STUDY PLAN FOR BIOLOGICAL SORPTION AND TRANSPORT

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ABSTRACT

The purpose of the Biological Sorption and Transport Study is to estimate the effects of the microbiological community found at Yucca Mountain on the movement of actinide waste elements through the unsaturated zone, as influenced by the chemical and physical characteristics of the subsurface environment. This study will address two important Information Needs: (1) site information to calculate the releases of radionuclides to the accessible environment (1.1.1), and (2) determination whether the site is or is not likely to be disqualified by the disqualifying conditions (1.9.1). Presented in this study plan is the purpose, rationale, description, and application of each of the individual analyses that will be used to measure the numbers, activity, and influence of the microbiological community at Yucca Mountain.

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1.0 PURPOSE AND OBJECTIVES OF THE BIOLOGICAL SORPTION AND TRANSPORT STUDY

1.1 Objectives of the Study

The goal of the biological sorption and transport study is to determine the effect, either positive or negative, that microorganisms may have on the transport of actinide elements through the tuffaceous material surrounding the potential site of the high-level nuclear repository and to relate this information to other studies that are part of the site characterization (see Section 4.0).

The possible importance of microorganisms at Yucca Mountain first received attention when millions of gallons of drilling fluids were used during initial characterization of the geology and hydrology of the potential site for a high-level nuclear waste repository (SCP, p. 8.3.1.3-80). Within a 3-km radius of the exploratory shaft, it is estimated that nearly 15,000,000 gallons of drilling fluids were released into the block (potential site) or in the immediate area (Hersman, 1987). The drilling logs from these wells indicate that fluids were lost in the Topopah Spring Member, which is the proposed horizon of the repository. The Topopah Spring Member has been characterized as a welded tuff with low permeability and a high content of fractures (Montazer and Wilson, 1984). They have therefore suggested that in this member, lateral movement may exceed downward rates. This statement is consistent with the observation of an apparent flow of drilling fluids from borehole USW G-1 to the vicinity of borehole USW UZ-1, which was air-drilled without fluids (Whitfield, 1985). This possible occurrence of fluid movement between the two wells is indicated by the presence at USW UZ-1 of polymers that were used in the drilling of USW G-1 (Spengler et al., 1981). USW UZ-1 is located 305 m northwest of USW G-1 and was drilled approximately 3 years after USW G-1. Its drilling was limited to the unsaturated zone at a depth approximately equal to that proposed for the repository (SCP, p. 8.3.3-15). The presence of mobile drilling fluids could significantly affect the numbers and distribution of microorganisms found in the block area.

In addition to those drilling fluids, several other forms of organic materials will be introduced into the block. During the construction of the exploratory shaft, items such as diesel fuels and exhausts, hydraulic fluids, and additional drilling fluids will be introduced routinely into the surrounding environment. A complete list of materials proposed for use during the construction of the Exploratory Studies Facility (ESF) has been prepared by West (1988). It is also anticipated that many of the same materials will be used during the construction of the repository itself. The potential impacts on the site by these materials have been discussed and evaluated in SCP Section 8.4.

Organic material and drilling fluids could have a significant influence on the indigenous population of microorganisms. Previous studies have demonstrated that several species of microorganisms isolated from Yucca Mountain are capable of utilizing drilling fluids as a nutrient for growth (Hersman, 1987). A specific concern is that these fluids and organic materials may be utilized as growth substrates by large numbers of microorganisms, which in turn may influence the transport of radioactive wastes away from the repository. In particular, microbial effects would become significant if the primary containment fails.

Microorganisms can affect transport of radioactive wastes in one or more of the following ways:

1) by altering the composition of the groundwater chemistry through changes in pH or Eh and production of metabolites such as CO_2, H_2S, NH_3, and NO_3;

2) by producing chelating agents that can solubilize radioactive elements;

3) by transporting the radionuclide via biological movement;

4) by transporting the radionuclide via colloidal dispersion;

5) by sorption of the radionuclide onto a nonmotile solid phase, thereby retarding the transport of the radionuclide; and/or

6) by plugging pores in the host sediment, thereby retarding movement of groundwater.

The purpose of this study, presented in the Department of Energy (DOE) Site Characterization Plan (SCP) Section 8.3.1.3.4.2 (DOE, 1988), is to estimate the effects of the microbiological community found at Yucca Mountain on the movement of actinide waste elements through the unsaturated zone. The actinide elements to be studied are neptunium, plutonium, and americium; initial experiments will focus on plutonium, with all the experiments to be repeated using neptunium and americium. Four major areas of research activity outlined in the SCP are

1) sorption on microorganisms and data on steady state, V-max, actinide speciation, and cellular location

2) data indicating potential for the transport of radioactive wastes by microorganisms and microbial byproducts, data on colloidal properties and mobility of microorganisms

3) understanding of the magnitude of microbial activity of retardation and transport of radionuclides

4) identification of the microorganisms

Since preparation of the SCP, progress has been made in each of the above areas of research. For example, sorption of plutonium by microorganisms is nearly complete (Hersman, 1986), with the exception of describing the cellular location of sorbed actinides (discussed in Subsection 1.1.3). No studies will be done on V-max or actinide speciation (Area #1), because these investigations are beyond the needs of this study. Other sections have been reorganized to better achieve the goal of this study. The potential for transport of radioactive waste (Area #2) is discussed in Subsections 1.1.2, Chelation, 1.1.3, Sorption, and 1.1.4, Microbial Effects on Colloidal Dispersion and Actinide Transport. Data on colloidal properties are described in Subsection 1.1.4, while the mobility of microorganisms is discussed in Subsection 1.1.3. Area #3, Understanding of the Magnitude of Microbial Activity of Retardation and Transport of Radionuclides, is discussed in Section 4.2, Interfaces With Other Site Characterization Studies. Specifically, the information generated from these studies will be used to address the information needs (Site Information to Calculate the Releases of Radionuclides to the Accessible Environment) discussed in Section 4.1. Finally, Area #4, Identification of the Microorganisms, is discussed in Subsection 1.1.1. The following subsections have been reorganized from the initial SCP Areas of Research Activity to better achieve the overall goal of this project.

1.1.1 *Indigenous Microorganisms*

Until recently it was believed that soil microorganisms, both in number and metabolic activity, were largely confined to the "near surface" environment of the earth, located within a few meters of the earth's surface. This was due in large part to the relative inaccessibility and remoteness of

the subsurface. As stated by Ghiorse and Wilson (1988), the "out-of-sight, out-of-mind" aphorism "aptly describes the traditional view of terrestrial subsurface held by generations of human beings." However, recent findings of the DOE Deep Microbiology Subsurface Program have shown that significant numbers of microorganisms exist, independent of depth, to at least 500 m below the earth's surface (Balkwill and Wobber, 1989). Also, the increasing depth did not appear to affect the diversity or metabolic activity of the microorganisms. In a related study performed at LANL, microorganisms were found in significant numbers in very dry tuffaceous earth at depths to 55 m (Hersman et al., 1988). These studies serve to demonstrate that microorganisms are found in extreme geologic environments previously believed to be devoid of life and therefore may exist at depth in Yucca Mountain.

Due to their suspected influence on the transport of actinide elements, it will be essential to determine the numbers and activities of indigenous microorganisms found at the location of the potential high-level nuclear repository. It will also be important to relate microbial information to the chemical and physical characteristics of Yucca Mountain in order to understand better what parameters control microbial numbers and activities.

The numbers and activities of indigenous microorganisms in Yucca Mountain will also be used to determine the potential effect of each of the laboratory studies. The laboratory studies will be performed under optimal conditions to maximize a potential effect. For example, optimal growth conditions will be used to culture microorganisms for the colloidal interaction studies, Subsection 1.1.4. It will be necessary to use optimal growth conditions so that the results can be determined within a reasonable amount of time. Optimal growth conditions do not exist in Yucca Mountain, rather the growth conditions in Yucca Mountain are believed to be less than optimal. By determining what the numbers and metabolic activities of the indigenous species in Yucca Mountain are, it will be possible to apply the results of the laboratory studies to Yucca Mountain. For example, if the laboratory studies were performed with 10^8 cells per ml, but it was later found that only 10^5 cells per gram (dry wt. soil) exist in Yucca Mountain, then the overall effect of the laboratory studies could be reduced by 10^3, or be expected to have only 1/1000 the effect.

