Metabolic Network Analysis of an Anaerobic Microbial Community: Potential for Syntrophic Methane and Hydrogen Production

Adam, Z.[5], Bell, T.[1,3], Camilleri, L.[1,3], Connolly, J.[1,2], Hunt, K.[1,2], Michaud, A.[4], Smith, H.[1,4], Tigges, M.[6], Carlson, R.[1,2], Fields, M.[1,3], Foreman, C.[1,4], Gerlach, R.[1,2], Inskeep, W.[4]

Montana State University, Bozeman MT: [1] Center for Biofilm Engineering, [2] Chemical and Biological Engineering, [3] Microbiology, [4] Land Resources and Environmental Science, [5] Earth Sciences, [6] Chemistry and Biochemistry

INTRODUCTION

The essential role of microbial communities for driving nutrient cycles and producing useful products is increasingly appreciated by ecologists, geobiologists, and bioprocess engineers. Even when individual microbial community members are well understood, comprehending community-level behavior is challenging. Community level functioning is a result of both system members and their interactions. Due to this complexity, computer (*in silico*) models are essential. By computing possible metabolite fluxes between individual community members and their role on community-level behavior, *in silico* methods can be used to interpret and generate hypotheses related to how environmental conditions influence community structure and function. This presentation utilizes *in silico* techniques to build metabolic models representing six fully sequenced anaerobic organisms and their combined community potential for syntrophic methane and hydrogen production.

Waste → **Anaerobic Microbial Community** → **Biofuel**
- Methane
- Hydrogen

TRANSFORMATIVE HYPOTHESES

- Genomic analysis of community organisms will predict competitive strategies for essential nutrients
- Syntrophic relationships will develop through metabolite exchange
- Biofuel production can be optimized through community and nutrient manipulation

QUESTIONS AND OBJECTIVE

QUESTIONS

Natural anaerobic environment
- What drives microbial interactions?
- What metabolic and social strategies are probable?

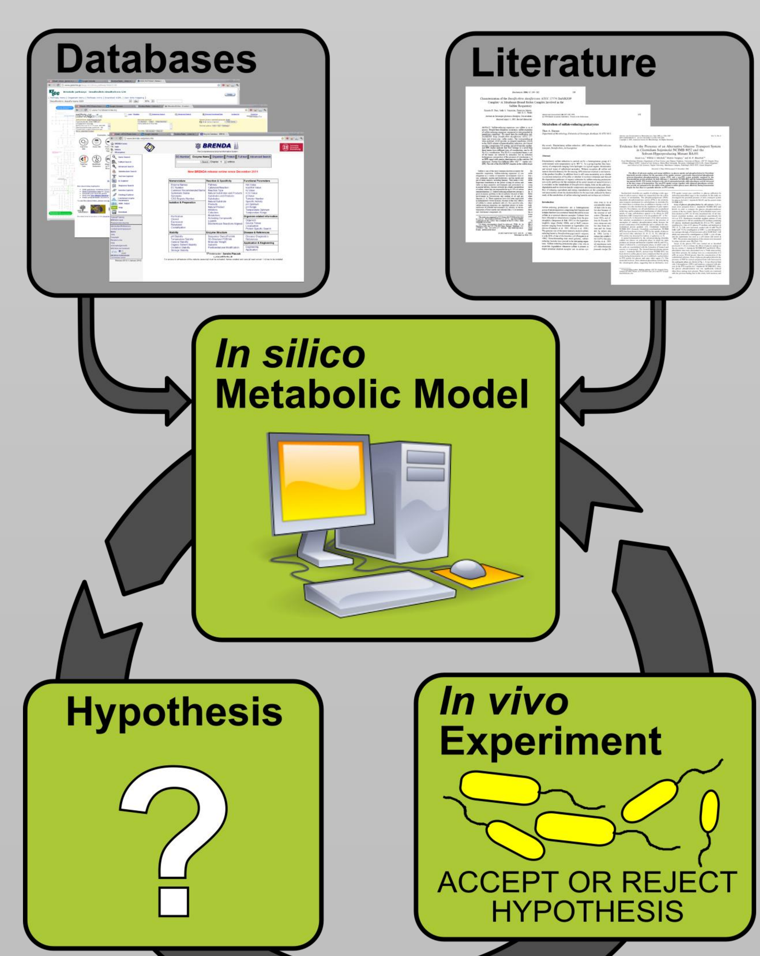
Bioreactor for fuel production
- How can microbial interactions be optimized for biofuel production?
- How can byproducts be controlled?

