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BIOMASS-BASED APPROACH TO DETERMINE THE CAPMIC BIO-COLLOID PARAMETER FOR THE WIPP MOBILE ACTINIDE SOURCE TERM

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EXECUTIVE SUMMARY

A description of the biomass-based approach used in the compliance recertification application (CRA) -2014 and planned for CRA-2019 to determine the biocolloid parameter CAPMIC is provided in this report. This change replaced the toxicity-based approach used prior to this time in the Waste Isolation Pilot Plant (WIPP) performance assessment(PA). It was implemented to reflect our current understanding of the WIPP microbial ecology and the desire to move the basis of this parameter to more WIPP-relevant conditions. This report is in response to a commitment made at the February 13-15, 2018 WIPP technical exchange between Department of Energy (DOE) and the Environmental Protection Agency (EPA) to provide additional information on the method used to determine the CAPMIC parameter in the WIPP actinide source-term model.

The biocolloid parameters CAPMIC and PROPMIC, together, define the approach used to establish the contribution of bioassociated actinides to the actinide source term. In this context, bioassociation refers to any association that an actinide may have with a microorganism that could affect the mobility of the actinide through the dissolved brine release (DBR) scenario. It is potentially comprised of cell-surface sorption, mineralization, and internal uptake processes. This CAPMIC value, defined as the maximum actinide concentration that can be associated with mobile cells, sets an upper limit for the microbial contribution.

The CRA-2014/2019 biomass-based CAPMIC approach involves determining the maximum actinide concentration that could be associated with cells under optimal, but repository-relevant, conditions (keeping in mind both inventory and solubility limits to the bioavailable concentration and the appropriate actinide/biomass ratio) and then multiplying this value by a maximum possible biomass concentration obtained under idealized growth conditions. This approach integrates pre-CCA data with our currently-available measurements and understanding to establish the maximum associated concentration and remains a defensibly conservative approach to this colloidal contribution to the actinide source term.

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CLARIFICATION OF TERMS

The terms that refer to microbial-actinide interactions are often used interchangeably and this, over time, has led to some confusion in how the biocolloid parameter is understood. The following are the operational definitions used by the DOE in this report as well as pending CRA documentation (e.g., appendix SOTERM and supporting documents):

Bioassociation

In this report, the term "bioassociation" will be used to refer to all types of microbial-actinide associations, whether internal or external and whether transient or accumulative. These may include surface sorption, bio-mineralization, or internal uptake. If the specific mode of interaction is known, it will be named. Ultimately, the nature of the association is important in determining the long-term fate of the actinide given the lifetime of the WIPP.

Surface sorption

Surface sorption is a metabolism-independent process in which metals interact with functional groups at a cell's surface, either by ion-exchange or electrostatic interactions. This process can be reversible depending on the pH and the presence of strong complexing agents. It is generally pH-dependent, as functional groups become deprotonated with increasing pH. At normal physiological pH, cells generally carry a net negative surface charge. Surface sorption can occur on live and dead cells, on live but inactive cells, and onto cell debris. It can lead to either the mobilization or immobilization of an actinide.

Biomineralization

Biomineralization, or the process by which microorganisms can generate actinide or analog minerals, can occur both internally and externally to the cell. Generally, it involves the association of metal with phosphate or carboxylate groups. Once the metal has bound, it can serve as a nucleation site that furthers the progression of mineralization. Often, the release of phosphate is a toxic response by the organism to sequester the metal. This can be at the cell's surface or internally within phosphate granules. Mineralization is usually a result of active cells, but cells do not necessarily need to be growing for this to occur. In general, it leads to the immobilization of an actinide, as cells or the actinide solid phase precipitate from the matrix; however, this may not always be the case.

Internal uptake

Internal uptake is the incorporation of the metal/actinide within the cell. This is more difficult to predict holistically because it will depend on the organism and the metal/actinide in question. Most cells will respond to metal exposure with an active efflux system. This is less costly than sequestration that may require induction of a complexant, such as phosphate. Still, internalization as a sequestered metal is possible and can lead to high concentrations of the metal inside the cell. If the actinide is bound to a ligand that is biodegradable, its fate is uncertain. It could be taken up into the cell but could also be ejected again. Thus, uptake can be either transient or accumulative. Finally, there are some radionuclides (Cs and Sr) that can substitute for biological cations (K and Ca, respectively) without inducing a toxic effect. Internal uptake can lead to either mobilization or immobilization.