This approach is being proposed because it is very difficult to conduct the laboratory experiments under the field conditions of Yucca Mountain. It would take an unreasonable amount of time (several years) to observe an effect. Therefore, we believe that by using the numbers and metabolic activity of Yucca Mountain to adjust the laboratory data, it will be possible to predict the potential effects of microorganisms on actinide transport.

Finally, it must also be stated that this study addresses only indigenous species. As mentioned in Section 1.1, several million gallons of fluids have been and may be introduced into the block area. In addition to providing nutrients to indigenous microbial species, these fluids will serve as an inoculum for introduced microbial species. The extent to which these introduced species will affect the transport of radioactive wastes is unknown, and an estimation of these species is beyond the scope of this investigation.

1.1.2 Chelation

A very important principle of microbial ecology is that microorganisms can strongly influence the movement of metals through soils. One mechanism is the microbial production of powerful chelating agents, called siderophores, that solubilize the very insoluble Fe(III) cation. In the last three decades, over 80 siderophores have been isolated and characterized, and binding constants have been reported to be as high as 10^{52} (Neilands, 1974). "At pH 7, the equilibrium concentration for ferric iron (as free [Fe(III)]) is approximately 10^{-18} M"(Latimer, 1952). "For microorganisms

such as enteric bacteria, which need at least a total concentration of iron of 5×10^{-7} M for optimal growth, this concentration is many orders of magnitude too low. Only powerful chelating agents such as the siderophores can mobilize iron from the environment and facilitate transport of iron into the microbial cell" (Raymond et al., 1984). Neilands (1981) believes that because Fe(III) and Pu(IV) are similar in their charge/ionic-radius ratio (4.6 and 4.2, respectively), Pu(IV) may possibly serve as an analog to Fe(III) and could therefore be solubilized by siderophores. It is possible, therefore, that some actinides could be transported in the environment via the siderophore transport system. Such transport would probably occur over long periods of time involving many generations of microorganisms.

1.1.3 Sorption

Microorganisms are able to sorb a variety of metals as a means of protection against the toxic effects of the metals. The simplest mechanism used is the deposition of the metal on the outer surface of the microorganism. The binding of metals has been shown to be specific; the charge, ionic radius, and coordination geometry are the predominant factors. The deposition of the metal occurs because of the activities of membrane-associated sulfate reductases (Seigal, 1983) or through the biosynthesis of oxidizing agents such as oxygen or hydrogen peroxide (Wood, 1983). In this way, metals such as iron are deposited on the surfaces of many species of bacteria; uranium is deposited on the surface of fungi; and iron, nickel, copper, aluminum, and chromium are deposited on the surface of algae (Wood, 1983). A second means of surface accumulation occurs through the production of extracellular ligands that complex metals outside the cell and prevent their uptake (Wood and Wang, 1983). Both cyanobacteria and green algae have been found to concentrate nickel on their surface to 3,000 times greater than the concentration of the culture medium. Another measure adopted by microorganisms to prevent metals from reaching toxic levels is the biosynthesis of intracellular traps for the removal of metal ions from solution. One example is the biosynthesis of the sulfhydryl-containing metallothionein protein to bind cadmium and copper (Wood and Wang, 1983). Similarly, poly(γ, glutamylcysteinyl)-glycines produced by Schizosaccharomyces pombe (a fission yeast) bind cadmium (Murasugi et al., 1981, and Robinson and Jackson, 1986).

By depositing metals, internally or externally, microorganisms are not only protecting themselves from the toxic effects of the metal ions but are also, in effect, concentrating the metal in the biosphere. Relative to the Yucca Mountain Site Characterization Project, the concern is that microorganisms may be capable of sorbing actinides. The literature indicates that microorganisms are capable of sorbing radioactive wastes (for example, Strandberg, 1982; West et al., 1982; Champ et al., 1980; Treen-Sears et al., 1984; Christofi et al., 1985; Rice and Willis, 1959; Honda et al., 1971; Strandberg et al., 1981; Dreher, 1981; Sakaguchi et al., 1978; Horikoshi et al., 1979; Aoyama et al., 1976; and Marchyulene, 1978). Most notably, Strandberg and coworkers (1981) described the intracellular accumulation of uranium by Pseudomonas aeruginosa. According to Strandberg, the uptake of uranium is quite rapid, as if mediated by an active transport system, even though a uranium transport system has not yet been described.

Regardless of the means by which actinides are sorbed by the bacteria, bacteria are able to remove actinides from solution, as demonstrated by experiments performed at Los Alamos (Hersman, 1984, 1986). In these experiments, the bacteria, on a per-gram-dry-weight basis, sorbed Pu(IV) nearly 10,000 times greater than crushed tuff. What remains to be determined is the overall effect that this biological sorption may have on the movement of radioactive wastes away from a high-level nuclear repository. In most classical saturated flow conditions, bacteria will be removed from suspension by soil or rock. It is unclear, however, what effect the surrounding rock will have on the bacteria, depending on whether saturated, fracture flow, or unsaturated flow conditions

predominate. Most bacteria are motile and therefore are not confined to an isolated location. It is possible that microorganisms may strongly sorb significant quantities of actinide elements and transport those elements over great distances via microbial motility. In fact, preliminary studies performed at LANL demonstrated that microorganisms were able to penetrate 4.0-mm-thick wafers of tuffaceous rock taken from the Calico Hills formation. Due to lack of available core material from Yucca Mountain, this experiment was performed only a few times; however, in every replicate, microorganisms were able to penetrate the core material. Therefore, it will be important to continue these studies as soon as additional core material becomes available. The information generated from these studies will be used to describe the overall effect that sorption and biological activity have on the transport of actinide elements.

1.1.4 Microbial Effects on Colloidal Dispersion and Actinide Transport

Colloidal dispersion has been implicated as a means of transporting toxic wastes and heavy metals (Buddemeir and Hunt, 1987). However, colloidal dispersion can be a viable means of transporting actinides only where the colloids remain as individual particles suspended in an aqueous solution. If the colloids become attached to one another, forming agglomerates, then these particles would not be available to participate in colloidal dispersion processes. Agglomerates would tend to settle out of solution or become entrapped in small pore openings in a rock matrix. The purpose of this activity is to determine the role of microorganisms in the agglomeration process.

Many natural habitats have a low nutrient status, therefore solid surfaces such as the surfaces of colloids are potential sites of nutrient concentration (as ions and macromolecules) and, consequently, of intensified microbial activity on a macro-scale. The movement of water across a surface provides increased opportunities for microorganisms to approach solid-liquid interfaces. In addition, there are many operative physicochemical and biological attraction mechanisms in the immediate vicinity of interfaces. They are chemotaxis, electrostatic attraction, electrical double-layer effects, and cell-surface hydrophobicity.

Once attracted to a surface, microorganisms are capable of strongly adhering to that surface. The adhesion of bacteria to inanimate surfaces is widely recognized as having enormous ecological significance, such as the fouling of manmade surfaces (hulls of ships, pipelines, fuel storage tanks, etc.). Most microorganisms adhere to surfaces by means of surface polymers, including lipopolysaccharides, extracellular polymers and capsules, pili, fimbriae, flagella, and more specialized structures such as appendages and prosthecae. Microorganisms are known to adhere to the surface of colloidal particles. The adsorption of colloidal clays to microbial surfaces has been studied by Lahav (1962) and Marshall (1968). In recent years, several processes that take advantage of microbial sorption of clays have been patented for the flocculation of clay, particularly clays derived from phosphate benefication.

Microorganisms are known to sorb to colloidal clays and to sorb strongly onto surfaces, therefore microorganisms may affect the colloidal dispersion of actinide elements. The sorption of microorganisms to clay particles would cause the clay particles to lose their colloidal characteristics.

Preliminary studies conducted at LANL have demonstrated that in the presence of microorganisms, the formation of agglomerates was significantly accelerated (Hersman, 1988b). It is therefore entirely possible that, in the presence of microorganisms, the colloidal dispersion of actinide elements would be much less than it would be in the absence of bacteria. Because of the possibility of transport of actinides away from the potential site of the high-level nuclear waste repository, it will be essential to document the effect of microorganisms on the colloidal transport

of actinide elements. The results of this study would be used to determine the overall effect microorganisms will have on the colloidal transport of actinide elements. This information is directly related to Activity 8.3.1.3.4.1.4 (Sorption onto Colloids) and would be used for evaluation in Activity 8.3.1.3.6 (Radionuclide Retardation).

