ARPA-E Rewiring Anaerobic Digestion Workshop

Thursday, 27th October, 2016

Microbiome shaping of anaerobic fermentation and chain elongation to increase product specificity

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the angenent lab

We are moving

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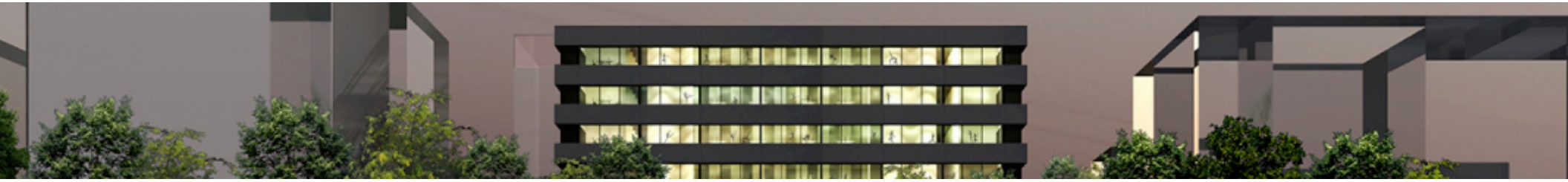
Carbon recovery, electric power storage, and microbial electrochemistry



Outline – 4 questions

1. Is the reactor microbiome of AD stable and predictable? 7 slides
2. Why does AD produce methane at such high specificities? 3
3. What else can we produce with microbiomes at high specificities? 15
4. What parameters we can use to shape the reactor microbiome? 1

Introduction to Paul Weimer: chain elongation pathways

Outline – 4 questions

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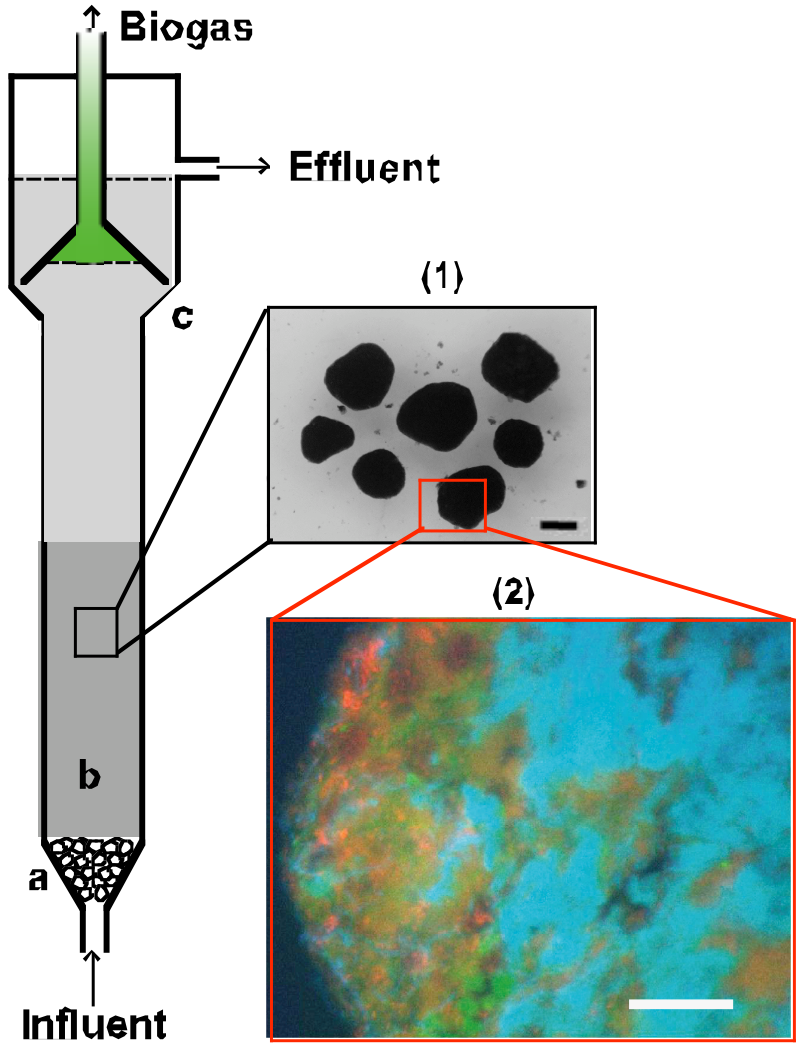
Time series of 9 full-scale granular upflow digesters treating brewery wastewater: 112 samples

Werner, J. J., D. Knights, M. L. Garcia, N. B. Scalfone, S. Smith, K. Yarasheski, T. A. Cummings, A. R. Beers, R. Knight and L. T. Angenent (2011). "Bacterial Community Structures are Unique and Resilient in Full-Scale Bioenergy Systems." Proceedings of the National Academy of Sciences **108**(10): 4158-4163.