OBJECTIVE

Identification of metabolic modes that contribute to cooperative resource allocation and optimize biofuel production

APPROACH

1. Six *in silico* metabolic network models were developed to represent the behavior of individual organisms.
2. Genomic databases and literature were mined for model input.
3. Models were used to generate hypotheses about community interactions.
4. Hypotheses were tested or rejected and the model refined.



COMMUNITY OVERVIEW

Fermenters	***Clostridium beijerinckii* NCIMB 8052** Basic fermentation of a wide variety of sugars, producing mixed acids, alcohols and gases ***Clostridium ljungdahlii* DSM 13528** Acetogenic fermentation of a wide variety of sugars, producing acetate and ethanol
Sulfate Reducers	***Desulfovibrio alaskensis* G20** Incomplete oxidizer of lactate to reduce sulfate, producing acetate, H_2 and sulfide. ***Desulfotomaculum acetoxidans* DSM 771** Complete oxidizer of acetate to reduce sulfate, producing acetate, H_2 and sulfide.
Methanogens	***Methanospirillum hungatei* JF-1** Strict CO_2/H_2 methanogenesis, oxidizing H_2 without cytochromes ***Methanosarcina barkeri* Fusaro** Utilizes CO_2/H_2 or acetate for methanogenesis, using cytochromes in electron transport