1.2 Regulatory Rationale and Justification

1.2.1 Resolution of Performance Issues

Performance allocation was used by the Yucca Mountain Site Characterization Project to establish the appropriate issue resolution strategies for performance issues in the Yucca Mountain Project Issues Hierarchy. A general discussion of the performance allocation approach is provided in Subsection 8.1 of the SCP, and issue resolution strategies for each performance issue are provided in Subsection 8.3.5. Confidence in performance goals specified for both the total system performance (SCP, Subsection 8.3.5.13) and actinide transport (SCP, Subsection 8.3.1.3.6) is directly related to the certainty with which the rates of actinide movement in the unsaturated zone can be established (Issue 1.15). This work is also related to Issues 1.1, 1.2, 1.3, 1.5, 1.8, and 1.9. Results of the microbiological studies could provide important information for modeling sorption (SCP, Subsection 8.3.1.3.4.3) and for the calculation of retardation mechanisms (SCP, Subsection 8.3.1.3.7.1).

1.2.2 Tie to Regulations

As indicated in the discussion of performance allocation, this study could provide support concerning compliance with the applicable environmental standards for radioactivity as established by the Environmental Protection Agency (EPA), as required in 10 CFR 60.112. Secondly, those microbiological studies would provide support concerning compliance with the Environmental Standards for the Management and Disposal of Spent Nuclear Fuel, High-Level and Transuranic Radioactive Wastes (EPA, 40 CFR 191).

Studies of microbiological effects will address the following Information Needs and investigations:

1.1.1	site information to calculate the releases of radionuclides to the accessible environment,
1.9.1	determination that the site is not now nor likely to be disqualified for each of the disqualifying conditions,
1.9.2	determination that the site meets and is likely to continue to meet the qualifying conditions of the technical guidelines,
8.3.1.3.1	water chemistry within the potential emplacement horizon and along potential flow paths,
8.3.1.3.5	radionuclide retardation by precipitation processes along flow paths to the accessible environment,
8.3.1.3.6	radionuclide retardation by dispersive/diffusive/advective transport processes along flow paths to the accessible environment, and
8.3.1.3.7	radionuclide retardation by all processes along flow paths to the accessible environment.

2.0 RATIONALE FOR BIOLOGICAL SORPTION AND TRANSPORT STUDIES

As discussed in Section 1.1, there are several ways that microorganisms can influence the movement of actinide elements. Given the limited resources of the Yucca Mountain Site Characterization Project, it is beyond the scope of this study to investigate all of the possible interactions and influences of microorganisms in the Yucca Mountain environment. It is therefore the approach of the microbiological subtask to select those activities considered to be significant. The specific tests that are to be used to measure these activities have been selected based upon their acceptance among microbiologists, their applicability to unique characteristics of Yucca Mountain, and the difficulty associated with each test.

All of the tests to be used in this study are laboratory tests. No field tests are planned at this time. Samples will be collected from the ESF and drill holes, as needed. Therefore, the approach taken for the microbiological testing is to look for the maximum possible effect in the laboratory, i.e., the worst case scenario. Laboratory parameters will be designed such that it may be assumed that a given microbial effect will be more significant in the laboratory than in nature. If we determine, however, that microorganisms may affect the performance of the repository, then it may be necessary to do an appropriate field analysis. We feel that this approach will give us the most useful and meaningful results.

2.1 Technical Rationale and Justification

2.1.1 Determination of the Presence of Indigenous Microorganisms

The microbiological studies of Yucca Mountain have two major areas of emphasis. The first area is the laboratory investigations, which will determine the potential effect of each of the types of microbial interactions (listed in Section 1.1). The second area is the determination of the indigenous microbial population, the types of microorganisms present in the study site and their metabolic activities. The determination of the indigenous species population and metabolic activity is essential to this project. The indigenous species studies will be used to adjust the laboratory studies. As discussed in Subsection 1.1.1, the laboratory studies might demonstrate that chelation will affect the movement of actinide elements. However, through studies of indigenous species, we may find that the laboratory studies were performed with microorganisms having three orders of magnitude higher metabolic activity than exists in the field. One could then reduce potential effect of chelation by 1/1000. Total microbial populations will be determined by direct microscopic techniques and plate counts.

Other laboratories will be subcontracted to perform the remainder of the indigenous species studies. These studies will include, but not be limited to, the following:

1) General physiological types of bacteria will be determined, e.g., aerobes, anaerobes, spore formers, nitrifiers, nitrate and sulfate reducers, methanogens, and

2) Metabolic activity as determined by labeled substrate uptake, CO_2 evolution, adenosine triphosphate (ATP) analysis, and enzyme analysis (phosphate and pectinolyase).

The proposed studies would involve established laboratories using standard and accepted microbiological techniques. The methods used by these laboratories would be taken from Standard Methods for the Examination of Water and Wastewater, from the Manual of Methods for General Bacteriology, or from journal publications.

Samples will be collected from both radial boreholes in the exploratory shaft (ES) and from rubble removed from the ESF during its construction. Overall emphasis will be placed on achieving a general understanding of population distribution, diversity, and microbial activity of the subsurface environment as it relates to the physical and chemical parameters of Yucca Mountain.

2.1.2 Chelation

As stated earlier, siderophores have been implicated in the solubilization of actinide elements. To evaluate the significance of chelation, it is necessary to determine the formation constant of the siderophore/actinide complex. It is preferable to work at near-neutral pH in order to maintain relevance to environmental conditions found at Yucca Mountain. For Pu(IV), the best way to avoid spontaneous polymerization at neutrality is to work with very dilute solutions such as 10^{-8} M or lower. Classically, these solutions have been prepared by diluting an acidic Pu(IV) feed solution. Hobart et al. (1986) are currently using carbonate complexation to stabilize the oxidation state of the actinide. Regardless of the preparation method, the plutonium ion must be diluted to avoid polymerization. Because of the low actinide concentration, classical spectrophotometric and electrochemical analysis of siderophore/Pu(IV) complexation cannot be used to determine the formation constant of the complex.

For that reason, competition experiments between the siderophore and a known metal-chelating agent were chosen to measure formation constants. Chelex 100 is a chelating ion exchange resin that shows unusually high preference for copper, iron, and other heavy metals. Chelex is a styrene-divinyl benzene copolymer matrix to which iminodiacetic acid residues are attached, and therefore, its selectivity for metals corresponds to that of iminodiacetic acid. Raymond et al. (1984) have described the atomic similarities of Fe(III) and Pu(IV). It is therefore appropriate to assume that Chelex will bind to Pu(IV) in a manner analogous to that of Fe(III) and therefore can be used to determine the formation constant between siderophore and Pu(IV). The formation constant of the Pu(IV)/Chelex will have to be determined experimentally.

2.1.3 Sorption

The determination of the cellular location of actinide deposition would provide additional information regarding the mechanism(s) of actinide sorption. This information would provide for a better understanding of the strength of the binding between the bacterial cells and actinide elements. For example, one would assume that stronger and longer-term binding would occur if the actinide element is being sorbed internally as compared with external deposition. Deposition of the metals outside the cell can be assumed to occur from precipitation resulting from membrane-associated enzymes or from the production of extracellular ligands that complex the metal. If an actinide is deposited externally, then one would also assume the absence of an active transport mechanism(s). If, however, the actinides are deposited internally, an investigation of membrane transport mechanisms would be appropriate. Related studies would include an investigation of the mechanism(s) of deposition (i.e., is there an internal protein associated with actinide binding or is the actinide precipitated in a vacuole, is it precipitated along the membrane, is it sheathed, is it crystallized or amorphous). One could begin to address these issues once the cellular location of deposition is determined. This determination can be accomplished by performing a series of simple experiments. First, the external constituents of the cells need to be examined for activity. Cells would be washed with mild buffers or mild acids and examined. Next, the cells would be ruptured, and, using differential centrifugation, the constituents of the cell (membrane, ribosomes, DNA, cytoplasmic material) would each be examined for activity. Finally, a mass balance of the actinide could be performed to determine the proportion of actinide located on the external membrane, the internal membrane, and among the cytoplasmic constituents.

