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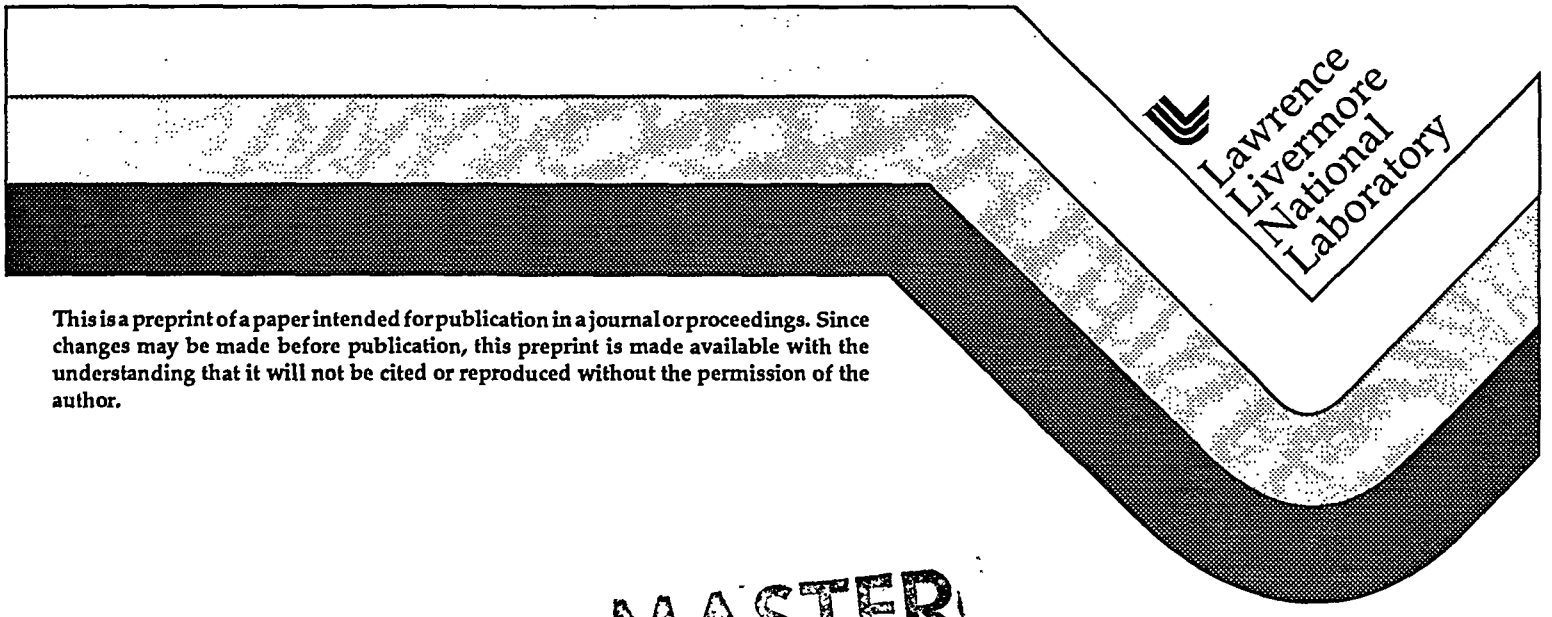

Initial Studies to Assess Microbial Impacts on Nuclear Waste Disposal

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I. INTRODUCTION

The impacts of the native and introduced bacteria on the performance of geologic nuclear waste disposal facilities should be evaluated because these bacteria could promote corrosion of repository components and alteration of chemical and hydrological properties of the surrounding engineered and rock barriers. As a first step towards investigating these potentialities, native and introduced bacteria obtained from post-construction Yucca Mountain (YM) rock were isolated under varying conditions, including elevated temperature, low nutrient availability, and the absence of available oxygen. Individual isolates are being screened for activities associated with microbially induced corrosion of metals (MIC). Preliminary determination of growth rates of whole YM microbial communities under varying conditions was also undertaken.

II. METHODS

Samples of Topopah Springs tuff collected from a mined Fran Ridge outcrop, and rock excavated during construction of the Exploratory Studies Facility tunnel (Yucca Mountain, NV) were collected aseptically. Microorganisms were isolated both aerobically and anaerobically from whole and aseptically crushed (1.7-2.4 mm) rock samples at room temperature by plating onto low nutrient R2 agar (Difco). Organisms that survive in nutrient-depleted environments and at elevated temperature (50°C) were isolated from crushed samples (1.0g) using low nutrient R2[1] broth. The resulting whole microbial communities were grown for extended periods (aerobic incubation, 72 h.; anaerobic incubation, 17 days) at room temperatures and 50°C. After extended cultivation, samples were incubated on R2 agar at the temperature of previous growth. A representative group of morphologically distinct individual isolates were purified by repeated streaking of single colonies, which were identified primarily using fatty acid analysis. The growth of microbes that possess sulfate-reducing and iron-oxidizing capabilities was encouraged from crushed rock samples using other specialized growth media.

