Methanogenic Community Dynamics from Natural and Synthetic Systems

Orkun S Soyer

AD Research Colloquium
Manchester, 24 January 2019

OSS LAB

Bio Electrical Engineering Innovation Hub @ Warwick

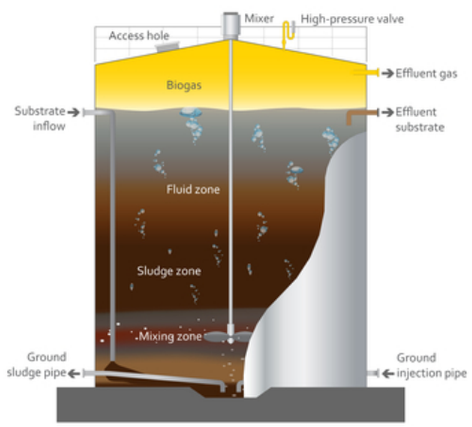
WARWICK
THE UNIVERSITY OF WARWICK

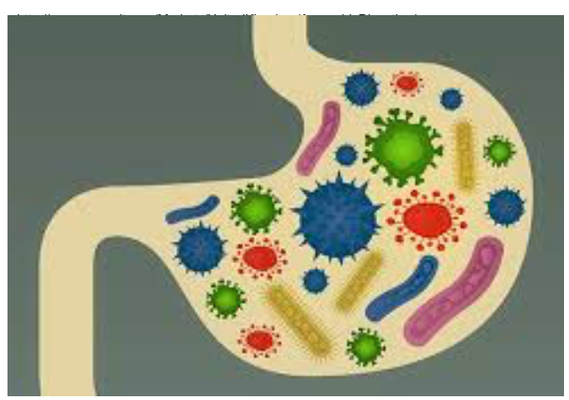
WARWICK CENTRE FOR
INTEGRATIVE SYNTHETIC BIOLOGY

Microbial Communities

High industrial and medical relevance

BIOTECH | AGRI-TECH
WATER | MEDICAL





Scientific frontier with a multitude of open questions

How can we explain diversity in microbial communities?

What is the function (if any) in a given microbial community?

Does community stability relate to diversity?

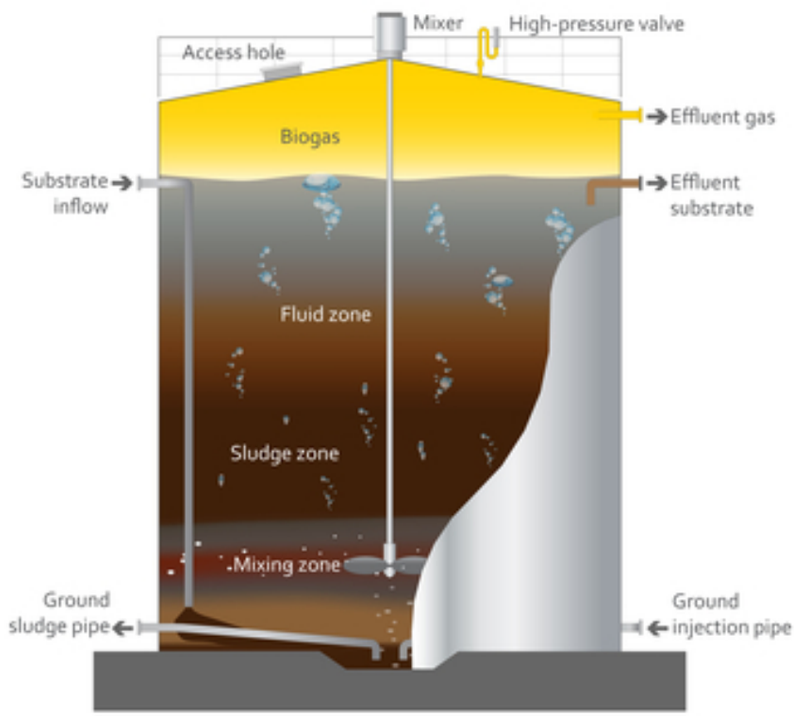
What are the key interactions in microbial communities?

What is the relation between ecological and evolutionary processes?

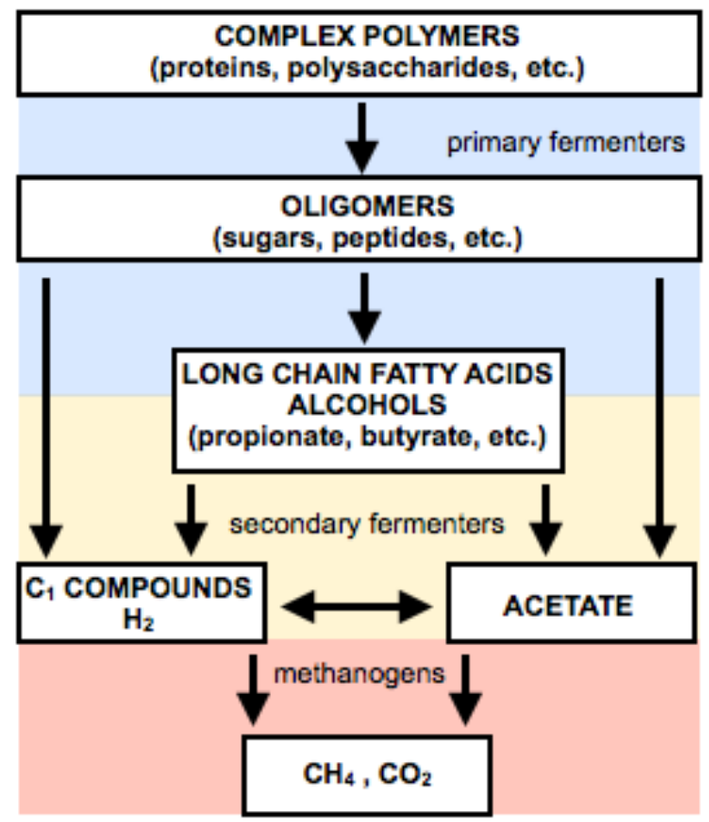
Widder S. *et al*, ***ISME J*** 10:11 (2016)

A Functional/Tractable Microbial Community?

Anaerobic Digestion (AD)



http://www.mannvit.com/Markets/UnitedKingdom/AnaerobicDigestion/



Schink B *Microbiol Mol Biol Rev* 61:2 (1997)

BBSRC bioscience for the future

sLoLa
2013-2018

WARWICK THE UNIVERSITY OF WARWICK | UNIVERSITY OF EXETER | Imperial College London | Newcastle University | TGAC The Genome Analysis Centre™

Engineering Synthetic Microbial Communities for Biomethane Production

http://osslab.lifesci.warwick.ac.uk/adLola_rsrcs.html

£3.86M

10 PIs
7 PDRAs
6 PhDs

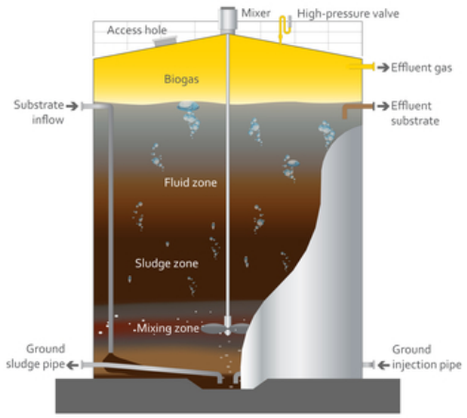
>24

4 sci.
2 ind.
2 videos

1 IPA
1 TRDF
1 Spin-out

Our Approach

Decipher complex, natural systems to identify general trends



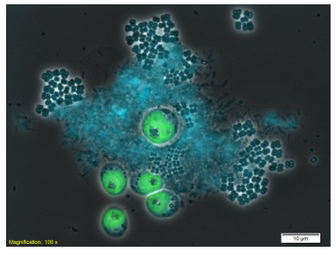
http://www.mannvit.com/Markets/UnitedKingdom/AnaerobicDigestion/

TOP-DOWN

? Insights and Applications ?

BOTTOM-UP

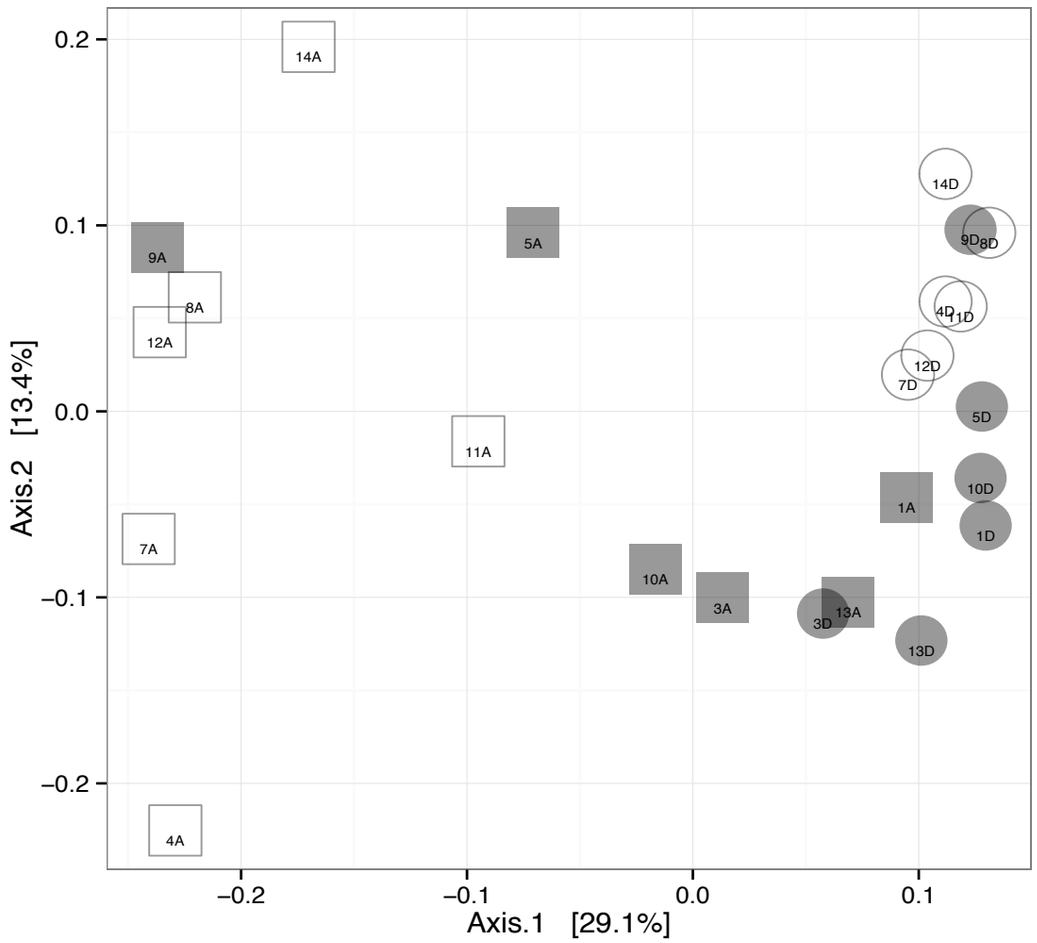
Engineer minimal systems to learn about biochemical basis of communities



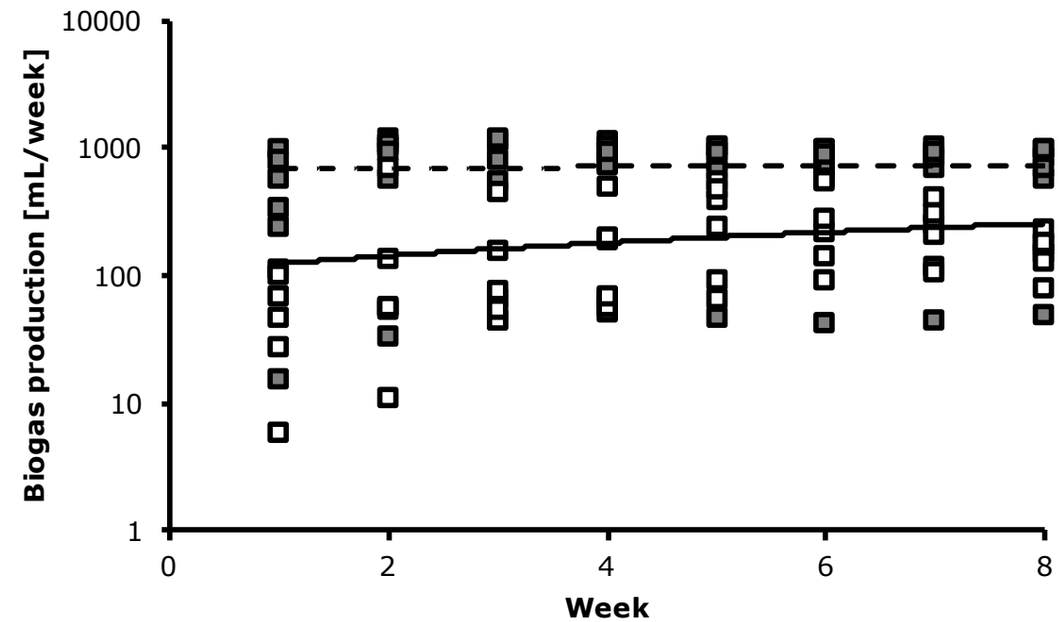
‘Top-down’ insights from natural and AD communities

Communities are different but not

Work led by Angus Buckling *et al*



Degradation performance, ie biogas production differs among communities from AD reactors vs. natural



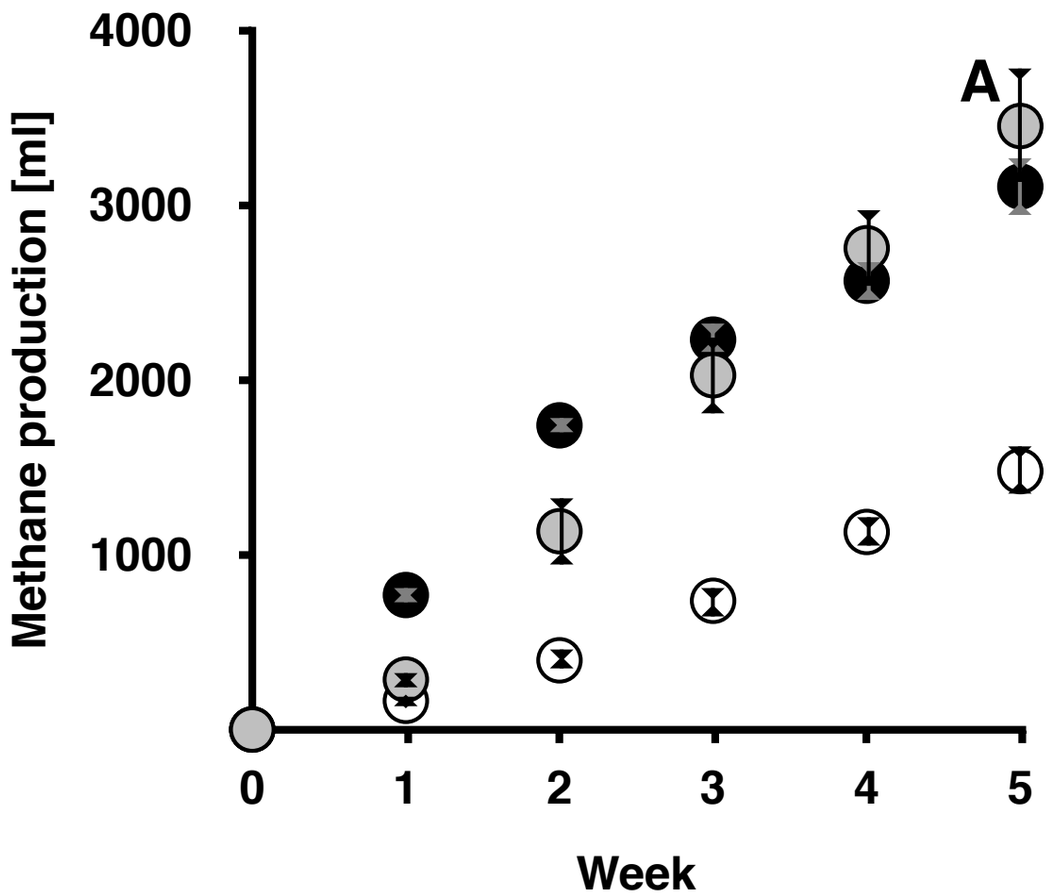
Community composition (and biogas production) converges upon adaptation to same feedstock