Upflow anaerobic digesters

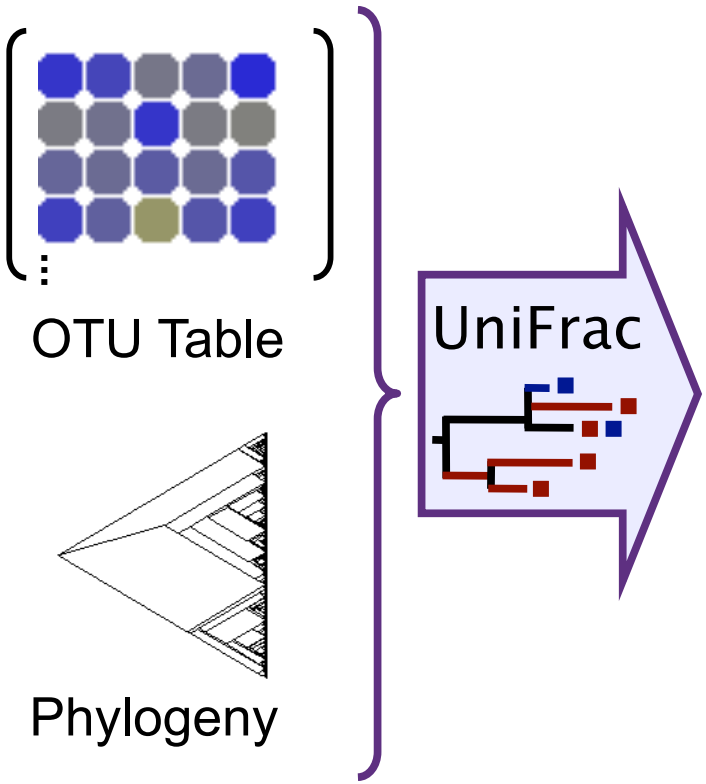


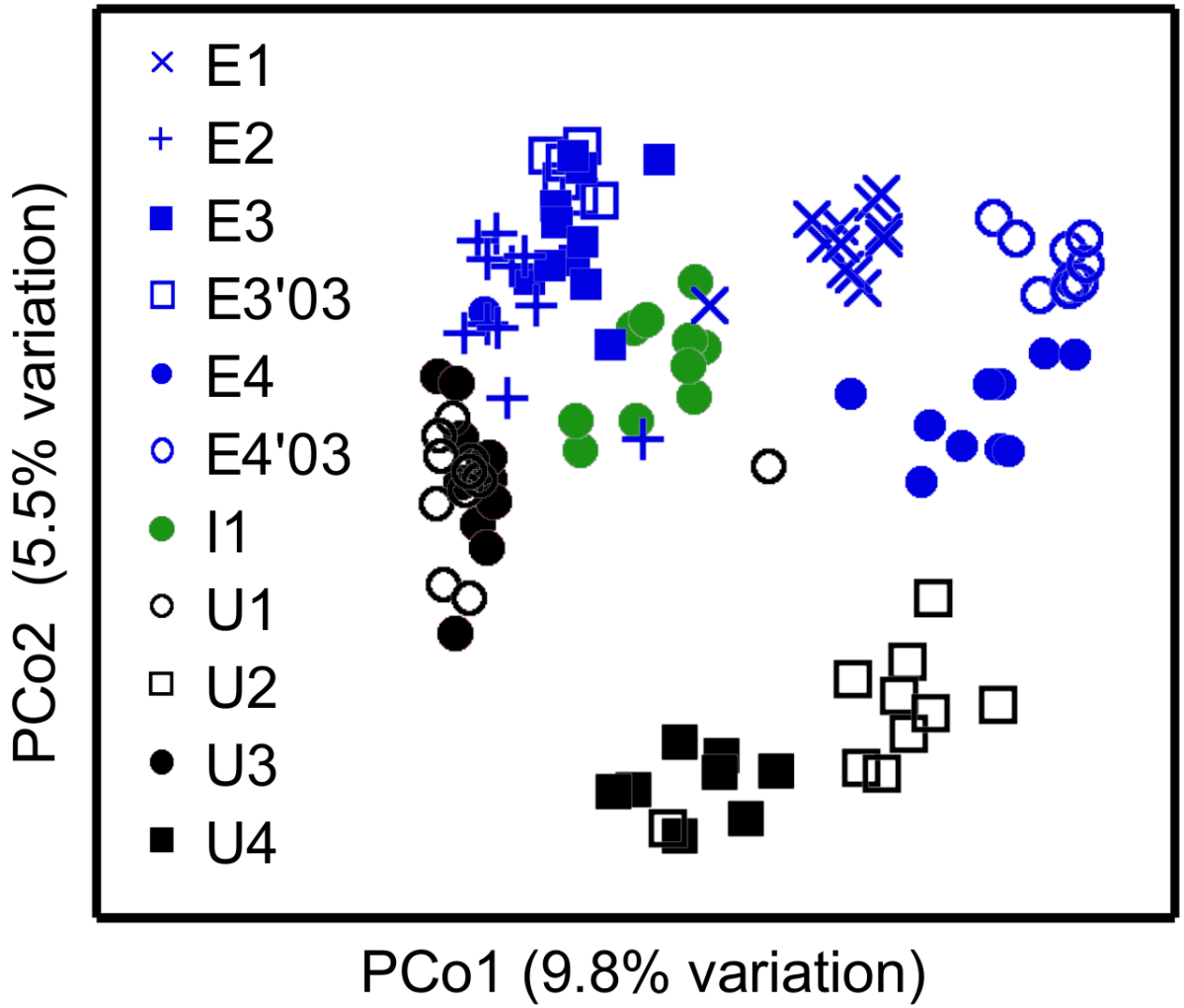
Work performed by Dr. Jeff Werner (now at SUNY Cortland)

Angenent *et al.* (2004) *Nature Immunology*

Brewery offsets ~ 20% of all boiler fuel with methane from biogas

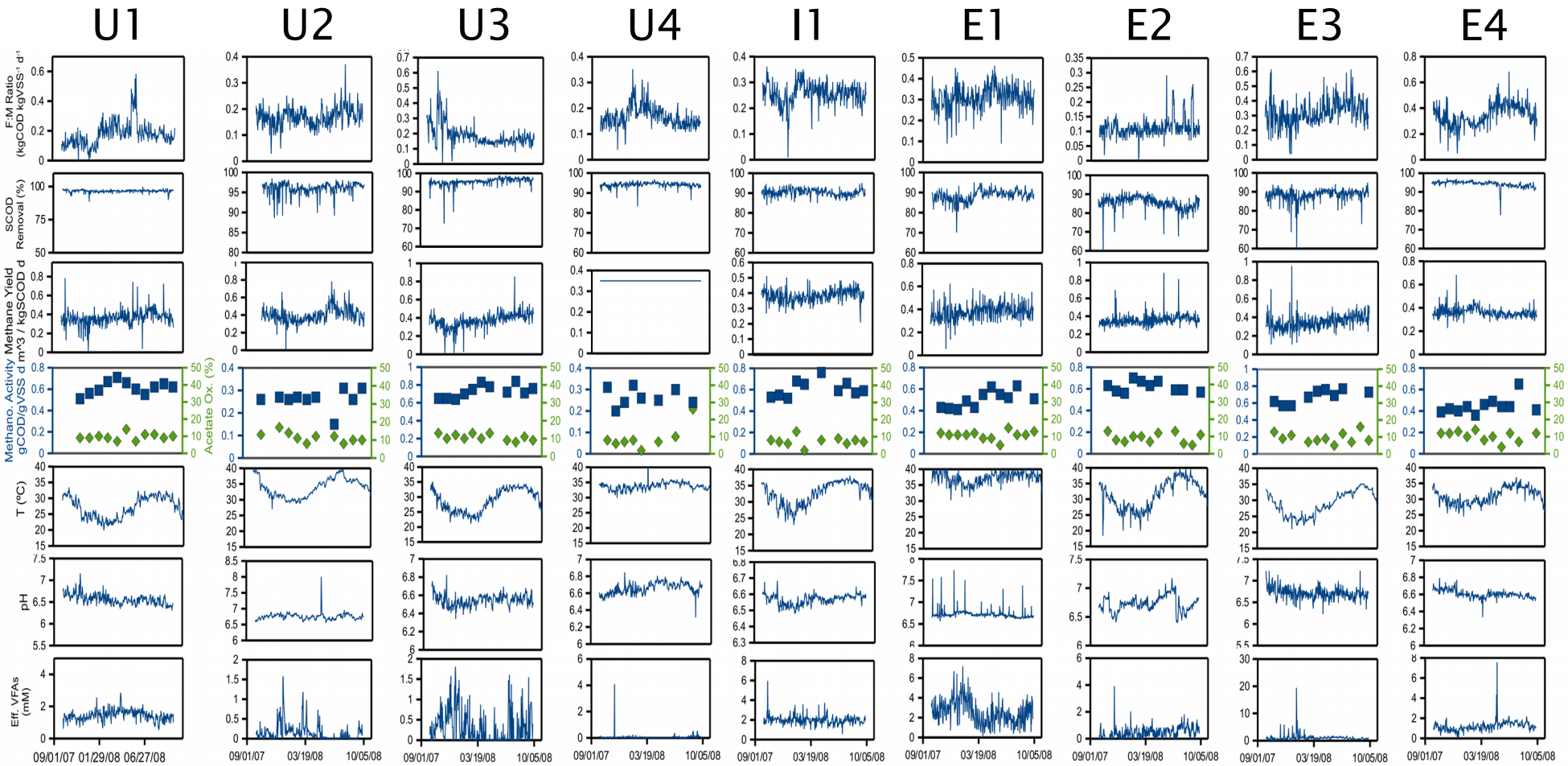
β diversity: comparing between microbiome samples with 16S rRNA gene sequencing (>420,000 seqs)





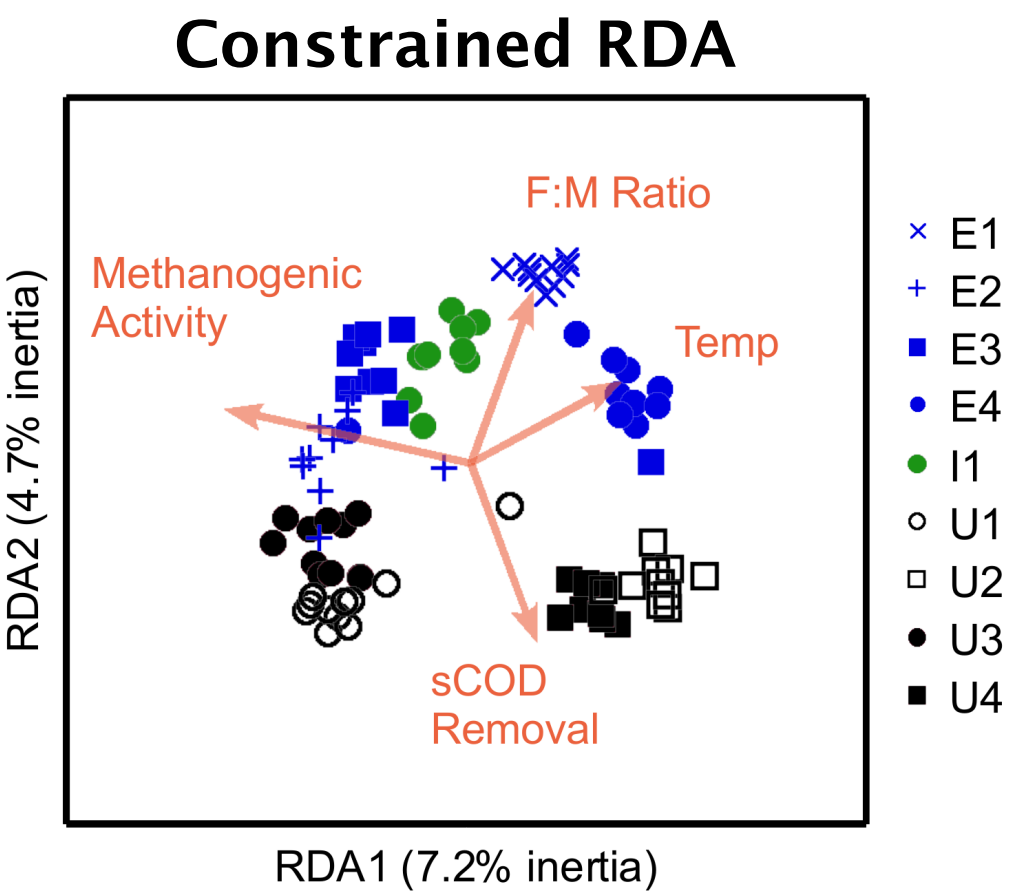
Each bioreactor has a unique and stable microbiome

