REVIEW OF THE DOE STUDY PLAN FOR BIOLOGICAL SORPTION AND TRANSPORT (8.3.1.3.4.2)

by
Paul Bertetti and David R. Turner, Center for Nuclear Waste Regulatory Analyses
Matthew Alexander, Southwest Research Institute
June 18, 1993

1.0 INTRODUCTION

Within the past few years, the potential impact of subsurface microorganisms on the geochemistry, hydrology and mineralogy of geologic materials has gained increased recognition. Specifically, microorganisms can retard or mobilize geochemical constituents. Enhancement of radionuclide transport can be influenced by:

- microbiological effects on groundwater chemistry;
- enhanced radionuclide solubility due to metabolic activity;
- transport by biological movement, and;
- stabilization of colloidal phases.

Conversely, microbial activity may enhance retardation by:

- plugging of porosity by microbes, and;
- sorption of radionuclides on an immobile biological phase.

Evidence for the existence of microbes in deep subsurface unsaturated environments, coupled with the introduction of large amounts of nutrient laden substrate, emphasize the need for assessment of microbial activity at the proposed high-level nuclear waste repository at Yucca Mountain. If significant microbial activity is present at Yucca Mountain, disregarding the influence of microbial processes could have adverse effects on the accuracy of modeling transport phenomena. Because predicting the ability of the geologic setting to retard possible migration of radionuclides from a high-level waste repository to the accessible environment during postclosure is one of the key measures of repository performance assessment, evaluating microbial activity is therefore an important aspect of the site characterization program. The data obtained in the proposed study is designed to provide information on types of organisms present at Yucca Mountain, radionuclide sorption on microorganisms, microorganism-mediated transport, and effects on colloidal stability (page 5 of the Study Plan).

2.0 REVIEW BACKGROUND

A Preliminary Review of the Study Plan for Biological Sorption and Transport, Revision 2.0 (8.3.1.3.4.2) was completed on March 16, 1993 (HLWM, 1993; HLPD, 1993) using the Review Plan for NRC Staff Review of DOE Study Plans, Revision 2 (NRC, 1993). The preliminary review determined that since the work outlined in the Study Plan (SP) involved only laboratory analyses, there will be no adverse effects on repository performance, and no objections were identified for any of the proposed activities. However, the review also determined that there were a number of aspects of the SP that warranted detailed comments and/or questions.

Although Study 8.3.1.3.4.2 was not formally divided into activities in the Site Characterization Plan (SCP; DOE, 1988), four general areas of research were described:

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 on retardation and transport of radionuclides, and;

4) Identification of the microorganisms.

The current Study Plan also has four major areas of research, but has reorganized these from what was originally described in the SCP. The study plan states that Area 1 is substantially complete, and further states that V-max and actinide speciation studies are beyond the needs of the current study (pg. 5 of the Study Plan). Cellular location will be covered in Section 1.1.3 (Sorption). Area 2 is discussed in Sections 1.1.2 (Chelation), 1.1.3 (Sorption), and 1.1.4 (Microbial Effects on Colloidal Dispersion and Actinide Transport). Area 3 is also folded into the results obtained from Sections 1.1.2, 1.1.3, and 1.1.4. Area 4 (Identification of the Microorganisms) has remained as a major area of research (Section 1.1.1).

In terms of open items, the Site Characterization Analysis (SCA; NRC, 1989) contained one comment (#29) directly related to Study Plan 8.3.1.3.4.2.

3.0 REVIEW CRITERIA

The detailed review of this Study Plan is based on the Review Plan for NRC Staff Review of DOE Study Plans, Revision 2 (NRC, 1993). Specifically, the detailed review considers:

- Whether the objectives of the study plan are consistent with the investigation presented in the SCP and are technically defensible in the context of the overall site characterization program;
- Whether the activities, tests, and analyses proposed in the SP are able to provide the data necessary for licensing;
- Progress towards resolution of open items.

4.0 DISCUSSION

The stated objectives of the SP are 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." (pg. 4 of the SP). The SP recognizes that the study of microorganisms is not, in and of itself, the goal of the study, and that any data obtained is only useful in terms of evaluating the effect on radionuclide transport. The SP concentrates on the microbes present at Yucca Mountain, and on the actinides plutonium, americium, and neptunium.

The SP is divided into four major areas of research:

1) Indigenous Microorganisms - collection, cultivation, isolation and identification of microorganisms found at Yucca Mountain, and an examination of their metabolic activity;

2) Chelation - evaluation of the effect of microbially produced chelates (siderophores) on actinide transport/sorption;

3) Sorption - determination of the cellular location of sorbed actinides, and an assessment of the mobility of actinides due to mobility of microbes, and;

4) Microbial Effects on Colloidal Dispersion and Actinide Transport - assessment of the influence of microorganisms on the agglomeration of colloidal particles.

Organization of the SP allows for the grouping of tasks so that effects on sorption and transport due to chelation, cellular location and microbial movement, and colloidal dispersion can be characterized independently. The SP is likely to provide most of the data identified in the SCP. It is of particular interest in that it attempts to measure effects of chelation and microbial transport in unsaturated column experiments.

As mentioned above in section 2.0, the activities outlined in the SP do not in general coincide with those originally outlined in the SCP. Also listed in the SCP, but not considered in the Study Plan, are experiments relating microbial sorption to actinide speciation and V-max. Another task described in the SCP, determination of growth of microorganisms on fluids (e.g. drilling fluid) is also not discussed in the current Study Plan. In the introduction to the SP, drilling fluids are mentioned as a possible nutrient and reason for concern with microbial activity. However, despite this, the proposed experiments do not follow through with studying the role of drilling fluids as a nutrient, or their effect on microbial activity.