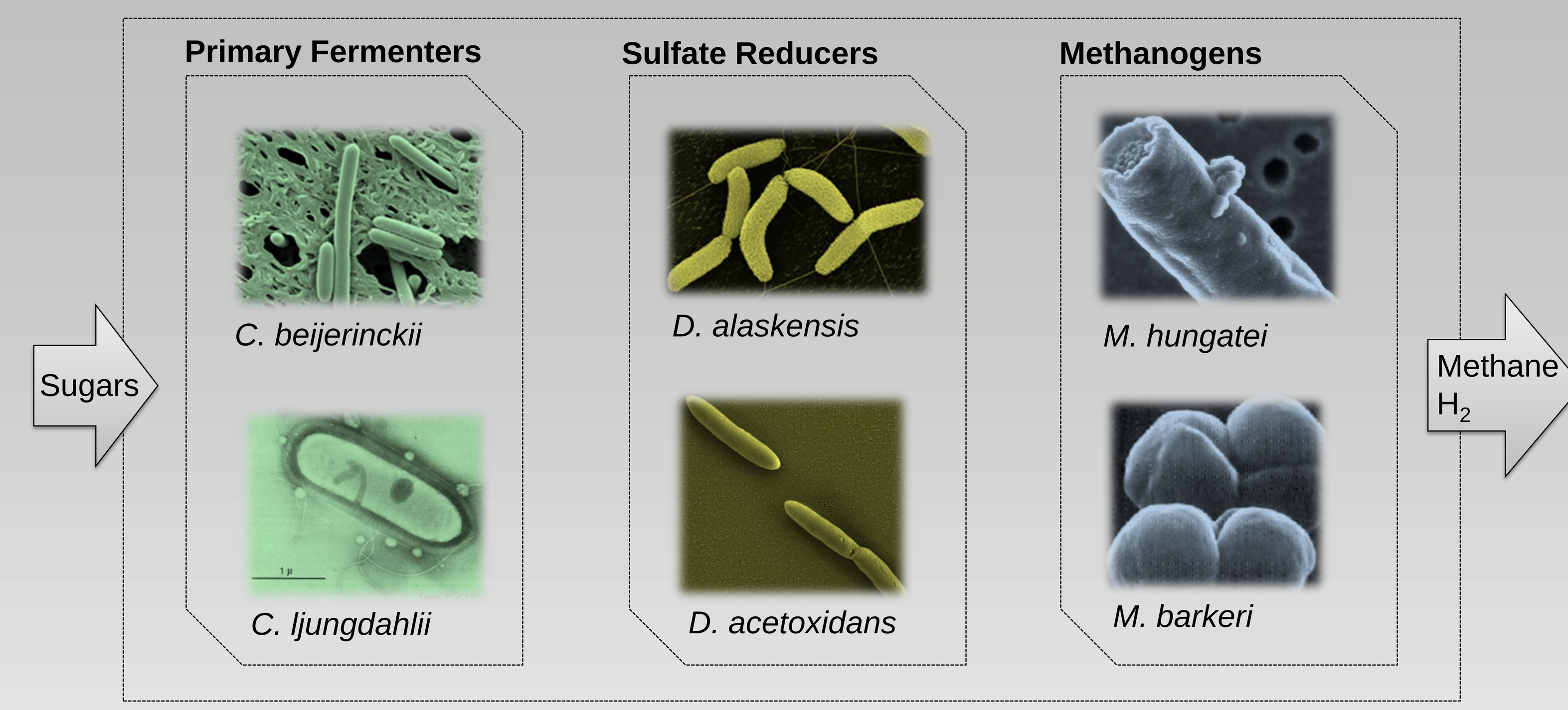


Figure 1: Representative anaerobic community comprised of six organisms with sequenced genomes that group into three functional guilds.

Information Sources

Genome-Based Data
Predicts internal metabolic function based on genes identified in each organism's genome.

Literature
Experimental observations noted in literature ensure that the models are grounded to observed functionality.

Protein and Pathway Databases
Metabolic potential is not always clear based on the analysis of an individual organism's genome. Protein sequences with known function were compared to the genome of interest to provide evidence for a pathway that is likely to be present but not annotated in the genome.