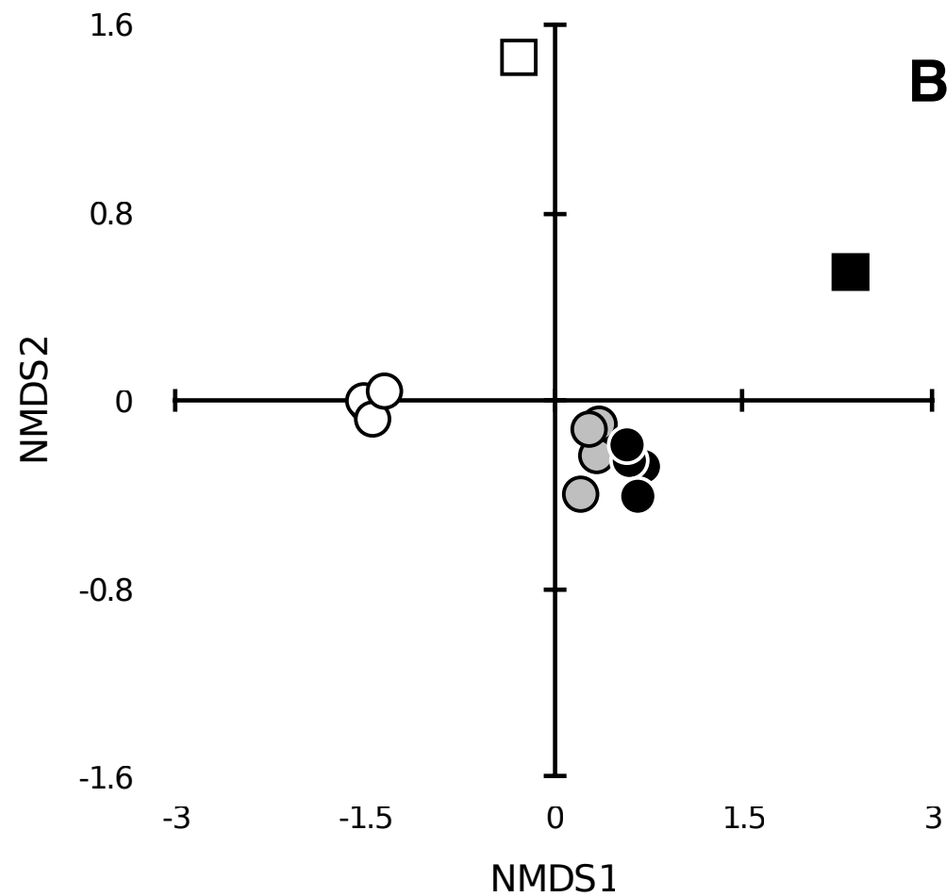
Sierocinski P et. al. ***Mol. Ecology*** (2018).

Communities are entities…
Mix to get the best?

Work led by Angus Buckling *et al*

Communities' mixtures are dominated by top performing community

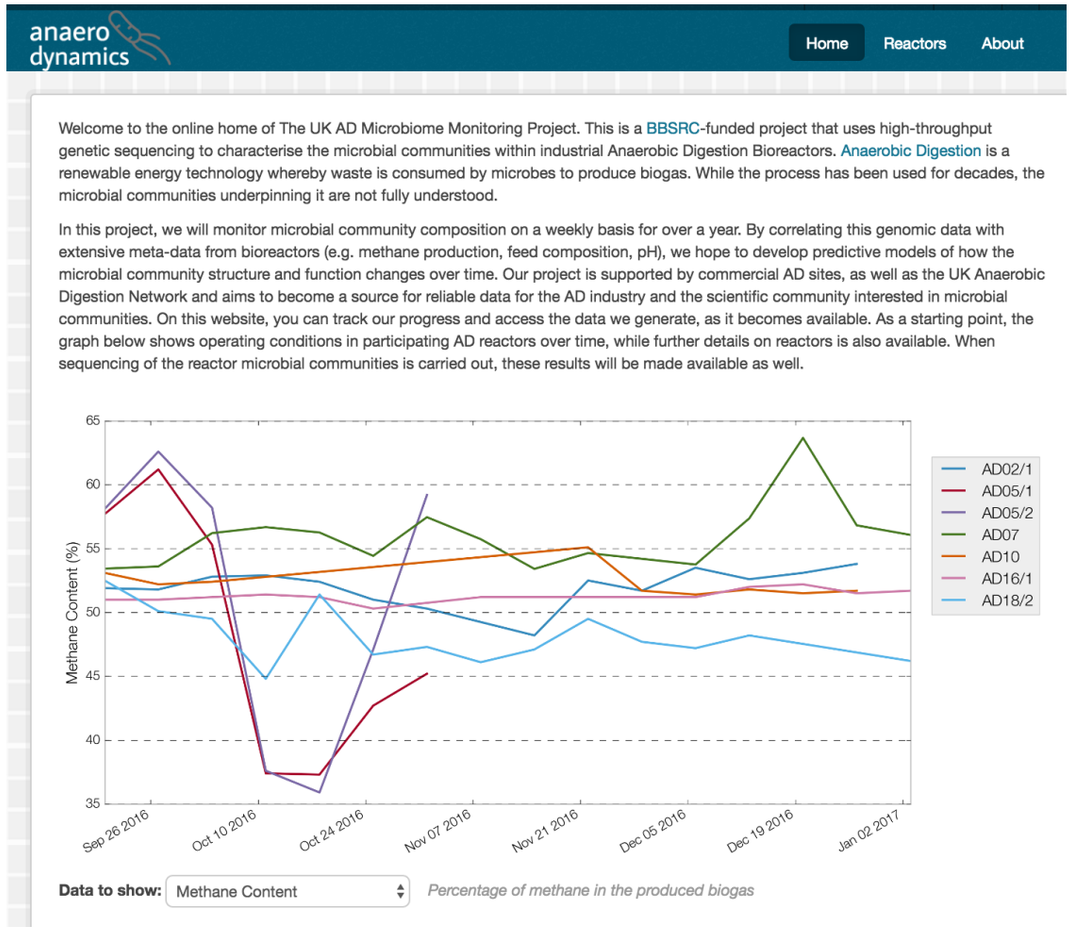




Sierocinski P et. al. ***Curr. Biology*** (2017).

Offshoot: Temporal analysis of industrial AD reactors

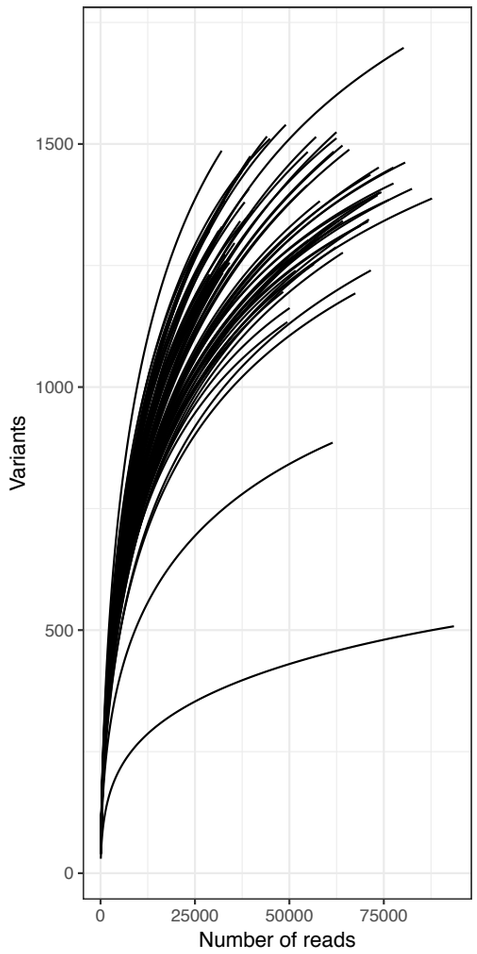
www.anaerodynamics.com

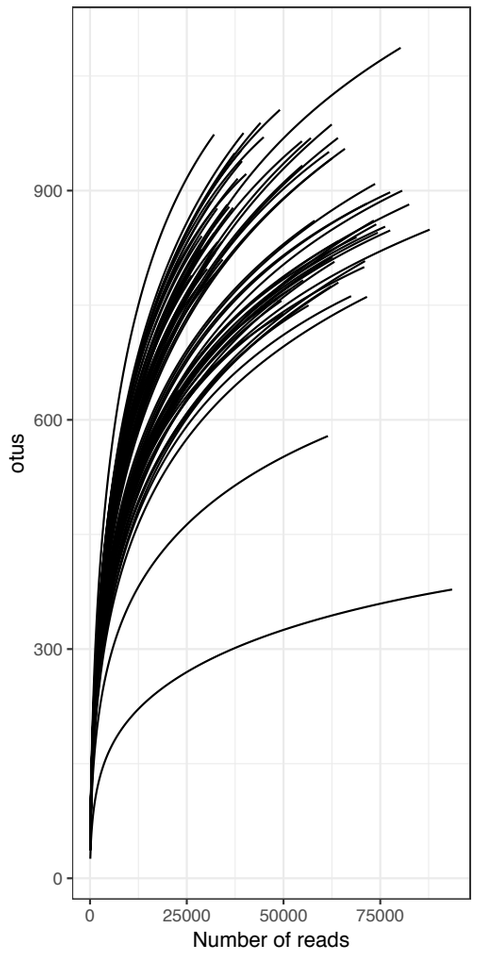


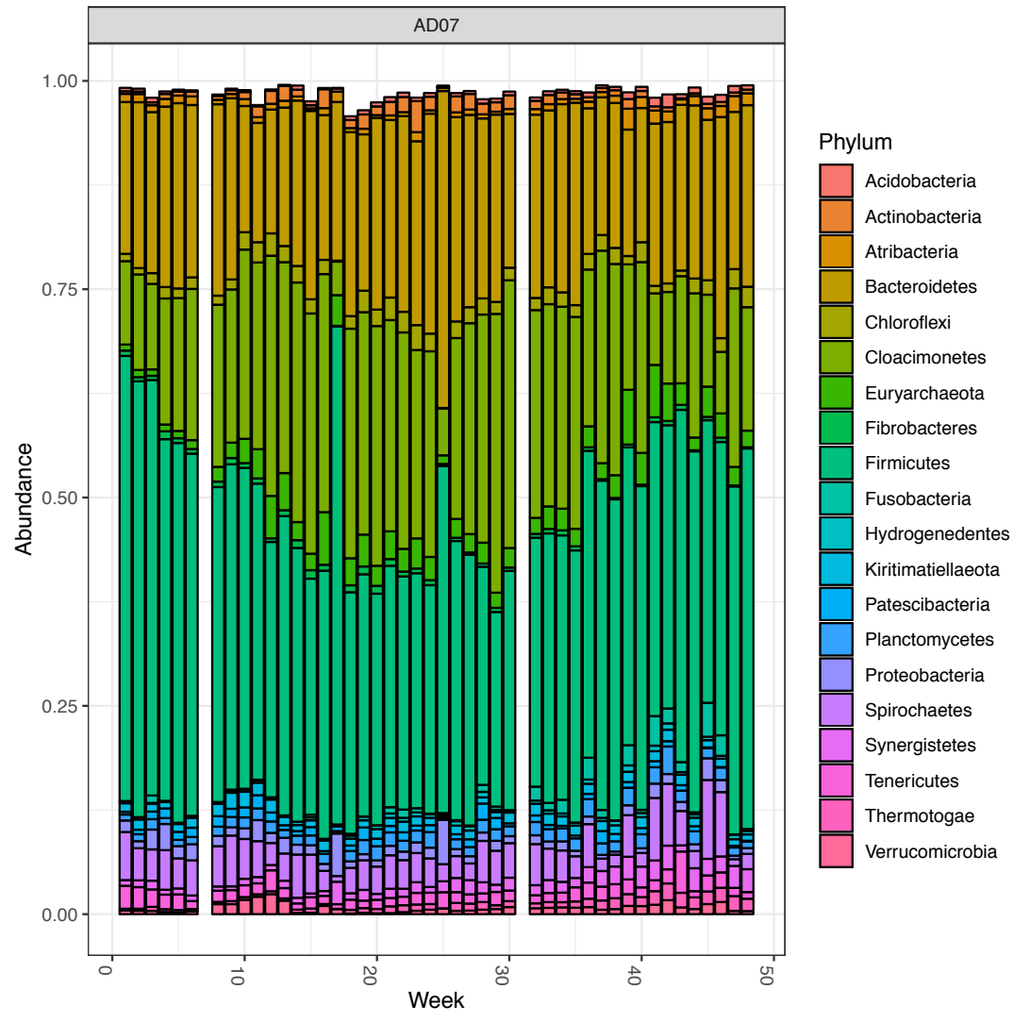
In collaboration with Chris Quince & Sebastien Raguideau

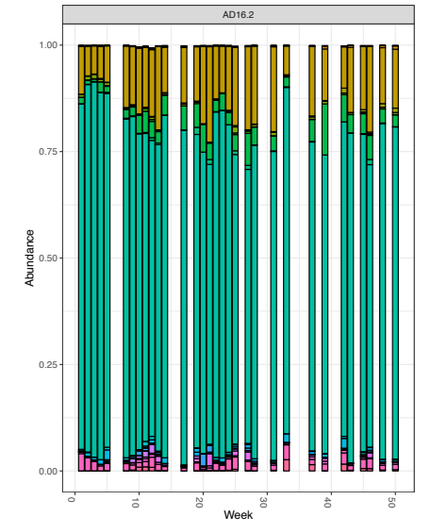
- Weekly samples over a year
- 16s (V4 region) for bacteria and archea
- Metagenomics
- Metadata on methane, pH, and (in some cases) feed. **Coverage could have been better! :(**

The most comprehensive AD sequencing?









Sample, sample, sample!

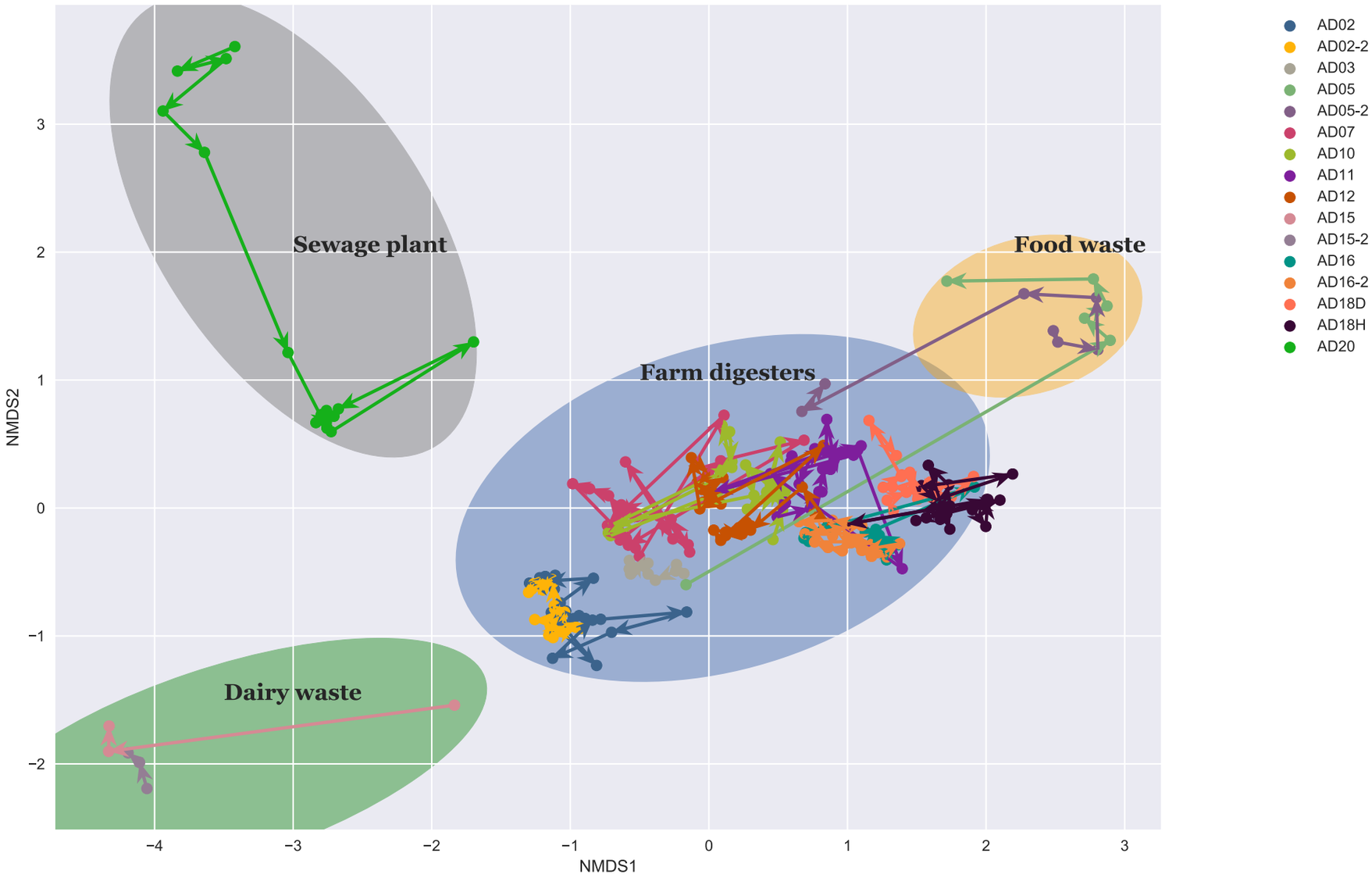
16s rarefaction curves & temporal coverage for reactor AD07

Metagenomics. Pooling samples over time gives ~**3.8 giga bp** of assembly for AD07, comparable to studies on gut (~3.3 giga bp)

How many MAGs?