The work focuses on using indigenous microorganisms in the Yucca Mountain environment. Only laboratory experiments are planned, and the proposed activities will not affect the repository block. Extrapolation of laboratory results to field scale will involve a proportionality constant called the indigenous index in the Study Plan. The SP does not offer much justification for this simple linear extrapolation, nor is there much detail on how this index will be determined (See Comment #1). Samples are to be collected from the Exploratory Studies Facility (ESF), and timetables will be controlled to some extent by the construction schedule for the ESF. Unless there is a delay in constructing the ESF, there is no proposal for sampling wells or drill core, which seems to limit the representativeness of the sampling program. The application of these results is described in Section 4.0 of the SP.

The experiments proposed in the SP focus on the actinides, especially plutonium, americium, and neptunium. However, despite the importance of uranium in the inventory at Yucca Mountain (Kerrisk, 1985), and the evidence cited in the study for microbial uptake of uranium (e.g., Strandberg et al., 1981; 1982; Dreher, 1981), references to uranium studies are absent from the SP (See Question #2).

With regard to open items, Specific Comment #29 in the SCA (NRC, 1989) is directed at this Study as originally proposed in the SCP. Among other things, the comment is concerned chiefly with "...the effects of radioactive decay heat, the radiation field, and the effect of non-site specific microorganisms (introduced during site construction) on microbial activity and ecology..." (NRC, 1989; pg. 4-32). The comment recommends that activities, procedures and methods be included in the study which consider these effects. In the current SP, there is no direct response to this open item, and no clear attempt to

address the issues raised by Comment #29. The reviewers must therefore recommend to leave the item open.

4.1 Research Area #1 - Indigenous Microorganisms

Overall, the sampling and processing methodology is weakly documented and not well supported with references. Considering the importance of defensible numbers when applying the indigenous index (pg. 21-22), this aspect of the study should be clearly outlined. Details on techniques to ensure field sample integrity and quality assurance (QA) are not provided (See Question #3).

Additionally, little information is given with respect to how microbial activity in field samples will be determined (See Question #6). Those references cited do not contain appropriate procedures for estimation of environmental microbial activity, and the mention of other journal publications seems rather vague (note: the edition of "Standard Methods..." referenced is out of date; the most recent edition contains major revisions in the areas covering microbial analyses).

The difficulties of inducing environmental microbes to grow on laboratory media are discussed. This discussion includes methods to determine the ideal means for resuscitation and cultivation, but it does not describe where such work will be performed and on what samples these methods will be tested. Additionally, information regarding the factors that have the most effect on stimulation of microbial activity is not mentioned as being used to identify critical areas in the field (See Question #1).

An indigenous index is described for linear extrapolations from laboratory results to field conditions. This index is described as being applied based on numbers of organisms counted (pg. 6) and, later, based on relative differences in activity (pg. 21). These parameters are not necessarily equivalent, and there is no clear indication of which one will be used to determine the index (See Comment #1).

It is noted that the procedure outlined on page 15 for acridine orange direct count (AODC) calls for 5.0 L of suspension to be transferred to clean microscope slides. It is assumed that a smaller amount is intended, perhaps 5.0 mL or 5.0 μL.

4.2 Research Area #2 - Chelation

Part of the concern is based on the observed solubilization of Fe^{+3} by siderophores and a presumed analog between Fe^{+3} and Pu^{+4}. Procedures for the investigation of the influence of chelating agents produced by microbes on the transport of actinides are described in detail. Because the Study does not consider actinide speciation, only one oxidation state of each actinide will be studied (e.g. Pu^{+4}). However if Pu, Am or Np species distribution is sensitive to chemical changes brought on by microbial metabolism, this may affect species available to be chelated. Likewise, pH and Pu concentration are controlled to prevent polymerization of Pu, but the potential for polymerization data to be used in the sorption or colloid activities is not mentioned (See Question #7).

The siderophore to be used in initial experiments is isolated from a *Pseudomonas* sp. previously isolated from surface samples at the Nevada Test Site. The potential for a lack of siderophore producers to be collected and isolated from field samples, or the fact that some siderophore producers are anaerobic is not included in the discussion (See Question #4).

An especially promising aspect of Research Area #2 is the unsaturated column experiment. Data gathered in this area would be of extreme benefit to the assessment of chelate mediated transport at Yucca Mountain. The SP does not give any indication of how initial conditions for the apparatus to be used in the unsaturated column experiment were selected or how sterilized tuff may have differing sorption characteristics than native tuff (Jenneman et al., 1986).

4.3 Research Area #3 - Sorption

Research Area #3 seeks to identify the location of sorbed actinides with respect to cell structure, and seeks to evaluate the potential for actinide transport due to the mobility of microbes containing sorbed actinides. Experimentation to identify the cell location of sorbed actinides is straightforward and should provide information regarding the "intensity" of binding to the microbe.

Examination of transport due to movement of microorganisms is based on the premise that microbes will be available for movement within liquid medium. It would be expected however, that given the environmental stress on the Yucca Mountain population, most microbes will be attached to geological substrate (Kjelleberg *et al.*, 1983; Pringle and Fletcher, 1986). If most organisms are attached and not readily available for transport in the liquid phase, experiments with free floating bacteria may result in an overestimation of the influence of the mobility of organisms on transport (See Question #11).