INDIVIDUAL MODEL CONSTRUCTION and ANALYSIS

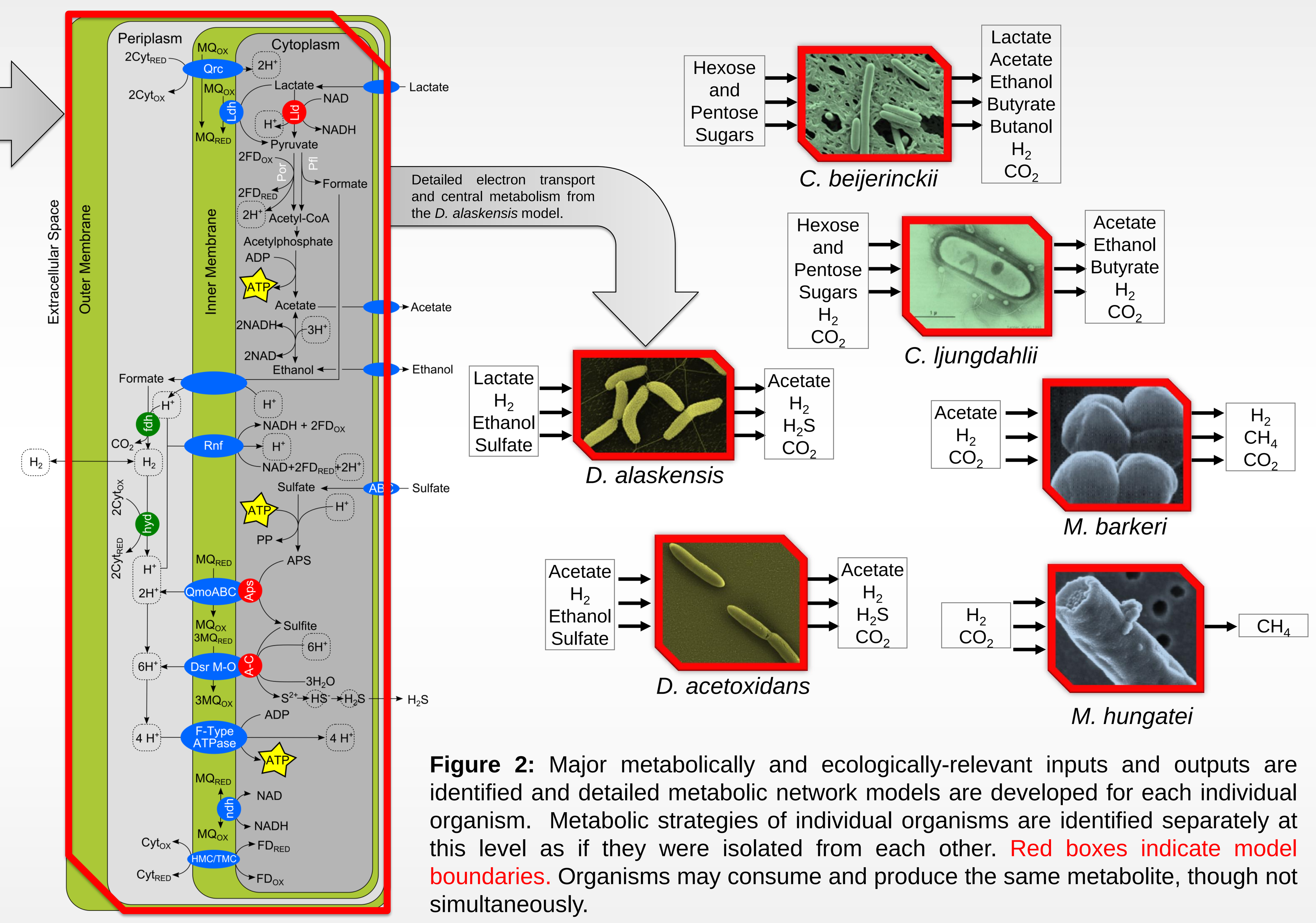


Figure 2: Major metabolically and ecologically-relevant inputs and outputs are identified and detailed metabolic network models are developed for each individual organism. Metabolic strategies of individual organisms are