How will OTUs & MAGs compare?

Communities are entities?



Reactor microbiomes are different, *possibly* correlating with their 'food' source

unpublished results

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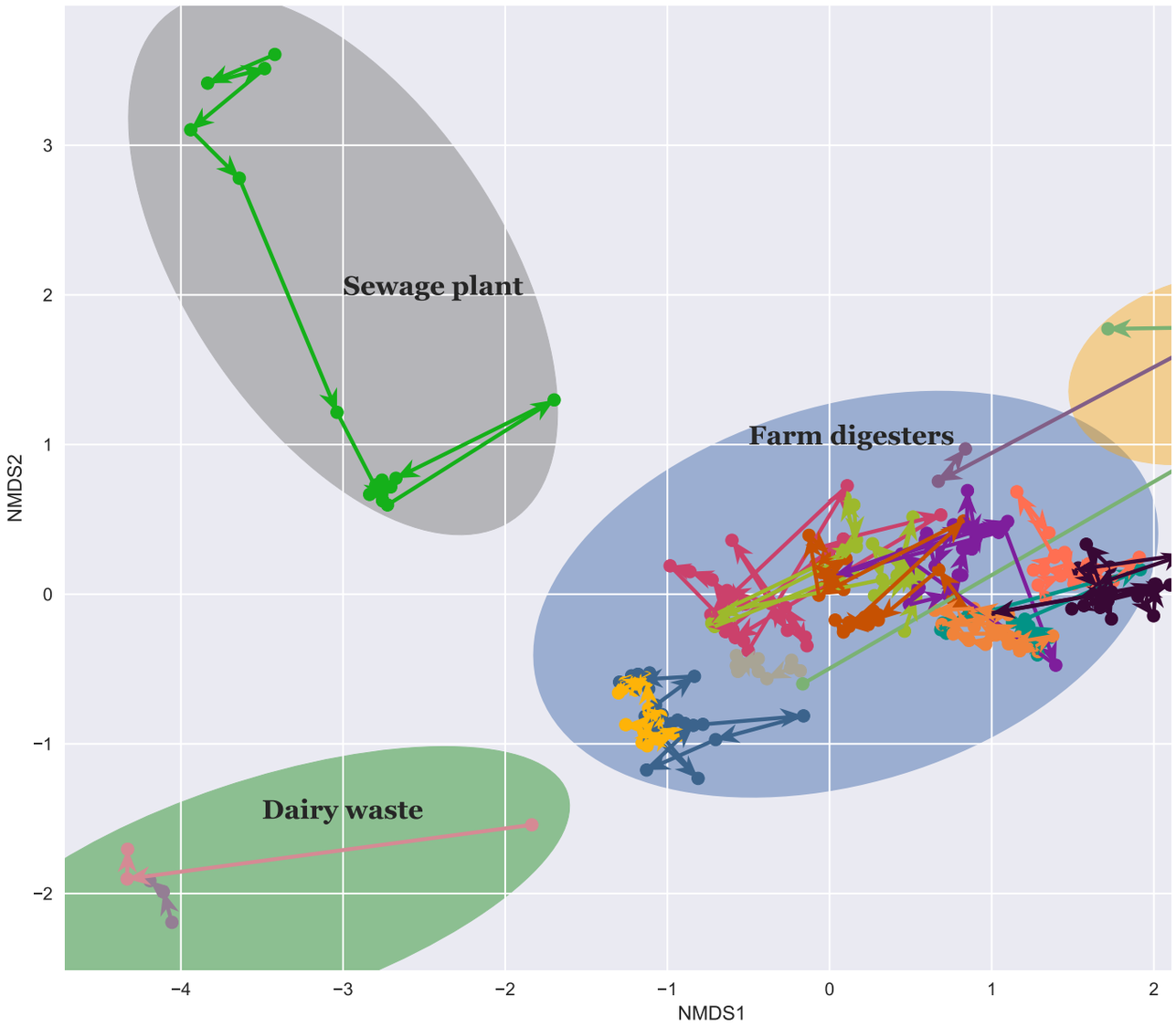

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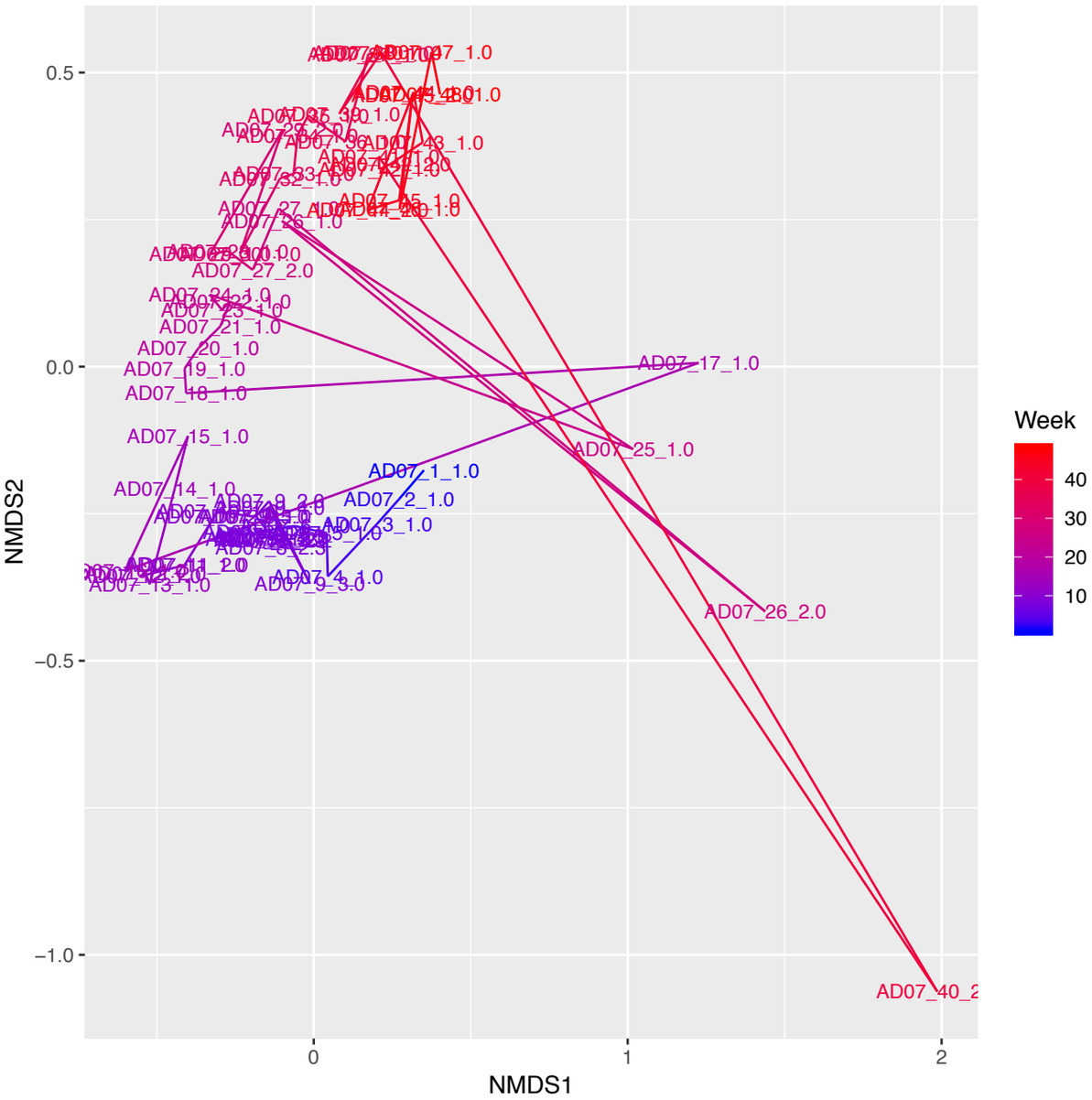

Microbiome composition changes over time, but how significant is this?

unpublished results

Is there hope for predictive models?!

Correlations exists between meta-data and microbiome at OTU-level

AD07:

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
gas	1	0.4850	0.48496	6.1235	0.06215	0.001	***
temperature	1	1.4584	1.45842	18.4150	0.18690	0.001	***
o2	1	0.4643	0.46429	5.8625	0.05950	0.001	***
h2s	1	0.4267	0.42673	5.3882	0.05469	0.001	***

AD12:

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
gas	1	0.6418	0.64176	11.3233	0.15107	0.001	***
ph	1	0.6578	0.65781	11.6065	0.15485	0.001	***

unpublished results

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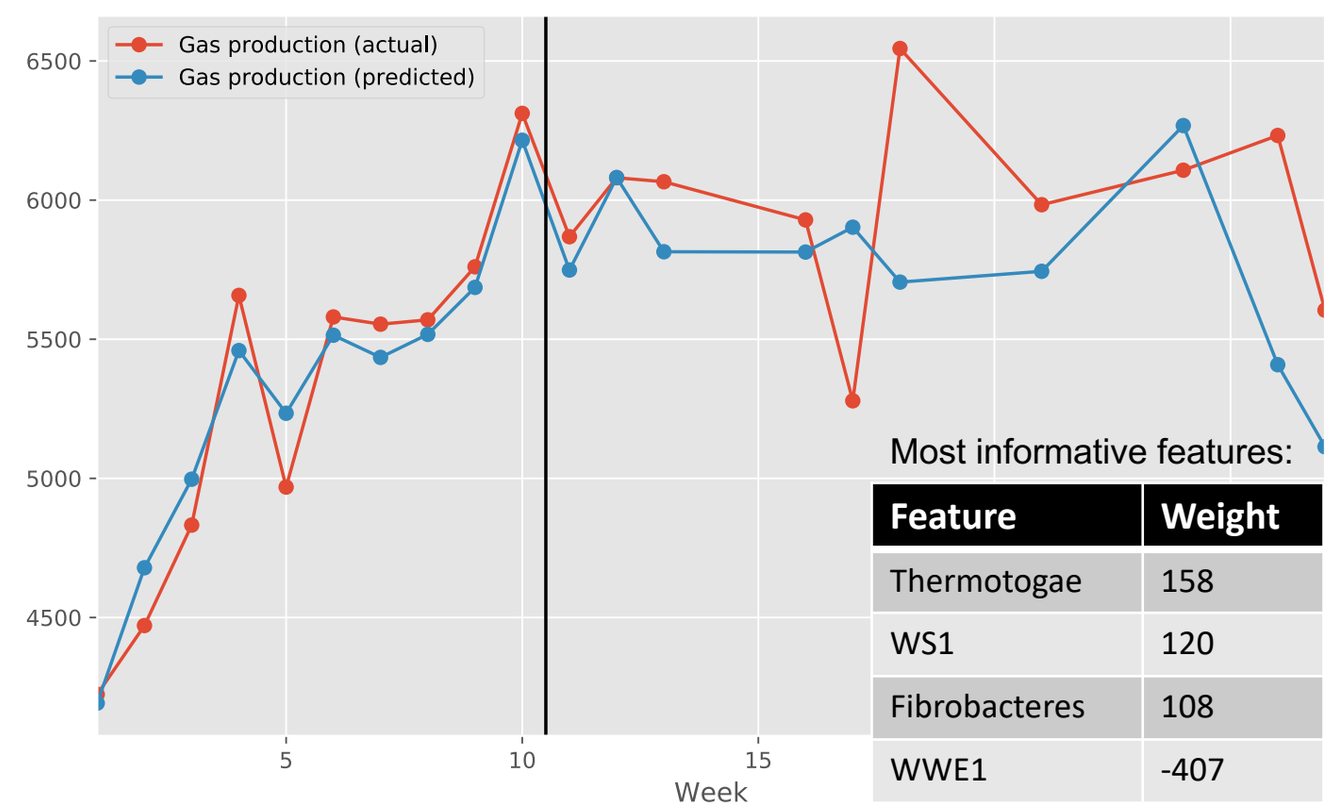
This might allow 'learning' models that can predict methane production

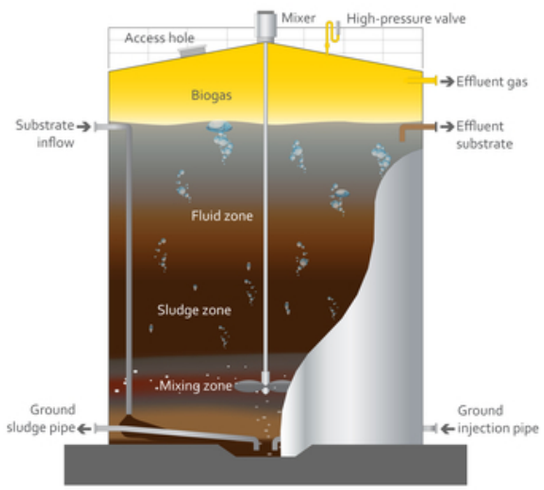


Most informative features:

Feature	Weight
Thermotogae	158
WS1	120
Fibrobacteres	108
WWE1	-407

unpublished results

What about biological insights?



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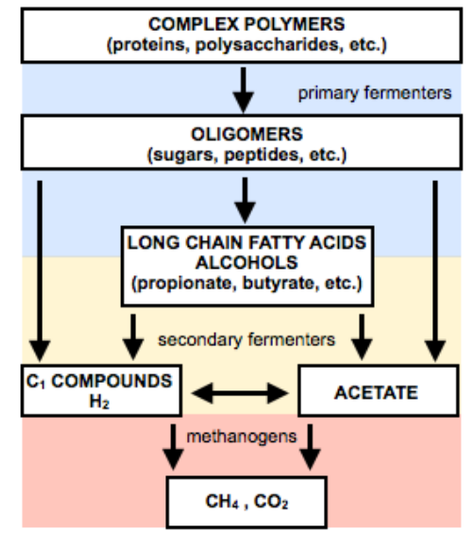
OTUs

