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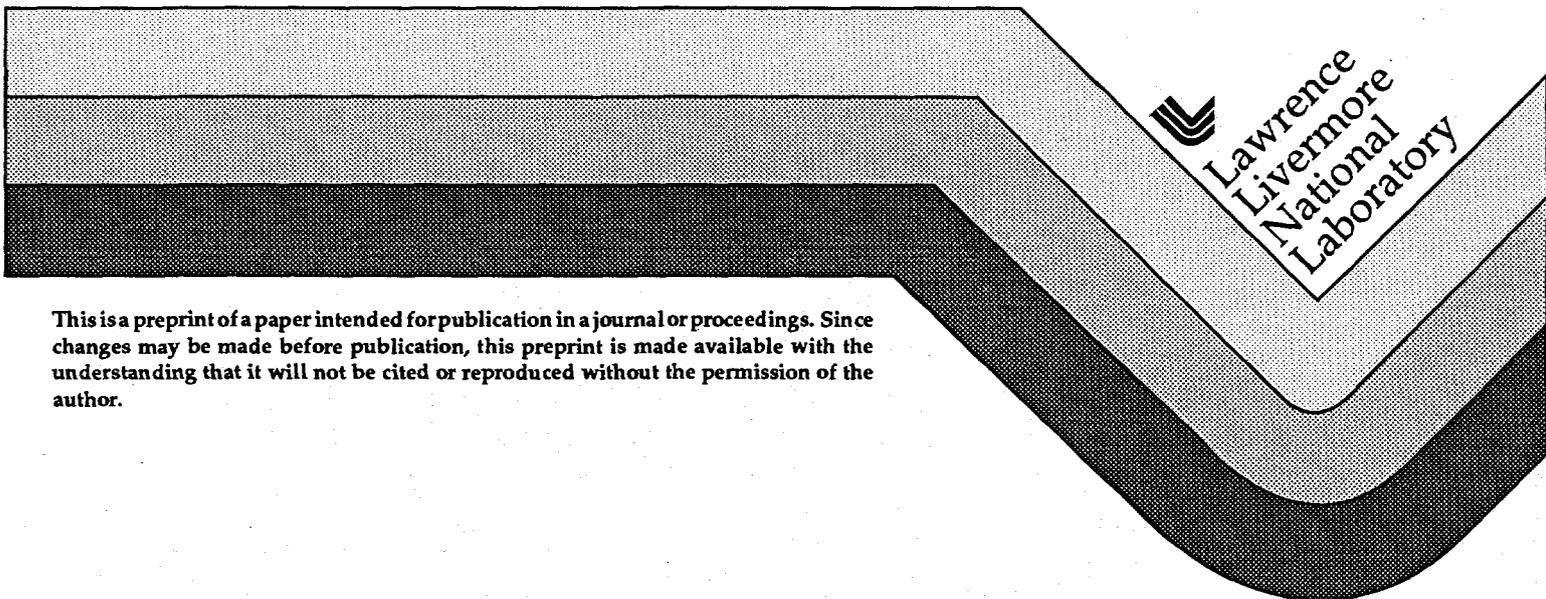
A Program to Assess Microbial Impacts
on Nuclear Waste Containment

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A PROGRAM TO ASSESS MICROBIAL IMPACTS ON NUCLEAR WASTE CONTAINMENT

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I. INTRODUCTION

Microorganisms are ubiquitous in distribution and have diverse metabolic capabilities. They can survive and maintain activity under extremely harsh environmental conditions. Biotic factors may directly contribute to waste package or repository component failure through microbially induced corrosion (MIC), or indirectly impact containment through alteration of the surrounding chemical environment. Microbes could also facilitate the transport of radionuclides from the near-field, and impact the hydrologic properties of geologic and engineered barriers.

In this paper we discuss aspects of a comprehensive program to identify and bound potential effects of microorganisms on long-term nuclear waste containment, using as examples, studies conducted within the Yucca Mountain Project.