identified separately at this level as if they were isolated from each other. Red boxes indicate model boundaries. Organisms may consume and produce the same metabolite, though not simultaneously.

COMMUNITY NETWORK MODEL

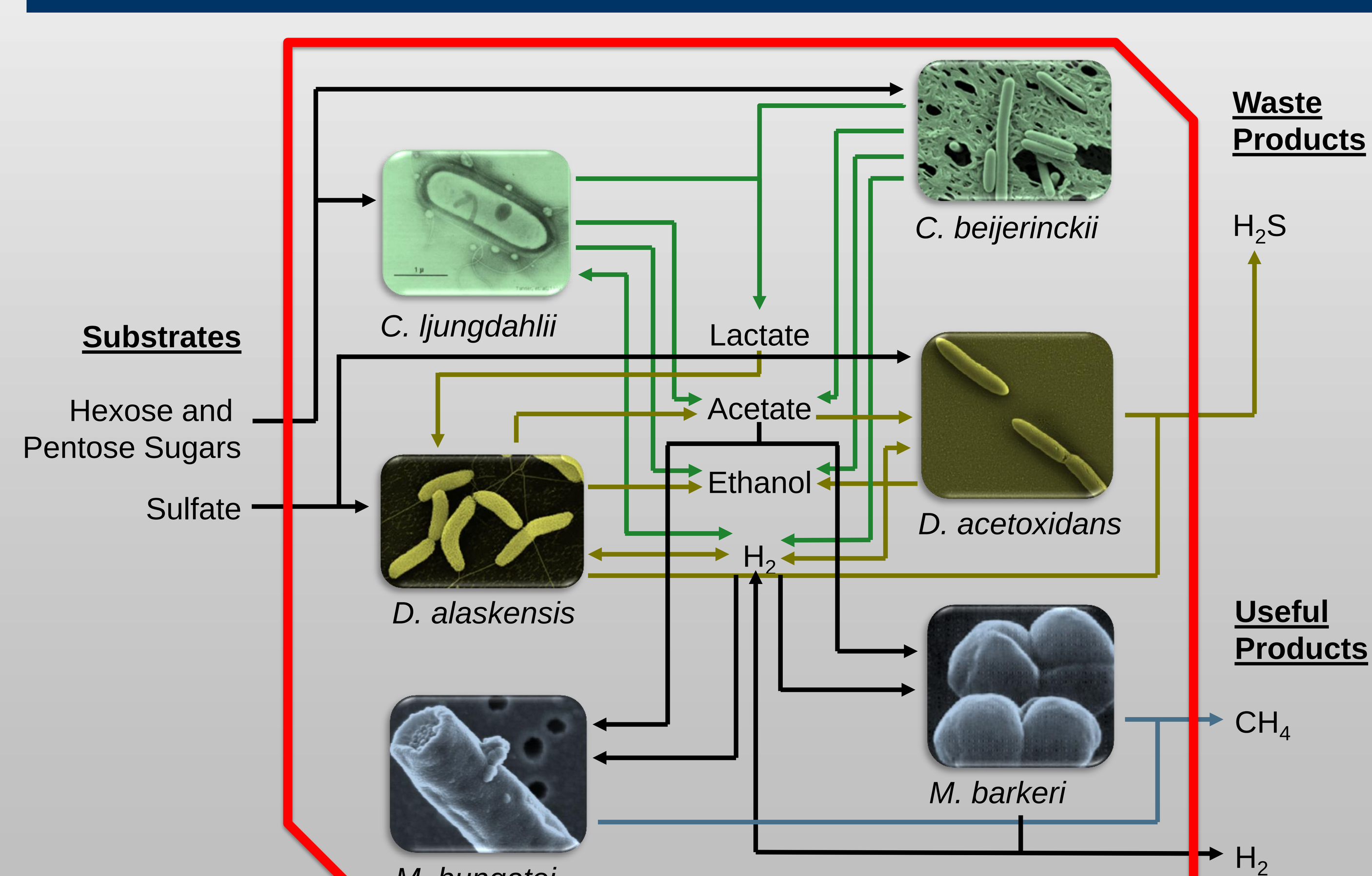
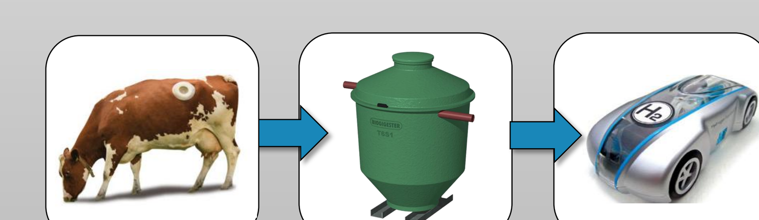