Once the mechanisms of sorption are determined, experiments can then be conducted to determine the transport or bioaccumulation of the actinide elements. It would then be appropriate to determine the transport rate of actinide elements bound to microorganisms through tuffaceous rock. First the rock wafer experiments, 2.1.4, would be continued. These experiments would provide basic information regarding the transport of microbially-bound actinides through tuff. Information gained from these experiments would then be used to design more definitive column experiments. The purpose of the column experiments is to provide for information regarding the microbially-mediated transport of actinide elements through tuff.

2.1.4 Microbial Effects on Colloidal Dispersion and Actinide Transport

The purpose of the proposed research is to describe the mechanism(s) by which microorganisms influence the agglomeration of colloidal particles. As mentioned earlier, preliminary studies suggest that microorganisms are able to significantly accelerate colloidal agglomeration. Colloidal agglomeration is important to the Yucca Mountain Site Characterization Project from the standpoint that if colloids remain as individual particles, then these particles can participate in the colloidal dispersion of actinide elements. However, if agglomeration of the colloid particles occurs, then colloidal dispersion would not occur or it would occur at a reduced rate. Although the literature contains numerous studies that discuss the interactions of bacteria and colloids, none of the studies address colloidal agglomeration as affected by bacteria. Hence, there exists little information regarding (1) agglomeration rate, (2) agglomerate stability, (3) the threshold concentration of clay particles needed to initiate agglomeration, and (4) particle distribution within the agglomerate. It is believed that these issues are central to determining the interaction of microorganisms and colloids; therefore, the approach of future research is to conduct a series of experiments addressing each of these issues. It is essential that these issues be addressed; otherwise, models designed to predict the colloidal dispersion of actinide elements, that do not include biological interactions, may be inaccurate. Performance of these experiments would enhance greatly our understanding of the potential for the transport of actinides via colloidal dispersion.

Agglomeration Rate. These studies will be limited to clay colloids and to rock samples that are germane to the potential Yucca Mountain repository. Also, samples collected from Yucca Mountain will be used. A key to understanding bacterial influence of colloidal agglomeration is the determination of the kinetics of agglomerate formation. This information would tell us how quickly an agglomerate forms in the presence of bacteria. Although the preliminary experiments were useful from the standpoint of demonstrating the involvement of bacteria in the agglomeration process, they provided little in the way of detailed information. Several additional experiments must be performed to determine the exact rate of agglomerate formation. This information would be very useful in understanding the types of attractions that are occurring between the clay and bacterial particles. For example, if agglomerate formation is occurring concurrent with bacterial growth, then one could assume that agglomeration is simply a function of the number of bacterial particles. If, however, agglomeration is delayed until the stationary phase, then one would suspect that agglomeration is "threshold" in nature and depends upon some other parameter such as pH or the concentration of extracellular metabolic products.

Agglomerate Stability. Another critical aspect of the proposed research is to study the stability of the bacterial/clay agglomerate. From preliminary studies performed at Los Alamos, it has been observed that the number of particles within an agglomerate changes with time. For example, agglomerates containing 2–5 particles initially decreased, then increased with time; the 6–10 particle agglomerates increased with time; the 10–25 particle agglomerates initially increased, then decreased with time; and those agglomerates containing >25 particles also initially increased,

then decreased with time. It appears that the large agglomerates are forming early, followed by a breakdown into the smaller agglomerate units (2–5 and 6–10). However, such interpretation is only speculation. More detailed studies need to be performed to determine the interaction among these agglomerate units.

The long-term stability of the agglomerates has not been determined, leaving us with several questions. What is the eventual fate of the agglomerates? Is there one particular agglomerate unit that will dominate, or will there be an equal distribution of particles among all the agglomerate units, or will all the agglomerates eventually break down to individual particles? In order to understand the agglomeration process, these questions must be answered.

Threshold. It is necessary to determine if a threshold concentration of clay particles is needed to initiate aggregate formation. Only one clay concentration was used in the preliminary experiments; however, in the proposed experiments, the concentration of clay particles will be varied.

Particle Distribution Within the Agglomerate. The relative distribution of bacteria and clay particles within a single agglomerate should be determined. It is not known whether the ratio of bacteria to clay is uniform among all the agglomerate units or if this ratio changes with the size of the agglomerates. It may be that a single bacterium initiates agglomerate formation, or several bacteria near one another may be responsible for agglomerate formation. Each of these activities (agglomeration rate, agglomerate stability, threshold, and particle distribution) affects colloidal dispersion and actinide transport, because each activity is part of the agglomerate formation process. Before one can predict the impact of colloidal agglomeration on actinide transport, one must first understand the agglomerate formation process. Therefore the study of these activities is necessary to understand and predict actinide transport by colloidal dispersion.

2.2 Constraints on the Study

There are two constraints on the indigenous species study (2.1.1). First, all of the samples from the ESF must be collected aseptically. These techniques are discussed in Section 3.1. Second, much of the work to be performed is dependent upon the construction of the ESF. If there is significant delay in the construction of the ESF, then there will be a concomitant delay in the biological studies. If there is a significant delay, backup samples will be obtained from dry drillholes or from the G-Tunnel.

An overall limitation of each of the microbial studies is that it is impossible to duplicate field conditions in the laboratory. The types and numbers of bacteria, metabolic activity, drilling fluids, construction chemicals, natural colloids, and other conditions can only be estimated. Therefore, the microbiological studies will be a best approximation of the effect of microorganisms on actinide transport.

This study provides no impact on the Yucca Mountain site. The analytical accuracy and precision of the measurements are discussed in the technical procedures section (3.0), where appropriate.

3.0 DESCRIPTION OF TESTS AND ANALYSIS

3.1 Indigenous Microorganisms

Most of the methods used to detect microbial populations and activities in the Yucca Mountain tuff are standard and accepted techniques for microbial analysis of groundwater and soil. These methods are most commonly found in Standard Methods for the Examination of Water and Wastewater and in Manual of Methods for General Bacteriology. The Manual of Methods for General Bacteriology contains several sections on microscopy, which will be used extensively during the examination of samples. Standard Methods for the Examination of Water and Wastewater contains accepted methods for the general microbial analysis of surface waters. The methods also can be applied to the analysis of ground water or soils washed with water. These methods will also be augmented with the current literature. It is anticipated that all of the samples collected from Yucca Mountain will be aerobic; therefore, only aerobic analyses will be performed.

Sample Collection. Samples from the ESF will be collected from specially collected dry samples that are to be mined without using water for dust control. Samples should be collected from the upper, middle, and lower locations of each formation in Yucca Mountain and from the surface to bottom of the ESF. Therefore, a minimum of 15 samples should be collected (four major formations x three, plus three formation interfaces). Sample size will be determined based on the size of the rubble rock that can be subcored to yield approximately 500 g of sterile rock material. From each sample location, three replicates of 500 g each should be collected.

Samples will be immediately returned to the laboratory and placed in a sterile, humidified glove box. In the glove box, subsamples will be taken using a nitrogen-cooled drill bit and drill press. These subcores will then be crushed aseptically and prepared for microbial analysis. For most analyses, it is anticipated that direct microscopic analysis will be used. Typically, an epifluorescence microscope is used to examine samples that have been stained specifically for living material such as DNA. Acridine orange is a fluorescent stain that is used routinely for the analysis of soil samples and has been used successfully to examine samples collected for the deep-probe studies conducted at the Savannah River Laboratory (SRL). It is specific for DNA, the results are reproducible, and the method is widely accepted among the scientific community.

Microbiological Analysis. The acridine orange direct count (AODC) method, with some recent modifications to improve sensitivity, will be employed in the project. The modifications include heating the microscope slides used to prepare sediment smears at 450–500°C for 48 h to combust background "slide" bacteria. Light microscopic characterization of the samples will be done routinely during the AODC procedure. The morphological characteristics of bacteria in the samples will be recorded by photomicrography of representative fields. In addition, it may be possible to utilize a new film flotation procedure, developed by Balkwill at Florida State University, to release bacteria from the sediment samples before staining with acridine orange or other fluorescent dyes. This flotation procedure has been used successfully on the SRL samples to determine morphology of indigenous bacteria, yeasts, and fungal spores by epifluorescence light microscopy. Electron microscopy of the bacteria released by this method also is possible but depends on a relatively high population density.