Lots of performance and operating data (metadata)



Constrained redundancy analysis (RDA):

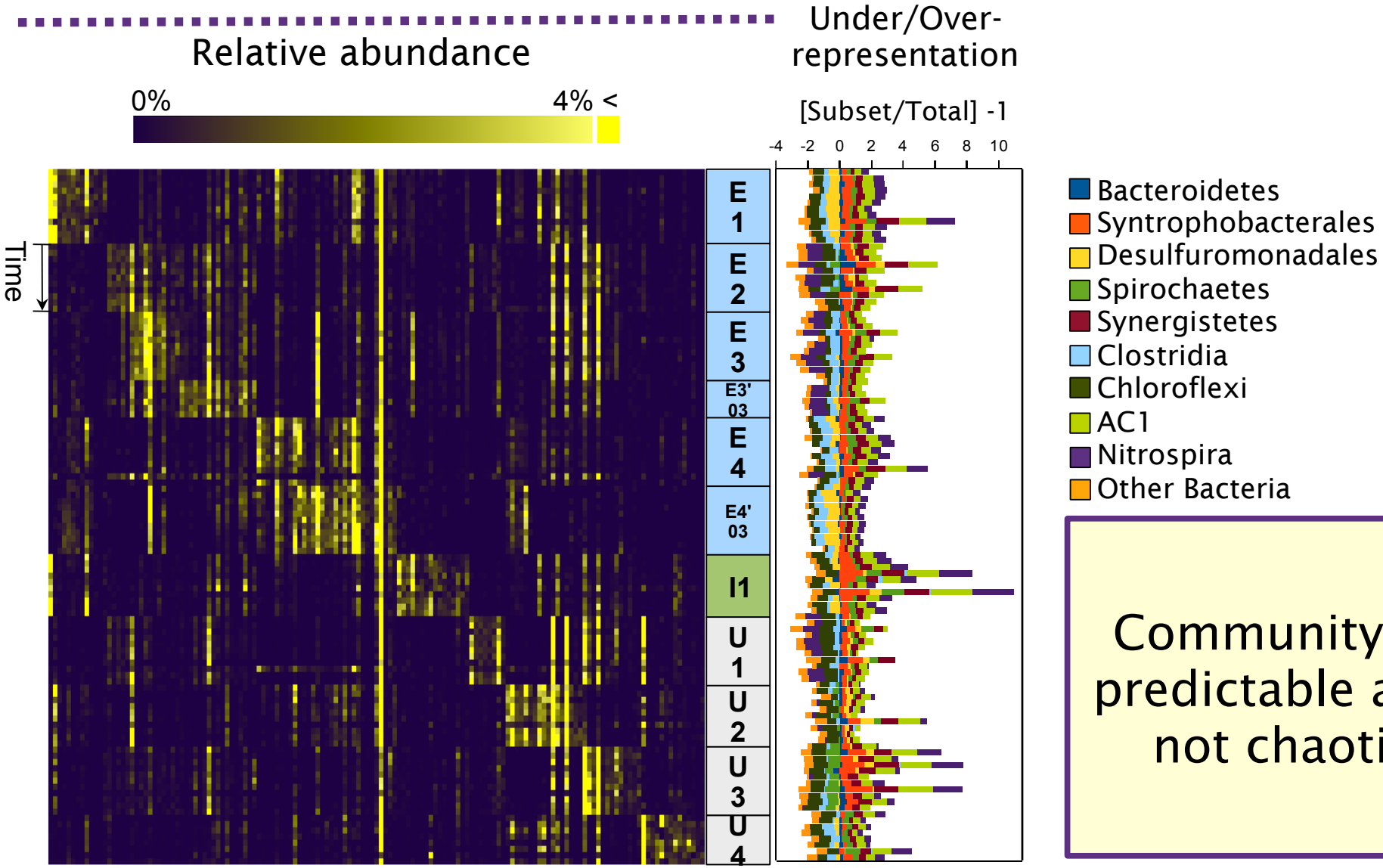
Phylogenetic structure was explained by function and environment



Constrained redundancy analysis (RDA) fits the variation in UniFrac data with the variation in the metadata matrix (UniFrac data is constrained by the metadata)

Predictive OTUs (145) selected using machine learning

97% accuracy for predicting facility location with synthrophs



Community is predictable and not chaotic

Werner, J. J., D. Knights, M. L. Garcia, N. B. Scalfone, S. Smith, K. Yarasheski, T. A. Cummings, A. R. Beers, R. Knight and L. T. Angenent (2011). "Bacterial Community Structures are Unique and Resilient in Full-Scale Bioenergy Systems." Proceedings of the National Academy of Sciences **108**(10): 4158-4163.

Answer 1

Reactor microbiomes are predictive rather than chaotic

Outline – 4 questions

1. Is the reactor microbiome of AD stable and predictable?
2. Why does AD produce methane at such high specificities?
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AD = extreme fermentation

Fermentations: no external e^- acceptor present:

CH_4 (8) = most reduced carbon: C in methane has an oxidation state of -4 *or* oxidation number of 8 (8 e^- from O_2 to fully oxidize to CO_2)

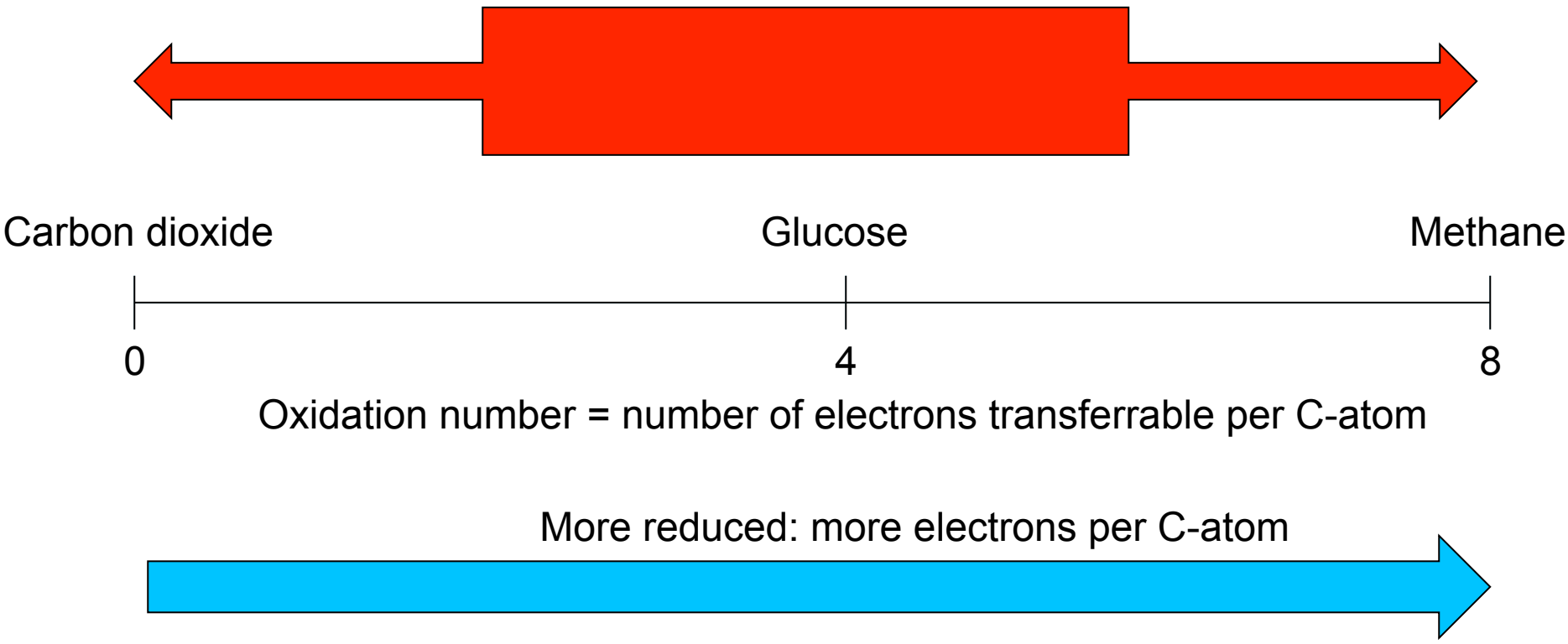
- CH_4 not further reducible: most energy for microbiome!*
- "Stable" end product
- Gaseous end product, bubbles out

