Thermodynamics and H_2 Transfer in a Methanogenic, Syntrophic Community

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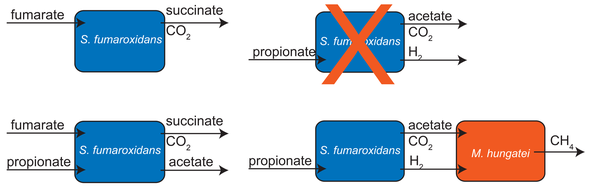
Introduction

- In syntrophic systems, two organisms combine forces to perform a function neither alone can carry out
- In these systems, metabolic reactions are barely thermodynamically favorable, with low energy yields
- Syntrophic association between *Syntrophobacter fumaroxidans* (*S. fum*) and *Methanosprillum hungatei* (*M. hun*) has been extensively characterized
- **Can we use a thermodynamic model to describe the syntrophic association between these two organisms?**

Thermodynamics in Syntrophic Systems

- In monoculture, *S. fum* grows on fumarate (fum) or fum + propionate (prop)
- Growth on propionate alone only occurs in the presence of *M. hungatei*

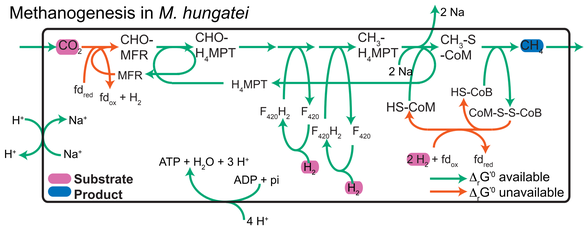


- Free energy estimates suggest a thermodynamic basis for the metabolic shift
- H_2 consumption by *M. hungatei* prevents a thermodynamic bottleneck

	Stoichiometry	$\Delta_r G'^0$ (kJ/mol)
Fum Only	7 fum + 4 H_2O + 2 H^+ → 6 succ + 4 CO_2	-374.3
Fum + Prop	prop + 3 fum + 2 H_2O → ac + CO_2 + 3 succ	-154.0
Prop Only	*S. fum*: prop + 2 H_2O → ac + CO_2 + 3 H_2	139.5
	M. hun: CO_2 + 4 H_2 → CH_4 + 2 H_2O	-196.8
Overall	prop + 0.5 H_2O → ac + 0.25 CO_2 + 0.75 CH_4	-8.1

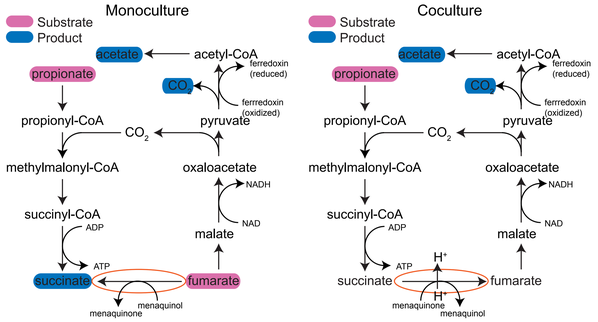
Metabolic Reconstruction of M. hungatei

- Built using RAVEN toolbox using sequence homology to existing models
- Curation of features unique to methanogens lacking cytochromes
- Stoichiometric and thermodynamic validation of energy conservation



Metabolic Reconstruction of S. fumaroxidans

- Built using RAVEN toolbox from KEGG database
- Curation of carbon and electron transfer mechanisms



- Stoichiometric and thermodynamic validation of energy conservation
  - H_2ase (CHYD): 3 H + NADH + 2 fd_{red} → 2 H_2 + NAD + 2 fd_{ox}
  - Formate deH_2ase (FDH): NADH + CO_2 + 2 H + 2 fd_{red} → NAD + formate + 2 fd_{ox} + H_2
  - Formate-hydrogen lyase (FHL): CO_2 + H_2 ↔ formate

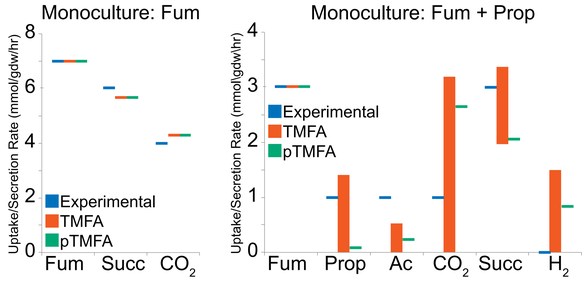
Thermodynamic Modeling of S. fumaroxidans in Monoculture

- Two-step, parsimonious thermodynamic metabolic flux analysis (pTMFA) used to predict extracellular flux distributions