Community/ Sequences



Biochemical Functions

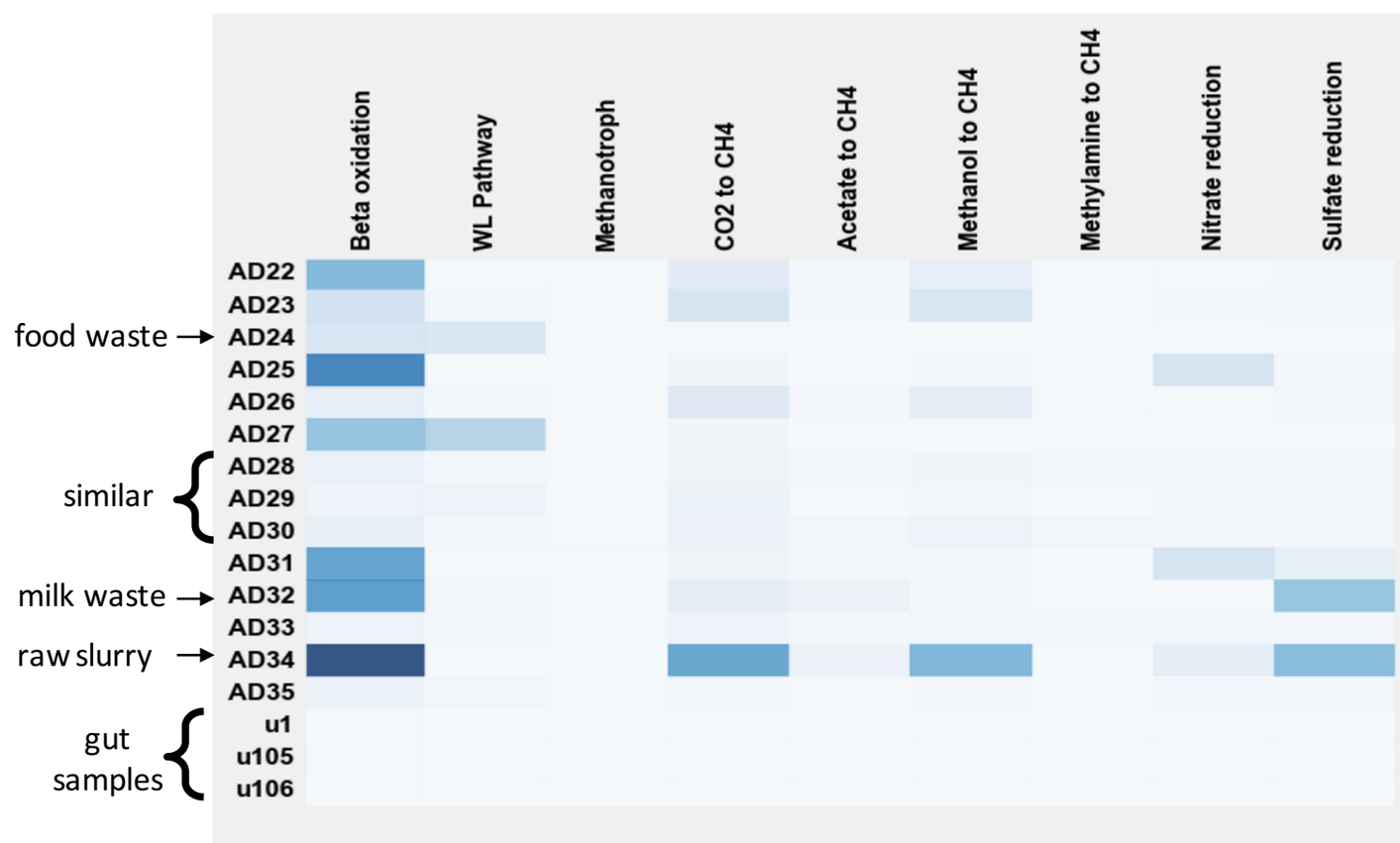
MetQy

For mapping of sequences to KEGG modules

https://www.biorxiv.org/content/early/2017/11/16/215525

Classifiers for specific functions. Currently, based on Faprotax[1]. More physiological data please!!!

https://www.biorxiv.org/content/early/2018/04/25/307157



[1] Louca et al. **Science** 353:1272-1277 (2016)

‘Top-down insights’ from AD communities

Microbiome composition/diversity differs across communities and determines community function (methane production), at least initially(!), but adaptation to feedstock occurs

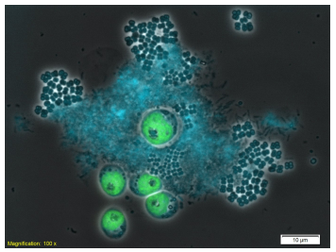
Mixing of communities leads to (sorting of?) best performing communities

=> *interactions within communities are important and perhaps optimised through co-adaptations*

Temporal sequencing of communities and associated metadata *might* allow predictive models and functional understanding at community level

‘Bottom-up’ insights from ‘synthetic’ communities

? Insights and Applications ?

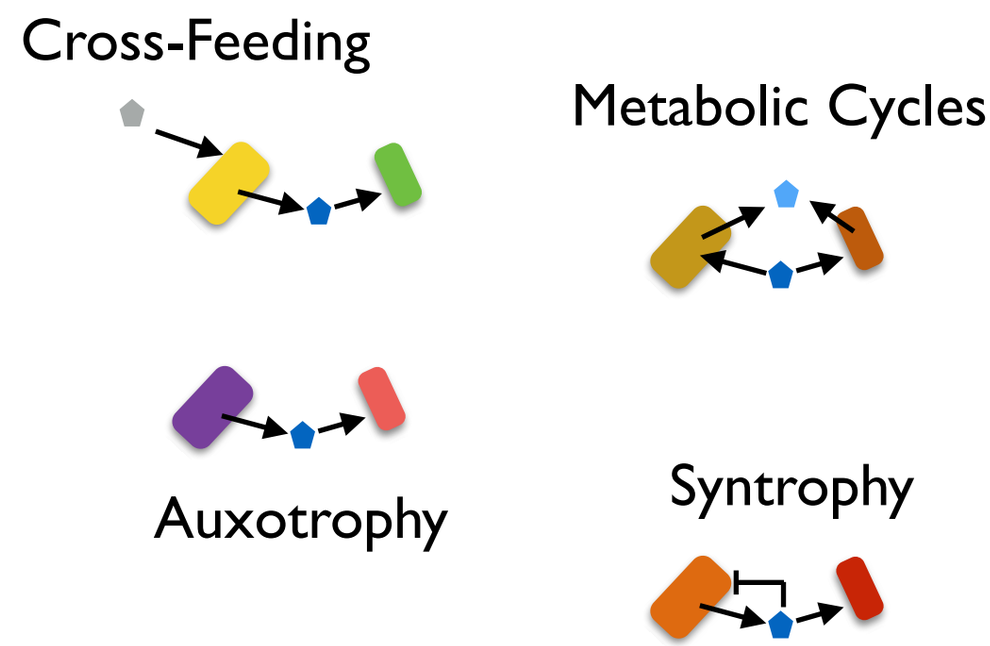


BOTTOM-UP

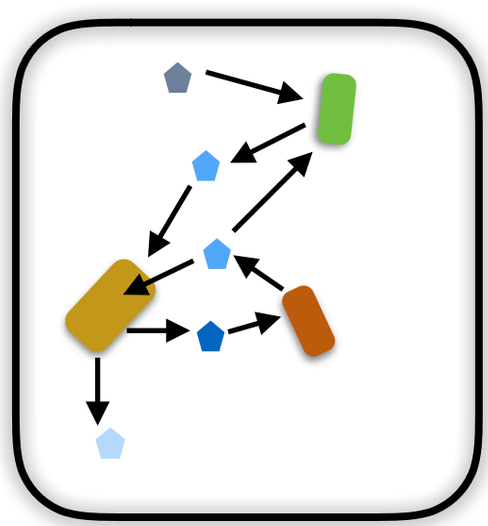
Create/engineer synthetic communities to learn about biochemical basis of communities

design principles ??

modules

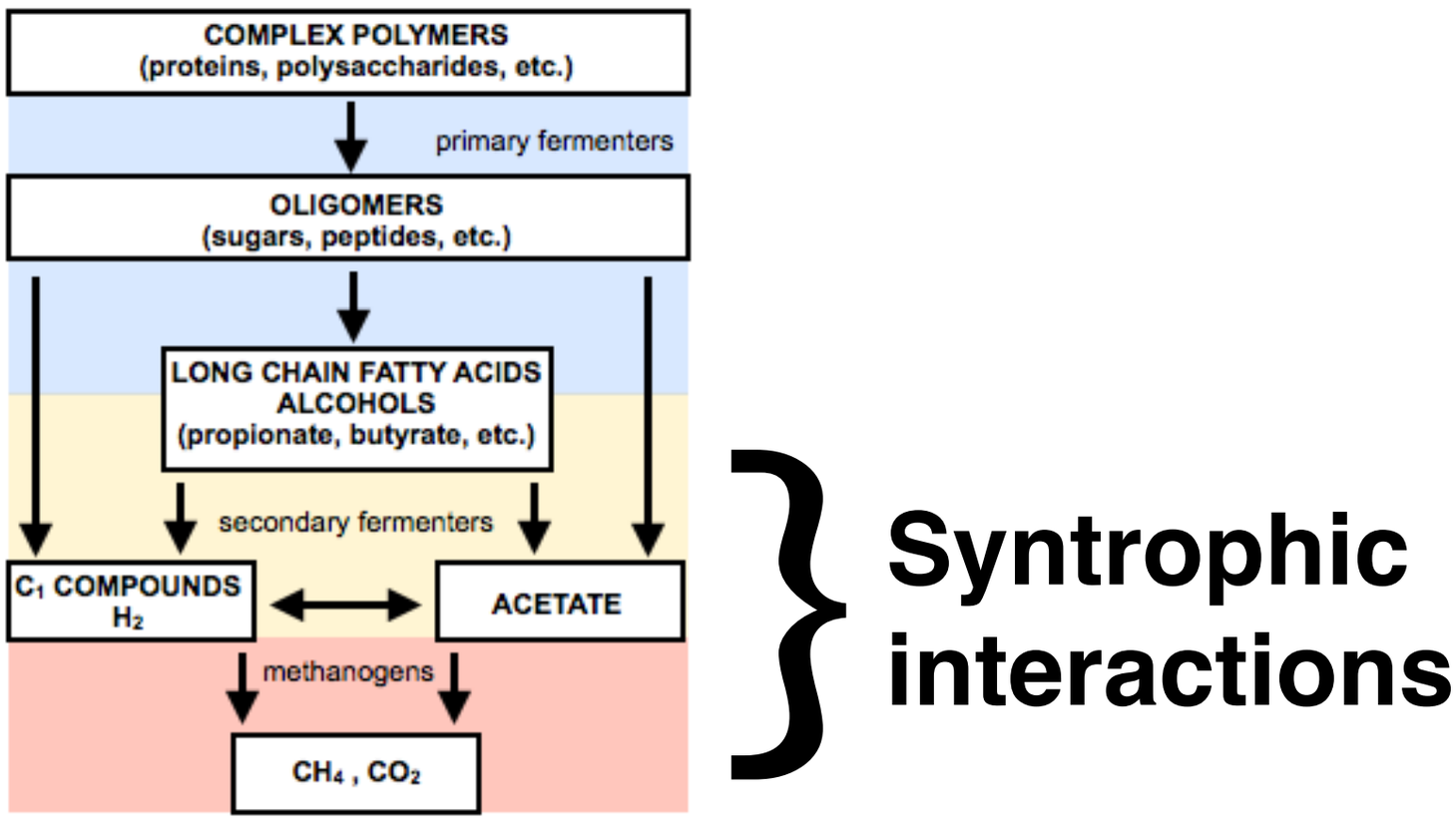


functional systems

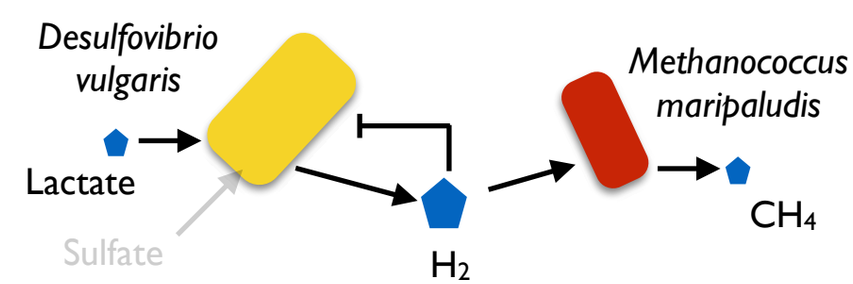


Grosskopf & Soyer, ***Curr. Op. Biotech*** (2014)

Syntrophy: Crucial in systems lacking strong terminal electron acceptors (TEAs)



Schink B *Microbiol Mol Biol Rev* 61:2 (1997)

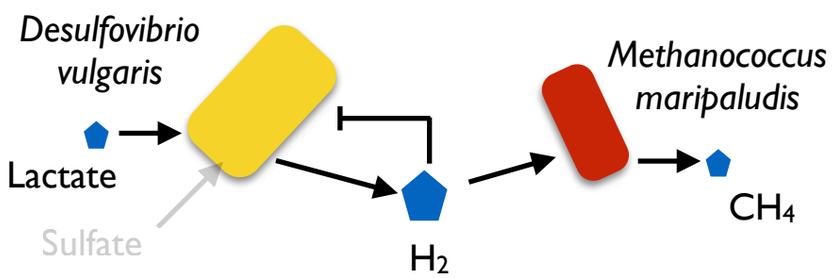


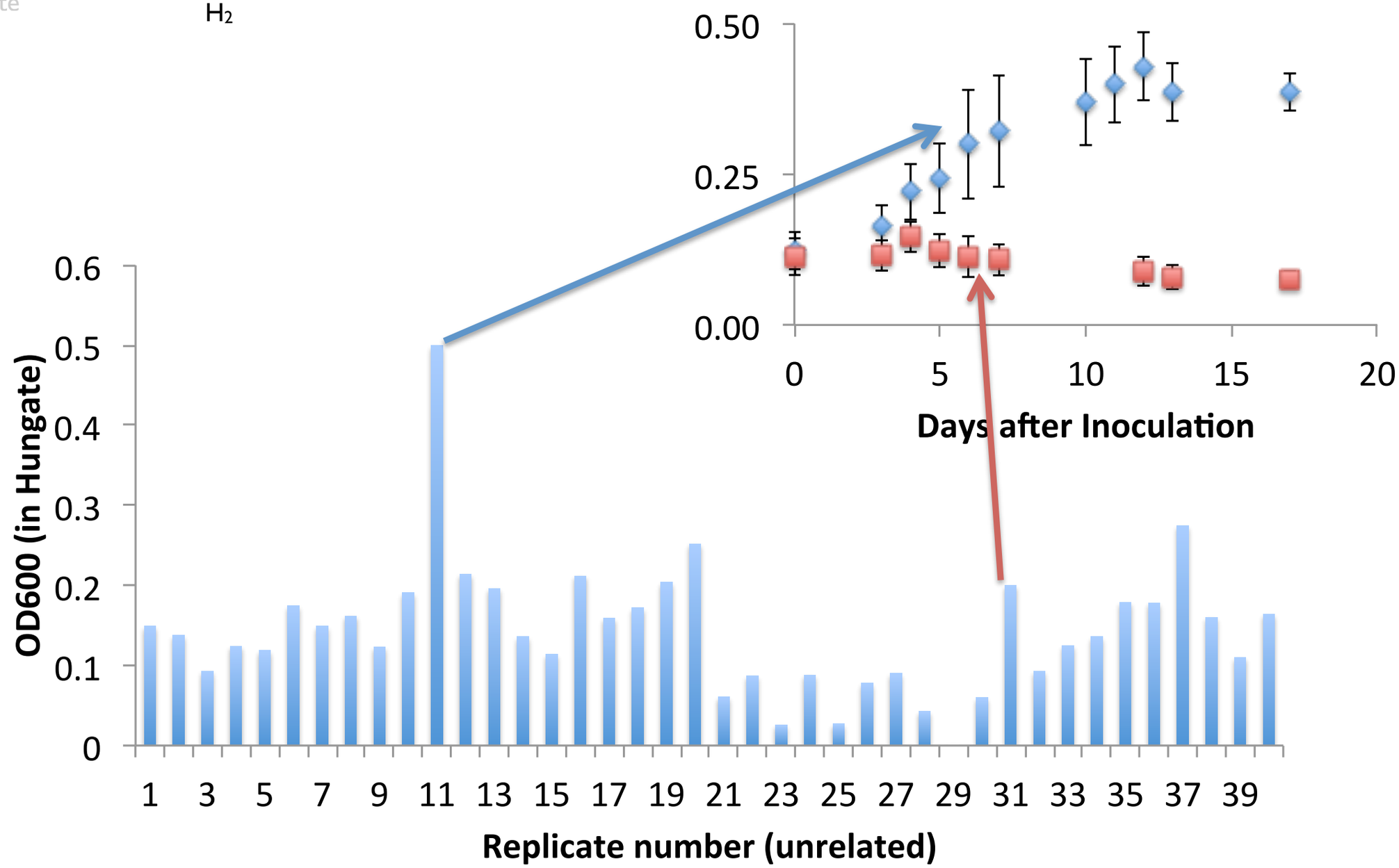
$$\text{Lactate} + 2\text{Sulfate} + 3H^+ \longrightarrow 3CO_2 + 2HS^- + 3H_2O \qquad \Delta G_0 = -259.09 \text{ kJ/mol}$$

$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + CO_2 + 2H_2 \qquad \Delta G_0 = -8.79 \text{ kJ/mol}$$