Culturing of microorganisms from a condition of extreme desiccation and starvation may require special treatment. Although spores will readily germinate when suddenly placed in rich organic media of high water potential, starved, desiccated, vegetative cells may be adversely affected by such treatment. Very low carbon media (1–15 mg C l^{-1}) are generally more appropriate for resuscitation of starved microorganisms. Similarly, it may be that a gradual increase in water potential may be more effective in resuscitating extremely desiccated vegetative cells. This gradual increase in water potential will be achieved by equilibration with an atmosphere

saturated with water vapor. This pre-incubation of core material will be at 5°C for 12 to 24 h to minimize growth but allow for gradual rehydration. A low-nutrient growth medium will then be introduced. This procedure may result in a more accurate measure of total microbial populations and species diversity.

Assessment of viability of the microbial population in the samples will depend to a large degree on selecting the proper nutrient media and growth conditions to encourage dehydrated and dormant cells to grow and form colonies. At present, several low-nutrient media formulations can be employed for routine plate counts of bacteria and fungi. However, based on current information, the present viable cell count protocol and media are not entirely adequate for the viable counting of bacteria in these very dry samples. Therefore, it will be necessary to improve plate count protocol and the formulation of new media to encourage bacterial growth.

Total biomass will be measured by ATP assays. Activity of microbial communities will be measured as total respiration (CO_2 production). Other microbial responses that will be measured under varying water potential conditions include uptake and respiration of radiolabeled substrates.

Previous experience with other dry samples from the high clay layers at the Savannah River Plant sites and from the vadose zone at the Pajarito Plateau site suggests that only a small fraction of the population, usually 0.0001% or less of the total population as determined by AODC, was able to grow on the media we employed (Hersman et al., 1988). Such a lack of recovery of bacteria from very dry samples was not unexpected. In fact, lack of recovery of bacteria from environmental samples is a very common problem in environmental microbiology; its solution is not trivial.

The challenge will be to identify important factors affecting survival and growth during the initial rehydration (resuscitation) period. Water potential, nutrient content, salt concentration, pH, and other environmental factors will be manipulated, one at a time and in combination, during the resuscitation period in order to identify the important factors. Microscopic assays will be employed to monitor growth of indigenous bacteria. These assays will be done with a vital fluorescent dye procedure to stain cells and observe growth or by adding a cell-division-inhibiting antibiotic (nalidixic acid) to a sample amended with growth substrates and then looking for elongated cells. An indirect assay of metabolic activity will also be used. This can be done by common radiorespiration techniques using likely carbon-14 labeled substrates and measuring $^{14}CO_2$ produced. The environmental factors that stimulate cellular growth and/or metabolic activity in the samples can then be identified. Knowledge of these factors ultimately will be used to plan strategies for resuscitation growth of bacteria in the sample.

Listed below are typical procedures for AODC and plate counts. These procedures will be used in initial experiments and prototype studies.

AODC:

1) 2.5 g of subsample are mixed with 22.5 ml 0.1% sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$), pH 7.0.
2) Flasks are agitated at 160 rpm, 25°C for 30 min.
3) After settling, portions of the suspension are fixed with glutaraldehyde (50% aqueous). Final glutaraldehyde concentration is 0.5% in each vial.
4) 1.0 ml of 1% aqueous Noble agar is added to each vial.
5) 5.0 l of suspension is transferred to a clean microscope slide with a delineated area of 1.0 cm^2.
6) To dried smears, filter-sterilized 0.01% aqueous acridine orange solution is added, and the slides are rinsed and blotted dry.
7) AO-stained smears are observed in an Olympus Vanox microscope using phase contrast and epiillumination.

Plate Counts:

1) 10 g of core material is transferred aseptically into dilution bottles containing 90 ml of diluent (2.2 mM KH_2PO_4 and 4.0 mM K_2HPO_4, pH 7.0).
2) Viable bacteria are enumerated by spread plate techniques on dilute peptone-tryptone-yeast extract-glucose (PTYG) agar.
3) Viable fungi are counted on 20-fold diluted potato dextrose agar, pH 5.6.
4) Plates are incubated for 2 weeks at 26°C in the dark.

As indicated in Subsection 2.2.1, other standard methods such as respiration, ATP, CO_2 evolution, and enzyme analysis will be performed by subcontractors. In addition, the general physiological types of bacteria will also be determined by subcontractors.

3.1.1 Quality Assurance Requirements and Required Technical Procedures

The data from this activity are expected to be used for the analysis of long-term sorption and desorption activity to be used in the repository license application. Therefore, the data from these activities will be graded in accordance with Project Office guidance, and the resulting grading reports will be submitted to the Project Office for review and approval. The work will be performed in accordance with the LANL Quality Assurance Program Plan, LANL-YMP-QAPP. All procedures will be approved and in place 30 to 60 days prior to the initiation of the work, including the following standard procedures:

1) DP 401, Maintenance of Culture Collection
2) a procedure for the collection of samples from the ESF,
3) a procedure for the shipment of samples collected from the ESF, and
4) procedures for the microbial analysis of samples collected from the ESF, to include procedures used by subcontractors.

3.2 Chelation

As stated in Subsection 1.1.2, through the production of siderophores, microorganisms are capable of solubilizing very insoluble metals. The experimental approach will be to isolate and purify siderophores from several microorganisms indigenous to Yucca Mountain and then to determine the formation constant of the siderophore and plutonium. This information can then be used to determine the potential for enhanced transport of actinide elements by chelation. The procedures that will be used have been taken from the literature and modified in our laboratory to yield the maximum production and purity of siderophores (Hersman, 1988a).

The medium used for most of the experiments consists of ($g \cdot l^{-1}$) K_2HPO_4, 6; KH_2PO_4, 3; $(NH_4)_2SO_4$, 1; $MgSO_4 \cdot 3H_2O$, 2; succinic acid, 4; pH = 7.0. Iron is removed by passing all of the constituents of the medium through a Chelex 100 column. The concentration of iron is adjusted to 1.0 mM. For large preparations, cultures are incubated at room temperature (22°C), while smaller preparations are incubated at 36°C. The presence of the siderophore is detected by the addition of an equal volume of $Fe(ClO_4)_3$ (5.0 mM $FeCl_3$ in 0.14 M $HClO_4$) to 1 ml of the spent medium. The sample is then centrifuged at 1,800 g for 5 min; a deep amber color in the supernatant indicates the presence of siderophore (Hersman, 1988a).

Three 2-l batches of the succinate medium are inoculated with a 24 h culture of a Pseudomonas sp. (isolated from the NTS) and incubated at room temperature under constant agitation (TWS-LS2-DP-401). After 40 h of incubation, the cultures are centrifuged (4,000 g, 30 min at 4°C) and the cell-free supernatant is evaporated under reduced pressure to approximately 100 ml. The concentrated material is then extracted with an equal volume of chloroform/phenol solution (1:1, v/v). The organic phase is treated with equal volumes of water and ether. The aqueous phase of this second partition is extracted two additional times with an equal volume of ether. The aqueous

phase of the final partition is further concentrated to about 10 ml. This concentrated aqueous phase is loaded onto a CM-Sephadex-C25 column and eluted with 0.1 M pyridine/acetic acid (pH 6.5; flow rate, 20 drops per min). Ten milliliter fractions are collected, and absorbance is measured at 280 nm. The fractions containing the siderophore are further purified on a Bio-Gel P2 column and eluted with glass-distilled water.

Siderophore Assay. The presence of siderophore is indicated by the presence of a deep amber color upon the addition of $Fe(ClO_4)_3$ to the spent medium. For the uninoculated controls, no color change is observed. This deep amber color also suggests the presence of a hydroxamate-type siderophore, as opposed to a catecholate-type, because according to Neilands (1981) and Emery (1971), upon addition of ferric iron to the spent medium, trishydroxamates turn orange-colored, while triscatacholates turn wine-colored.

High-Pressure Liquid Chromatography (HPLC) Purification. Approximately 50 mg of freeze-dried siderophore is reconstituted in 1 ml of HPLC-grade water and injected into an HPLC, equipped with a CN Bondapack column. The solvent gradient is 15 min of isocratic flow (H_2O), and 2 h linear gradient from 100% solvent A (100% H_2O) to 100% solvent B (50% H_2O, 50% acetonitrile). Each of the peaks is tested for siderophore content using the $Fe(ClO_4)_3$ assay.