Saturated wafer experiments designed for Research Area #3 specifically select against samples containing fractures and are performed under saturated conditions. In the wafer experiment it is not clear how microbial presence will be detected in the tubes. The presence of light could affect transport/movement of the organisms used. Moreover, although the wafer is saturated with nutrient media, no investigation for studying the effects of the media on the tuff (surface wetting, etc.) is presented (See Question #12).

Like Research Area #2 - Chelation, an unsaturated column experiment is designed to be run. In Research Area #3 the experiment evaluates transport due to sorption of actinides onto microbes. One of the initial conditions for the experiment is that an inoculated column be used. There is no discussion of how the column will be inoculated or how the presence of nutrient media in the column might affect results.

4.4 Research Area #4 - Colloidal Dispersion

This activity seeks to evaluate the effects of microorganisms on the agglomeration of colloidal particles and to evaluate the stability of those agglomerates. It is mentioned that microbes are known to absorb to clays (which presumably make up the majority of colloidal particles, besides microorganisms, in nature), but no mention is made of the expected distribution of clays or other particles (colloids) at the site (See Question #14). (Note: The particle sizes listed in the SP do not reflect clay size fractions. Does the author really mean 0.2 to 2.0 micrometers instead of 0.2 to 2.0 mm?)

Spent media is used in the experiments to provide the products thought to produce microbially induced agglomeration. There is no mention of how different growth medium may influence the production of metabolic by-products which cause agglomeration or how a mixed culture might better represent the indigenous population's effect at Yucca Mountain (See Question #13). A threshold level of particle concentration necessary for agglomeration is investigated, but there is no discussion on attempts to evaluate a threshold for bacterial population or by-product concentration. Agglomerate stability is then tested versus several physical parameters (e.g. agitation). The J-13 well water (to be used as a dispersant medium) is sterile, but there is no mention of filtration to remove cells in the water that may affect

results. The final portion of the colloid experiments describes agglomerate particle characterization with respect to association of cells, yet filtered spent media is used in the agglomeration experiments. It is not clear where the cells appear in the process (See Question #15).

An unsaturated column experiment as described in Research Areas #2 and #3 is not included to evaluate the transport of agglomerates.

5.0 SUMMARY

The SP proposes a series of laboratory experiments to determine the effects of four major microbial processes on actinide transport in Yucca Mountain. The usefulness of the results depends on the application of these data to radionuclide transport issues at Yucca Mountain. To correlate laboratory data to field conditions the use of a factor, or indigenous index, is proposed. Unfortunately, the basis on which the factor will be derived is not explicitly stated. This vagueness could lead to wide disparities in interpretation of how the laboratory results have been applied. Additionally, the integration of the SP with other related projects is only briefly mentioned. The results of the individual components of this Study could have significant impact on the investigations of transport by inorganic processes. For example, information regarding the nature of colloids at Yucca Mountain (Activity 8.3.1.3.4.1.4) could be used to set the initial conditions for Research Area #2 - Chelation. The results of the agglomeration tests could then be transferred directly to colloid transport studies. Likewise, information from Research Area #2 could be directly input to studies of sorption of actinides on tuff.

6.0 REFERENCES

Department of Energy (DOE). 1988. *Site Characterization Plan: Yucca Mountain Site, Nevada Research and Development Area, Nevada.* Office of Civilian Radioactive Waste Management (OCRWM), DOE/RW-0199. Washington, D.C.

Dreher, K.T., 1981. *Removal of Uranium and Molybdenum from Uranium Mine Wastewaters by Algae.* M.S. Thesis, New Mexico Institute of Mining and Technology, Socorro, New Mexico.

HLWM. 1993. Memorandum to Joseph Holonich from Margaret Federline on the Preliminary Review of Study Plan 8.3.1.3.4.2, Biological Sorption and Transport. 03-16-93.

HLPD. 1993. Letter to Dwight E. Shelor from Joseph J. Holonich on the Phase I Review of U.S. Department of Energy (DOE) Study Plan "Biological Sorption and Transport." 03-25-93.

Jenneman, G.E., M.J. McInerney, M.E. Crocker, and R.M. Knapp, 1986. Effect of sterilization by dry heat or autoclaving on bacterial penetration through Berea Sandstone. *Applied and Environmental Microbiology*, 51:39-43.

Kerrisk, J.F. 1985. *An Assessment of the Important Radionuclides in Nuclear Waste.* Los Alamos National Laboratory. LA-10414-MS. Los Alamos, New Mexico.

Kjelleberg, S., B.A. Humphrey, and K.C. Marshall, 1983. Initial phases of starvation and activity of bacteria at surfaces. *Applied and Environmental Microbiology,* 46:978-984.

U.S. Nuclear Regulatory Commission (NRC). 1989. *NRC Staff Site Characterization Analysis of the Department of Energy's Site Characterization Plan, Yucca Mountain Site, Nevada.* NUREG-1347. Office of Nuclear Material Safety and Safeguards. Washington, D.C.

U.S. Nuclear Regulatory Commission (NRC). 1993. *Review Plan for NRC Staff Review of DOE Study Plans, Revision 2, March 10, 1993.* Division of High-Level Waste Management, Office of Nuclear Material Safety and Safeguards. Washington, D.C.

Pringle, J.H. and M. Fletcher, 1986. Influence of substratum hydration and adsorbed macromolecules on bacterial attachment to surfaces. *Applied and Environmental Microbiology*, 51:1321-1325.

Strandberg, G.W., S.E. Shumate, and J.R. Parrot, 1981. *Applied and Environmental Microbiology*, 41: 237-245.