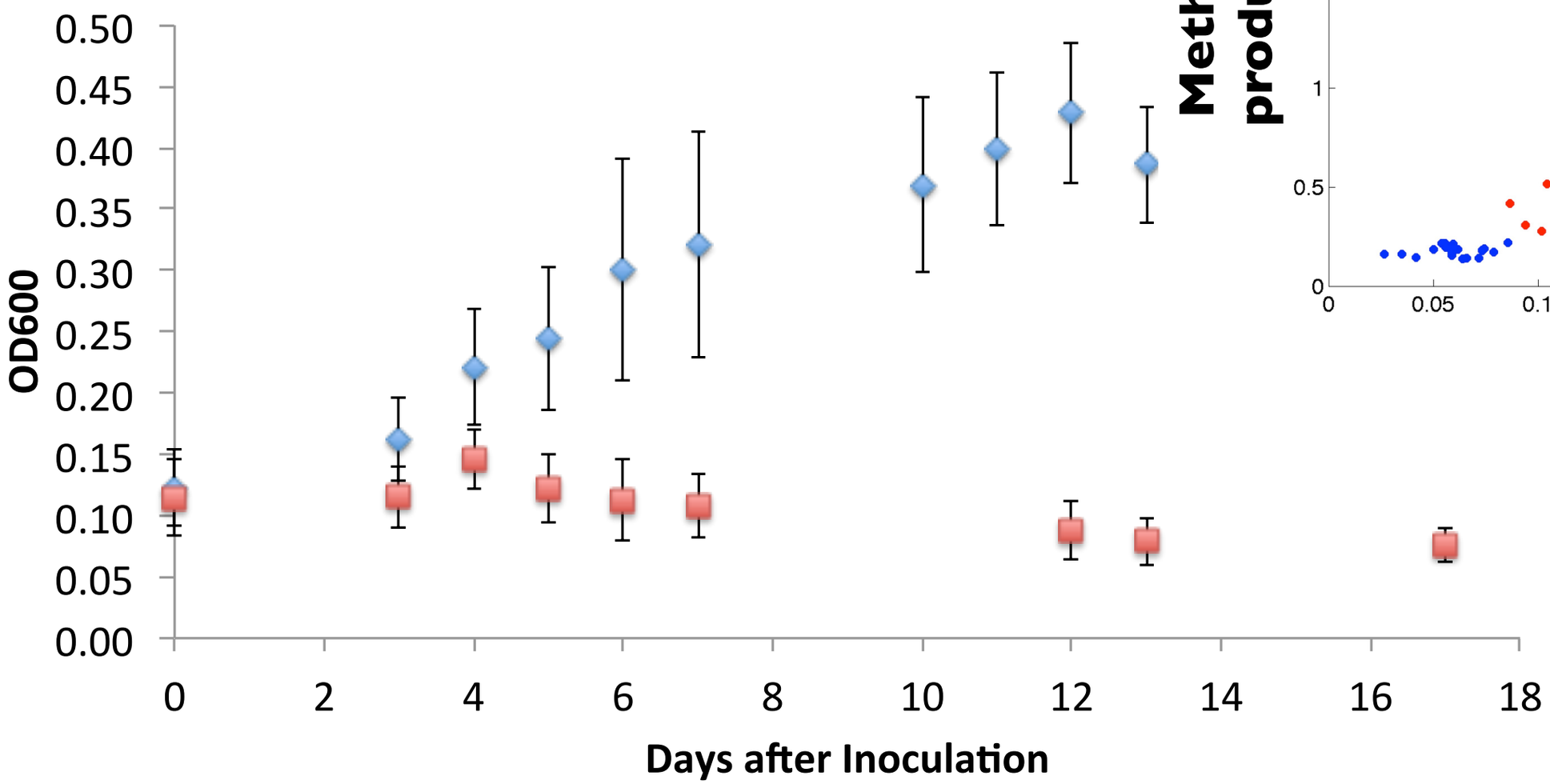
$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + 0.5CO_2 + 0.5CH_4 \qquad \Delta G_0 = -74.19 \text{ kJ/mol}$$

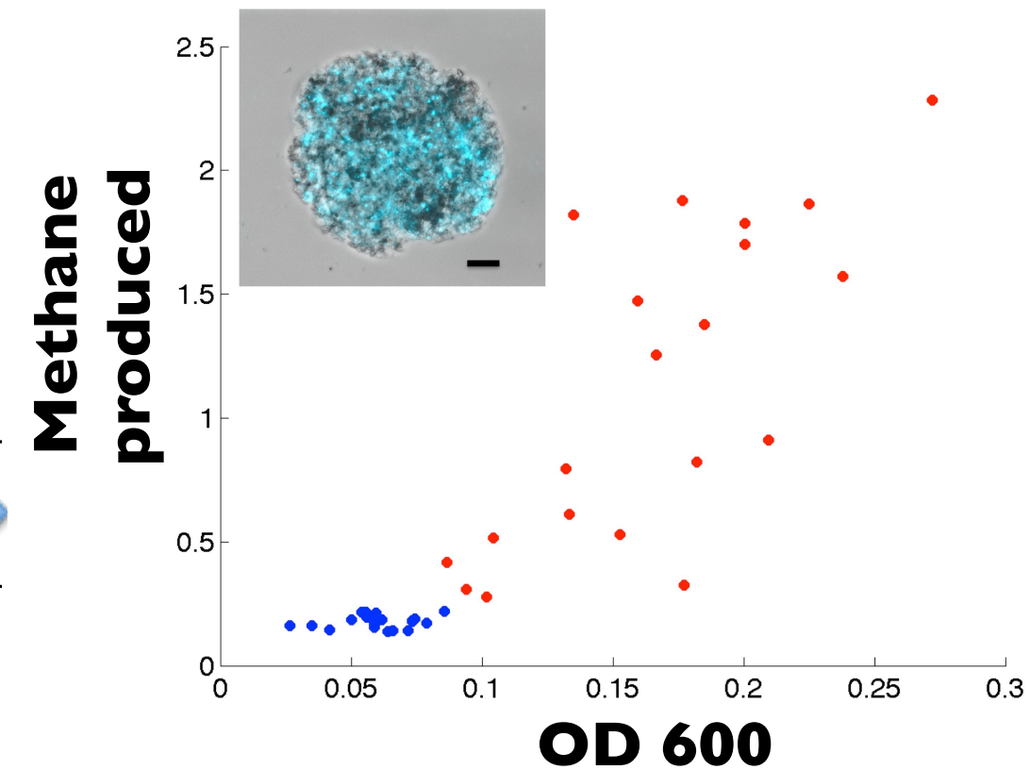
Are there genetic drivers of syntrophy? I.e. can evolutionary adaptation to syntrophy happen?





Isolates from co-culture are consistently "syntrophic", while those from wild type are not





Co-cultures using isolate from working co-culture

Co-cultures using isolate from *Dv* stock culture

Thermodynamics basis for genetic drivers of syntrophy?

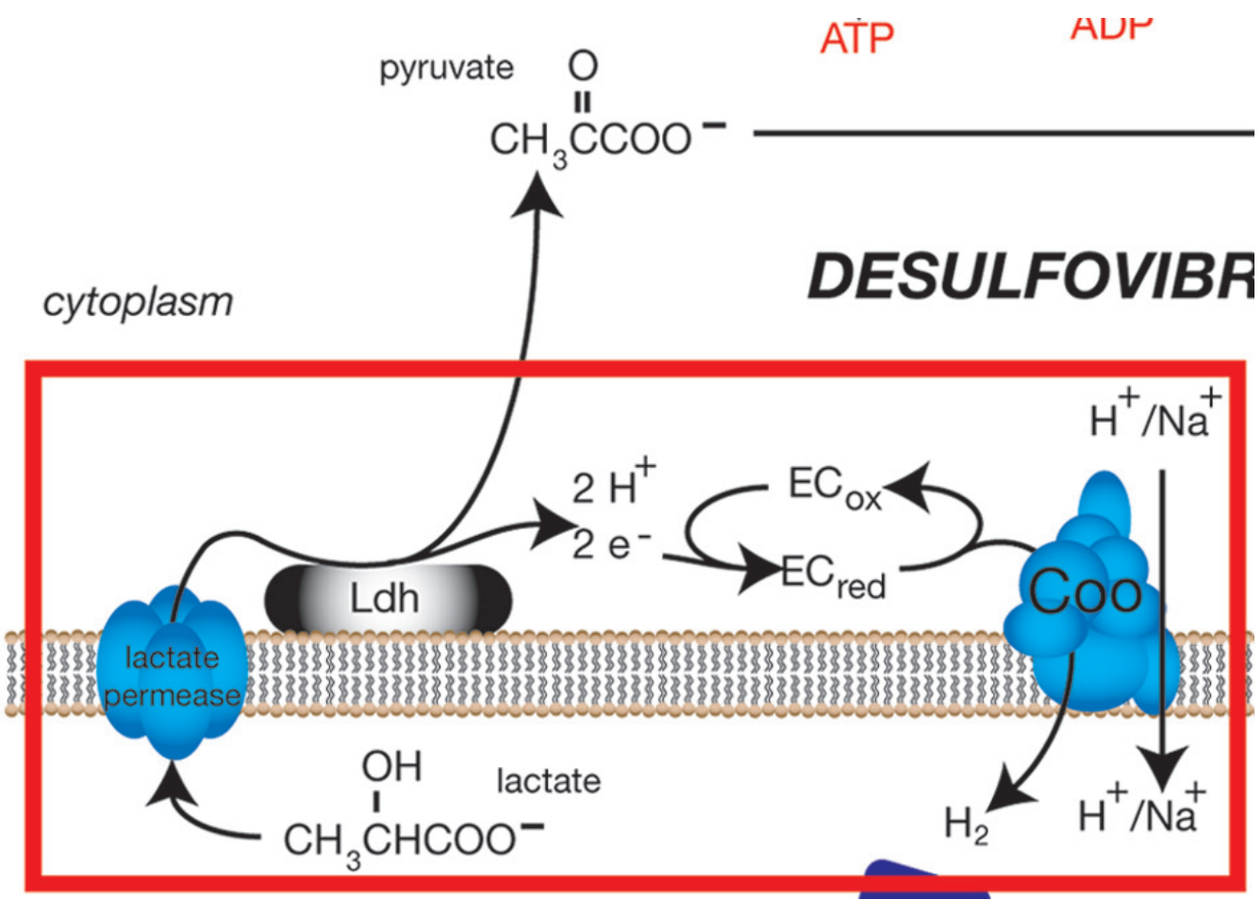
<u>Hypothesis:</u> Syntrophy enabling mutation allows energy investment to overcome thermodynamic hurdle:

$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + CO_2 + 2H_2 \qquad \Delta G_0 = -8.79 \text{ kJ/mol}$$

?

$$\text{Lactate} \longrightarrow \text{Pyruvate} + H_2$$

$$Na^+ \text{ (out)} \longrightarrow Na^+\text{(in)}$$



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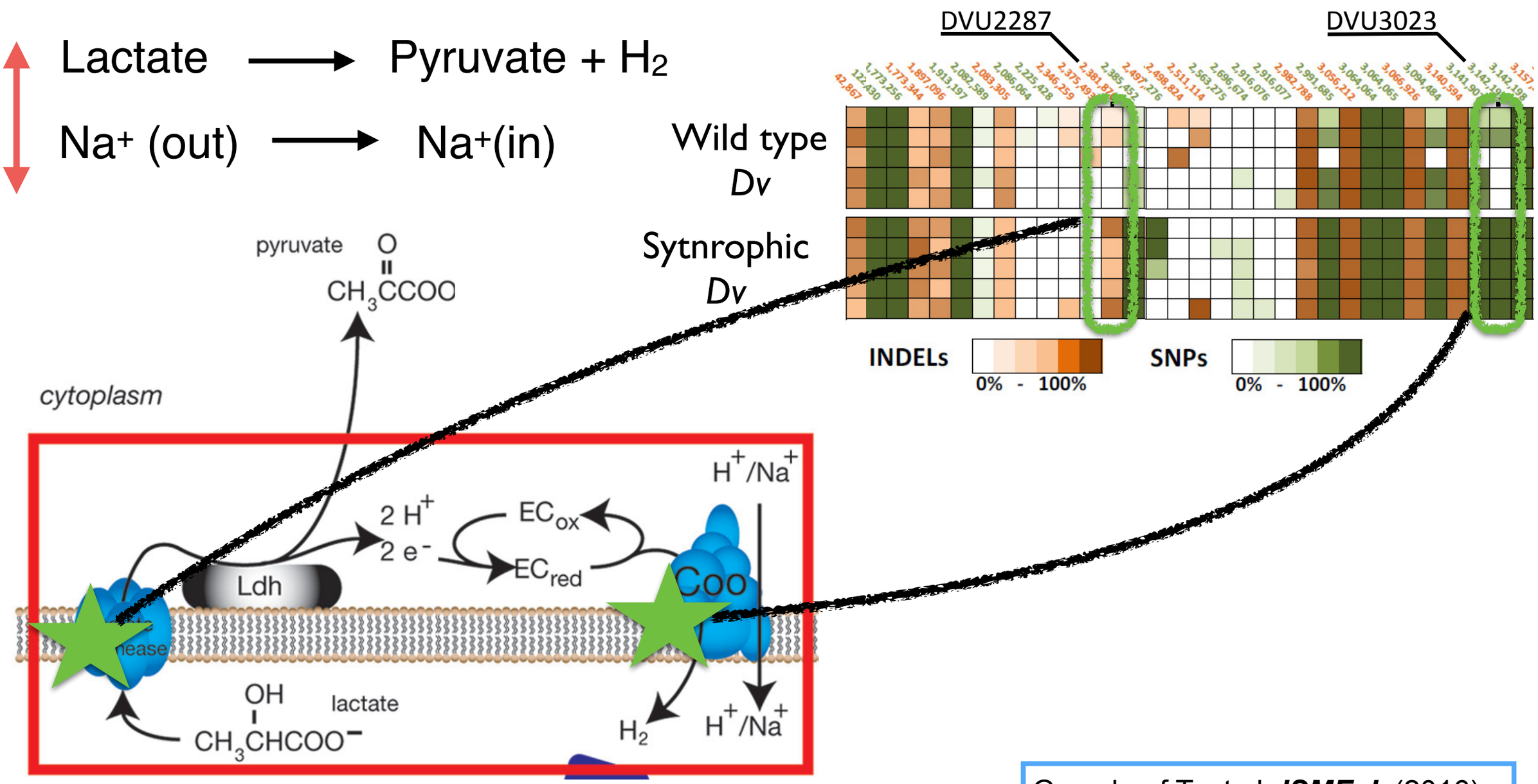
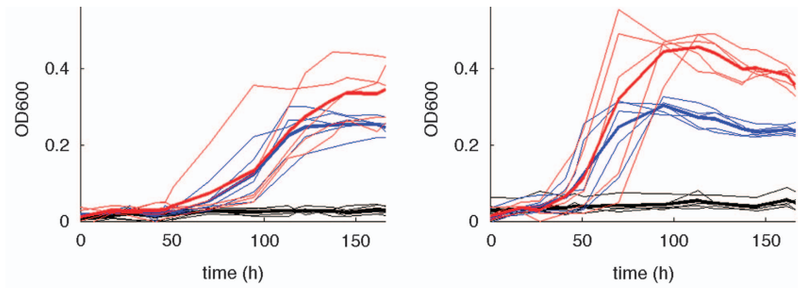
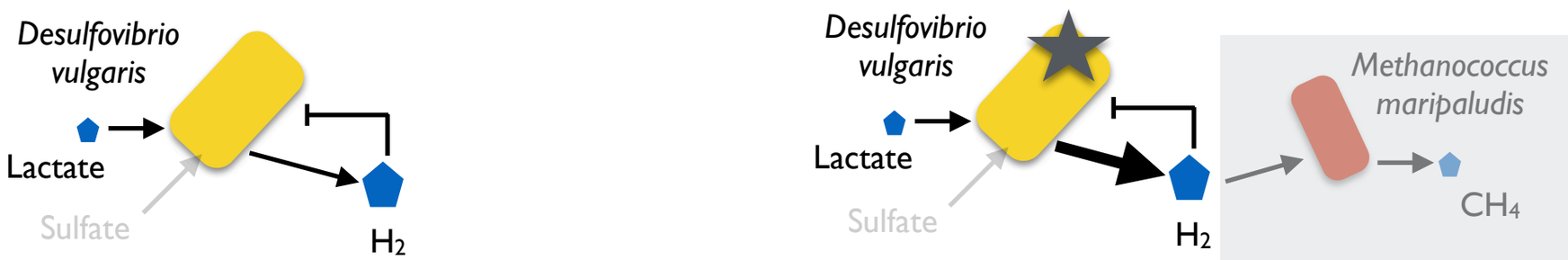


