REE Bioadsorption from buffer solution by Caulobacter biofilms. *Caulobacter* biofilms were grown in polystyrene wells of 24-well plates.  Following exposure to a simple Tb solution, cells grown in 24-well plates adsorbed ~18-22 μmoles Tb/m2, exceeding the biomass assumption (5 μmoles Tb/m2) used in the prior TEA analysis in the proposal (Figure 1).

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|  | **Figure 1. Biofilm adsorption capacity test.** Control and LBT-displayed *Caulobacter* biofilms were formed by inoculating cells at low cell density into bacterial growth medium and incubating for 3 days.  Planktonic or unattached cells were removed each day and fresh media was added with adhered cells used to seed further growth. Adsorption experiments were performed in MES buffered (pH 6) solution with 15 μM TbCl3 for 30 min. |

REE Bioadsorption from mock geothermal fluids by *Caulobacter* biofilms. Control and LBT-displayed *Caulobacter* biofilms were formed by inoculating cells at low cell density into bacterial growth medium and incubating for 3 days.  Planktonic or unattached cells were removed each day and fresh media was added with adhered cells used to seed further growth. Bioadsorption experiments were performed using synthetic Great Salt Lake (GSL) brine and real Blue Mountain geothermal fluid with control and LBT-displayed *Caulobacter* biofilms. Tb was spiked in at 100 and 1000 ppm since Blue Mountain geothermal fluids lack quantifiable REEs. Tb and major elements (Na, K, Mg, and Ca) were quantified by ICP-MS. Both control and LBT-displayed *Caulobacter* biofilms performed well for Tb extraction from the Blue Mountain brine (Figure 2). LBT-displayed cells extracted ~9 μmoles/m2 Tb while major elements, present in the initial feedstock at ~17 ppm, were below the detection limit, with the exception of Na added during the desorption step with citrate (Figure 2). Future work will examine whether high TDS is deleterious to REE extraction from biofilms. Nevertheless, the successful extraction of Tb spiked into the Blue Mountain geothermal fluid provides initial support for a biofilm-based extraction approach from geofluids.

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| Macintosh HD:Users:dmpark:Desktop:Desktop_Folder:Untitledmetal.pdf | **Figure 2: REE Bioadsorption from Blue Mountain (BMT) fluids spiked with Tb by *Caulobacter* biofilms.** Control and LBT-displayed *Caulobacter* biofilms were formed by inoculating cells at low cell density into bacterial growth medium and incubating for 3 days.  Planktonic or unattached cells were removed each day and fresh media was added with adhered cells used to seed further growth. Adsorption experiments were performed in MES buffered (pH 6) BMT solution with 1000 ppb TbCl3 for 30 min. Following adsorption, the remaining solution was remove, cells were washed once with MES pH 6, and then adsorbed metals were desorbed using 5 mM citrate pH 6. Metal concentrations were determined using ICP-MS. |

REE biosorption capacity and its temperature dependence with Mutag Biochips. Using the optimized sterilization approach, we were able to consistently seed *Caulobacter* biofilms onto the Biochips and characterize the REE adsorption capacity. Briefly, Mutag biofilm chips were seeded with LBT-displayed *Caulobacter* biofilms by inoculating cells at low cell density into bacterial growth medium and incubating for 6 days.  Planktonic or unattached cells were removed each day and fresh media was added with adhered cells used to seed further growth.

Successful biofilm colonization was confirmed using a crystal violet assay and by determining the biofilm mass per chip (~1 mg cells /chip). The latter facilitates the comparison with data obtained with another form of cell immobilization method -- cell encapsulation. The REE adsorption capacity of biofilm chips was determined by incubating chips in a synthetic saline solution with increasing concentrations of Nd. The data indicate that biofilm chips adsorbed ~30 μg Nd per chip, which translates to ~ 7.5 g Nd /m3. This equates to an adsorption capacity of ~32 mg Nd / gram of dry cell weight, which is in good agreement with the ~29 mg Tb /dry cell weight determined previously for batch scale tests with free-floating (planktonic) cells.

The effect of temperature on REE adsorption capacity of*Caulobacter* biofilms was tested by performing biosorption assays in synthetic saline solution at room temperature and 70 °C, based on the promising batch-scale data described in the submitted ES&T manuscript. We observed a ~2 fold increase in REE adsorption with dLBT biofilms compared to the biofilms produced with *Caulobacter* lacking LBT (Figure 3). An additional ~3-4 fold increase in Tb adsorption was observed with both strains at 70 °C compared to room temperature, supporting an increased adsorption capacity at higher temperature. Future experiments will examine the effect of temperature on Tb adsorption under flow through conditions.

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|  | **Figure 3. Temperature dependence of REE biosorption onto *Caulobacter* biofilms.**Wild-Type *Caulobacter* or LBT-engineered Caulobacter biofilms were pre-formed and Tb adsorption was performed at room temperature and 70 °C in MES buffered (pH 6) solution with 20 μM TbCl3 for 30 min. Strain NA1000 is a negative control strain of Caulobacter that does not form biofilms, 723 is the base strain (S-layer with no LBT) that was used for LBT bioengineering, and dLBT contains a double LBT (dLBT) at position 723 of RsaA. |