*Hanselmann, K. W. (1991). "Microbial energetics applied to waste repositories." Cellular and Molecular Life Sciences **47**(7): 645-687.

But, electrons cannot be lost or gained in AD without external e^- acceptors:

.....

$$C_6H_{12}O_6\ (4) \rightarrow 3\ CO_2\ (0) + 3\ CH_4\ (8)$$



Answer 2

Thermodynamic reasons for high specificity with methane:

Methane is the most reduced product:

Stable
Most energy for microbiome

Bubbles out (product removal)

Outline – 4 questions

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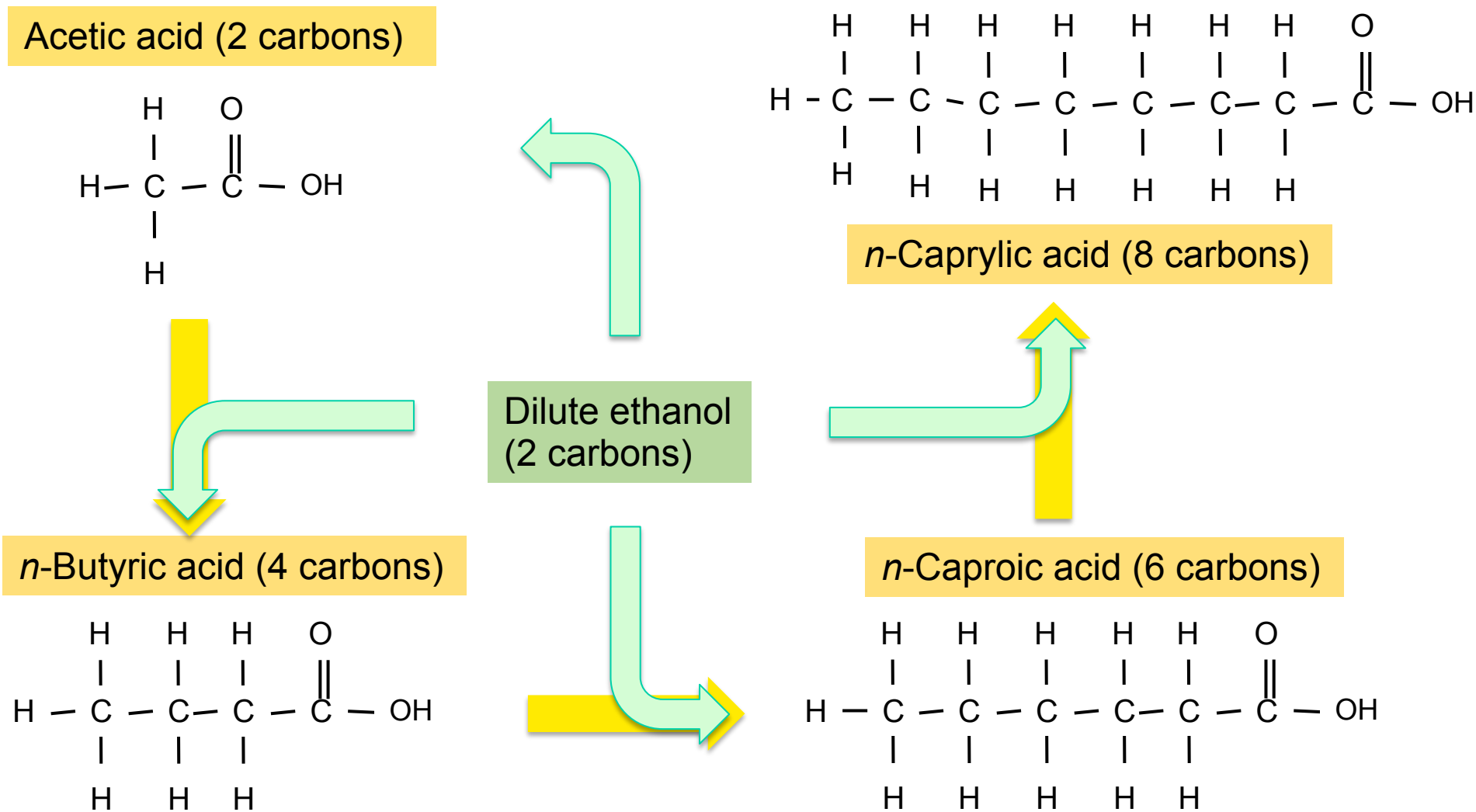
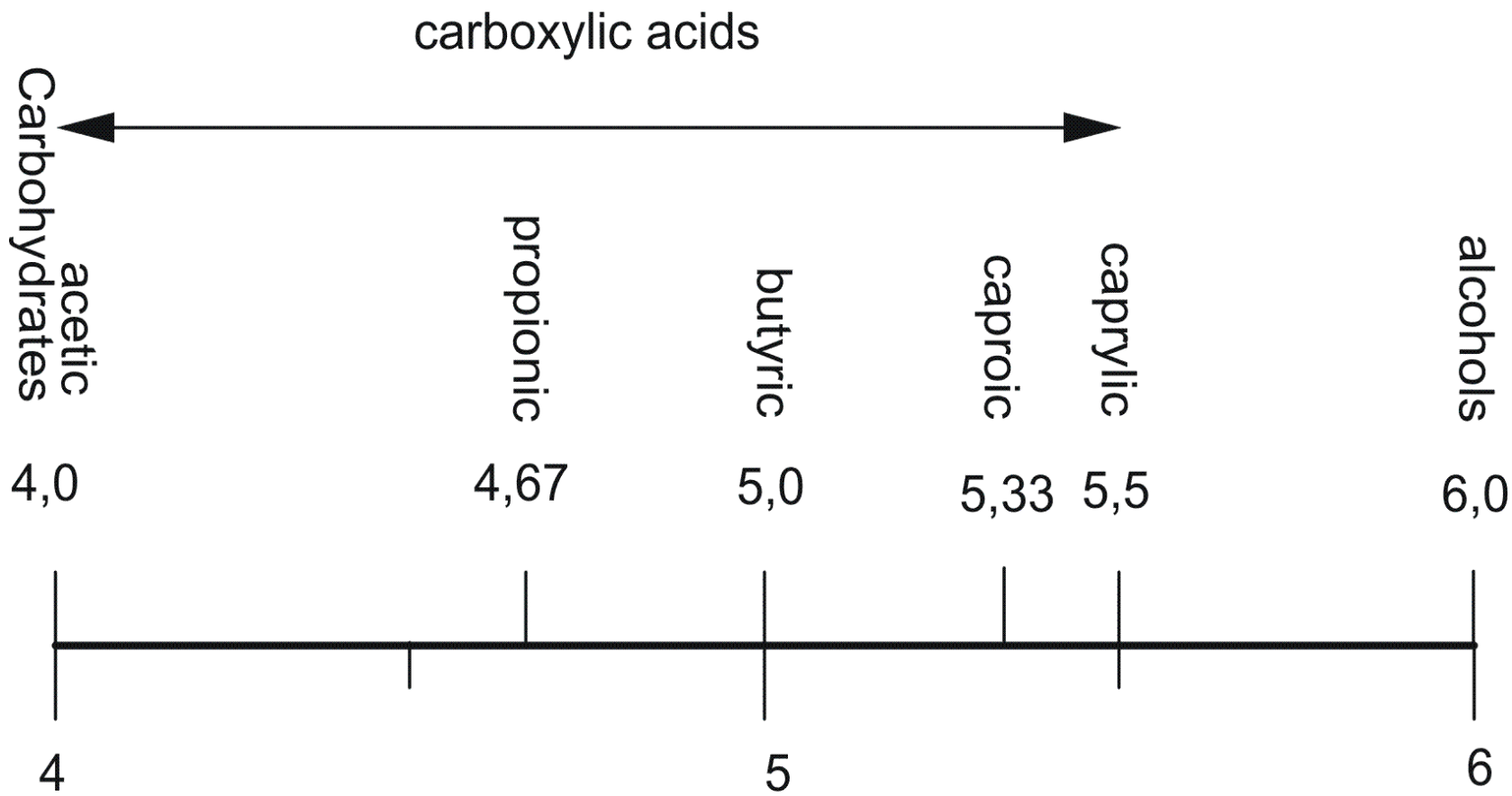
Chain elongation with reactor microbiomes