II. METHODS

The potential impact of microbes on a subsurface radioactive waste repository are described in Horn and Meike¹, the salient issues of which are outlined in Figure 1. Here we present a program, composed of three interconnected elements (Fig. 1), designed to assess these issues.

The aim of the first program element is to identify microbes and their associated metabolic paths that operate or are enhanced by the modified environmental conditions expected after subsurface waste emplacement. In this element, extant microbes (both indigenous and those introduced as a result of construction activities) are identified and evaluated for specific potential metabolic activities, and a library of microbes are developed to provide a source of isolated test organisms. The identification process uses a broad range of detection techniques, since no single method has been shown to be fully representational. The techniques include traditional culturing as well as direct molecular techniques, which

rely on signatures resident in ribosomal DNA² (rDNA) and membrane fatty acids³ specific to given classes of organisms for identification. Both DNA and fatty acids are extracted directly from the excavated site material without the need to grow organisms, the principal limitation of culturing approaches. Libraries, composed of native microorganisms^{4,5,6} and organisms from disturbed areas^{7,8} representative of post-construction environments, are presently being assembled for the Yucca Mountain Project.

The second program element is intended to quantify specific microbial activities that modify aqueous chemistry or corrosion rates relevant to waste isolation in a subsurface repository. Microbial activity is likely to occur in two episodes: an initial response to the influx of nutrients and water due to construction, and a second episode during which starvation-resistant bacteria predominate after nutrients have been exhausted. The impact of microbes on the repository during these episodes will be determined by two related phenomena: thermal history and water availability. The effect of a thermal pulse is complex. It can augment microbial activity and favor thermophilic and even hyperthermophilic bacteria⁹. However, beyond a certain threshold, heat causes cessation of activity.

In this second program element, the extent and rate of microbially-mediated chemical reactions are assessed for both episodes of microbial activity in long term growth studies. Experimental microcosms using site material inoculum and historical analogs¹⁰ in which combinations of conditions (heat, relative humidity, radiation flux, nutrient availability, chemistry) relevant to a given repository environment, will be analyzed. These combinations of conditions are altered while measuring specific activity rates, to identify both rates and limiting conditions. Experimental assessment of the starvation-resistant fraction of the microbial community may be most difficult because activity may only be detected after prolonged growth under highly dilute nutrient conditions.

Such experiments are fundamentally important because the starvation-resistant fraction may represent the most prominent microbial activity in a repository setting over a long period of time.

Assays to determine microbial activities that alter metal and radionuclide speciation, and generate acids and gases (e.g., hydrogen sulfide, methane), will be performed *in vitro* under standardized conditions, using microbial isolates from the assembled library, with the appropriate substrate, and measurement of product accumulation or substrate disappearance⁷. Coordinately, extraction and quantification of proteins and mRNA that have been specifically identified with the catalysis of relevant biochemical reactions will be undertaken. Well-established extraction techniques are combined with either immunodetection (for proteins) or nucleic acid hybridization (for mRNA) protocols to provide a direct measure of the extent of a given microbial activity *in situ*.

The third program element is intended to determine the effect of microbes on some physical properties of the repository horizon. Growth rates, biofilm production, and radionuclide sorption by microorganisms and their by-products are determined under relevant conditions, both in batch cultures and rock samples. The resulting data will be used for three different applications. First, to correlate with flow rates through crushed and whole geologic media to model the effect of microbial growth on hydrologic properties of the surrounding geologic barrier^{6, 8}. Second, to predict the availability of microbially-generated colloids (both cells and biofilm materials). Finally, to assess the significance and effects of microbially induced alterations of localized relative humidity.