Formation Constant With Pu(IV). Experiments first will be performed to test the feasibility of using Chelex 100 in competition experiments. Competition among Chelex and ethylenediaminetetraacetic acid (EDTA) for ferric iron is tested. A similar experiment is performed using siderophore instead of EDTA. The purpose of this experiment is to determine if siderophore will compete with Chelex for ferric iron. The concentration of siderophore is adjusted to approximately 0.014 M, based upon the assumption that the molecular weight of the siderophore is approximately 1,000 daltons.

Preliminary experiments demonstrated that both the siderophore and Chelex bind to Pu(IV). However, these experiments were very crude and demonstrated only that these competition experiments are feasible. Before the formation constants can be determined, several modifications of the preliminary experiments need to be performed.

First, although the procedures used to produce and purify the siderophore were taken from the literature, HPLC analysis of the siderophore suggested that a contaminant had mixed with the siderophore. The presence of a contaminant would cause an underestimation of the activity of the siderophore because either the contaminant diluted the activity of the siderophore or it interfered with the binding of the siderophore to Pu(IV), or both. Purification of the siderophore can be achieved by passing the siderophore through an HPLC column and by spectrophotometrically analyzing each fraction for a binding affinity with Fe(III).

Other modifications of the preliminary experiments will include the following:

1) Approximately 1.0×10^{-3} M Pu(IV) feed solutions will be prepared both in $NaNO_2$ (as done previously) and in carbonate buffers. Spectrophotometric analysis will be performed to determine the presence of colloids or changes in oxidation state.

2) Larger volumes of Chelex beads will be used in future experiments. In the preliminary experiments, a large percentage of the experimental error could be attributed to the measurement of the beads. In all probability, the variation did not result from the inaccuracy of pipetting, but rather from the variation in exchange capacity among the beads themselves. Pipetting a small number of beads can lead to a high variability of the exchange capacity. An easy way to reduce the error is to increase the volume of beads used in any given experiment.

3) By varying the concentrations of Chelex, siderophore, and actinide, sorption isotherms can be constructed. From these isotherms one can determine a formation constant of the siderophore/actinide element complex at a given pH and temperature. In order to construct isotherms, the concentrations of two parameters are held constant while the concentration of the third parameter is varied. The experiment is then repeated, varying the concentrations of the second parameter while holding the concentration of the first and third parameters constant. Finally, the experiment is repeated a third time, where the concentration of the first parameter is varied. This entire process is repeated two additional times to ensure reproducibility of the experiment. At the end of the experiment, a formation constant of the siderophore/actinide element can be determined. Then the process is repeated for another actinide element.

4) Several batch experiments will be performed to determine the ability of siderophores to affect the distribution of waste package radioactive wastes. These experiments will be designed to determine the relative capabilities of various siderophores to solubilize actinide elements in slurries containing crushed tuff taken from the Yucca Mountain site.

   Secondly, experiments will be designed to determine the selectivity of siderophores for actinide elements among a mixture of other metals (such as iron, aluminum, etc.) in solution.

5) For column experiments, tuff collected from the Yucca Mountain site will be crushed and sieved and packed into 2.5 x 30 cm glass columns. The columns will be sealed and reduced pressure (approximately 12 in. of Hg) will be applied to the columns. Using a peristaltic pump, J-13 well water will be added at a rate of approximately 0.2 $ml \cdot min^{-1}$. Then Pu, chelated with either EDTA, Desferal (commercial siderophore), or siderophores produced by microorganisms isolated from the Yucca Mountain site will be added to the columns. Unchelated Pu(IV) (as a sodium bicarbonate complex) will be added to the control columns. The breakthrough times of the control versus the chelated Pu(IV) will be compared.

As stated earlier, a Pseudomonas sp. isolated from the Yucca Mountain site will be used for these studies as well as for the sorption (Section 3.3) and colloidal agglomeration (Section 3.4) studies. Once methodologies have been developed for each study, other species of microorganisms will also be used. The species tested will be representative (as determined by the indigenous species study, Section 3.1) of the Yucca Mountain site; however, the numbers of species that can be tested will be limited by time and budget constraints.

3.2.1 Quality Assurance Requirements and Required Technical Procedures

The data from this activity are expected to be used for the analysis of long-term sorption and desorption studies to be used in the repository license application. Therefore, the data from these activities will be graded in accordance with Project Office guidance, and the resulting grading reports will be submitted to the Project Office for review and approval. The work will be performed in accordance with the LANL Quality Assurance Program Plan, LANL-YMP-QAPP. The procedures used in the chelation studies are nonstandard procedures that are either modifications of procedures taken from the literature or developed in the laboratory. In addition, the following standard detailed technical procedures will be required:

1) DP 401, Maintenance of Culture Collection

2) a procedure for the determination of the formation constant between actinide elements and microbially-produced chelating agents. This procedure will be approved and in place 30 to 60 days prior to the initiation of the work.

3.3 Sorption

As stated in Subsection 2.1.3, the location of the actinide deposition will be determined by performing a series of simple experiments. First, the external constituents of the cells need to be examined for activity. Cells would be washed with mild buffers or mild acids and examined. Next, the cells would be ruptured, and using differential centrifugation, the constituents of the cell (membrane, ribosomes, DNA, cytoplasmic material) would each be examined for activity. Finally, a mass balance of the actinide could be performed to determine the proportion of actinide located on the external membrane, the internal membrane, and among the cytoplasmic constituents.

Although previous studies have demonstrated that a microorganism isolated from the NTS strongly sorbs Pu(IV), no information exists regarding the fate of the microbial/actinide complexes (Hersman, 1986). It is not known if the sorption of an actinide by a microorganism increases or decreases the transport of the actinide through the environment. The following experiments will address this question.

First, using welded tuff, 4-mm tuff wafers will be placed between two "L"-shaped tubes, 2-cm internal diameter. The tuff wafer will first be examined for fractures using a light microscope, low power (100x). If fractures are observed, the wafer will be discarded. Nonfractured tuff wafers will be held between the two tubes by epoxy glue, and the entire unit will be sterilized by repeated cycling between 200°C for (24 h) and room temperature (22°C for 24 h) for one week. Each tube, on either side of the wafer, will be filled with 10 ml of sterile nutrient broth. To ensure that sterile conditions are established within the unit, it will be incubated at room temperature for 72 h. Then, one of the two tubes will be inoculated with a 24-h culture of a microorganism. The unit will again be incubated at room temperature, until the microorganisms are detected in the second tube.

In the second experiment, a series of column studies using crushed rock collected from either or both the Calico Hills and Topopah Spring Members of the Yucca Mountain formations will be performed. The unsaturated transport of actinide elements through sterile columns will be compared with transport rates through columns inoculated with microorganisms. These experiments will be conducted similarly to the column experiments described in Section 3.2. Crushed rock will be used in preliminary experiments, intact soil cores will be used in subsequent experiments.

3.3.1 Quality Assurance Requirements and Required Technical Procedures

The data from this activity are expected to be used for the analysis of long-term sorption studies (8.3.1.3.4.1) to be used in the repository license application. Therefore, the data from these activities will be graded in accordance with Project Office guidance, and the resulting grading reports will be submitted to the Project Office for review and approval. The work will be performed in accordance with the LANL Quality Assurance Program Plan, LANL-YMP-QAPP. The procedures used in the sorption studies are nonstandard procedures that are either modifications of procedures taken from the literature or developed in the laboratory. In addition, the following standard detailed technical procedures will be required:

1) DP 401, Maintenance of Culture Collection
2) a procedure for the sorption of actinide elements to microorganisms. This procedure will be approved and in place 30 to 60 days prior to the initiation of work.

3.4 Colloidal Dispersion

The purpose of this research is to describe the mechanism(s) by which microorganisms influence the agglomeration of colloidal particles. Colloidal particles have been implicated in the transport of actinide elements (Buddemeir and Hunt, 1987); however, in order for colloids to transport actinide elements, the colloids must remain in suspension as individual particles. If the colloids begin to agglomerate, forming aggregates, then colloidal dispersion would no longer occur. As stated earlier, preliminary results indicate that the presence of microorganisms significantly increases the agglomeration rate. Using particle-size separation procedures, experiments will be designed to study agglomeration rate.

The methods that will be used represent the easiest and most cost-effective means of measuring particle distribution. In initial experiments, Wyoming bentonite clay will be used because it is well-studied and readily available. Later, crushed tuff samples, collected from the Yucca Mountain site, will be used. Clay particles, ranging in size from 0.2 mm to 2.0 mm will be equilibrated in J-13 well water for 48 h. Clay particles will then be added to solutions of spent growth medium, and the change in particle size distribution over time (1 to 200 h) will be compared with clay particles added to sterile, uninoculated growth medium (control).