Acetic acid (e^- acceptor) and ethanol (e^- donor) to gain energy

- CH_4 production from acetate is inhibited
- CH_4 production from hydrogen is limited by CO_2



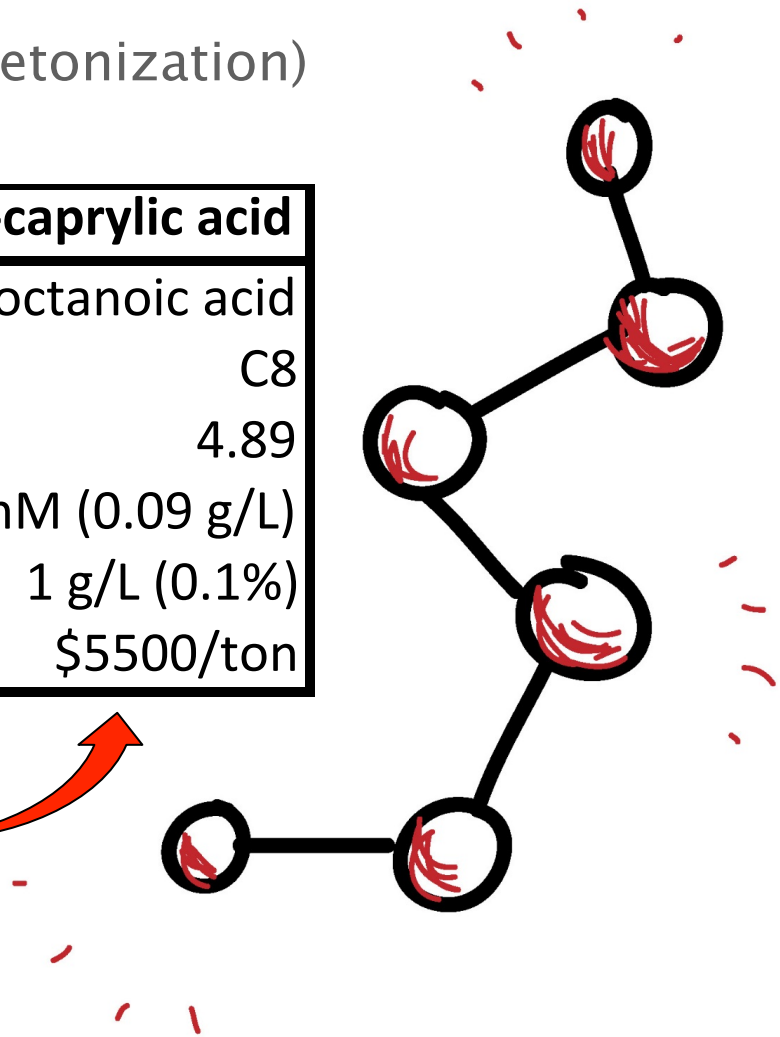
Oxidation number = number of transferable electrons per C-atom.

Caproic acid and caprylic acid

Medium-chain carboxylic acids:

- Antimicrobials for animal husbandry
- Biofuel precursor (Kolbe electrolysis or ketonization)

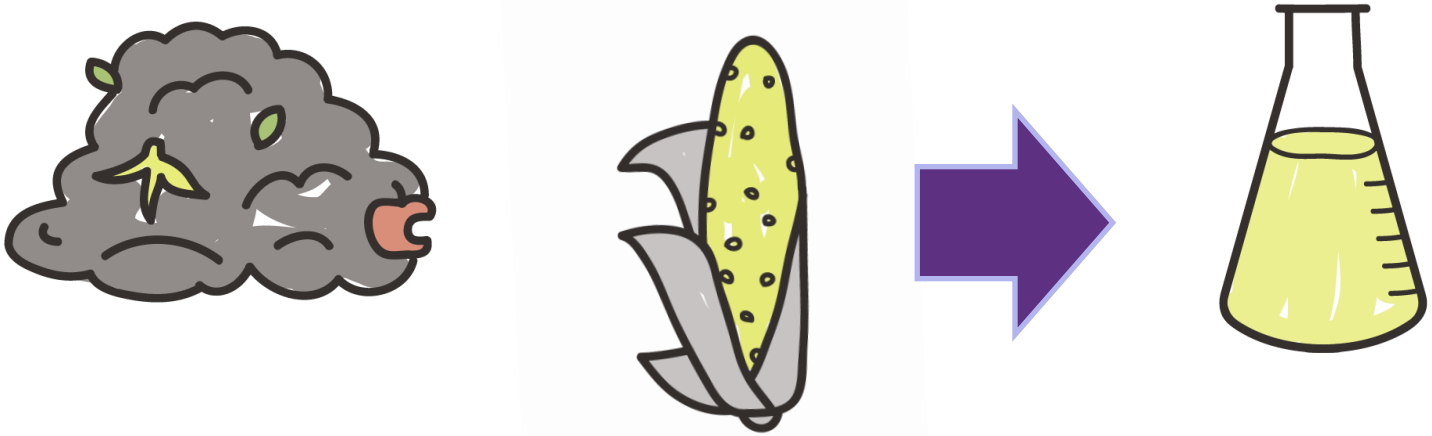
	***n*-caproic acid**	***n*-caprylic acid**
Chemical name	*n*-hexanoic acid	*n*-octanoic acid
Carbon atoms	C6	C8
pK*a*	4.88	4.89
Toxic concentration	10.7 mM (1.2 g/L)	0.63 mM (0.09 g/L)
Max. solubility	10 g/L (1%)	1 g/L (0.1%)
Bulk price	$3000/ton	$5500/ton



Price ~ doubles with each 2 carbons elongated

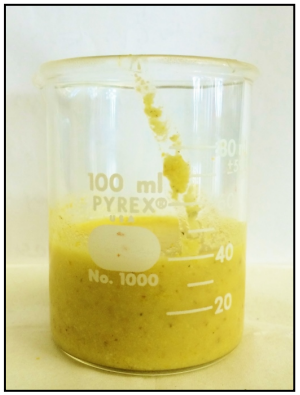
Caprylic acid is 35x more valuable per carbon atom than methane

Feedstock to microbial oil



Wasted biomass + undistilled ethanol

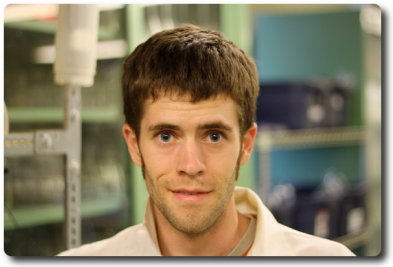
Specialty oils



Corn kernel-to-ethanol fermentation beer (15% Etoh)

Medium-chain carboxylic acid (MCCA) oil (>90% MCCAs)

Corn Ethanol Industry (50 billion L year^{-1})



Work performed by: Dr. Matt Agler (now at Max Planck Institute, Köln)