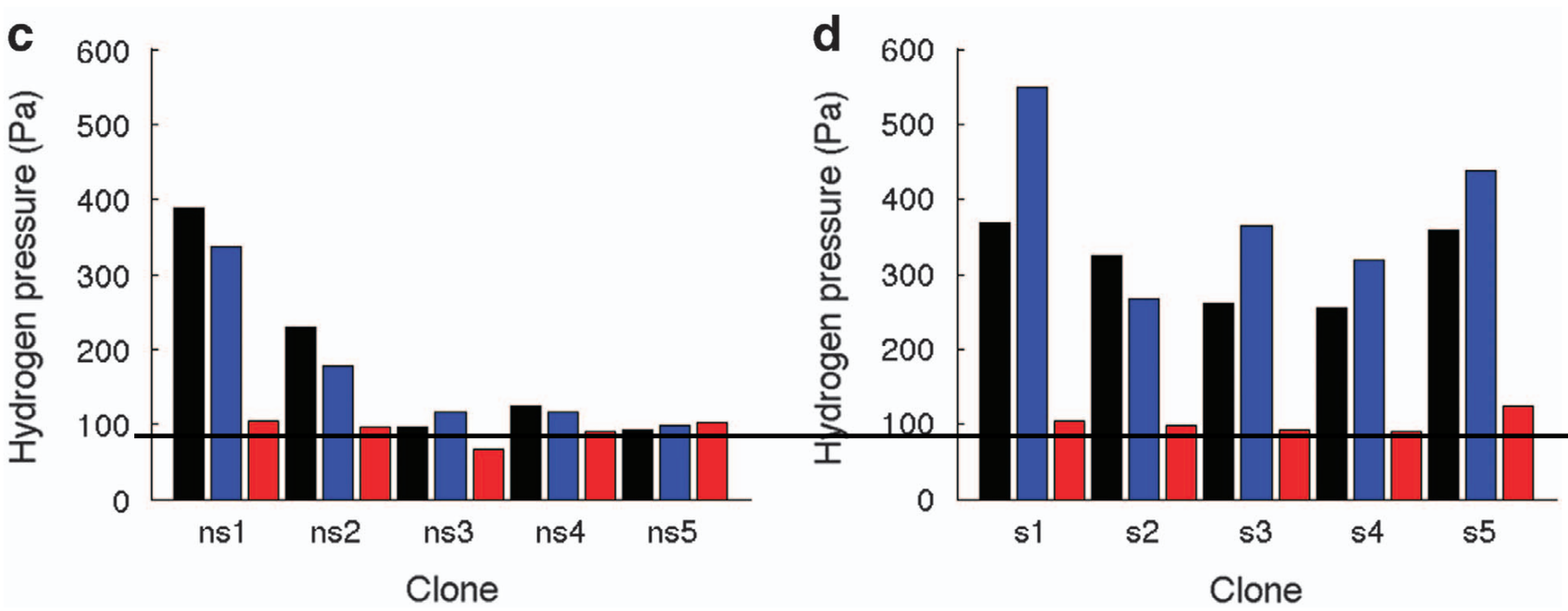
Grosskopf T. et al. ***ISME J.*** (2016).

‘Syntrophic isolate’ produces more H_2, enabling establishment of interaction with methanogen

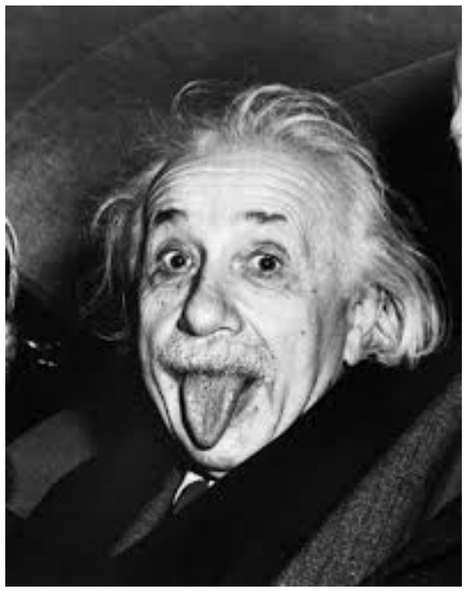
Investing (membrane potential ?) into H2 production - an evolutionary insurance policy?







Background H_2



It’s thermodynamics baby!

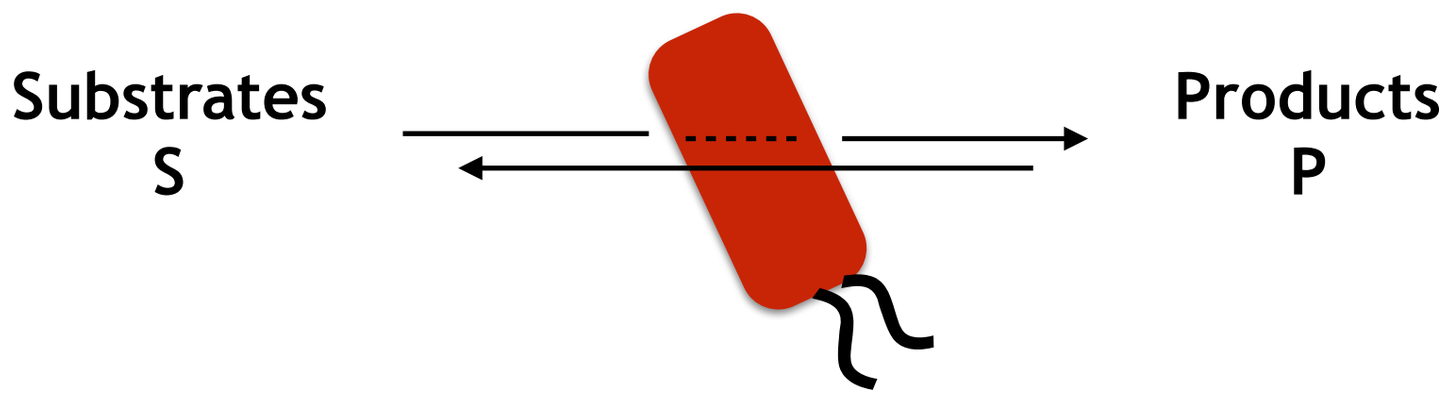
Thermodynamic limits not only drive syntrophic interactions but also influence genetic adaptations in species

Broader implications on diversity/evolution?

Metabolism and therefore metabolic interactions are highly versatile at thermodynamic limits.

Implications for modelling and sequence interpretation?

Kinetic vs. Thermodynamical modelling of microbial metabolism/growth



Substrate uptake kinetics $\frac{v_{\max} \cdot [S]}{K + [S]}$

&

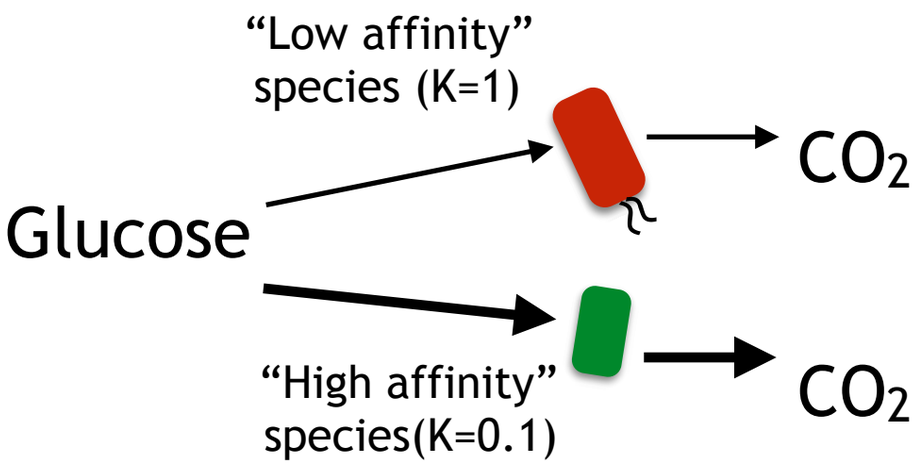
Reaction free energy $\Delta G_{rxn} = \Delta G_0 + RT \ln\left(\frac{[P]}{[S]}\right)$

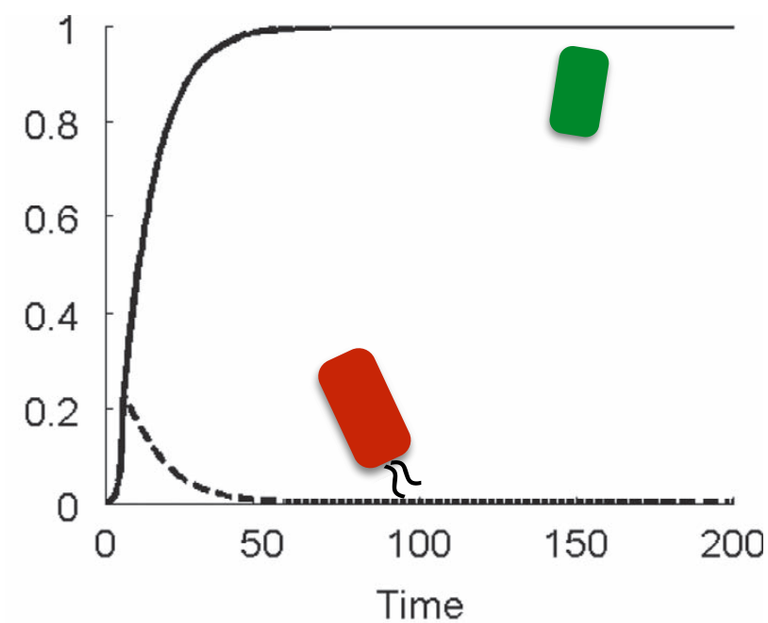
} Growth

Hellingwerf, K J, et al *FEMS Microbiol. Lett* 15 (1982);
Rodríguez J et al, *Trends in Biotech* 26:7 (2008);
Hoh CY and R Cord-Ruwisch. Biotech & Bioeng 51:5 (1996);
Qusheng J and Bethke CM. *American J. of Sci* 307:4 (2007)

Purely kinetic models predict low diversity in microbial species (and metabolic pathways)!

Kinetic model of microbial growth: $$v = \frac{v_{\max} \cdot [S]}{K + [S]}$$





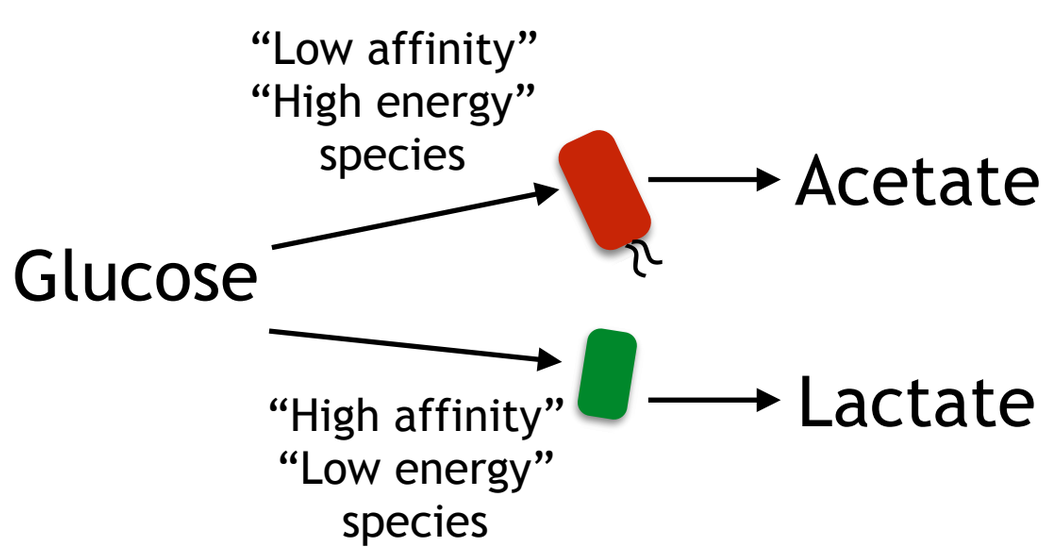
Diversity not possible on single substrate!

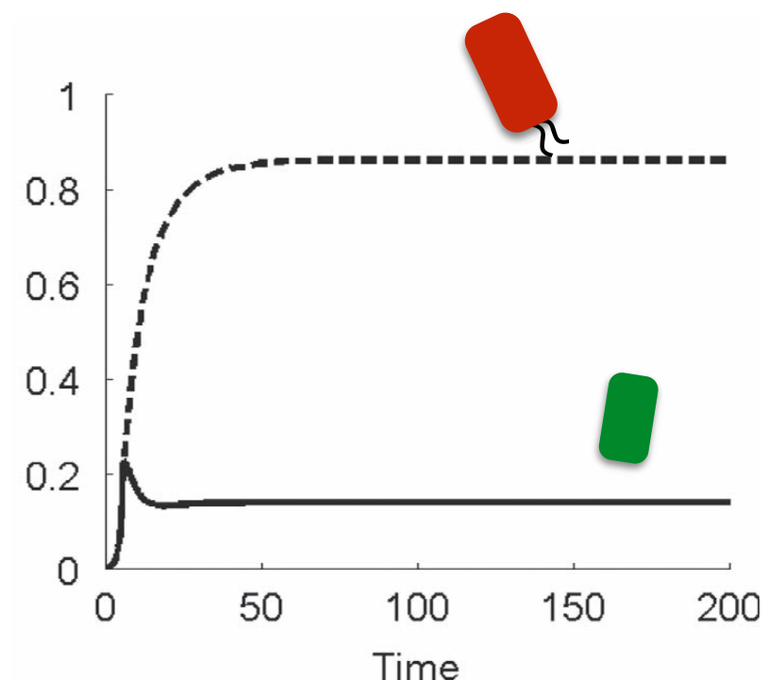
KINETIC GROWTH THEORY (EXCLUSION PRINCIPLE)

Kinetic/thermodynamic model *allows* high diversity in metabolic pathways and species

Thermodynamic model of microbial growth:

$$v = \frac{v_{\max} \cdot [S] \cdot \left(1 - \exp(\Delta G_{rxn})\right)}{K + [S] \cdot \left(1 + k_r \cdot \exp(\Delta G_{rxn})\right)}$$



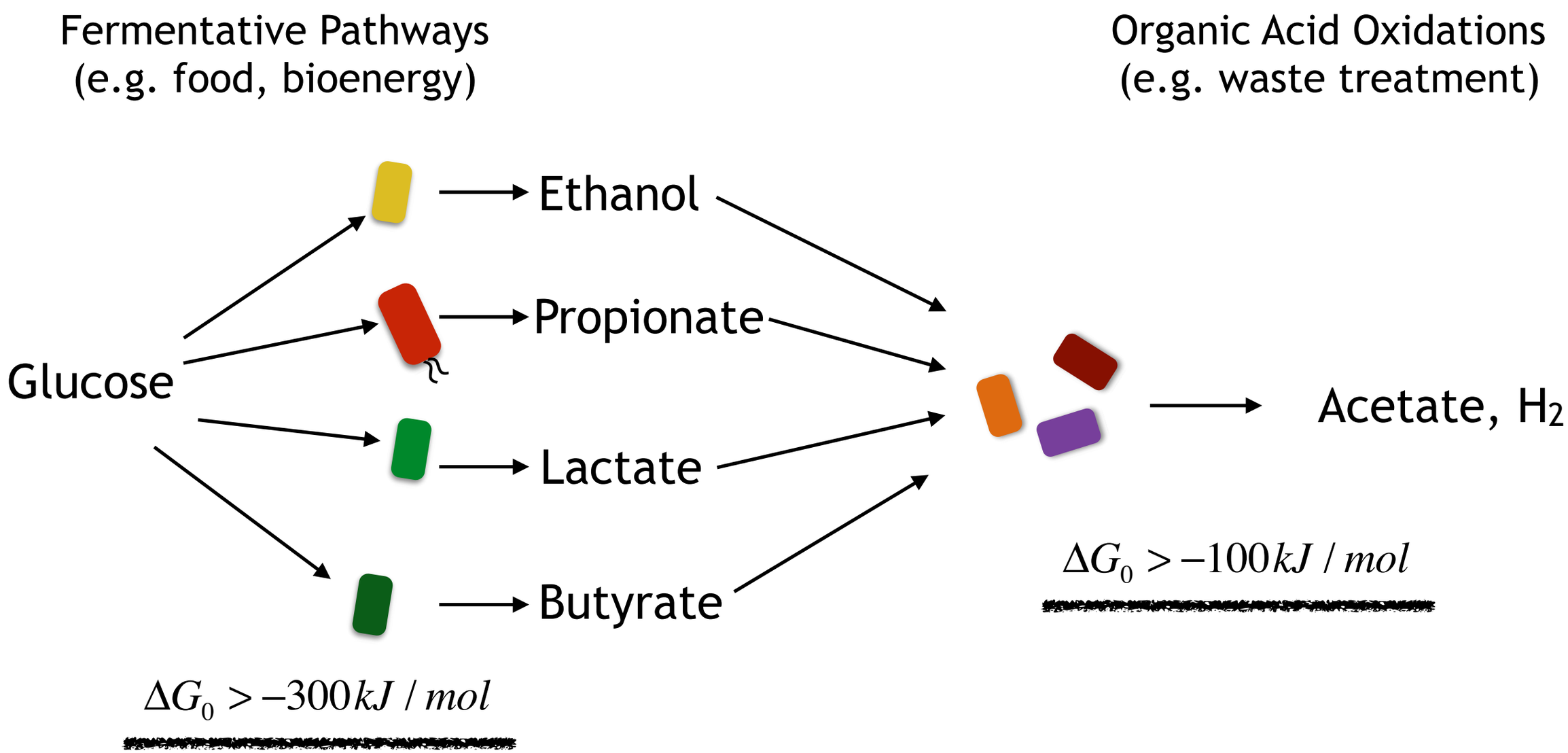


Diversity possible on single substrate with different metabolic pathways!