4 SPECIFIC OBJECTIONS, COMMENTS, AND QUESTIONS

4.1 Objections

There are no objections to this Study Plan

4.2 Comments

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport

Comment 1

The approach described in the SP for using an indigenous index to relate the results of laboratory experiments to actual field conditions is a logical approach for the circumstances of this study, but it is oversimplified as explained in the SP.

Basis

In paragraph 3 of Section 1.1.1 (pg. 6), the statement is given that "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." An example is given for reducing laboratory results by 1000-fold in the case that experiments are performed with 10^8 cells/ml when only 10^5 cells/gram dry soil are found in the field sample.

A similar example of proportional scaling is given regarding microbial activity and chelation of actinides in the first paragraph of Section 2.1.1 (pg. 10). The ability to extrapolate between laboratory and field results is posed as a basis for constructing "worst case scenarios" (pg. 10).

Finally, Section 3.6 (pg. 21), states "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." The concept is represented as an equation given at the top of page 22, namely that:

- Laboratory results × Indigenous index = Predicted effect.

The SP intends to construct a linear relationship between lab derived data and field activity (pg. 22), however, it is not clear what will influence the indigenous index. The application of lab results clearly depends on the factor used, and the SP makes no statement that details how the indigenous index will be calculated or derived. Vague or nonspecific formulation of the index can only lead to speculation about how the results of the study are to be applied.

First, the description of this approach strongly implies a simple proportional relationship between laboratory results and possible field effects. This may be suitable in some cases, but a working knowledge of bacterial metabolism, especially considering the complexities of field environmental conditions, would strongly suggest that the relationships may be more complex in many cases.

Are numbers of organisms or measured metabolic activity more important? Neither has a proven linear relationship with *in situ* activity. Are possible weighting criteria involved? Items that might affect weighting are: the overall estimated metabolic activity based on CO_2 respiration, total biomass, number of cells per dry weight soil, activity of dominant organisms found (number and biomass), the influence of anaerobes, and activity along preferred flow paths. Issues related to inorganic chemistry might further confound data interpretation to derive an indigenous index.

A good example of greater complexity between field and laboratory conditions is given by the case of siderophore chelation of actinides. The production of siderophores is induced by the lack of available solubilized iron (Neilands, 1981). It is stated that the laboratory microbial experiments will be carried out under optimal growth conditions (paragraph 3 of Section 1.1.1, pg. 6)--implying plentiful iron, whereas the field environment is very likely to have little iron directly available for bacterial growth. Therefore, bacteria in the field could have a significantly higher cell-specific rate of siderophore production than those tested in the laboratory. This is opposite to the implication made with regard to chelation and metabolic activity in the first paragraph of Section 2.1.1 (pg. 10).

This relation of field and laboratory effects of actinide chelation is further complicated in that the SP (in any sections on chelation or on indigenous microorganisms) does not provide any details on the measurement of metabolic activities related to siderophores or siderophore levels of the indigenous microorganisms which are needed to determine an indigenous index for the chelation process. For example, if siderophore-chelated actinides are transported without any microbial uptake, then the level of free siderophores in the field environment would have to be known, along with the laboratory determined formation constants, to predict the actual field effects.

The description for applying the indigenous index should also include some elaboration of which particular bacterial parameters (e.g. bacterial numbers, metabolic activity, alive or dead state of bacteria) are relevant to the various transport processes to be studied in the laboratory and then predicted for the field conditions. For example, if it is found that colloidal agglomeration is only affected by the number of bacteria present, then the indigenous index would only be a function of the measured bacterial numbers (and not of the metabolic activity). Conversely, if it is found that colloidal agglomeration is more dependent on the presence of extracellular metabolic products, then both bacterial number and metabolic activity may determine the indigenous index value.

Similarly, regarding the possible sorption processes and cellular location of sorbed actinides, if actinides adsorb only externally on the cell, then bacterial numbers (perhaps even both dead and alive) will be the only factor in determining the indigenous index. If actinides are localized inside the cell, then metabolic activity may be the greatest determining factor for the indigenous index of the sorption process.

Recommendation

Provide some basis for justifying a linear extrapolation. Make the calculation of the indigenous index as specific as possible. The application of the indigenous index to different actinide transport processes considered in the SP (colloidal agglomeration, actinide chelation, and actinide sorption) should include a more detailed consideration of the differences in metabolic activity which may exist between the laboratory bacteria grown at optimal conditions and the bacteria present in the field. Also, the SP should provide a more detailed explanation of which bacterial parameters may be most important (or even the only ones considered) for each of the transport processes which, with the laboratory results, are to be related to the possible field effects. Use several components of the indigenous species study to weight factors independently. For example if few siderophore producers are found, reduce the effect of chelation by more than just the ratio of activity levels.

Reference

Neilands, J.B. 1981. Iron Absorption and Transport in Microorganisms. *Annual Reviews in Nutrition*, 1:27-46.

4.3 Questions

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport

Question 1

Wouldn't the SP benefit from an investigation which includes nutrients from expected external sources (e.g. drilling fluids and construction fluids/materials)?

Basis

"The presence of mobile drilling fluids could significantly affect the numbers and distribution of microorganisms found in the block area" (pg. 4). "...several other forms of organic materials will be introduced..." (pg. 4). These materials may be used as growth substrates by indigenous organisms (pg. 4).

