppered test tube (2.5-cm diameter, 18-cm long) with a centrally located AISI 1020 carbon ribbon electrode (1-cm x 18-cm x 0.1-mm) through the stopper and into the cell electrolyte, aerated J-13 well water in Agar gel. The gel was chosen in initial studies to provide a viscous medium which retards rapid oxygen transport and leads to local formation of anaerobic conditions and growth of anaerobic corrosive bacteria. A platinum counter electrode and gel-solution bridge to a saturated calomel reference electrode are included in the cell also. Chloride additions to the gel-solution bridge were necessary to increase bridge conductivity for stable potential measurements. Dye additions to the gel-filled bridge showed that chloride leakage into the cell was insignificant.

Appropriate procedures were determined in abiotic dummy cells to measure corrosion rates electrochemically by polarization resistance. A potential scan rate of 0.05 mV per sec. was found acceptable to obtain the necessary near-steady-state conditions in the common potentiodynamic potential-sweep procedure selected for this investigation. Having established the most appropriate electrochemical procedures, we could measure MIC in the cells as a function of real time, microbiological composition, and alloy resistance.

A series of tasks were designed to address each factor in a logical sequence, each task building on the ones preceding it. Task descriptions are listed as follows.

TASK 1: Methods Development

Define electrochemical instrumentation and procedures for MIC testing at room temperature (RT), using low carbon steel

(LCS) as a prototype. LCS will be studied initially because it is known to be susceptible to MIC, it is a major candidate for the corrosion allowance overpack material for the multiple purpose storage (MPS) container, and it is ubiquitous in infrastructure components, such as rock bolts, drift-wall beam and shell supports, and concrete rebar. As far as possible, the base composition of test media will conform to J-13 well water with appropriate nutrients added, both aerobic and anaerobic.

TASK 2: Bacterial Environment

Using the methods developed in TASK 1, determine effects on MIC of LCS by four major bacterial types, individually and in combination, which are known to have detrimental effects on corrosion. These four have been identified as: sulfate-reducing bacteria (SRB), exopolysaccharide producing bacteria (EPS), iron oxidizing bacteria (FEOX), and acid producing bacteria (APB). It will be verified that each of the four are present in samples taken from YMP. Many if not most bacteria may qualify in more than one of the four types. For example, SRB often generate acidic H_2S and can be classified as APB; also, most aerobic bacteria, including FEOX also are EPS. Testing will be conducted in bacteria cultured from YMP samples and compared with effects of similar cataloged, commercially available, bacterial strains to see if there is anything unique about the YMP bacteria.

TASK 3: Alloys

Test MIC susceptibility of four additional alloys at RT--titanium-grade 12, nickel-base Alloy C-22, Cu-30Ni alloy 17500, and Alloy 825--using methods of Task 1. Results from Task 2 should establish the most appropriate bacteria or combinations of bacteria which will be the most aggressive. Review the literature and study possible bacteria and conditions which could foster MIC in the resistant alloys, Ti-gr12 and Alloy C-22.

TASK 4: Temperature, Low Carbon Steel

Adapting procedures developed in Task 1, determine effects of elevated temperatures, 60 and 90°C, on bacterial growth and MIC of LCS in the presence of those individual and combined bacteria which have been shown to cause MIC in Task 2. Thermophillic, heat-resistant, bacteria which may be present or imported in the YMP will also be identified and tested.

Thermophillic anaerobes, such as SRB, could be especially aggressive because maximum oxygen solubility near 80°C will feed anaerobic anodes developed under exopolymeric deposits.

TASK 5: Temperature, Alloys

With results from Task 4 as a guide, determine the effects of 60 and 90°C on MIC of the four alloys in Task 3.

TASK 6: Weldments and Galvanic Effects

Test welded specimens with minor adaptations of methods in Task 1, and effects of imposed potentials simulating galvanic effects in welds.

TASK 7: Concentration and Temperature

Determine effects on MIC of LCS and the four alloys in Task 3 in 20x concentrated J-13 well waters acidified to pH 2 and pH 12 at RT, 60°C, and 90°C.

Experimental Results

TASK 1 was essentially complete, and TASK 2 had just begun at the end of fiscal year 1995. The following summarizes the results through March 1996. In the abiotic control cell, corrosion rate decreased continuously from 2 to 1 mpy (mils per year) and remained steady thereafter through 100 day exposure. All three added individually increased the electrochemically measured corrosion rates. The increase was greatest for SRB to about 2.5 to 3 mpy after about 50 days exposure. Combinations in pairs increased corrosion rates somewhat, but a combination of all three produced corrosion rates increasing up to a maximum of 5 mpy after 20 days exposure. The corrosion rate decreased after 20 days down to about 3 mpy at 50 days exposure. Different types of bacteria are known to grow in consortia. FEOX oxidizes Fe^{2+} to Fe^{3+} which forms insoluble $\text{Fe}(\text{OH})_3$ surface deposits. EPS form slime films which harbor SRB in the anaerobic environment under the deposits. An occluded cell develops between oxygen reduction at outer $\text{Fe}(\text{OH})_3$ cathode surfaces and inner anode metal surfaces where corrosion is increased. Corrosion rates may decrease after 20 days because of oxygen starvation in the sealed cell. More detail will be available in FY1996 progress reports.

Future Work

Another series of tests is planned at UNLV for carbon steel at elevated temperature of 50°C to test the temperature tolerance of the YM-native bacteria used in this investigation (TASK 4 above). Similar tests are just getting started

for Type 304 stainless steel and Alloy 825 at LLNL (TASK 3). Additional bacterial isolates from the surface rubble piles adjacent to the ESF bore hole at YM are being processed for study and comparison. TASKS 5-7 will be started probably mid to late FY1997.

References

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