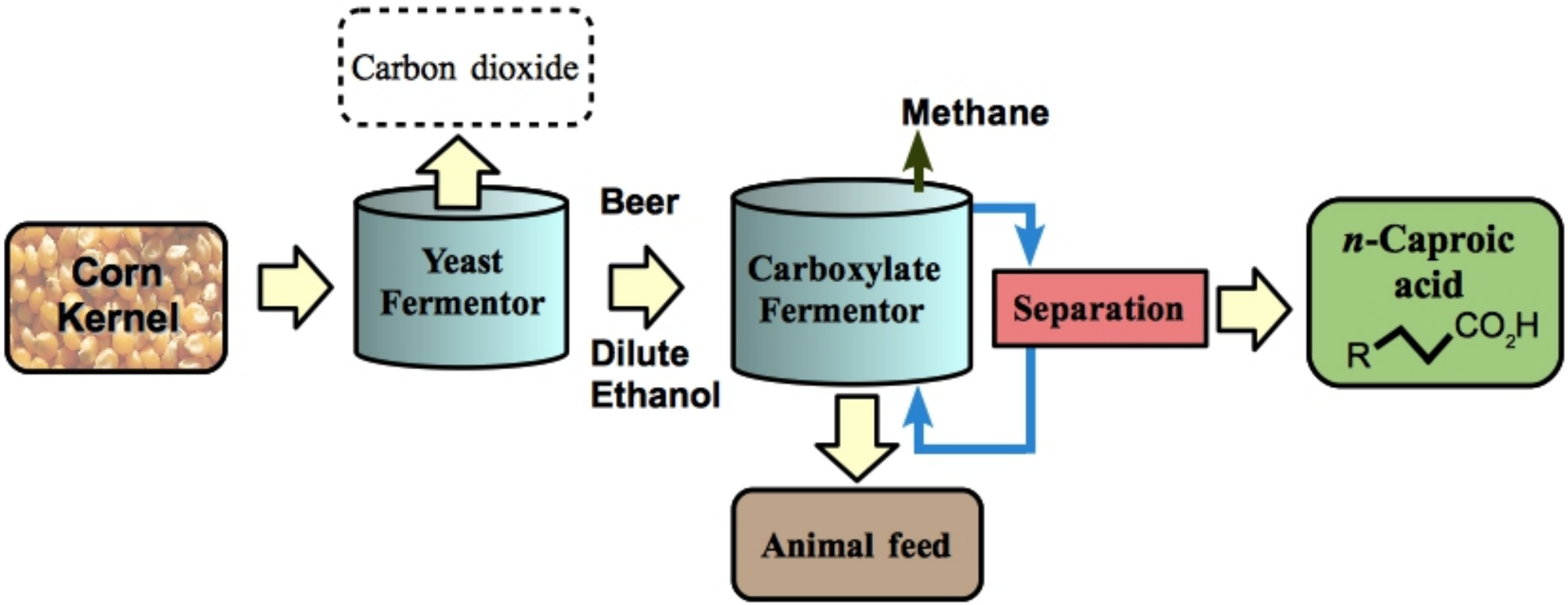
15-20% of energy value in ethanol itself is used for distillation (recovery)*

Here, we fed ethanol broth (fermentation beer with 15% ethanol) to our reactor microbiome: biomass and yeast into acetate and *n*-butyrate

* Shapiro et al. (2010) evaluated several dry milling plants based on 2008 data gathering.

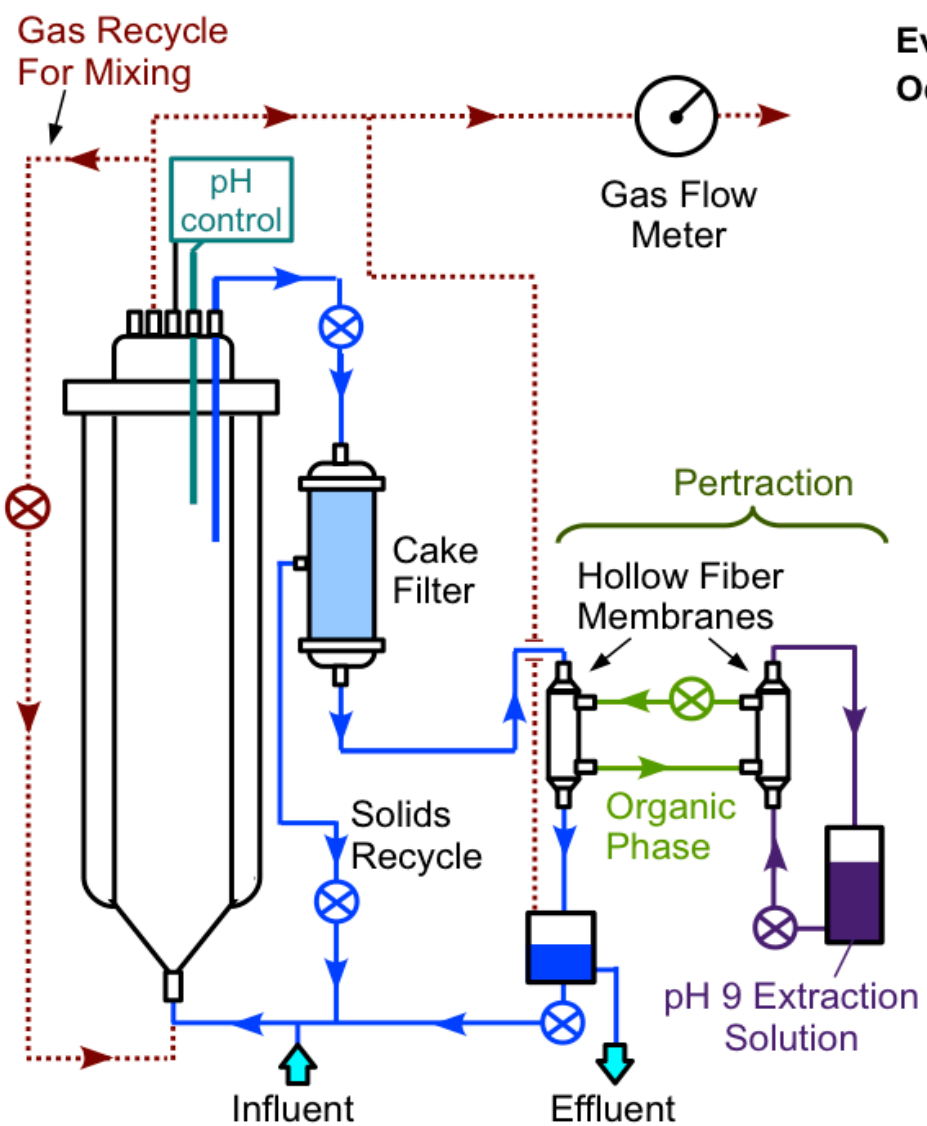
Agler, M. T., C. M. Spirito, J. G. Usack, J. J. Werner and L. T. Angenent (2012). "Chain elongation with reactor microbiomes: upgrading dilute ethanol to medium-chain carboxylates." Energy & Environmental Science **5**(8): 8189-8192..