It is expected that drilling and other fluids released would follow preferential flow paths through the Topopah Spring Member. With added nutrients, microbial activity and motility would increase along these paths. Releases of waste from the repository would also be expected to follow these paths. The combined effect would be an increase in the impact to transport of actinides due to microbial activity along the paths of travel. Without studying the interaction of these nutrient sources on collected indigenous samples, the most important effects of chelate production, motility, sorption and colloidal dispersion/agglomeration may not be seen at all.

Recommendation

Include experiments in each activity to evaluate the effects of external nutrient sources. Experiments of this nature would provide better interaction with related SCP activities. Examples might include investigations of:

1) The change in metabolic activity due to added nutrients.

2) Interaction of drilling fluids with chelates (e.g. competition of chelates and fluids for actinides, effects of the presence of drilling fluids on chelate mediated transport).

3) Competition of sorption of actinides on microbes vs. drilling fluids.

4) Increase in motility of organisms due to increased nutrient supply from varying sources.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport

Question 2

Are there any plans to study the effect of microbial activity on uranium transport?

Basis

"The actinide elements to be studied are neptunium, plutonium, and americium..." (pg. 5)

A number of the references cited in the SP indicate that microorganisms are capable of sorbing uranium (e.g., Dreher, 1981; Strandberg et al., 1981). In fact, uranium uptake for intracellular accumulation is quite rapid, "...even though a uranium transport system has not yet been identified." (pg. 7). Uranium has also been identified as an important radionuclide in the HLW inventory (Kerrisk, 1985). Given this, it seems that experiments to study uranium should be undertaken as well. This is especially favored since the chemistry of the uranium system has been studied extensively (e.g., Grenthe et al., 1992) and is much better understood than Pu, Am, and Np.

Recommendation

Expand the scope of the experiments to include uranium experiments. The same experimental procedures developed here for Pu should be applicable, at least to a similar extent to which they can be applied to Am and Np.

References

Dreher, K.T., 1981. *Removal of Uranium and Molybdenum from Uranium Mine Wastewaters by Algae.* M.S. Thesis, New Mexico Institute of Mining and Technology, Socorro, New Mexico.

Grenthe, I., J. Fuger, R.J. Lemire, A.B. Muller, C. Nguyen-Trung, and H. Wanner. 1992. *Chemical Thermodynamics Series, Volume 1: Chemical Thermodynamics of Uranium.* Nuclear Energy Agency, Organization for Economic Cooperation and Development. New York: Elsevier.

Kerrisk, J.F. 1985. *An Assessment of the Important Radionuclides in Nuclear Waste*. Los Alamos National Laboratory. LA-10414-MS. Los Alamos, New Mexico.

Strandberg, G.W., S.E. Shumate, and J.R. Parrot. 1981. *Applied and Environmental Microbiology*. 41:237-245.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Indigenous Microorganisms

Question 3

There is little mention of field sampling protocols to be used. What specific techniques will be used to ensure that samples are representative of indigenous organisms? What sample integrity and QA tests will be utilized?

Basis

It is stated in Section 2.2 that one of the primary constraints on the indigenous species study is that all samples be collected aseptically. However, specific aseptic techniques for handling samples are not discussed until samples are returned to the laboratory (pg. 14).

Samples are to be examined from the ESF "...from specially collected dry samples..." (pg. 14). If there is a delay in ESF construction, dry drillholes or the G-tunnel will be used (pg. 13). It is not clear that the sampling strategy described will provide representative samples.

The techniques used to collect samples and the location from which they are collected (i.e. drill hole or mine shaft wall) can have a significant impact on the potential contamination of the samples from airborne or surface microorganisms. Several methods have been described that provide accurate sampling and QA control (e.g. Amy *et al.*, 1992; Colwell *et al.*, 1992). Considering the importance of acquiring representative samples, detailed procedures should be developed for collection of samples.

Recommendation

Design detailed procedures that incorporate both aseptic methods and a means to evaluate the representativeness and quality of samples. Use techniques like surface swabs at the collection site and filtered air to provide Quality Assurance and minimize contamination respectively.

References

Amy, P.S., D.L. Haldeman, D.H. Hall, and C. Russell, C. 1992. Comparison of identification systems for classification of bacteria isolated from water and endolithic habitats within the deep subsurface. *Applied and Environmental Microbiology*, 58:3367-3373.

Colwell, F.S., G.J. Stormberg, T.J. Phelps, S.J. Birnbaum, J. McKinley, S.A. Rawson, C. Ververka, S. Goodwin, P.E. Long, B.F. Russell, T. Garland, D. Thompson, P. Skinner, and S. Grover. 1992. Innovative techniques for collection of saturated and unsaturated subsurface basalts and sediments for microbiological characterization. *Journal of Microbiological Methods*, 15:279-292.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Indigenous Microorganisms

Question 4

What if a significant number of anaerobic bacteria and/or spore formers are identified during the indigenous species study? Can the SP be adjusted to incorporate anaerobic activity into its evaluation of microbiological effects?

Basis

"It is anticipated that all samples collected will be aerobic; therefore, only aerobic analyses will be performed." (pg. 14)

Anaerobic bacteria (both stringent and non-stringent) are known to exist in many common soils along with aerobic bacteria and other microorganisms (Pelczar, 1986, pg 546-547). The cited statement says that all samples obtained for the indigenous microorganism studies will be aerobic, yet no basis is given for this assumption. Further, the statement implies that only aerobic bacteria will be investigated. Contradicting this is the statement about work to be subcontracted in Section 2.1.1, which includes "....physiological types of bacteria will be determined, e.g....anaerobes.."