"Microbial diversity arising from thermodynamic constraints" Großkopf T, **Soyer OS**, ISME J. 2016.

THERMODYNAMIC INHIBITION THEORY

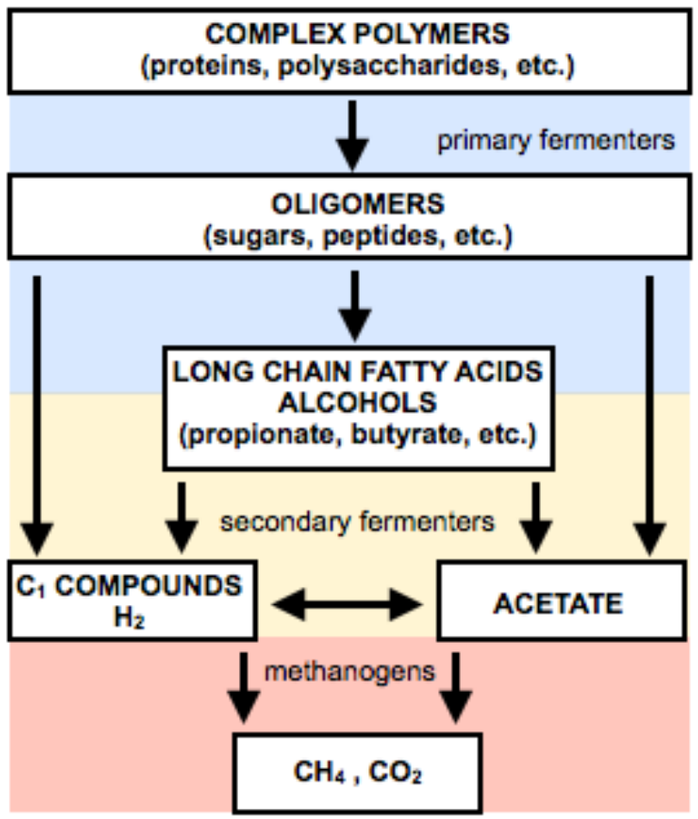
Diversity observed in low energy pathways is inline with thermodynamic theory



To avoid thermodynamic inhibition, organisms are expected to develop multiple pathways with different end-products and engage in syntrophies and cross-feeding.

"Microbial diversity arising from thermodynamic constraints" Großkopf T, **Soyer OS**, ISME J. 2016.

A thermodynamic view of AD



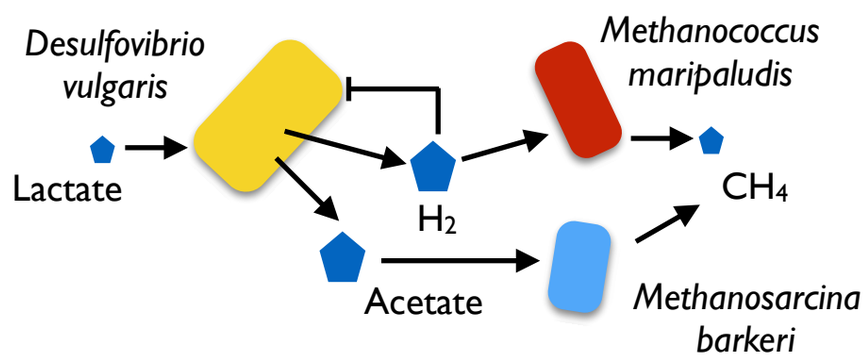
High |∂G| conversions
Kinetics dominates - low diversity

Low |∂G| conversions
Free energy dominates - high diversity

Hard to test - but have to talk to Tom Curtis!

Syntrophy and stability: Impact of electron acceptor (TEA) availability on different methanogens

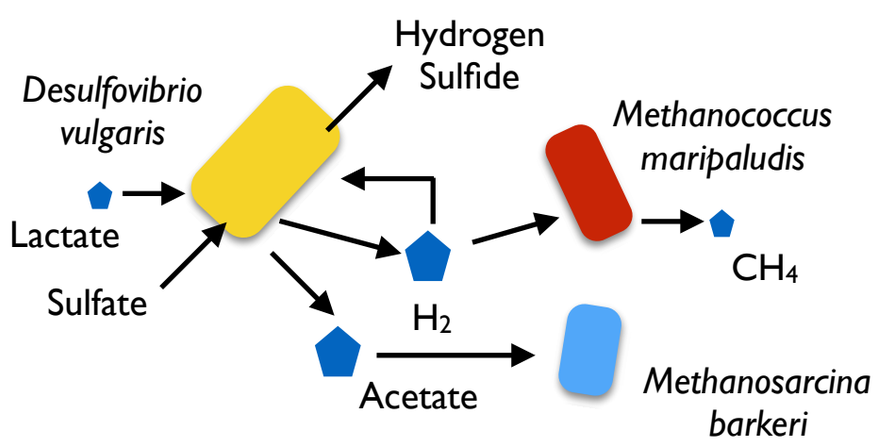
<u>Without Sulfate:</u>
No TEA, low ∂G for Dv



COOPERATION (SYNTROPHY)

CROSS-FEEDING

<u>With Sulfate:</u>
Good TEA, high ∂G for Dv



COMPETITION

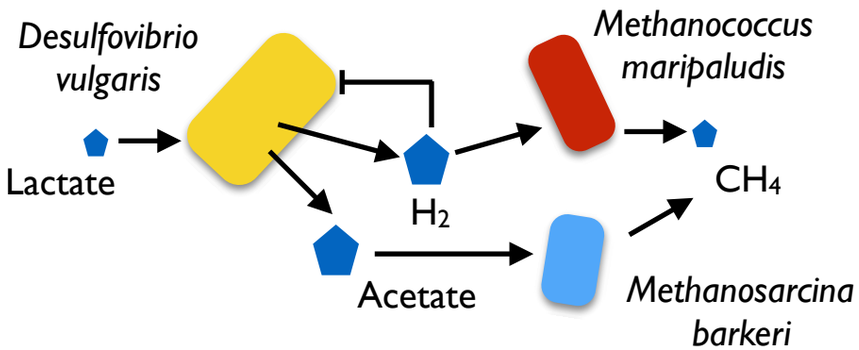
CROSS-FEEDING

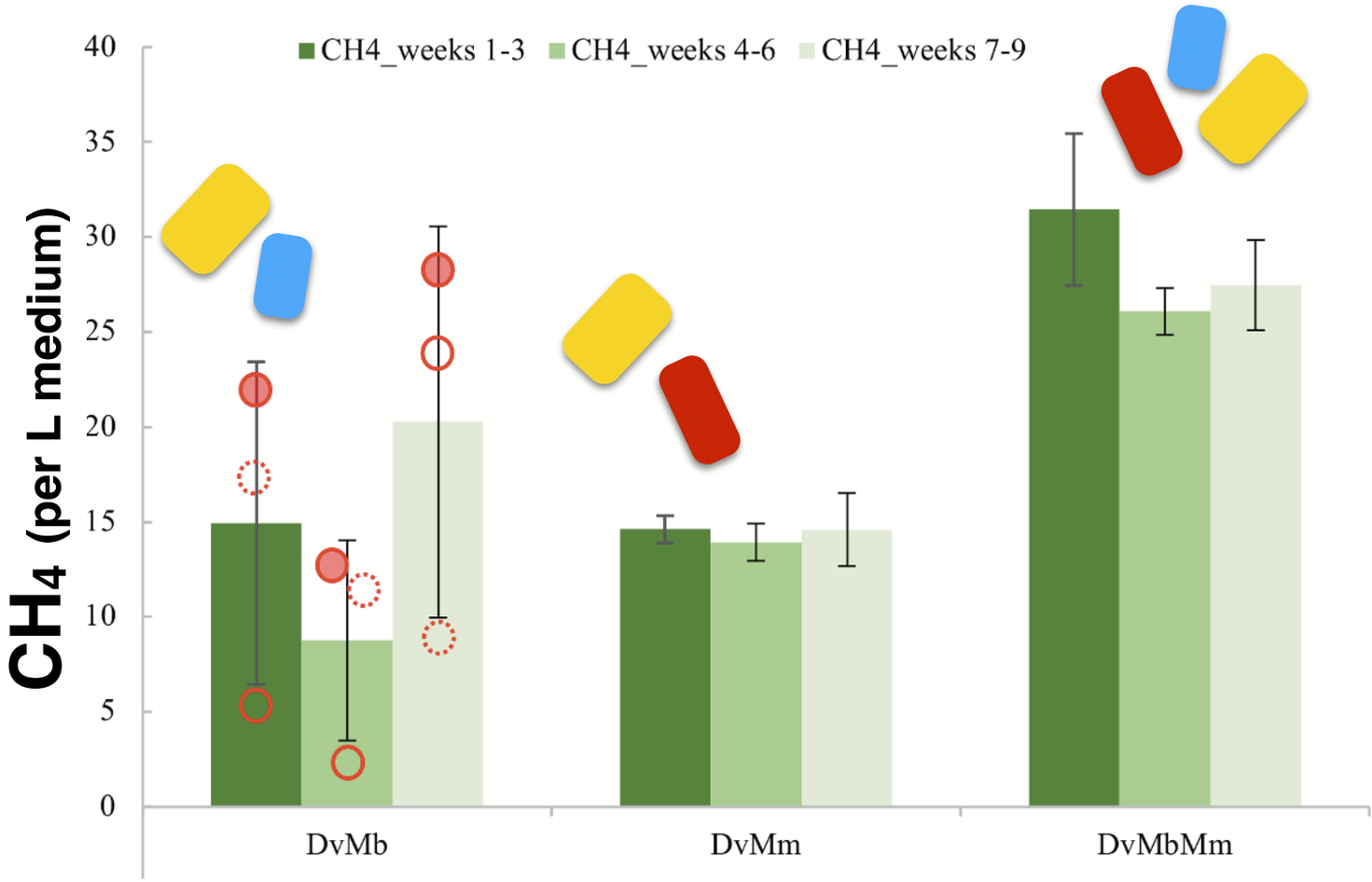
No sulfate: Different methanogens stably co-exist with sulfate reducer and increase methane production from lactate

Jing Chen

- Jan Dolfing
- Matthew Wade
- Hadrien Delattre

Extended Syntrophies





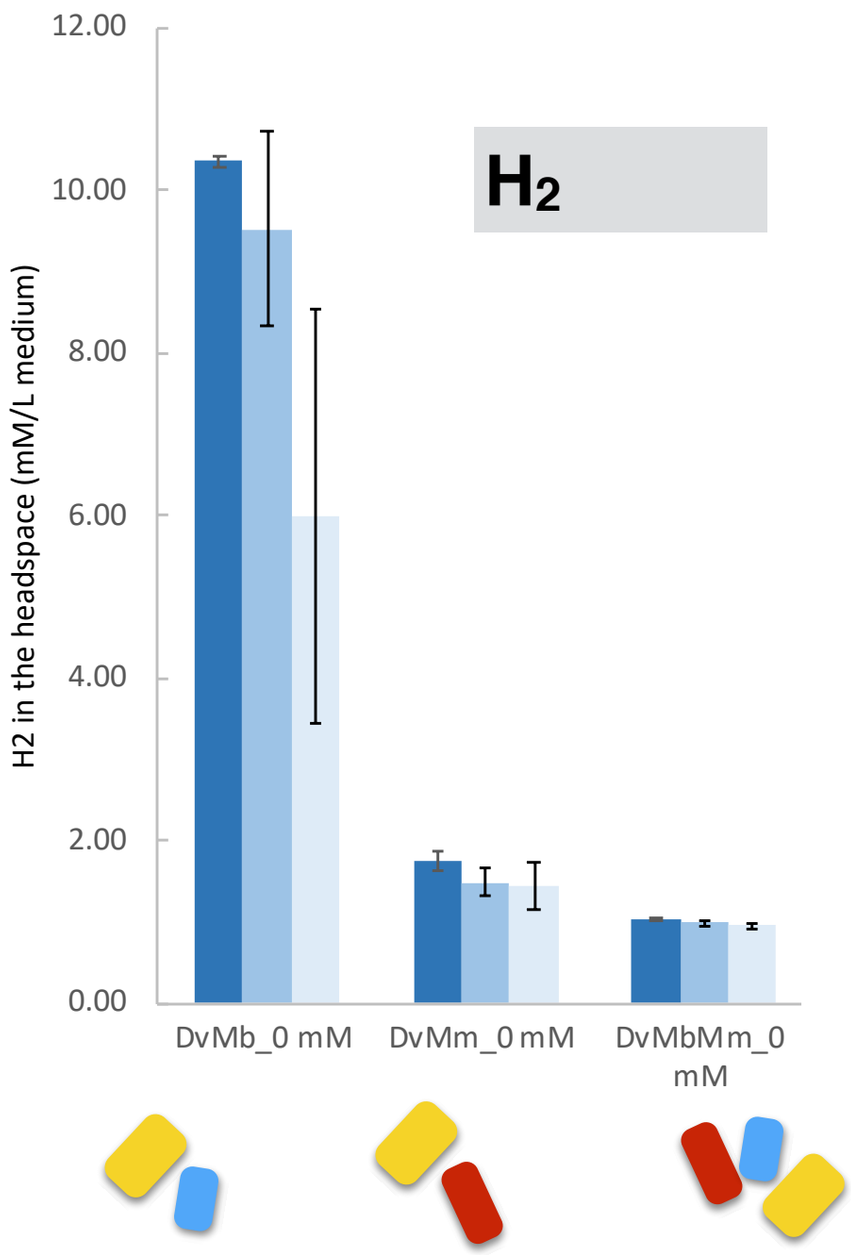
$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + 0.5CO_2 + H_2O + 0.5CH_4 \qquad \Delta G_0 = -74.19$$