Step 1: TMFA
max cellular growth
s.t. mass balance
flux bounds
all reactions bidirectional
$\Delta_r G' \cdot v < 0$

Step 2: pTMFA
min enzyme costs
s.t. mass balance
flux bounds
all reactions bidirectional
$\Delta_r G' \cdot v \leq 0$
cellular growth from Step 1

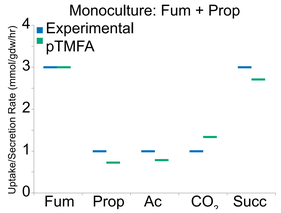
- TMFA and pTMFA accurately predict growth in monoculture on fumarate
- pTMFA incorrectly predicts by-product secretion during growth on fum + prop



Thermodynamics and H_2 Production

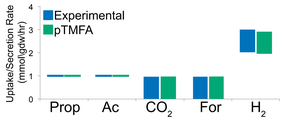
- Experimental observations suggest a thermodynamic explanation
  - Electron transfer genes are expressed in both mono- and co-culture
  - H_2 production in monoculture ceases at [10 Pa]
- Component contribution cannot compute $\Delta_r G'^0$ of electron transfer reactions
- Adding constraints on [H_2] and electron transfer direction enables the correct prediction

Reaction	Direction
H_2ase	Reverse as written
Formate deH_2ase	Forward as written
Formate-H_2 lyase	Reverse as written



Thermodynamic Modeling of S. fumaroxidans in Coculture

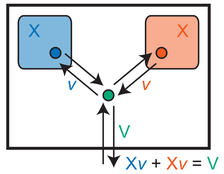
- pTMFA accurately predicts growth in coculture



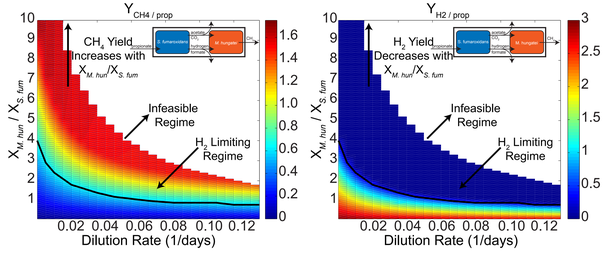
Exploration of Steady-State Continuous Culture

- We developed a novel community-level formulation with which we examine trade-offs between growth rate and species concentrations

min species-weighted enzyme costs
s.t. mass balance ∀ species
flux bounds ∀ species
all reactions bidirectional ∀ species
$\Delta_r G' \cdot v \leq 0$ ∀ species
dilution rate = growth rate ∀ species
reactor balance

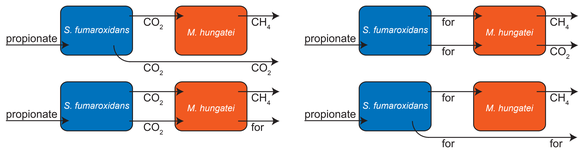


- Model predicts correct stoichiometry at onset of H_2 limitation
- Within H_2 limiting regime, methane yield increases with *M. hun* levels



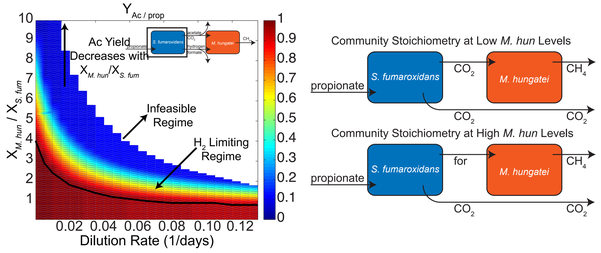
Carbon Transfer in H_2 Unlimited Regime

- The presence of a formate-hydrogen lyase in both *S. fum* and *M. hun* enables the free interconversion of CO_2 and H_2 to formate at no thermodynamic cost
- Flux distributions are a linear combination of the following four scenarios



Carbon Transfer in H_2 Limited Regime

- Stoichiometric shift in *S. fum* supports high *M. hun* levels
- *S. fum* with low *M. hun*: prop + 2 H_2O → ac + CO_2 + 3 H_2
- *S. fum* with high *M. hun*: prop + 2 H_2O → CO_2 + 2 formate + 5 H_2
- When H_2 is limiting, formate is preferred for carbon exchange



Conclusions

- Minimization of enzyme costs (pTMFA) predicts the observed extracellular flux distribution for both *S. fum* and *M. hun*
- Additional constraints required to model the role of [H_2] in enabling syntrophic behavior
- pTMFA predicts a shift in *S. fumaroxidans* extracellular flux distribution at high *M. hungatei* levels

Select References

Computational

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Experimental

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