If a large amount of anaerobe spores were present and stimulated aerobic activity, reduced O_2 levels or reducing conditions could form quite rapidly. This would have a dramatic effect on chemical composition of water in the immediate vicinity. If the estimated anaerobic activity potential were substantial, it would need to be included in the indigenous index, and information from other studies to help identify potential anaerobic/anoxic zones in Yucca Mountain would be needed.

Recommendations

Include available data on anaerobes from indigenous studies into experiments. Evaluate siderophores from anaerobes as well as aerobes. If anaerobes are to be completely excluded, provide justification.

References

Pelczar, M.J., E.C.S. Chan, and N.R. Krieg. 1986. *Microbiology*. McGraw-Hill Book Co., New York.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Indigenous Microorganisms

Question 5

Are releasing agents to be used in the viable plate count experiments?

Basis

The procedure for AODC lists the use of 0.1% sodium pyrophosphate followed by the use of agitation to release organisms (pg. 15). However, only a standard potassium phosphate buffer solution is listed under the plate count procedure, and no indication of any agitation is given (pg. 16).

It is standard practice to employ a releasing agent when processing environmental samples for plate counts because of the high number of attached organisms (e.g. Balkwill and Ghiorse, 1985). Studies have shown that not using a releasing agent may reduce the numbers of microbes cultured by more than one order of magnitude (Weirich and Schweisfurth, 1985).

Recommendation

Modify the plate count procedure to include both a releasing agent and a buffer solution. Process samples with and without the releasing agent and note the impact on plate counts.

References

Balkwill, D.L. and W.C. Ghiorse, 1985. Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma. *Applied and Environmental Microbiology*, 50:580-588.

Weirich, G. and R. Schweisfurth, 1985. Extraction and culture of microorganisms from rock. *Geomicrobiology Journal*, 4:1-20.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Indigenous Microorganisms

Question 6

Utilization of the laboratory derived data depends on a correction for the expected difference of laboratory based microbial metabolic activity as opposed to the metabolic activity as measured in field samples. What special precautions and procedures are to be used to evaluate field microbial activity?

Basis

Section 3.6 states that "...only when combined with the indigenous species studies will the laboratory studies become meaningful." And, "the indigenous species studies will provide information on the overall level of activity..."

The specific references mentioned with regard to metabolic activity determination (pg. 10 and pg. 28) do not provide methods for collection and measurement of environmental samples for *in situ* activity. Laboratory evaluations described (pg. 15) seem to refer to isolated and cultivated communities. Current literature does not provide "definitive" techniques on how to best estimate field activity (e.g. Moriarty *et al.*, 1985; Colwell, 1989). Additionally, *in-situ* activity and direct counts (e.g. AODC) do not necessarily have a direct correlation.

It is known that accurate metabolic activity analysis of field samples requires careful sample collection (e.g. quick cooling to -80° C) and minimal disturbance of the sample (including prolonged exposure to air). Common analytical techniques may not be sufficient.

Recommendation

Carefully evaluate and select procedures to measure field sample metabolic activity. Select several complimentary methods (e.g. phospholipid profiles) in addition to the planned CO_2 uptake and respiration and ATP studies. Attempt to measure activity in field samples that are disturbed as little as possible.

References

Colwell, F.S. 1989. Microbiological comparison of surface soil and unsaturated subsurface soil from a semiarid high desert. *Applied and Environmental Microbiology*, 55:2420-2423.

Moriarty, D.J.W., P.I. Boon, W.G. Hunt, I.R. Poiner, P.C. Pollard, G.W. Skyring, and D.C. White, 1985. Microbial biomass and productivity in seagrass beds. *Geomicrobiology Journal*, 4:21-51.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Chelation

Question 7

How may actinides be transported by siderophores?

Basis

In Section 1.1.2 (pg.7), the possibility is noted 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."

Generally, microbial siderophore systems operate in the following manner (Neilands, 1981): (1) low iron levels in the environment induce the production of siderophore compounds which are then excreted by the microbe, (2) the siderophore compounds chelate highly insoluble iron in the aqueous environment, (3) outer membrane receptors recognize siderophore compounds which contain chelated iron (ferrisiderophores), and (4) the ferrisiderophore compound is transported to the cell interior by specific transport proteins imbedded in the cells membranes. Section 1.1.2 (as well as other sections in this SP which describe chelation) does not discuss the means by which actinides may be transported by siderophores. There are two obvious means of transport that may occur, namely (1) solubilization of actinides by siderophores leading to actinide-siderophore transport with groundwater flow, or (2) solubilization of actinides by siderophores leading to actinide-siderophore uptake by microorganisms. Certainly, both processes are plausible and may enhance the transport of actinides in the Yucca Mountain environment. Also, the siderophore-mediated microbial uptake of actinides could lead to retardation of actinide transport in the environment in the case that the cells adhere to or become trapped in the pores of geologic material at the site. Apparently no experiments are planned to determine the possible importance of microbial actinide-siderophore uptake as another means of transport of actinides.

Recommendation

Consider and discuss the various means by which siderophore chelation of actinides may enhance or retard the transport of actinides.

Reference

Neilands, J.B. 1981. Iron Absorption and Transport in Microorganisms. *Annual Reviews in Nutrition*, 1:27-46.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Chelation

Question 8

Will both hydroxamate- and catechol-type siderophores or only hydroxamate-type siderophores be isolated and purified from indigenous microorganisms for the actinide/siderophore formation constant experiments? Is the procedure described for production and purification of siderophores specific only for hydroxamate-type siderophores?

