A Tailings Pond Clean Up Kit:
A Synthetic Biology Approach to Bioremediation of Tailings

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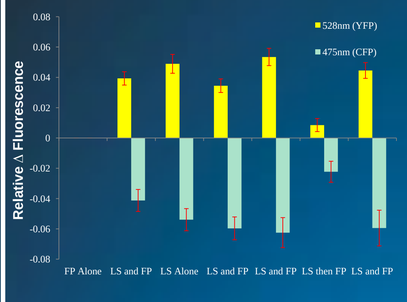
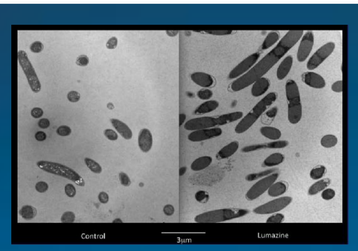
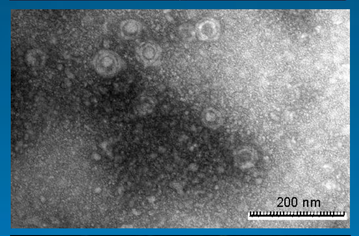
Managing byproducts of the extraction and refinement processes is a common problem in harvesting natural resources, such as oil. In most cases, tailings ponds are used for storing the toxic water byproducts, which not only have severe negative environmental impacts but by using current methods can take decades before they can be reclaimed. The current remediation methods need to be improved to provide economical, effective and efficient processes to decrease the negative environmental impact of the tailings ponds. The tailings ponds contain toxic organic compounds and fine clay particles, which require improved methods of treatment. We are working to produce a tailings pond clean up kit that uses environmentally safe methods to accelerate the decontamination of toxic organic molecules and settle fine clay particles at an increased rate. Toxic compounds will be degraded into metabolizable compounds at increased rates by using proteins that act within a common degradation pathway co-localized within a microcompartment in the form of an easily distributed dry powder. The rapid formation of fine clay sediments will be facilitated by the use of bacteria cell aggregates, increasing sedimentation rates from many decades to days or even hours. The kit will consist of either cell-free components or genetically modified organisms (GMO) that pose no threat to the environment as they will have been programmed with a method of rendering the cell inert and destroying its DNA once the desired action is completed. The methods within the tailings pond clean up kit will be applicable for large-scale treatment facilities as well as *in situ* tailings pond treatment.



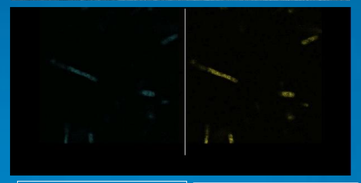
Fluorescence Resonance Energy Transfer (FRET) measurements of different expression patterns of lumazine synthase as well as tagged cyan and yellow fluorescent proteins. (CFP and YFP). Results suggest that co-localization efficiency is increased when the cargo proteins and microcompartment are co-expressed.



Transmission electron microscope image of *E. coli* DH5α cells before (left) and after expression of lumazine synthase (expression induced upon addition of IPTG). Homogeneous regions (right) not observed in the control experiment may indicate formation of microcompartments.

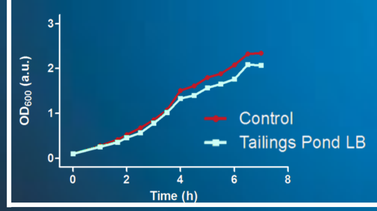


Transmission electron microscope image of enhanced lumazine synthase microcompartments (Hitachi H-7500 transmission electron micrscope, 100X magnification). Polyhedral particles approximately 40 nm in length are consistent with the expected size of microcompartments.



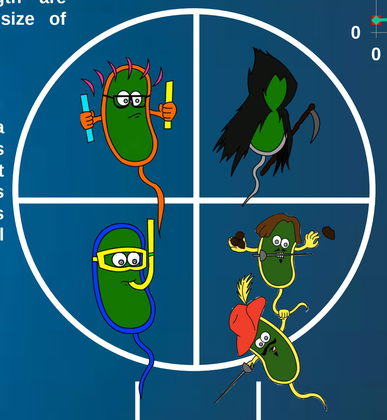
CFP Fluorescence | YFP Fluorescence

Confocal microscope image of *E. coli* DH5α cells expressing a lumazine synthase construct as well as cyan or yellow fluorescent proteins (CFP and YFP). Slides were viewed usingan Olympus FV1000 spectral confocal microscope (60X magnification).