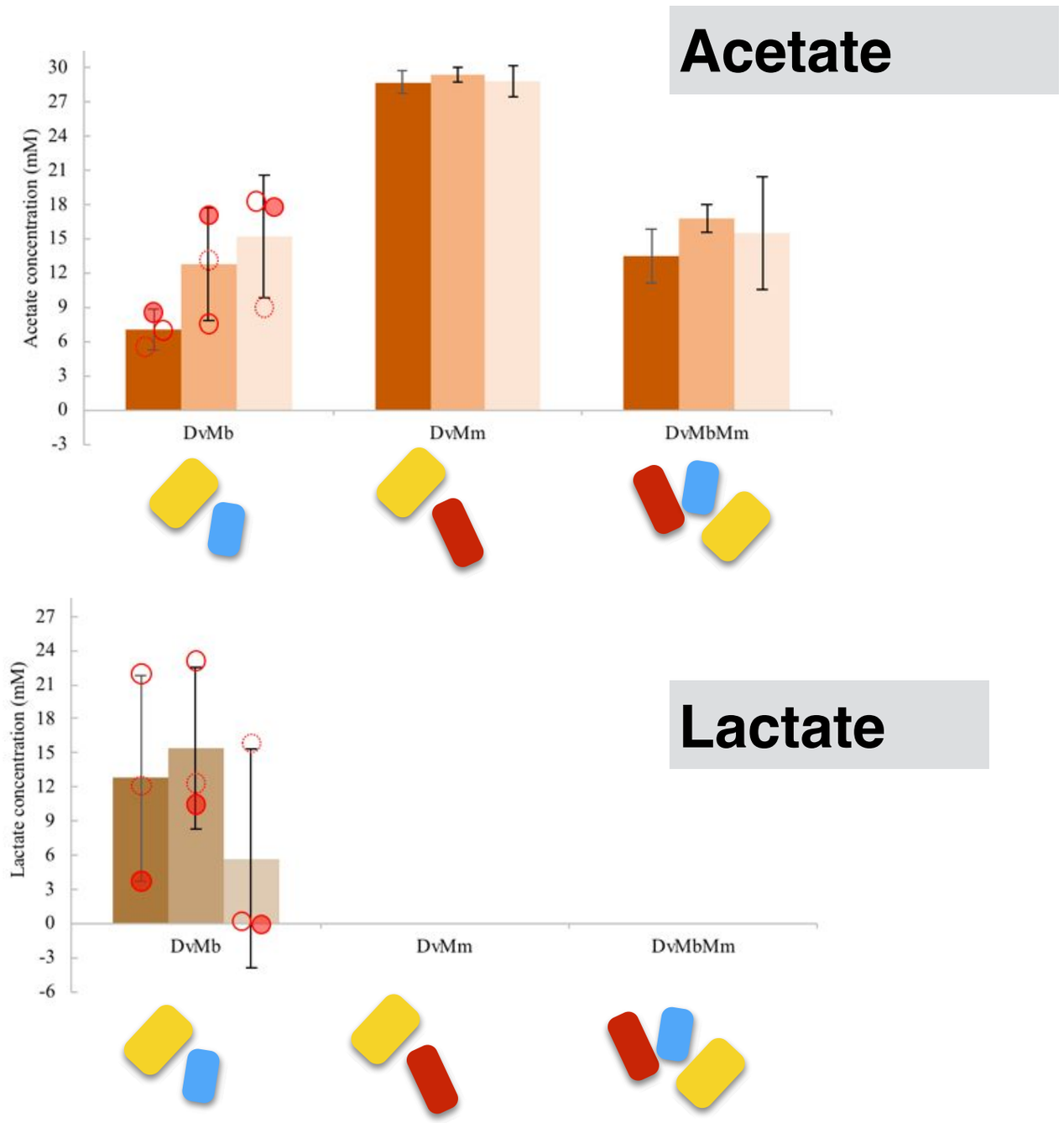
$$\text{Lactate} + H^+ + H_2O \longrightarrow 2CO_2 + 2H_2 + 1CH_4 \qquad \Delta G_0 = -44.69$$

$$\text{Lactate} + H^+ \longrightarrow 1.5CO_2 + 1.5CH_4 \qquad \Delta G_0 = -110.09$$

unpublished results

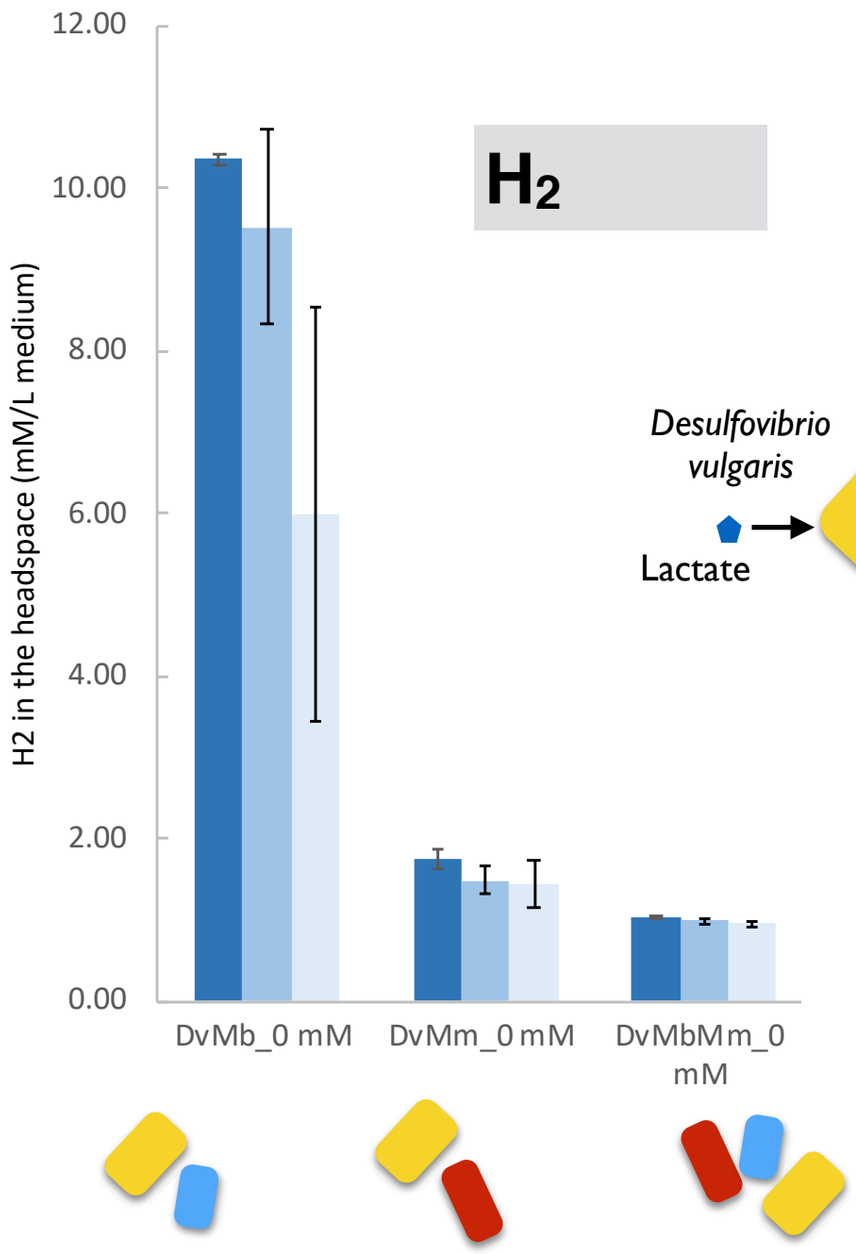
No sulfate: *Mb* does not perform fully acetoclastic methanogenesis, but relies on H_2 co-utilization



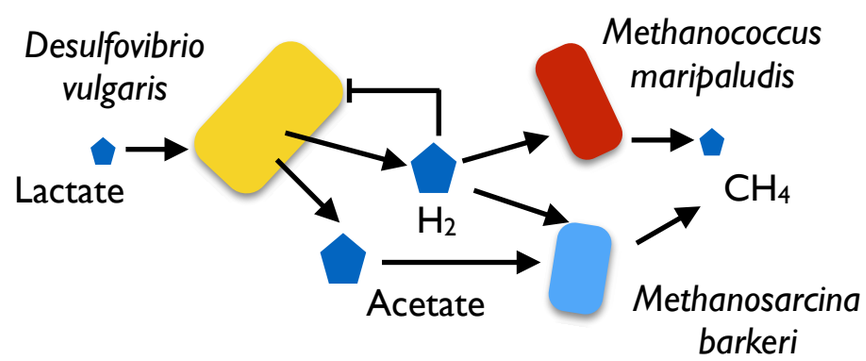


unpublished results

No sulfate: *Mb* does not perform fully acetoclastic methanogenesis, but relies on H_2 co-utilization



H_2



COMPETITION

CROSS-FEEDING

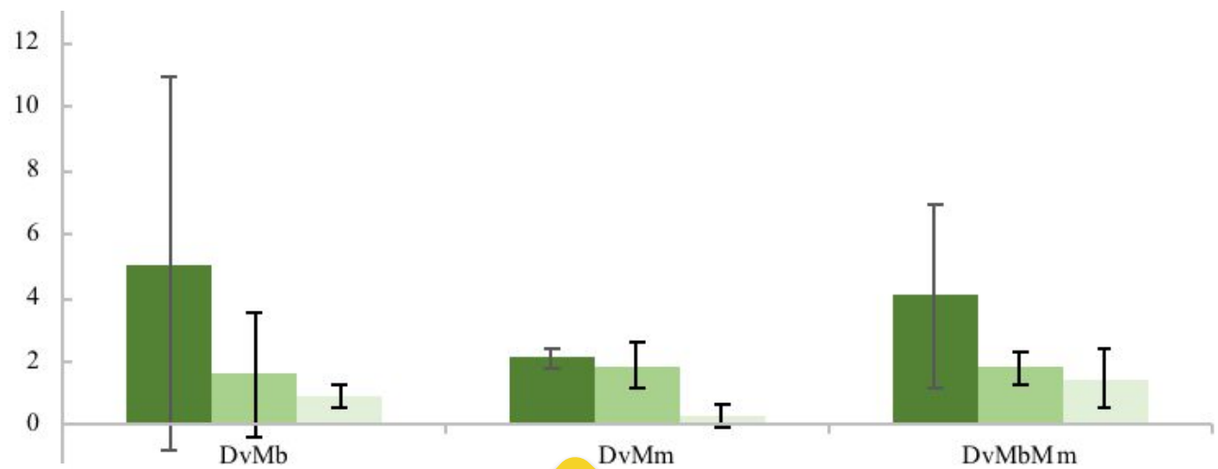
COMPETITION ??

unpublished results

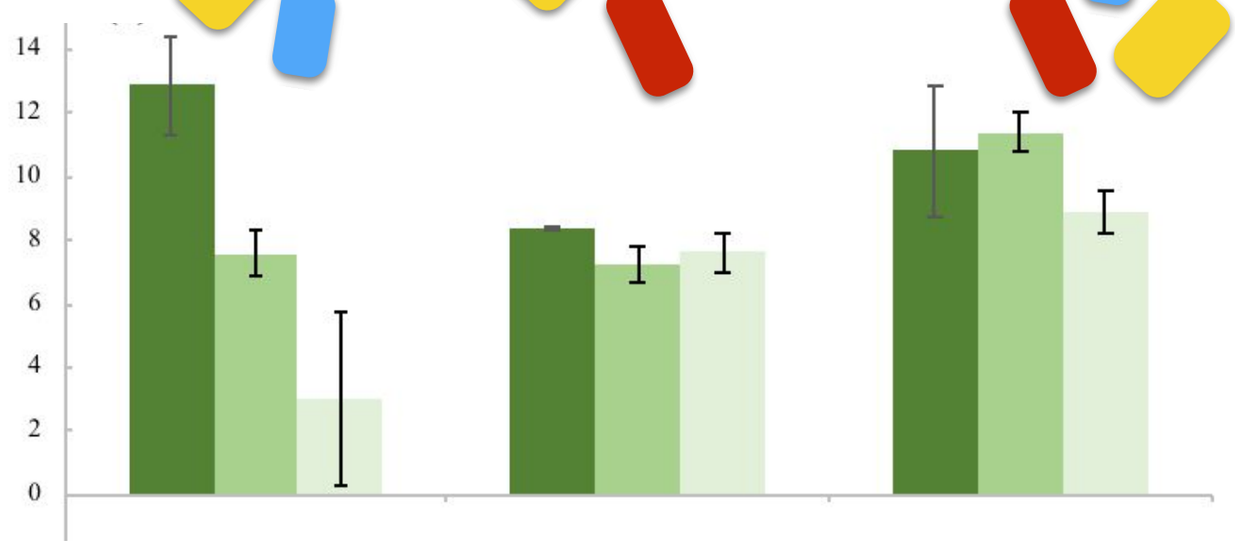
Sulfate: Different methanogens show differential stability against sulfate reducer with increasing sulfate levels

Sulfate equivalent for lactate conversion

Full:



Half:



H_2 competition in presence of sulphate is more harmful to *Mb* (mixotroph) than it is to *Mm* (hydrogenotroph)

unpublished results

‘Bottom-up’ insights synthetic communities

Metabolism and syntrophic (metabolic) interactions are versatile at thermodynamic limits.

=> *Thermodynamics need accounting in modelling and thinking of AD communities (all communities!)*

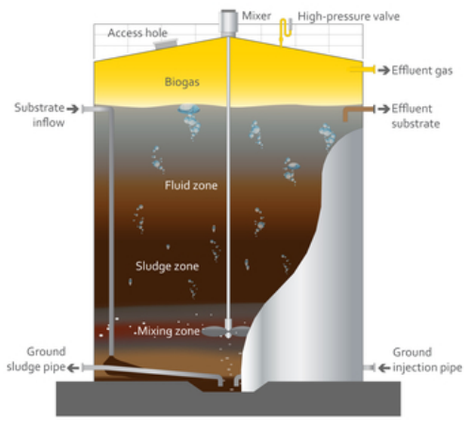
Organisms have (or can) adapt to thermodynamic limitations, which shift with environmental conditions and H2-dynamics.

=> *Evolution or physiology?*
=> *Basis of improvement of H2 addition?*

Thermodynamic inhibition can contribute significantly to high diversity (especially in 2ary degraders?)

=> *Control terminal electron acceptor availability to control diversity?*

Towards a Theory of Metabolism For Cellular and Community Engineering

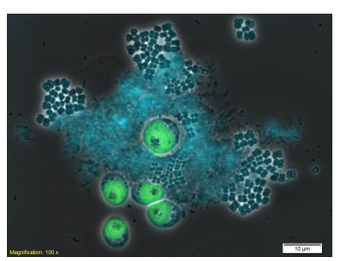


http://www.mannvit.com/Markets/UnitedKingdom/AnaerobicDigestion/

TOP-DOWN

? Insights and Applications ?

BOTTOM-UP



=> *More **temporal** sequence- and meta- data*

=> *Systems of 'in between' complexity*

Ecology & Evolution

Biophysical Drivers

=> *More **physiological** data*

=> *Systems of 'in between' complexity*

More info on the poster floor:

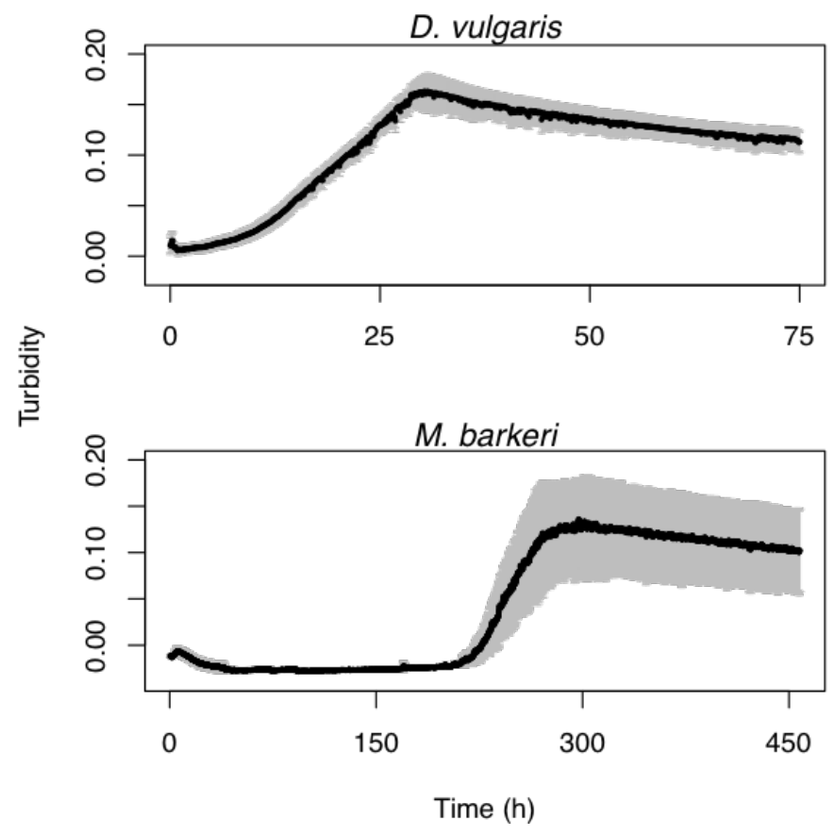
=> More **physiological** data.

*Can **DIY Science** be the answer;*

Kalesh Sasidharan

MicrobeMeter

available to download/buy
Follow progress at:
Humanetechnologies limited



=> More **systems** of 'in between' complexity.

Visit Jing's poster

Jing Chen

THANK YOU

OSS LAB

http://osslab.lifesci.warwick.ac.uk

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Craig McBeth

Hadrien Delattre
Clare Hayes
Connah Johnson
Kelsey Cremin

Funders

BBSRC bioscience for the future

EPSRC Pioneering research and skills

WARWICK THE UNIVERSITY OF WARWICK

[dstl]

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Angus Buckling (University of Exeter)

Jan Dolfing, Matt Wade (University of Newcastle)

David Swarbreck (TGAC)

Initiatives

www.anaerodynamics.com

Bee Bio Electrical Engineering Innovation Hub @ Warwick

WCISB WARWICK CENTRE FOR INTEGRATIVE SYNTHETIC BIOLOGY