Basis

In Section 3.2 (pp. 16-17), the second and fourth paragraphs emphasize siderophore detection by the amber color which develops upon the addition of ferric perchlorate. Catechol-type siderophores can also be detected with this reagent, leading to a wine color.

The descriptions given in the second and third paragraphs of Section 3.2 (pp. 16-17) appear to be siderophore production and purification procedures developed specifically for a *Pseudomonas* species which the principal investigator has already worked with. No identification is given that these procedures are generally applicable.

Recommendation

Clarify whether both hydroxamate- and catechol-type siderophores will be investigated, and whether the microbial growth conditions and the siderophore isolation procedures will be used in all cases or whether they will be modified for various microorganisms and possible catechol-type siderophores.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Chelation

Question 9

The siderophore/actinide chelation experiments first require fermentations to be performed to produce and isolate siderophores needed for these experiments. Is the iron level cited for these fermentations appropriate for efficient production of siderophore?

Basis

The minimal salts medium used for the siderophore-producing fermentation of a *Pseudomonas* species is given in the second paragraph of Section 3.2 (pg. 16). The cited iron level is 1 mM.

The production of siderophores by bacteria is a metabolically-regulated process (Neilands, 1981). This process is induced by the presence of low iron levels in the environment in which the bacteria are present. For most microorganisms, the "turning on" of the siderophore production process does not occur until

environmental iron levels are on the order of 0.1 - 1.0 μM (Neilands, 1984), far below the value cited above. Even though there is some low constituent level of siderophore production at iron levels associated with optimal growth, the most efficient production of siderophore surely occurs at conditions in which the siderophore producing enzymes are highly expressed, namely at iron levels around 0.1 - 1.0 μM.

Recommendation

Clarify the reason for using such a high iron concentration for the microbial production and purification of siderophores.

References

Neilands, J.B. 1981. Iron Absorption and Transport in Microorganisms. *Annual Reviews in Nutrition*, 1:27-46.

Neilands, J. B. 1984. Methodology of Siderophores. *Structure and Bonding (Berlin)*, 58:1-24.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Sorption

Question 10

In determining the cellular location of microbially-adsorbed actinides, how will the material external to the cell be determined, i.e. by direct measurement or by difference? How will a mass balance be applied to give the distribution of actinides among the different cell components?

Basis

The first paragraph of Section 3.3 (pg. 19) describes the series of simple experiments which will be used to determine the location of actinides in the cells (this same description is repeated at the end of the first paragraph of Section 2.1.3, page 11). In this description, the measurement of the actinides located externally on the cells is not clear. It is simply stated that "Cells would be washed with mild buffers or mild acids and examined." There is no explanation as to the phase (the wash or the remaining solids) into which the actinides are expected to partition.

The last sentence in the first paragraph of Section 3.3 (pg. 19) states "..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." There are generally two methods in which the mass balance approach is employed, (1) all contributing terms in the mass balance equation are known, and thus it is used as a 'check' or 'percent recovery' value against the sum total, and (2) one of the contributing terms is not known, and the mass balance equation is used to calculate that value by difference. The statement cited above does not clarify how the mass balance equation will be applied and thus what is gained from this approach.

Recommendation

Provide more detail about the individual measurements of actinides associated with certain cell constituents, and how these results will be incorporated with a mass balance approach to further describe the cellular sorption of actinides.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Sorption

Question 11

Transport of actinides by sorption onto motile/floating bacteria is investigated under Research Area #3 - Sorption. Microbes in the field will most likely be attached to the solid phase and not free to float in the liquid phase. How will the Study account for this?

Basis

Experiments evaluating transport by sorption onto microbes are designed to be performed using healthy, suspended microbes in saturated and unsaturated conditions (pg. 19).

Microbes at Yucca Mountain are not likely to be suspended in a liquid phase. Indeed, microbes experiencing low levels of water activity and nutrient supply are under significant stress. They tend to attach to the mineral phase as a survival mechanism (Kjelleberg *et al.*, 1983; Pringle and Fletcher, 1986). Additionally, they tend to be smaller in size (Amy *et al.*, 1992).

Recommendations

Design and perform experiments with stressed microorganisms to facilitate attachment and small microorganisms to evaluate breakthrough of microbes of reduced size. This should give more applicable results (within lab limits) relative to field conditions and lessen the dependence on an index factor.

References

Amy, P.S., D.L. Haldeman, D.H. Hall, and C. Russell, 1992. Comparison of identification systems for classification of bacteria isolated from water and endolithic habitats within the deep subsurface. *Applied and Environmental Microbiology*, 58:3367-3373.

Pringle, J.H. and M. Fletcher, 1986. Influence of substratum hydration and adsorbed macromolecules on bacterial attachment to surfaces. *Applied and Environmental Microbiology*, 51:1321-1325.

Kjelleberg, S., B.A. Humphrey, and K.C. Marshall, 1983. Initial phase of starvation and activity of bacteria at surfaces. *Applied and Environmental Microbiology*, 46:978-984.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Sorption

Question 12

How does the presence of artificial nutrient media influence the sorptive properties and transport characteristics of the tuff?

Basis

Waters in sorption experiments are saturated with nutrient broth (pg.19). Unsaturated experiments require inoculated columns (pg. 19). Spent media is used to determine agglomeration of colloids (pg. 20).

Composition of growth media varies with the product used (Tryptic Soy Broth, PTYG, nutrient broth). Each may contain chemical species that alter the characteristics of the tuff relative to J-13 or *in situ* pore waters.

