Sequestration of Cs by Na- and H-birnessite from pH 3 to 11 as measured with time-resolved synchrotron X-ray diffraction

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Low-level nuclear waste storage tanks at the DOE Hanford site in Richland, Washington have leaked more than 1 million gallons of solution that is extremely basic (pH > 13) with high ionic strength and concentrated in radioactive Cs-137 (2 x 10^{10} Bq/L, equivalent to 0.04 mmol/L). The underlying Ringold Formation consists of poorly consolidated clays, silts, and sands rich in Fe and Mn oxides, including the phyllomanganate birnessite. Interlayer cations in birnessite are highly exchangeable, and it has been demonstrated that millimolar concentrations of aqueous Cs^+ will rapidly exchange for Na^+ in the birnessite interlayer in neutral solutions [1]. For the first time, we have explored diadochic substitution of Cs^+ for Na^+ in birnessite over a wide pH range. The cation exchange products of *in situ* reactions are characterized with time-resolved synchrotron X-ray diffraction (TR-XRD) and inductively coupled plasma-mass spectroscopy (ICP-MS) at pH values ranging from 3 to 11. In these experiments, the rate of exchange of Cs^+ for Na^+ decreased as pH increased. However, the birnessite structure transformed from triclinic to hexagonal symmetry at pH 3. Thus, at low pH, aqueous H^+ outcompeted with Cs^+ and Na^+ in partitioning into the birnessite interlayer.

Hexagonal H-birnessite also readily exchanged interlayer H^+ for Cs^+ from pH 3 to 11, as revealed by *in situ* TR-XRD and ICP-MS. As with Na-birnessite, the rate of Cs^+ cation exchange decreased with higher pH. A transformation of hexagonal H-birnessite to triclinic symmetry at pH 13 and 0.1 M CsOH is reported for the first time; the mechanism of this transformation has yet to be determined.

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Untangling the true phylogeny of *Leptothrix ochracea* with single cell genomics and FISH

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Leptothrix ochracea is noted for its ability to rapidly accelerate the biofouling of iron contaminated water systems by producing large masses of Fe-oxyhydroxide encrusted microtubular sheaths. For the past 120 years this bacterium has been regularly described in the scientific literature, always being noted for its association with freshwater iron seeps. Its taxonomy has been based solely on its morphology, and it is considered the type species for the *Leptothrix* genus. Despite this long history, *L. ochracea* has proven refractory to laboratory culture, and attempts to discover its true phylogeny have failed. The recent development of single cell DNA sequencing used in combination with florescence *in situ* hybridization (FISH) have finally made it possible to address *L. ochracea*'s phylogeny.

Taking advantage of *L. ochracea's* filamentous growth and sheath formation, flow cytometry was used to select sheath-associated cells from a freshly formed iron mat. After flow-cytometric isolation, genomic DNA from individual, cell-containing sheath particles was amplified using multiple displacement amplification (MDA). The MDA products were then used as templates in PCR-amplification and subsequent sequencing of the 16S rRNA gene (40 sequences obtained). Alternatively, environmental iron mats were sieved through 8 micron filters (to retain sheath material and enrich *L. ochracea* cells). The 16S rDNA gene sequences were obtained from the filters using cloning methods (65 clones total). Phylogenetic analysis identified a cluster of sequences (29%) that deduction suggested might be *L. ochracea*. These were related to other *Leptothrix-Sphaerotilus* spp, but were sufficiently divergent that a highly specific FISH probe (Lepto175) was developed that bound to canonical *L. ochracea* but not *L. cholondii* or *S. natans*. Use of this probe with freshly formed iron mats, indicated *L. ochracea* accounted for between 31%-85% of total bacteria.

These data confirm the morphological identification of *L. ochracea* and suggest it belongs to a distinct clade of the *Sphaerotilus-Leptothrix* group. Obtaining the genome of *L. ochracea* will ultimately allow us to understand its physiology.