Corn-kernel-to-ethanol beer to *n*-caproic acid

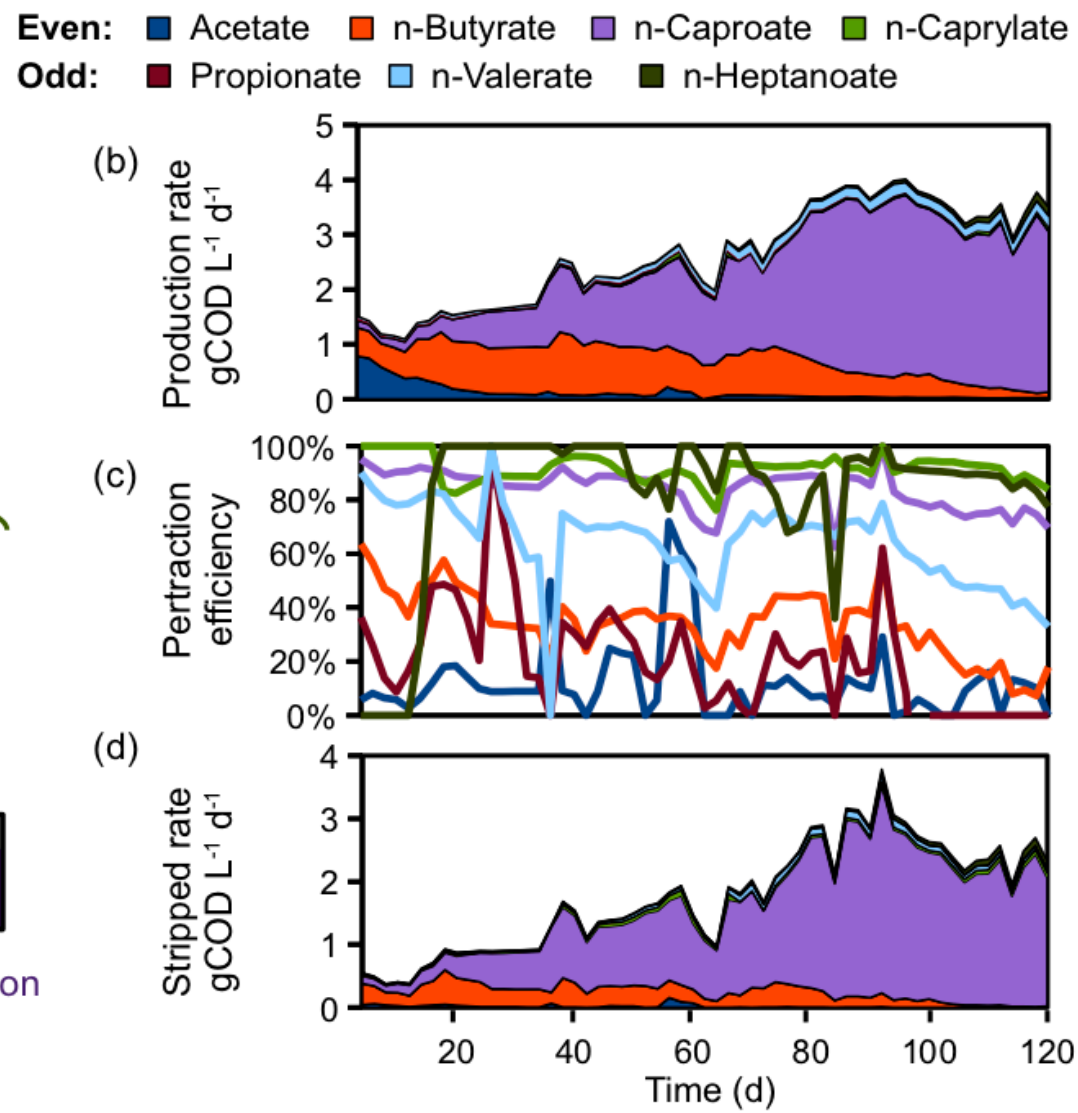


Complex substrates

n-Caproate production and specificity is similar to methane production



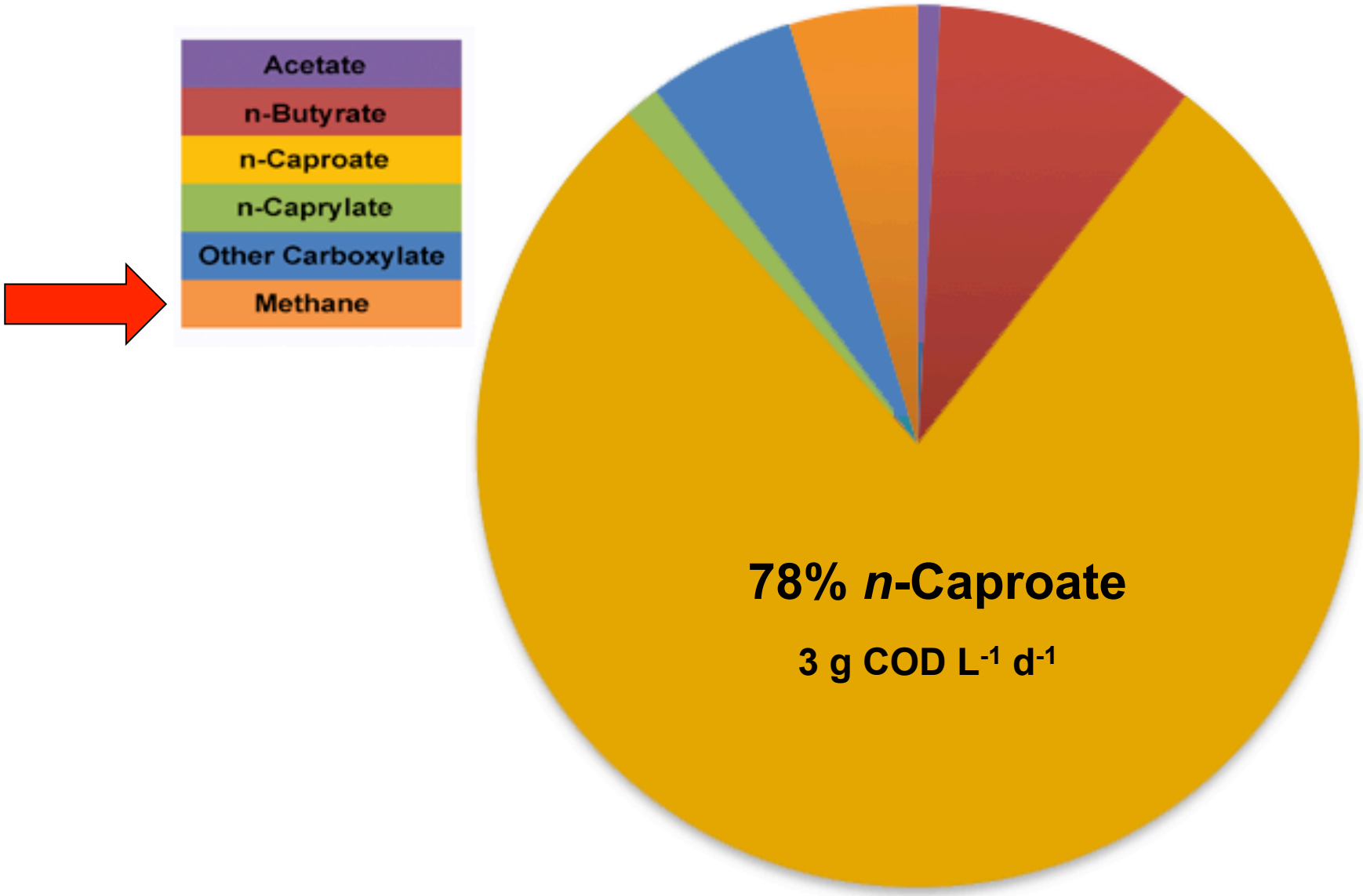
a. Current Bioreactor and Pertraction System



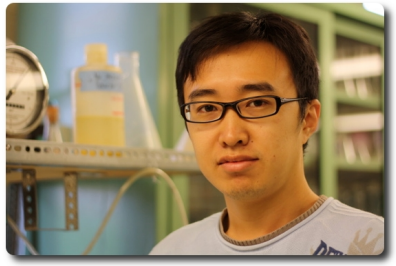
b-d. Carboxylates Produced

Bioreactor: 30 degrees C; pH 5.5; ethanol beer (15% Etoh); pertraction = extraction

