Review of: "Risk of colloidal and pseudo-colloidal transport of actinides in nitrate contaminated groundwater near a radioactive waste repository after bioremediation"

Yoshihiko Inagaki, Hieu Dang , RODGERS MAKWINJA, Habineza Elias

Potential competing interests: The author(s) declared that no potential competing interests exist.

The authors evaluated the water quality of groundwater samples taken near a radioactive waste repository at three different times, namely (i) before in situ bio-remediation was initiated at the site, (ii) one year, and (iii) two years after the bio-remediation had started. The samples were labeled as ‘Sample 1’, ‘Sample 2’, and ‘Sample 3’, respectively. In addition, using the raw water samples (referred to as natural water, denoted as NW) or diluted ones (referred to as model water, denoted as MW), they conducted laboratory tests, in which they applied two different methods for microbial stimulation: the supply of hydrogen gas or the addition of organic materials such as glucose and sodium acetate. The latter included further addition of clay or ferric iron.

First of all, the title of the article begins with the noun "risk". However, this word does not really match the main content of the article when the authors only evaluated the transport of actinides between particle fractions during microbiological stimulations. The title perhaps should start with "Assessment" to be more appropriate. In addition, the authors should provide an elaborated explanation of why they need to use model waters. Model waters were basically the dilutes of NW, although they were supplemented with four mineral compounds (those materials, however, were already contained sufficiently in the NW). It is also not clear why the authors did not investigate the NWI and NWIO (NW with iron and/or organic compounds).

Besides, there are a number of issues in terms of accurate descriptions of lab experiments and results as the following:

1) There are statements that do not reflect the data presented in Table 1: it is stated in the text that a TOC concentration did not exceed 5.9 mg/L, the data presented in Table 1, however, showed all above that concentration. According to a statement in the Materials and Methods, sample 2 was taken in the middle of 2016. It means it had been more than a year since sample 1 was taken and the bio-remediation must have been started for at least 12 months. However, it was unclear about the Eh values (page 4) when the authors stated that they "remained in the reduction region (-175 mV) as they were 3 months after bio-remediation. So, when exactly did the values measured? When was sample 2 taken, and the Eh data for sample 2 (table 1) taken from when?

2) It is very difficult to understand the purpose of presenting SEM-EDX images in figure 2. It is hard to say the distributions of certain elements on the filter cake were uniform, as claimed by the authors. At least for Si, it did not indicate so. Some

clusters of materials appeared on the image.

3) Even though the authors stated that only Samples 1 and 3 were used for the laboratory experiment #1, in which they used hydrogen gas as an electron donor, the results for Sample 2 were presented in Table 2 and discussed in the text for this experiment. Also, the experimental conditions, such as how much and how long (continuous or intermittent) hydrogen gas was supplied, was not described. Furthermore, the authors should have presented results of the control (with no hydrogen gas addition) to ensure that microbial activation was driven by hydrogen gas. Results obtained in this experiment are isolated from other experiments and not mentioned at all in the abstract and conclusion sections; thus application of hydrogen gas do not seemingly add values to the overall conclusion of this research. In addition, the authors perhaps should provide a clear terminological definition for colloidal or pseudo-colloidal materials in terms of sizes for easier following of the readers.

4) Regarding data presented in Table 3, the authors found "a low protein content" in the sample NWO (NW with organic compounds), but it is not clear how the author considered it low, because protein contents in the sample NWO are at similar levels with other samples. The authors should provide a discussion on why polysaccharide and protein concentrations decreased after the peaks.

5) The following statements were unpersuasive or inconsistent with data presented in Table 4:
- Again, there is an inconsistency between the text (“...were obtained on days 3,7,14,21, and 28”) and the information provided in the table (incubation time: days 5, 10, 15, 20, and 30).
- "[The observation of particle formation in MW at day 30] was probably due to the transformation of colloidal matter originating from the natural water aliquot or as a result of low microbial activity.": This statement is speculative. No evidence supporting this was presented.
- "In the presence of glucose and acetate, the emergence of the colloidal phase and a gradual increase in particle size were observed from the fifth day of incubation.": This is true for NWO, but not for MWO (MW with organic compounds).
- "In the presence of clay, stable colloids with the average hydrodynamic radii of 80–90 nm were formed." This radius was only observed at days 10 and 15, but not at other days.
- "The addition of iron to the model system resulted in the formation of the particles with hydrodynamic radii of ~ 100 nm.": Regardless of iron additions, hydrodynamic radii of 100 nm were achieved (for example, MWClO).

6) For the zeta potential, as presented in Table S2, zeta potentials of MWO and NWO were -14.4 and -11.7 mV at day 30, which means that "a shift in charge of particles towards zero and positive values" was canceled, indicating organic matter addition does not assure stabilization of particles in solutions.

7) For figure 4, the authors should have put a label for each panel [like (a) to (f)] so that they could point out a specific panel with a relevant statement. Otherwise, it is very hard for the reader to connect statements with corresponding data on the figures. Again, some statements are not consistent with data. For example, the author states "In the model water

Pu(IV) forms true colloidal associates (up to 50%) due to deep hydrolytic polymerization.", but it is not clear how they got 50% for which sample. Also, for the statement, "In the model water, increased pH and decreased Eh result in the occurrence of 99% Pu, 30% Np, and 10% U within large colloidal particles.", those percentages were clearly not derivable from Figure 4.

8) Actinides concentrations set at lab experiments #2 (i.e., 10^8 mol/L) seems to be different from those set at PhreeqC simulations. In the simulations, the amount added was 500 ug, which was supposed to be added to 50 mL, but in the later text, the author described this as 500 ug/L. The authors should have resolved these discrepancy. Additionally, simulations were supposed to be done for Sample 1, according to Table S1 in the Supplementary materials, which presents conditions of laboratory experiments. However, in the text as well as in Table 5, Sample 2 was mentioned.

9) The author suggested the addition of bivalent cations such as calcium and magnesium ions during bio-remediation for enhanced coagulation. First, no data supporting this statement is presented. In addition, those compounds were already included in high concentrations in NW so that the rational of further additions is questionable.

Overall, the data, data interpretation, and discussion in the article are quite fragmentary, not showing a good bonding between experiments. The arrangement and organization of data make it difficult for readers to understand what the authors want to express in the document.