Spent growth medium will be prepared by inoculating microbial growth media, such as trypticase soy broth, nutrient broth, etc. with microorganisms isolated from the Yucca Mountain site. Spent medium is being used for these studies because it contains extracellular metabolic products that are believed to facilitate agglomeration. Cell growth will be monitored photometrically until the cells have reached stationary phase. The cells will be removed by centrifugation, and the supernatant (spent medium) will be filter sterilized. The tube centrifugation methods detailed in Soil Chemistry Analysis, Advanced Course will be used to separate and to characterize size distribution of the colloidal particles and agglomerates.

Agglomerate Stability. Agglomerate stability will be determined by subjecting the agglomerates to disruptive forces such as sonication, surfactants, grinding, and agitation. Information gained from these studies would be used to assess the stability of agglomerates in the environment.

Threshold. It will be important to determine the particle density that is needed to initiate agglomeration. This can be accomplished by varying the particle concentration and measuring agglomerate formation. The information obtained from this study will be essential to determine if agglomerate formation would occur in the field (i.e., are there enough particles in the field to initiate agglomerate formation?).

Particle Distribution. Particle distribution within the agglomerates will be determined by analyzing the individual particles that are obtained from the agglomerate stability studies. Following disruption, all of the particles will be accounted for to determine the ratio of bacteria to clay colloids. This can be accomplished by differential staining and light microscopy. For example, acridine orange fluorescent stains are used routinely to differentiate bacterial cells from soil particles. The laboratory is equipped to perform AODC using epifluorescence microscopy.

3.4.1 Quality Assurance Requirements and Required Technical Procedures

The data from this activity are expected to be used for the analysis of long-term sorption and desorption studies to be used in the repository license application. Therefore, the data from these activities will be graded in accordance with Project Office guidance, and the resulting grading reports will be submitted to the Project Office for review and approval. The procedures used in the colloidal agglomeration studies are nonstandard procedures that are either modifications of procedures taken from the literature or developed in the laboratory. The work will be performed

in accordance with the LANL Quality Assurance Program Plan, LANL-YMP-QAPP. In addition, the following standard detailed technical procedures will be required:

1) DP 401, Maintenance of Culture Collection
2) a procedure for the determination of microbial effects on colloidal agglomeration. This procedure will be approved and in place 30 to 60 days prior to the initiation of work.

3.5 Equipment and Services

The following equipment will be used during the Biological Sorption and Transport Studies:

1) nitrogen atmosphere glove box,
2) rock-coring equipment,
3) ceramic ball grinders,
4) epifluorescence microscope,
5) high-speed refrigerated centrifuges,
6) rotary evaporation equipment,
7) walk-in cold room,
8) inductively-coupled plasma atomic adsorption spectrophotometer,
9) scanning spectrophotometers (Near IR-VIS-UV),
10) integrating recorders,
11) lyophilizer,
12) high-pressure liquid chromatograph,
13) liquid scintillation counter,
14) vadose zone soil column apparatus, and
15) image analysis system.

In order to analyze samples taken from the ESF (Section 3.1), it will be necessary to first conduct the following prototype work:

1) test hole drilling at LANL (prototype),
2) measurement of direct counts (prototype),
3) measurement of dilution/plate counts (prototype), and
4) biochemical analysis (prototype).

All procedures will be approved and in place 30 to 60 days prior to the initiation of the ESF work.

3.6 Representativeness of Microbiological Effects on Actinide Transport

A question can be raised regarding the representativeness of laboratory versus field studies. It should be stressed that the laboratory studies investigate processes by which the performance of the repository may be compromised. Laboratory studies are performed under optimum conditions, i.e., physical and chemical conditions are designed to optimize microbial growth effects.

As stated in Subsection 2.1.1, laboratory studies are being performed in order to determine the potential for microbial participation in actinide transport processes. However, laboratory tests by themselves bear little resemblance to the microbial processes that are found within Yucca Mountain. It is only when combined with the indigenous species studies (Section 3.1) that the laboratory studies become meaningful. The indigenous species studies will provide information on the overall level of microbial activity in Yucca Mountain. This activity level, or index, will be used to modify the laboratory studies in such a way as to predict microbial effects. This concept is represented in the following equation:

Laboratory results x Indigenous index = Predicted effect.

It is therefore through a combination of laboratory studies and indigenous species studies that one can predict the effects that microorganisms may have on the transport of radioactive wastes.

It should be emphasized that indigenous species studies are not the same as field studies. Indigenous species studies are designed to determine the types, numbers, and metabolic activity of microorganisms in the environment, whereas field studies are direct measurements of the microbial effects on actinide transport, either in the field or under field conditions in the laboratory.

Field experiments are not planned for this study because field studies are very difficult to perform and are subject to large experimental error. Conversely, laboratory studies under field conditions are not planned because it is nearly impossible to reconstruct field conditions in the laboratory.

4.0 APPLICATION OF RESULTS

4.1 Resolution of Design and Performance Issues

Biological Sorption and Transport studies will address several Information Needs. The information gained from these studies will be used for 1.1.1 (site information to calculate the releases of radionuclides to the accessible environment), for 1.9.1 (determination that the site is not now nor likely to be disqualified for each of the disqualifying conditions), and for 1.9.2 (determination that the site meets and is likely to continue to meet the qualifying conditions of the technical guidelines).

4.2 Interfaces with Other Site Characterization Studies

The results of the Biological Sorption and Transport studies will be used by the Retardation Sensitivity Analysis Study, 8.3.1.3.7.1. Specific information on microbial interactions with actinide elements, such as effects on actinide solubility, colloidal distribution, and transport by microorganisms, will be provided to the Retardation Sensitivity Analysis Activity. This information will then be incorporated into an overall transport model to determine the significance of the microbial population with respect to the performance criteria.

The activities of microorganisms are influenced significantly by the chemical and physical conditions of the environment. Therefore, investigation 8.3.1.2.2 (studies to provide a description of the unsaturated zone hydrologic system at the site) and 8.3.1.2.3 (studies to provide a description of the saturated zone hydrologic system at the site) are directly related to the microbiological study, and will be a source of important information, such as pH, complexing agents, redox potential, etc.

5.0 SCHEDULE AND MILESTONES

The following is a list of milestones. Figure 5.1 is a graphic representation of the dates and activities.

Number	Description
3176	Procedure for the Determination of Formation Constants Between Actinide Elements and Microbially-Produced Chelating Agents
3177	Procedure for Determining Microbial Effects on Colloidal Agglomeration (slide)
3080	Chelation (publication)
3092	Colloidal Agglomeration (slide)
3178	Analytical Methods and Procedures for Exploratory Studies Facility
3179	Microbial Effects on Transport: Lab Studies
3018	Sorption of Actinide Elements by Microorganisms Indigenous to Yucca Mountain
3094	Microbiological Activity and Its Influence on Sorption
T537	Microbial and Colloidal Transport Models