Recommendation

Design, perform and incorporate studies of the effects on tuff of the nutrient media used in experiments. Identify how wetting of surfaces or sorption characteristics change when nutrient media is used.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Colloidal Dispersion

Question 13

How do chemical changes affect the stability of agglomerates?

Basis

"Agglomerate stability will be determined by subjecting the agglomerates to disruptive forces such as sonication, surfactants, grinding, and agitation" (pg. 20).

In the unsaturated flow regimes of Yucca Mountain, chemical changes in pore water during contaminant release are likely to be abrupt. The experiment evaluates surfactant influence on agglomerates (in nutrient media) but does not investigate other chemical phenomena such as pH and ionic strength changes.

Recommendation

Redesign agglomerate stability tests to include influence due to changes in factors such as pH, Eh, salt content, and ionic strength.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Colloidal Dispersion

Question 14

Will particles other than clay be considered? What concentrations of particles (clay, tuff, and bacteria) will be used in the agglomeration rate studies?

Basis

Section 2.1.4 (pg. 12) states that Agglomeration Rate studies will be "...limited to clay colloids and to rock samples that are germane to the potential Yucca Mountain repository."

Section 3.4 (pg. 20) indicates that Wyoming bentonite clay and crushed tuff samples collected from Yucca Mountain will be used. Detailed information is provided on the origin, size, and preparation of particles, as well as on the preparation of the spent growth medium for the agglomeration rate tests, yet no information is provided on the particle concentrations to be tested and the relation of such concentrations to the actual environment at Yucca Mountain. Also, will these particles be sterilized in any fashion?

Although clays are important, other types of particles have been reported from waters in the vicinity of Yucca Mountain (e.g., Kerrisk, 1987; Buddemeier and Hunt, 1988; Kingston and Whitbeck, 1991). These include micas, amorphous silica, Fe-oxyhydroxides, quartz, and feldspar. These minerals all have different electrostatic properties (Stumm, 1992) and may respond to microbial activity differently.

Recommendation

Consider using other types of colloids for these studies. Provide preliminary information, such as from the previously run tests at LANL, about typical particle concentrations used in an agglomeration rate test.

References

Buddemeier, R.W., and J.R. Hunt. 1988. Transport of colloidal contaminants in groundwater: Radionuclide migration at the Nevada Test Site. *Applied Geochemistry* 3:535-548.

Kerrisk, J.F. 1987. *Groundwater Chemistry at Yucca Mountain, Nevada, and Vicinity*. Los Alamos National Laboratory. LA-10929-MS. Los Alamos, NM.

Kingston, W.L., and M. Whitbeck. 1991. *Characterization of Colloids Found in Various Groundwater Environments in Central and Southern Nevada*. Desert Research Institute. DOE/NV/10384-36, Publication # 45083. Las Vegas, Nevada.

Stumm, W. 1992. *Chemistry of the Solid-Water Interface. John Wiley Interscience*. New York.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Colloidal Dispersion

Question 15

One of the areas to be investigated is the effect of microorganisms on the agglomeration of colloidal particles onto which actinides may be adsorbed. Will the effects from the actual presence of microbes or from the presence only of extracellular metabolic products be tested in this SP?

Basis

Section 1.1.4 (pg. 8) cites results of preliminary studies at LANL which showed an effect of the presence of bacteria on colloidal agglomeration. In Section 2.1.4 (pg. 12 - Agglomeration Rate), a discussion is given regarding the interpretation of results from agglomeration experiments which may indicate agglomerate formation dependence on either number of bacteria particles or on extracellular metabolic products.

In Section 3.4 (pg. 20), the only agglomeration rate test described is one involving clay or tuff particles in the presence of spent growth medium.

In Section 3.4, no mention is made of the intent to perform any agglomeration rate tests involving both clay or tuff particles and bacteria, though such tests are mentioned previously in Sections 1.1.4 and 2.1.4. Therefore, Section 3.4 is confusing in light of the previous discussions.

Recommendation

Clarify the different variations (in the presence of bacteria and/or bacterial metabolites) under which the microbially influenced agglomeration of colloids will be studied.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Application of Results

Question 16

How are the results from the study to be applied to the geochemical investigations identified in Section 1.2.2 of the SP?

Basis

Several of the geochemical investigations (8.3.1.3.1, 8.3.1.3.5, 8.3.1.3.6, and 8.3.1.3.7) are cited as being addressed by the results of the proposed activities (Section 1.2.2 of the SP, pg. 9). However, with the exception of one study (8.3.1.3.7.1 - Retardation Sensitivity Analysis), these are not discussed in Section 4.0 - Application of Results (pg. 23). Instead, geohydrology investigations are mentioned as possible places where the results of the described work will be applied. While the investigations cited in both sections appear relevant, the discrepancy is not explained.

Recommendation

Reconcile the two sections and provide more detail in Section 4.0 on how the results of the Biological Sorption and Transport Study, 8.3.1.3.4.2 will be applied to geochemical and geohydrologic issues.

Study Plan 8.3.1.3.4.2 Biological Sorption and Transport - Schedule and Milestones

Question 17

Has the timetable been updated to reflect current schedules?

Basis

The Gantt chart shown in Figure 5.1 starts with 1990 and the schedule in Section 5.0 (pg. 24) lists several milestones (3176, 3177, 3080, and 3092) that are shown to be completed by the middle of CY 1992. Two of these milestones involve developing procedures critical to the work described in the SP.

Recommendation

Although it is understood that all timetables are tentative, it should be possible to update the SP to reflect new schedules. This is particularly true since some of the milestones involve procedures developed for the work described. Where possible, update the timetables to reflect current schedules.