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1.0 INTRODUCTION: OVERVIEW OF WIPP COLLOID MODEL

The potential impact of microbial-actinide interactions on the actinide source term is accounted for in WIPP PA (see Figure 1) by calculating the extent of bioassociation for each actinide. This can be a significant, albeit uncertain, contribution to the source term and general discussions of this microbial-actinide interactions can be found elsewhere (Banaszak et al, 199[illegible] Fredrickson et al., 2004; Wang and Francis, 2005; Reed et al., 2010;).

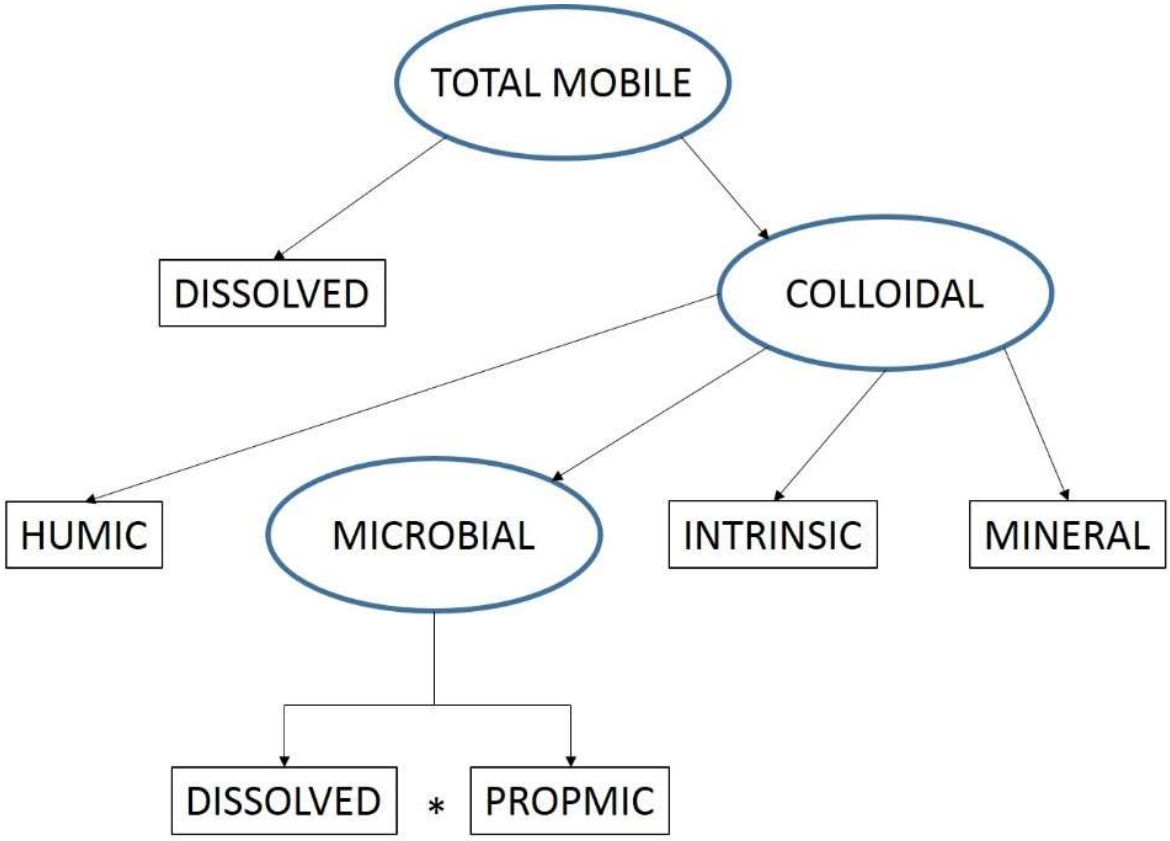

Figure 1. Schematic of the WIPP PA total mobile actinide model that shows the various colloidal contributions

In this view, the microbial contribution is defined as the dissolved actinide concentration multiplied by a proportionality constant, i.e., DISSOLVED * PROPMIC in Figure 1 above, and is one of four colloidal contributions evaluated. The CAPMIC parameter effectively sets an upper limit for this microbial contribution.

2.0 BIOCOLLOID MODEL AND ROLE OF THE CAPMIC PARAMETER IN WIPP PA IMPLEMENTATION

Conceptually, the microbial colloid contribution to the mobile actinide source term is the concentration of actinides that are associated with the microorganisms suspended in the brine and hence mobile under a DBR release scenario [U.S. DOE 1996; Papenguth 1996; Francis et al., 1998, Strietelmeier et al., 1999; U.S. DOE 2009; U.S. DOE 2014]. This is conceptually established by determining the biomass (correlated with the number of microorganisms per liter) that could be present and measuring/calculating the amount of actinide species that associate with this biomass. The amount of sorption is expected to be organism-specific and will reflect/follow the oxidation-state specific chemistry that is the basis of the WIPP actinide solubility model. In practice, this is evaluated generically for representative halophilic bacteria and archaea under WIPP-relevant conditions where bioassociation is the dominant process.

