Development of a Model, Metal-reducing Microbial Community for a System Biology Level Assessment of *Desulfovibrio vulgaris* as part of a Community

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Ecosystems and Networks Integrated with Genes and Molecular Assemblies

VIMSS Virtual Institute for Microbial Stress and Survival — http://vimss.lbl.gov/

Background

One of the largest experimental gaps is between the simplicity of pure cultures and the complexity of open environmental systems, particularly in metal-contaminated areas. These microbial communities form ecosystem foundations, drive biogeochemical processes, and are relevant for biotechnology and bioremediation. A model, metal-reducing microbial community was constructed as either syntrophic or competitive to study microbial cell to cell interactions, cell signaling and competition for resources. The microbial community was comprised of the metal-reducing *Desulfovibrio vulgaris* Hildenborough and *Geobacter sulfurreducens* PCA. Additionally, *Methanococcus maripaludis* S2 was added to study complete carbon reduction and maintain a low hydrogen partial pressure for syntrophism to occur. Further, considerable work has been published on *D. vulgaris* and the *D. vulgaris*/ *Mc. maripaludis* co-culture both with and without stress. We are extending this work by conducting the same stress conditions on the model community. Additionally, this comprehensive investigation includes physiological and metabolic analyses as well as specially designed mRNA microarrays with the genes for all three organisms on one slide so as to follow gene expression changes in the various cultivation conditions as well as being comparable to the co- and individual cultures. Further, state-of -the-art comprehensive AMT tag proteomics allows for these comparisons at the protein level for a systems biology assessment of a model, metal-reducing microbial community. Preliminary data revealed that lactate oxidation by *D. vulgaris* was sufficient to support both *G. sulfurreducens* and *M. maripaludis* via the excretion of H_2 and acetate. Fumarate was utilized by *G. sulfurreducens* and reduced to succinate since neither of the other two organisms can reduce fumarate. Methane was quantified, suggesting acetate and H_2 concentrations were sufficient for *M. maripaludis*. Steady state community cultivation will allow for a comprehensive, system biology level analysis of a metal-reducing microbial community.

Experimental Setup and Community Metabolism

Figure 1: Continuous cultivation of model communities was performed using bioreactors such as the New Brunswick Bioflo 110.

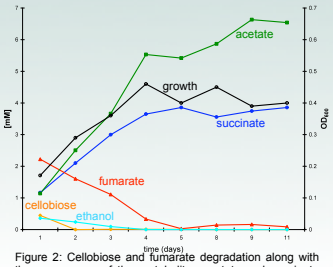


Figure 2: Cellobiose and fumarate degradation along with the appearance of the metabolites acetate and succinate during growth of the Clostridium led model community.

Multi-trophic Level Model Community

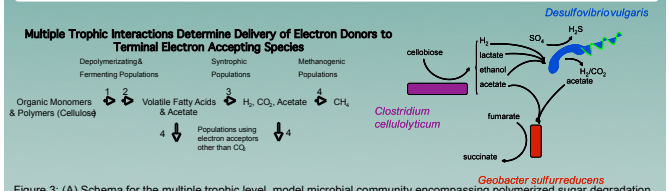


Figure 3: (A) Schema for the multiple trophic level, model microbial community encompassing polymerized sugar degradation which supports the overall community. (B) model community organisms and multiple carbon and electron pathways studied.

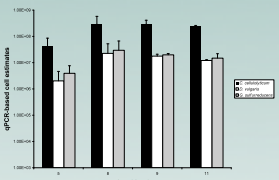


Figure 4: (A) qPCR based cell estimates showing the relative populations of the model community populations.

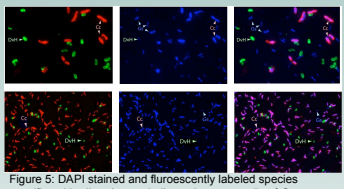
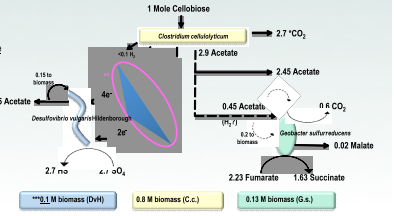


Figure 5: DAPI stained and fluroescently labeled species specific anitbodies. Arrows indicate the same cells of *C. cellulolyticum*, *C.c.*, *D. vulgaris*, DvH, and *G. sulfurreducens*, *G.s.*, imaged under different culture conditions.

Table 1: Fermentation Balance of the Multiculture

	Cells by PCR	Carbon balance (%)	Electron balance (%)	Energy in Products (%)
Overall	5.25	93	112	45
Clostridia	3.1	104	120	71
Geobacter	0.21	79	83	85
DvH	0.19	112	122	7

Proposed Model of Three Species Community Metabolism in Molar Units



Metal-reducing Model Community

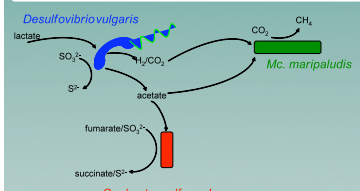


Figure 6: Schema for the metal-reducing model microbial community. This community differs from the multi trophic level community by forcing the community to be dependent upon the sulfate-reducer while following complete carbon reduction. This community will be explored for the interactions between the metal-reducers during syntrophic vs competitive growth conditions via the presence/absence of sulfite and assessing metal-reduction under these conditions.

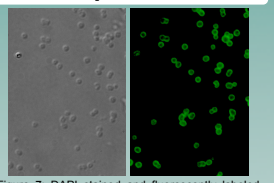


Figure 7: DAPI stained and fluorescently labeled species specific antibodies for *Methanococcus maripaludis* now developed (right) and compared to light microscopy (left).

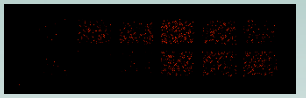


Figure 8: Multispecies microarrays have been developed and tested with negligable cross-species hybridization at ratios up to 20:1:1.

Conclusions and Future Work

Conclusions

- Technologies such as multi-species microarrays, fluorescent antibodies and qPCR are now in place and functioning.
-Initial metabolic model of the multi-trophic level microbial community shows incomplete energy usage and preference for the primary fermentor.
- construction and testing of the metal-reducing microbial community is nearly complete with new fluorescent antibodies for *Methanococcus maripaludis* S2.

Future work

-Completion of the metal-reducing community construction and analysis using all tools displayed here to determine the response by *D. vulgaris and Geobacter sulfurreducens* to different community attributes.
-The metal-reducing community will be analyzed under both syntrophic and competitive conditions to assess the response of each microorganism at the gene expression level.

ACKNOWLEDGEMENTS

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