Figure 3: Potential metabolite exchange amongst community members

CLEAN ENERGY PROSPECTS

- By feeding the modeled microbial community complex carbohydrates and controlling nutrients, end products can be optimized for biofuel production
- Hydrogen and methane both have substantial energy potential
  - Both can be used for heating, electricity and transportation
  - H_2 can be used in fuel cells to generate electricity



TRADE-OFF ANALYSIS

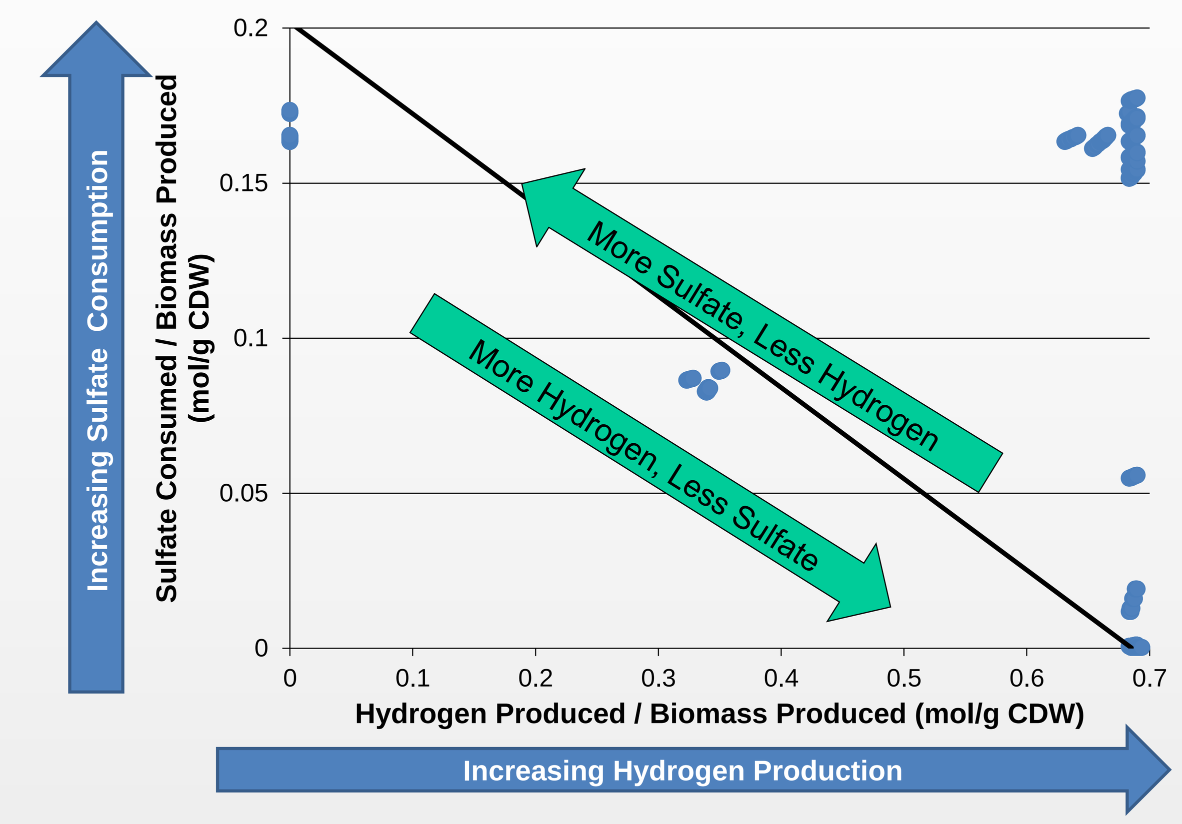


Figure 4: Trade off between hydrogen production and sulfate reduction. The line represents the relationship between the most efficient metabolic strategies for biomass production based on electron acceptor. Lower right strategies represent behavior in which hydrogen is produced, fostering cooperation with methanogens (Table 1). Upper left strategies represent behavior in which hydrogen availability to the community is unaffected. Additional strategies fall on this trade off envelope in which hydrogen is consumed as representative of a competitive strategy (Data not shown). Trade off curves such as these can be generated from model results for inter-species comparison (competitive strategies) or community analysis.

Table 1: Thermodynamic calculations at standard conditions for the phosphorylation of ADP to ATP and calculated free energies associated with the reduction of CO_2 to produce CH_4 at physiologically significant hydrogen partial pressures.

Stoichiometry	Partial Pressure H_2	ΔG' (kJ/Mole)	ATP/CH_4
ADP + P_i ←→ ATP	Standard Conditions	50	2.5 – 3
$CO_2 + 4H_2$ ←→ $CH_4 + 2H_2O$	1 Pascal	-17	0.34
	10 Pascal	-40	0.8

DISCUSSION

Depending on environmental conditions, individual metabolic strategies will either succeed or fail based on their thermodynamic favorability. As illustrated in Table 1 a methanogen with specialized energy conservation (ATP/CH_4) for survival at low partial pressures of hydrogen may outcompete other methanogens which require higher H_2 concentrations. These concentrations correspond with the upper limits of hydrogen production by SRBs. As shown in Figure 4, nutrient availability dictates community behavior. For example the role of SRBs will change from hydrogen consumers to hydrogen producers in sulfate deplete environments, thereby determining the optimal metabolic strategy for competing methanogens. From a community perspective, all six organisms exchange hydrogen as a metabolite (Figure 3), either utilizing it as an electron donor or producing it as a waste product. How the community shares this metabolite depends on the concentration of available alternatives such as sulfate. *In silico* models elucidate these trade-offs and provide a framework for system analysis of ecological and engineered systems.

FUTURE WORK

Develop focused experimental approaches to test additional hypotheses and verify model results.
- Genetic and biochemical techniques can be used to verify metabolic pathways of uncertainty.

Expand model-based results to *in situ* experimental systems facilitating model verification and microbial population analysis.
- Address scale and commercial feasibility for methane and hydrogen production.

SUPPORT and ACKNOWLEDGEMENTS

Support for this project provided by the NSF IGERT Program in Geobiological Systems (DGE 0654336) at Montana State University.