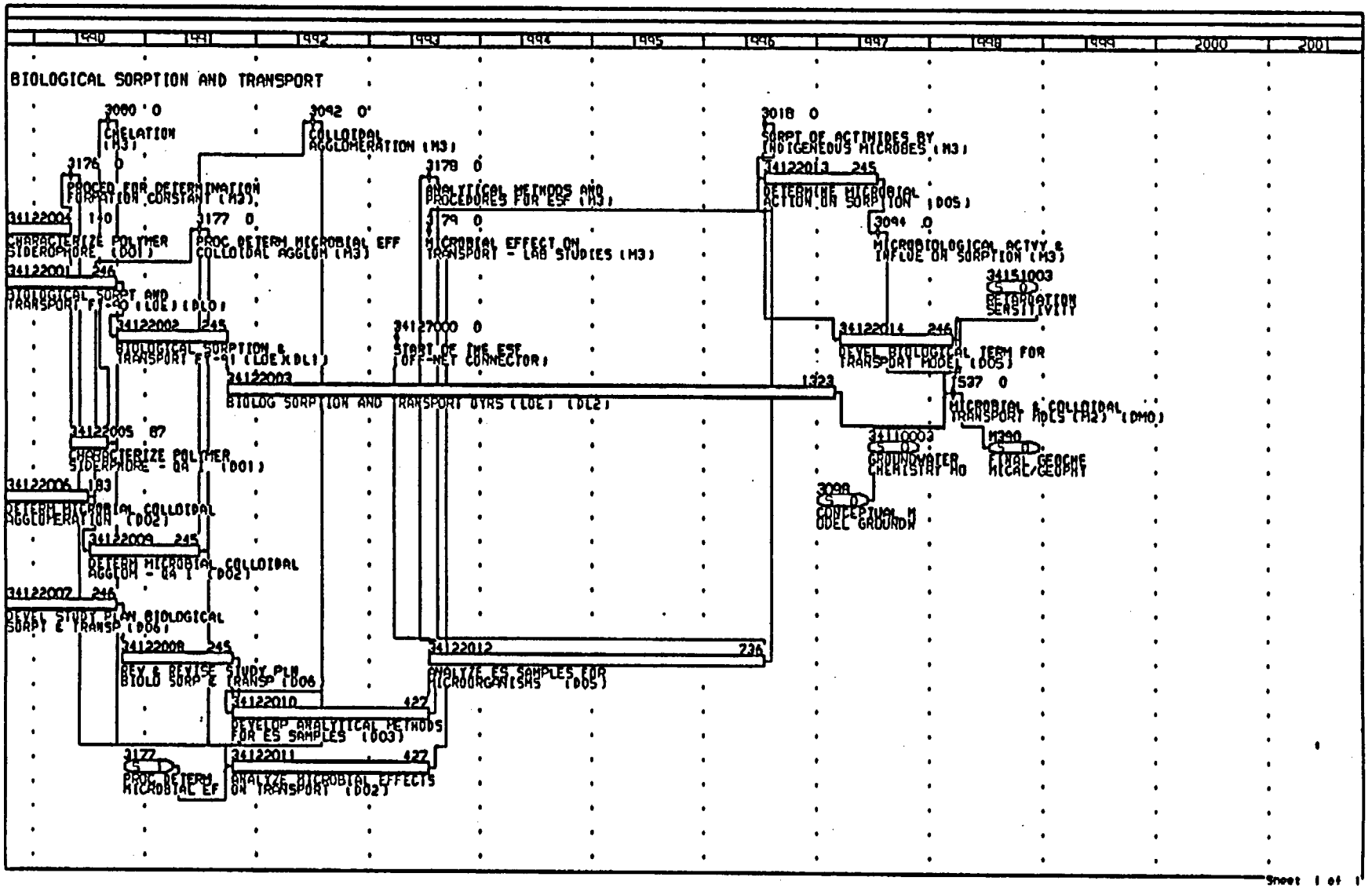

Figure 5.1 Schedule and Milestones for Biological Sorption and Transport

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APPENDIX A

QUALITY ASSURANCE SUPPORT DOCUMENTATION

NOTE: A quality assurance grading report for the study's WBS element will be prepared before the start of work in accordance with applicable YMP Office guidance.

TABLE A-1

APPLICABLE NQA-1 CRITERIA FOR SCP STUDY PLAN 8.3.1.3.4.2
AND HOW THEY WILL BE SATISFIED

NQA-1 Criteria	Documents Addressing These Requirements		Date of Issue (Anticipated)
1. Organization	The organization of the Office of Civilian Radioactive Waste Management (OCRWM) program is described in Section 8.6 of the SCP. The LANL QA organization is described in the LANL-YMP-QAPP.		
	TWS-QAS-QP-01.1	Interface Control	03/19/90
	TWS-QAS-QP-01.2	Stop Work Control	02/20/89
	TWS-QAS-QP-01.3	Conflict Resolution	03/04/89
2. QA Program	The LANL QA program is described in the LANL-YMP-QAPP and includes a program description addressing each of the NQA-1 criteria. An overall description of the YMP QA program for site characterization activities is described in Section 8.6 of the SCP. The LANL QA program contains quality implementing procedures (QP) further defining the program requirements.		
	TWS-QAS-QP-02.3	Readiness Review	03/19/90
	TWS-QAS-QP-02.4	Management Assessment	06/05/89
	TWS-QAS-QP-02.5	Selection of Personnel	03/02/90
	TWS-QAS-QP-02.6	Personnel Orientation and Indoctrination	08/17/90
	TWS-QAS-QP-02.7	Personnel Training	08/17/90
	TWS-QAS-QP-02.9	Personnel Proficiency Evaluations	03/02/90
3. Design and Scientific Investigation Control	This study is a scientific investigation. The following QPs apply:		
	TWS-QAS-QP-03.2	Preparation and Technical and Policy Reviews of Technical Information Products	05/09/89
	TWS-QAS-QP-03.3	Preparation and Review of an SCP Study Plan	05/24/89
	TWS-QAS-QP-03.5	Documenting Scientific Investigations	03/10/89

TABLE A-1

APPLICABLE NQA-1 CRITERIA FOR SCP STUDY PLAN 8.3.1.3.4.2
AND HOW THEY WILL BE SATISFIED
(continued)

NQA-1 Criteria	Document Addressing These Requirements		Date of Issue (Anticipated)
	TWS-QAS-QP-03.7	Peer Review	05/24/89
	LANL-YMP-QP-03.17	Reviews of Software and Computational Data	01/25/91
	LANL-YMP-QP-03.18	Creation, Management, and Use of Computational Data	01/25/91
	LANL-YMP-QP-03.19	Documentation of Software and Computational Data	01/25/91
	LANL-YMP-QP-03.20	Software Configuration Management	01/25/91
	LANL-YMP-QP-03.21	Software Life Cycle	01/25/91
	LANL-YMP-QP-03.22	Verification and Validation of Software and Computational Data	01/25/91
4. Procurement Document Control	LANL-YMP-QP-04.4	Procurement of Commercial-Grade Items and Services	12/10/90
	LANL-YMP-QP-04.5	Procurement of Noncommercial-Grade Items and Services	12/10/90
5. Instructions, Procedures, Plans, and Drawings	Applicable parts of this criterion are covered in Item 6.		
6. Document Control	LANL-YMP-QP-06.1	Document Control	11/16/90
	LANL-YMP-QP-06.2	Preparation, Review, and Approval of Quality Administrative Procedures	10/10/90
	LANL-YMP-QP-06.3	Preparation, Review, and Approval of Detailed Technical Procedures	10/10/90
7. Control of Purchased Items and Services	Applicable parts of this criterion are covered in Item 4.		

TABLE A-1

APPLICABLE NQA-1 CRITERIA FOR SCP STUDY PLAN 8.3.1.3.4.2
AND HOW THEY WILL BE SATISFIED
(concluded)

NQA-1 Criteria	Document Addressing These Requirements		Date of Issue (Anticipated)
8. Identification and Control of Samples and Data	TWS-QAS-QP-08.1	Identification and Control of Samples	10/10/89
	TWS-QAS-QP-08.2	Control of Data	08/23/89
9. Control of Processes	This criterion has been determined to be inapplicable to the scope of work of the LANL YMP.		
10. Inspection	This criterion has been determined to be inapplicable to the scope of work of the LANL YMP.		
11. Testing	This criterion has been determined to be inapplicable to the scope of work of the LANL YMP.		
12. Control of Measuring and Test Equipment	The control of instrument calibration and data collection is described in the technical procedures referenced in Section 3 of the LANL-YMP-QAPP. The following QP also applies:		
	TWS-QAS-QP-12.1	Control of Measuring and Test Equipment	02/20/90
13. Handling, Shipping, and Storage	TWS-QAS-QP-13.1	Handling, Storage, and Shipping Equipment	11/03/89
14. Inspection, Test, and Operating Status of Engineered Items	This criterion has been determined to be inapplicable to the scope of work of the LANL YMP.		
15. Control of Nonconformances	TWS-QAS-QP-15.2	Deficiency Reporting	04/03/90
16. Corrective Action	TWS-QAS-QP-16.2	Trending	06/20/89
17. Records	LANL-YMP-QP-17.3	Records Management	01/11/91
18. Audits	LANL-YMP-QP-18.1	Audits	03/01/91
	TWS-QAS-QP-18.2	Surveys	05/30/90
	TWS-QAS-QP-18.3	Auditor Qualification	05/30/90

The following is for the Office of Civilian Radioactive Waste Management Records Management purposes only and should not be used when ordering this document:

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