The specific definitions in the WIPP biocolloid model for the CAPMIC and PROPMIC parameters (Papenguth, 1996) are:

PROPMIC: the proportionality constant that describes "the amount of actinide element bound to mobile microbes" and calculated as "the ratio between the microbial actinide and dissolved actinide"

CAPMIC: defined as "the maximum concentration of actinide that can be associated with mobile microbes" and measured as the concentration "at which no growth was observed" in the toxicity studies.

There are in principle a number of possible ways and strategies to evaluate and/or measure each of these parameters. In this report we are focusing only on the biomass approach used to determine the CAPMIC parameter that is now being used.

3.0 CRA-2014 CAPMIC PARAMETER APPROACH

For CRA-2014, the DOE changed the approach to measure the CAPMIC value for the biocolloid contribution to the source term from a toxicity-based approach to a biomass-based approach. This was done for the following reasons:

- To reflect our current understanding within the WIPP project on what limits growth under WIPP-relevant conditions [See Ams et al., 2013; Swanson et al., 2013; Swanson et al., 2015; Swanson et al., 2016; Bader et al., 2017; Bader et al., 2018].
- Strengthen the defensibility of the approach.
- Improve the consistency of the approach with the current understanding of the actinide chemistry and speciation under WIPP-relevant conditons.

The overall result of this change was to add more realism, based on the current understanding of the microbiology at the WIPP, while maintaining an overall approach that is defensibly conservative.

Constraints on biomass concentrations: natural and man-made

The microorganisms that inhabit subterranean salt deposits are unique in their ability to survive at high ionic strength by osmotically balancing their internal and external environments. Two strategies exist by which organisms can do this: 1) by importing high concentrations of K^+ and Cl^- ions or 2) by accumulating low-molecular weight organics, called "compatible solutes". Extremely halophilic archaea (haloarchaea) and only two bacterial genera are capable of the first mode of osmoregulation. All other bacteria and eukaryotes employ the second mode. Both strategies require energetic input, but the second is much costlier than the first, especially if the solutes must be synthesized. As a result, the dominant microorganisms in hypersaline settings, such as those expected in the WIPP, are haloarchaea rather than bacteria.

Additionally, the energetic cost of osmoregulation also constrains which modes of metabolism are favorable. As such, many anaerobic modes are limited or even effectively eliminated at high salt environment. There are no documented extremely halophilic, anaerobic organisms from subterranean salt deposits. While these organisms have been detected in surficial hypersaline settings, their activity is still constrained by increases in salt concentration and by interactions with other naturally present halophilic organisms that provide them with other needed nutrients (e.g. algae, brine shrimp).

If organisms are capable of surviving the high ionic strength and anoxic conditions at the WIPP, there are still other constraints on their activity—for example, low water activity/high chaotropicity, the presence of potentially toxic radionuclides, and non-ideal substrates or pH. In summary, all of these constraints ultimately limit the biomass concentration possible under WIPP-relevant conditions.

Biomass concentrations at the WIPP

The number of cells currently present within the WIPP is indeterminate, and their numbers under WIPP-relevant conditions even moreso. Screening of raw WIPP-relevant samples resulted in the following cell counts:

Table 1. Cell Counts Observed in WIPP-relevant Experiments

Matrix	Francis and Gillow 1993; Francis et al 1998; Gillow and Francis 2006.	Vreeland et al. 1998	Swanson et al. 2013 and 2016
Halite	nd	nd	$0 – 10^2$ cells/g (mostly 0 counts)
Brine	7.2×10^4 cells/ml 3.4×10^6 cells/ml	nd	0 cells/ml

nd = not done

These counts are consistent with findings in other subterranean salt deposits and are generally less than counts obtained in surficial environments. The variation in halite cell counts is due to the heterogeneous distribution of cells within fluid inclusions or interstitial spaces. The variation in brine counts may be a function of brine source and exposure to open air and mine workings. For example, a fresh brine seep from WIPP had 0 cells (Swanson, unpublished); whereas, brine seeps from other boreholes contained many more (Gillow and Francis, 2006.).