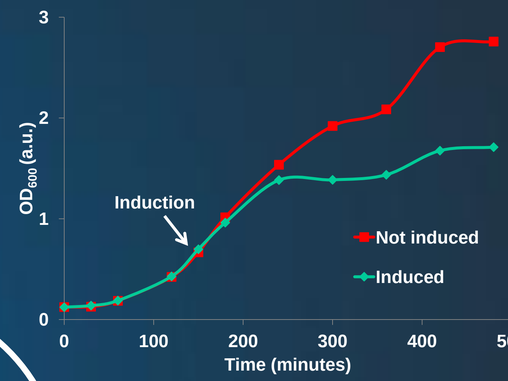
The OD_{600} of *E. coli* cultures grown in LB medium prepared with tailings pond water was compared to growth in standard LB medium. A slight loss in viability is seen, but this confirms that the growth of the selected chassis is not significantly impaired by contaminants tailings pond water.

Cell Viability: Surviving in the Tailings Ponds

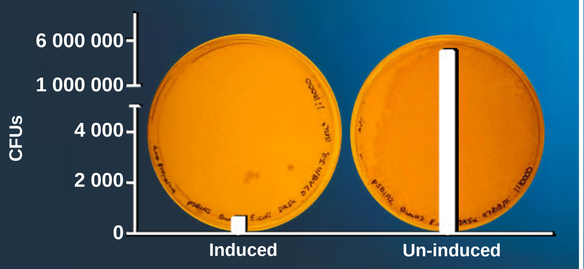


Compartmentalization: Degrading Toxic Compounds

Digestion of Recombinant DNA: Protecting the Environment



Growth curves of *E. coli* DH5α cells for expression of *Bam*HI. Expression of *Bam*HI causes a plateau in the growth of cells.



Colony forming units (CFU) grown from overnight cultures of *E. coli* DH5α induced for overexpression of *Bam*HI or uninduced. Expression of *Bam*HI significantly reduces CFU in comparison to uninduced cells.

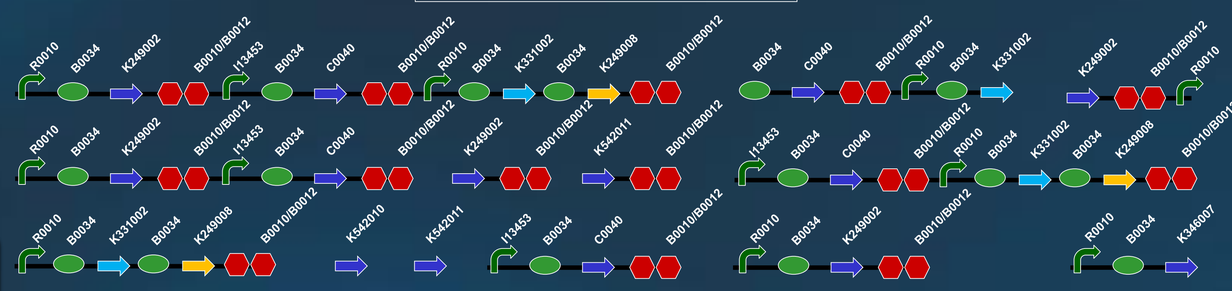
Aggregation of Sediments: Removing Fine Clay Particles

Sedimentation of *E. coli* DH5α cells induced for overexpression of Ag43 was compared to an uninduced sample and a blank control after 18 hours. Cells containing the construct displayed higher sedimentation than the blank control.

4h After Induction

18h After Induction

Parts Characterized and Submitted



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References:
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Viadiu H, Aggarwal AK (2000). Structure of BamHI bound to nonspecific DNA: a model for DNA sliding. Mol. Cell 5 (5): 889-895.