Growth rates were determined by adding 10g of crushed rock samples to 50 ml of R2 broth. Samples were incubated aerobically by shaking in covered flasks at ambient temperature, 30°C, and 50°C. Sterile controls were prepared by repeated cycles of autoclaving (120°C) and incubation. Growth was monitored by periodic live plating of appropriate dilutions on R2 agar.

Crude colorimetric screens were used to identify individual strains that produced the greatest quantity of a range of MIC-related capabilities. Acid producers were identified after growth of individual isolates in R2 broth containing pH indicators, the color change of which gave a qualitative indication of acid production. Production of hydrogen sulfide by individual isolates was detected by the ability to precipitate ferrous iron after growth in various media. Generation of exopolysaccharide capsular material was assessed after gross examination of colony morphology. Ferrous iron was assayed colorimetrically using ferrozine reagent[2].

III. RESULTS

While any given growth media permits only the growth of a small fraction of a microbial community, a multiplicity of microbial types were still detected from whole and crushed YM rock on R2 media; henceforth these were treated as a sample of the total microbial community present at this site. In general, the greatest diversity of those microorganims culturable on R2 arose from plated rock samples grown under aerobic conditions at room temperature, although 17 anaerobic strains were purified from rock samples incubated at ambient temperature under anoxic conditions. Extended growth at room temperature and 50°C showed a low diversity of microbial forms (one or two cell types). However, high cell numbers were reached after extended growth under aerobic conditions (2×10^8-10^9 cells/ml), while anaerobic conditions produced low cell densities (e.g., 140 cells/ml), and no growth was evident after extended anaerobic incubation at 50°C. In total, a group of over 60 isolates were preserved for further study. After one month, growth of iron oxidizers and sulfate reducers is not evident

in specialized media, although efforts continue to isolate these types of organisms.

Growth rates of whole communities of YM-derived microorganisms in low nutrient R2 broth varied depending on the temperature of incubation. While communities grown at room temperature or 30°C showed an average mean doubling time of 1.8 h., those growing at 50°C demonstrated doubling times of 3.2 h. over a 9 or 10 hour growth period. All cultures, however, demonstrated significant increases in cell numbers, ranging to over 20,000 cells/ml of media, at the conclusion of the 10 h. growth period.

Preliminary screening of over 60 YM-isolates shows that 27% produced enough acid to decrease the pH of the growth media (pH≤5.3) under aerobic conditions; 5 of these strains produced acid when incubated at 50°C. 44% of those examined after growth in anoxic conditions generated acid. Several isolates demonstrated marked production of capsular exopolysaccharide material. Studies aimed at screening isolates for sulfide production and iron oxidation are ongoing.

IV. CONCLUSIONS/DISCUSSION

We expect that both native microorganisms and those introduced as a result of construction activities are represented in our samples. While identification of *all* microbial community members requires application of alternative techniques (i.e., ribosomal DNA analyses), these studies examined that portion of the YM community capable of growth on R2 media under the conditions specified. These primary findings demonstrate that microbes present at the YM site are capable of survival and growth under conditions approaching those anticipated after waste deposition. Some examined members of the total microbial community can grow in the absence of oxygen and at temperatures of at least 50°C. Growth rates are measurable at ambient and elevated temperatures, and probable spore-forming organisms are even capable of surviving repeated exposure to 120°C. Further *in vitro* determinations will aid in determining *in situ* rates of growth, which can then be correlated with hydrologic flow rates. Depleted nutrient conditions favor the growth of only a select group of community members, but these are capable of reaching high cell densities under aerobic conditions, even at 50°C. Of those anaerobic isolates capable of growth on R2 media, it appears the combination of elevated temperatures and depleted nutrients are deleterious to growth.

Initial studies of MIC-associated activities demonstrate that YM microbial inhabitants possess the abilities to both produce acidic conditions and biofilm-generating materials. Production of biofilm "slimes" could facilitate the growth of organisms even at a very low relative humidity. Other microbial activities identified with MIC are currently being assessed and crude screening methods are being followed by more refined analysis to better characterize those isolates identified in primary screening protocols. Finally, microbial isolates that demonstrate the highest MIC-associated activity rates will be used to assess the MIC resistance of various alloys intended for use in waste deposition.

Clearly, further correlation of environmental conditions and their effects on relevant microbial activities is required to accurately predict the effects of microorganisms on waste containment. However, these studies provide evidence that microbial impacts are pertinent to risk assessment of nuclear waste storage facilities.

REFERENCES

1. Reasoner, D.J. and E.E. Geldreich (1985). A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49:1-7.

2. Stookey, L.L. (1970). Ferrozine:a new spectrophotometric reagent for iron. Anal. Chem. 42:779-781.

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