Cell numbers in actively growing cultures of WIPP-relevant samples are shown below:

Table 2. Cell Numbers in Actively Growing Cultures of WIPP-Relevant Samples

Matrix	Francis and Gillow 1993; Francis et al. 1998; Gillow and Francis 2006.	Vreeland et al. 1998	Swanson et al. 2013 and 2016
Halite	nd	0- 6.9 x 10^3 CFU/g halite, median 425 CFU/g (aerobic)	10^5-10^9 cells/ml in broth culture (aerobic); no growth (anaerobic)
Brine	0.1 – 1.0 x 10^4 CFU/ml, direct plating (aerobic)	30 CFU/ml to 1.0 x 10^4 CFU/ml, direct plating (aerobic)	0 cells/ml in enrichment culture (aerobic)
Air	nd	0 CFU after driving along WIPP drift with exposed agar plate (aerobic)	0 CFU after 24 hours of exposure to air in 3 locations with different air flow (aerobic)
Mixed matrix: muck pile salt, brine lake sediment and brine, halite	5.12 x 10^5 cells/ml (unamended, uninoculated) to 2.24 x 10^8 cells/ml (amended, inoculated, excess nitrate; anaerobic)	nd	nd

nd = not done

The important conclusions to draw from these summaries are:

- cell counts do not exceed 10^{10} cells/ml under the best of growth conditions
- the only inoculum matrix that yielded growth under anaerobic conditions comprised surficial sediments (n.b. anaerobic cultures have been obtained from groundwater samples)
- cell counts under anaerobic conditions were an order of magnitude less than optimal aerobic conditions (this is often true for anaerobic cultures, as energy production is less efficient)

Cell mobility

Another built-in conservativism of a biomass-based parameter is the fact that not all cells are mobile. If organisms are active enough, they could form biofilms. Many organisms grow in clumps in culture, leading to sedimentation as well as a reduced surface area for the sorption of actinides. Others may sediment from suspension with the added weight of mineralized substances on their surfaces or simply because they are dying. Thus, even if a maximum biomass concentration is reached, not all will be mobile.

Bioassociation as a function of biomass and actinide concentrations

Since CAPMIC is to measure the maximum concentration of mobile actinide, it should be a function of the maximum concentration of biomass available to associate with the actinide and the maximum concentration of actinide that is bioavailable.

In theory, bioassociation increases with increasing biomass to the point where all the actinide has associated (Figure 2) and increasing the concentration of the actinide will increase the amount associated until all sites are saturated.

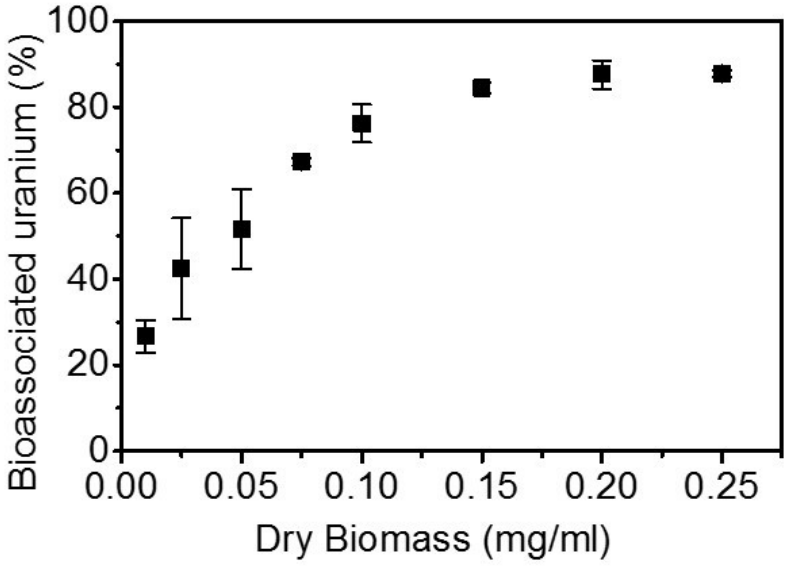

Figure 2. Biomass-dependent association of uranium with *Brachybacterium* sp. G1 (Bader et al., 2017). Dry biomass was correlated to optical density (OD) readings for these experiments.

Even so, biomass and actinide concentrations are not infinite. Bioavailable actinides will be limited by inventory, solubility, and the presence of ligands. By setting a maximum possible biomass concentration, the associated actinide concentration is overestimated. This maintains conservativism for the model.

4.0 CONCLUSIONS

Microbial growth in closed systems, like WIPP, is limited by the depletion of substrates and nutrients or by the build-up of inhibitory by-products of metabolism; therefore the biomass concentration cannot increase in perpetuity. Using biomass-based values in PA that were determined under optimal growth conditions in the laboratory is conservative relative to the stressed and nutrient-limited growth conditions that are realistically expected for WIPP-relevant conditions. This change in approach to the determination of an appropriate CAPMIC limit has the added benefit of more realistically representing our current understanding of the microbiology and actinide chemistry in the WIPP so there is added credibility in the safety case.

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