III. CONCLUSIONS/DISCUSSIONS

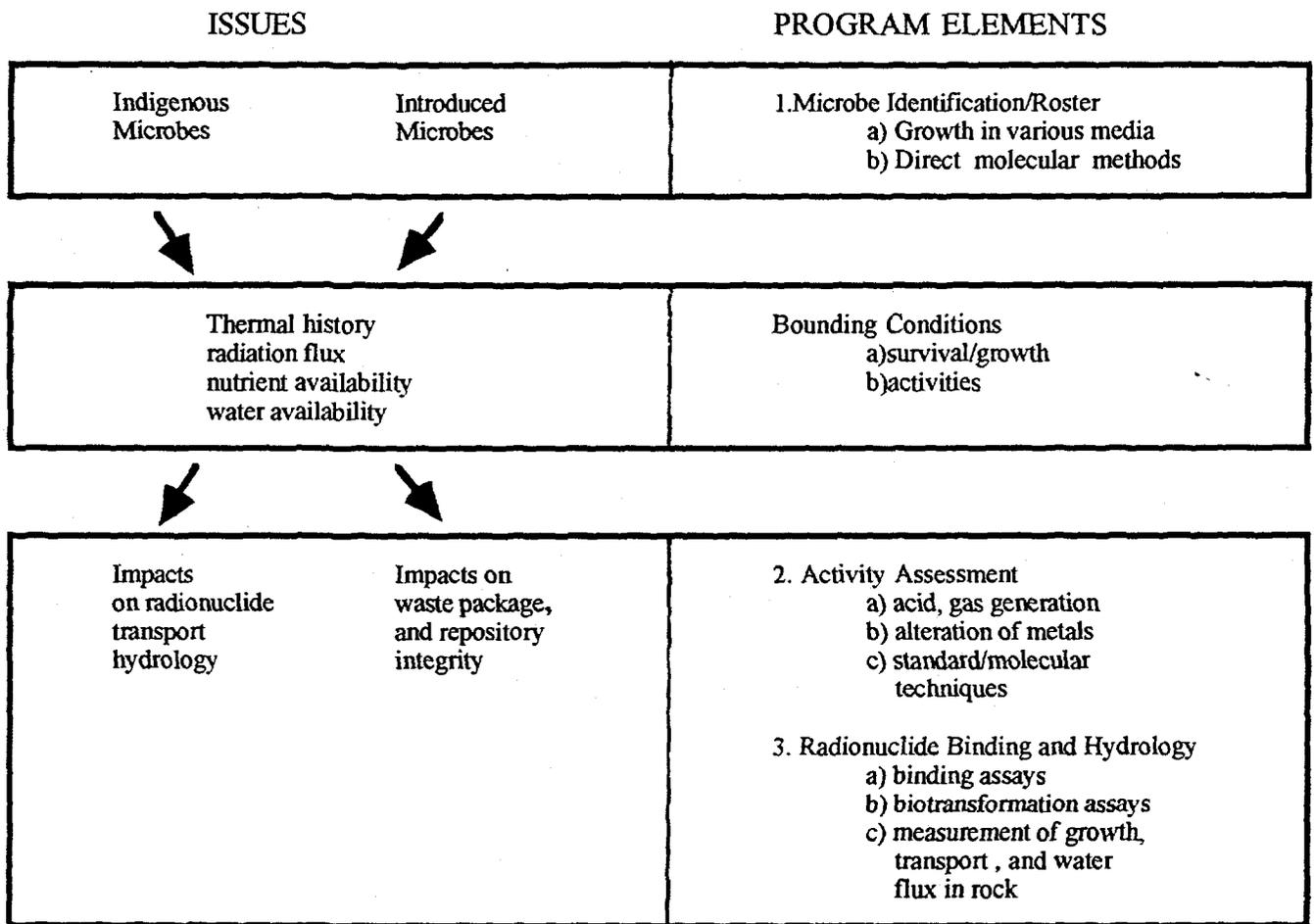
A comprehensive program has been formulated which cuts across standard disciplinary lines to address the specific concerns of microbial activity in a radioactive waste repository. Collectively, this program provides bounding parameters of microbial activities that: 1) modify the ambient geochemistry and hydrology, 2) modify corrosion rates, and 3) transport and transform radionuclides under conditions expected to be encountered after geological waste emplacement. This program is intended to provide microbial reaction rates and bounding conditions in a form that can be integrated into existing chemical and hydrological models. The inclusion of microbial effects will allow those models to more accurately assess long term repository integrity.

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FIGURE 1



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