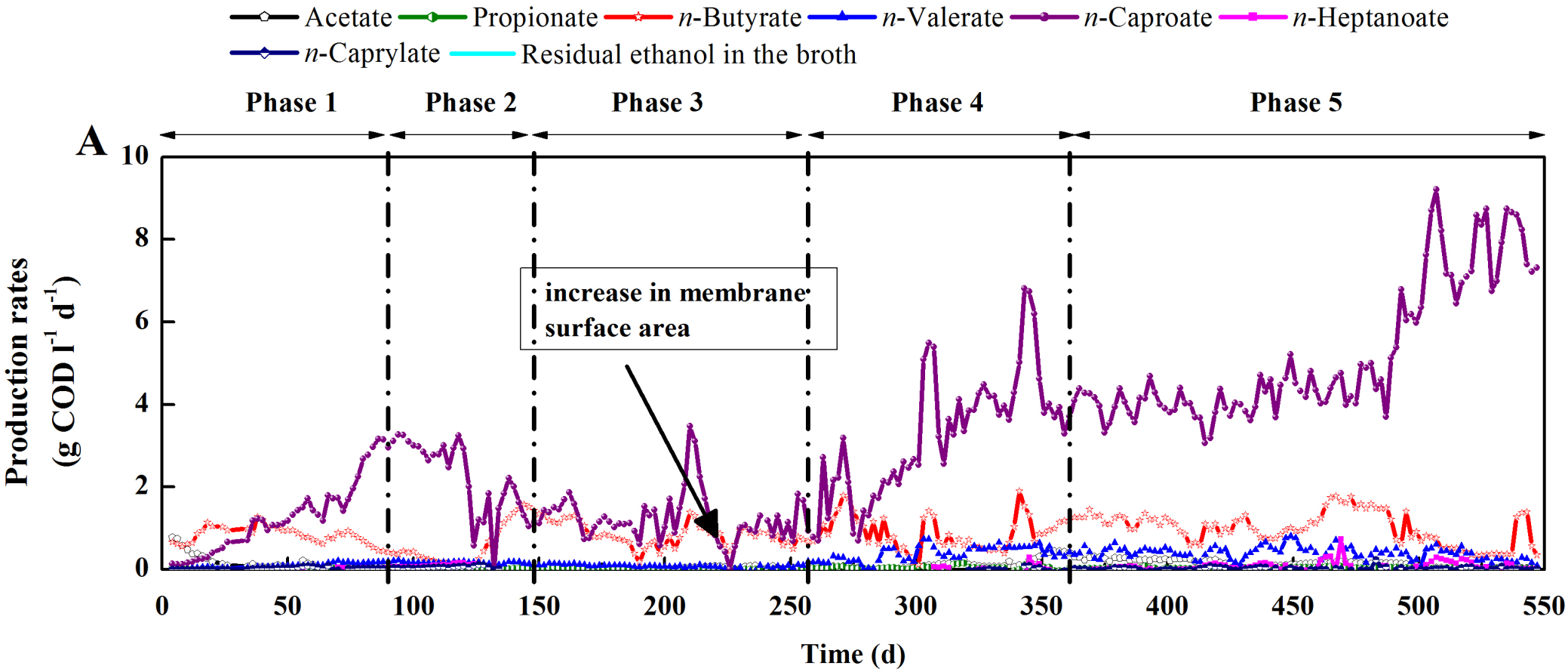
n-Caproate specificity is high



Long-term operation with > 300 days of extraction

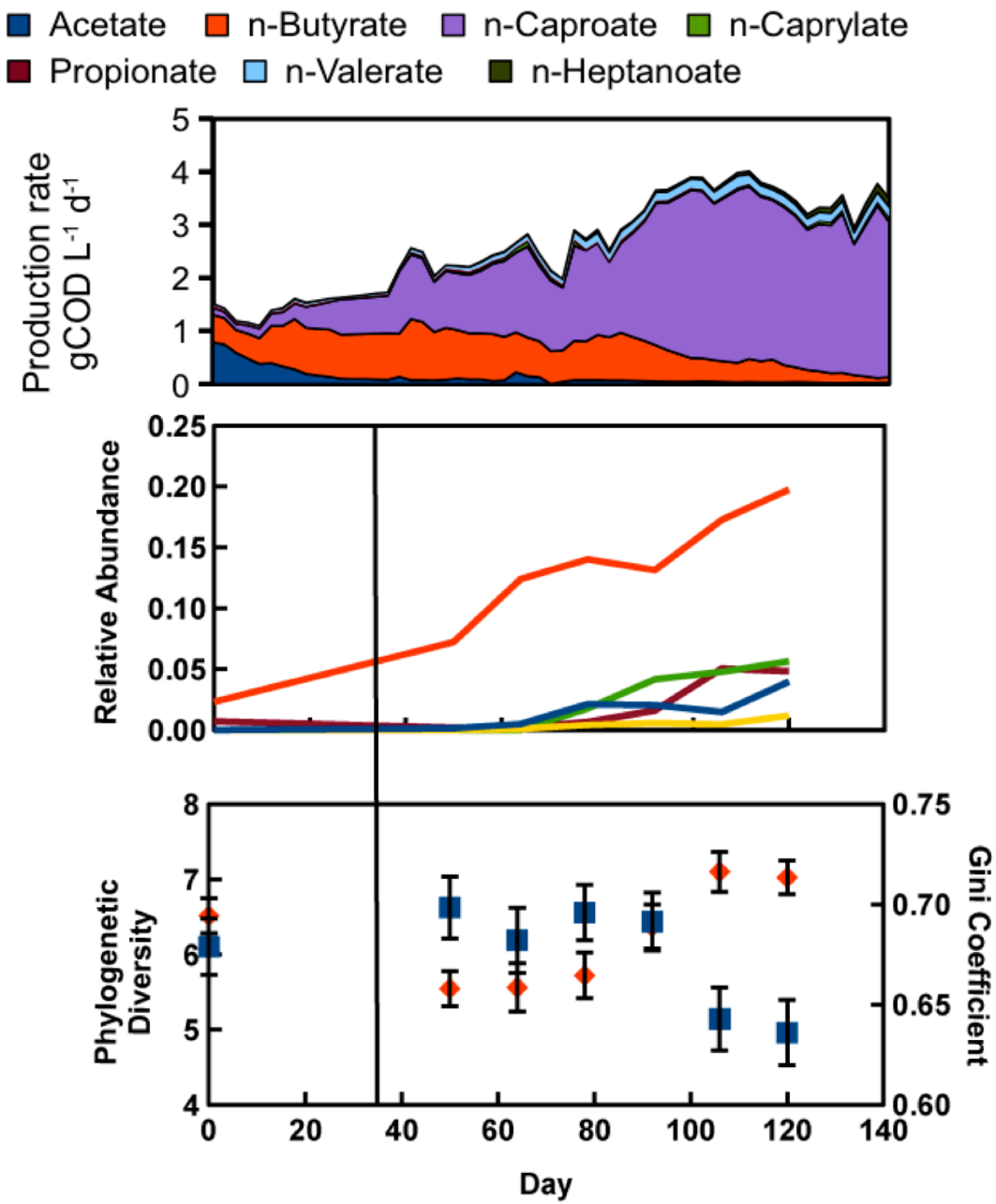


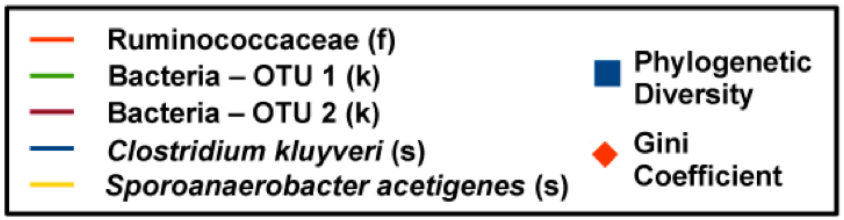
Work performed by Dr. Shijian Ge (now at Queen's University)

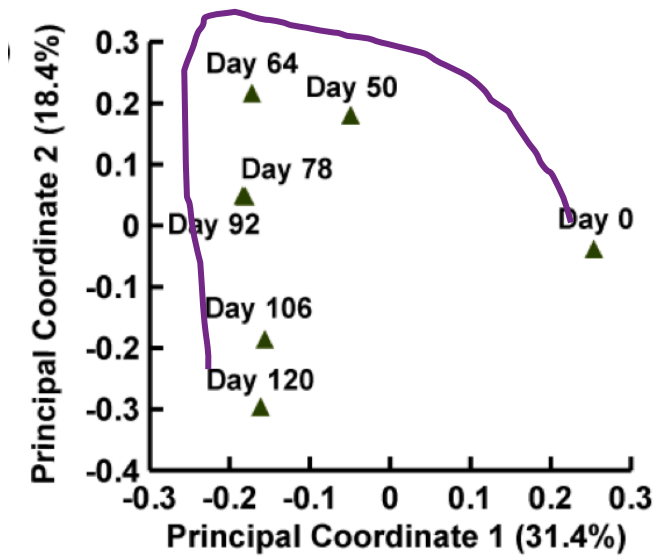


Ge, S., J. Usack, C. M. Spirito and L. T. Angenent (2015). "Long-term n-caproic acid production from yeast-fermentation beer in an anaerobic bioreactor with continuous product extraction." Environmental Science & Technology **49**(13): 8012-8021.

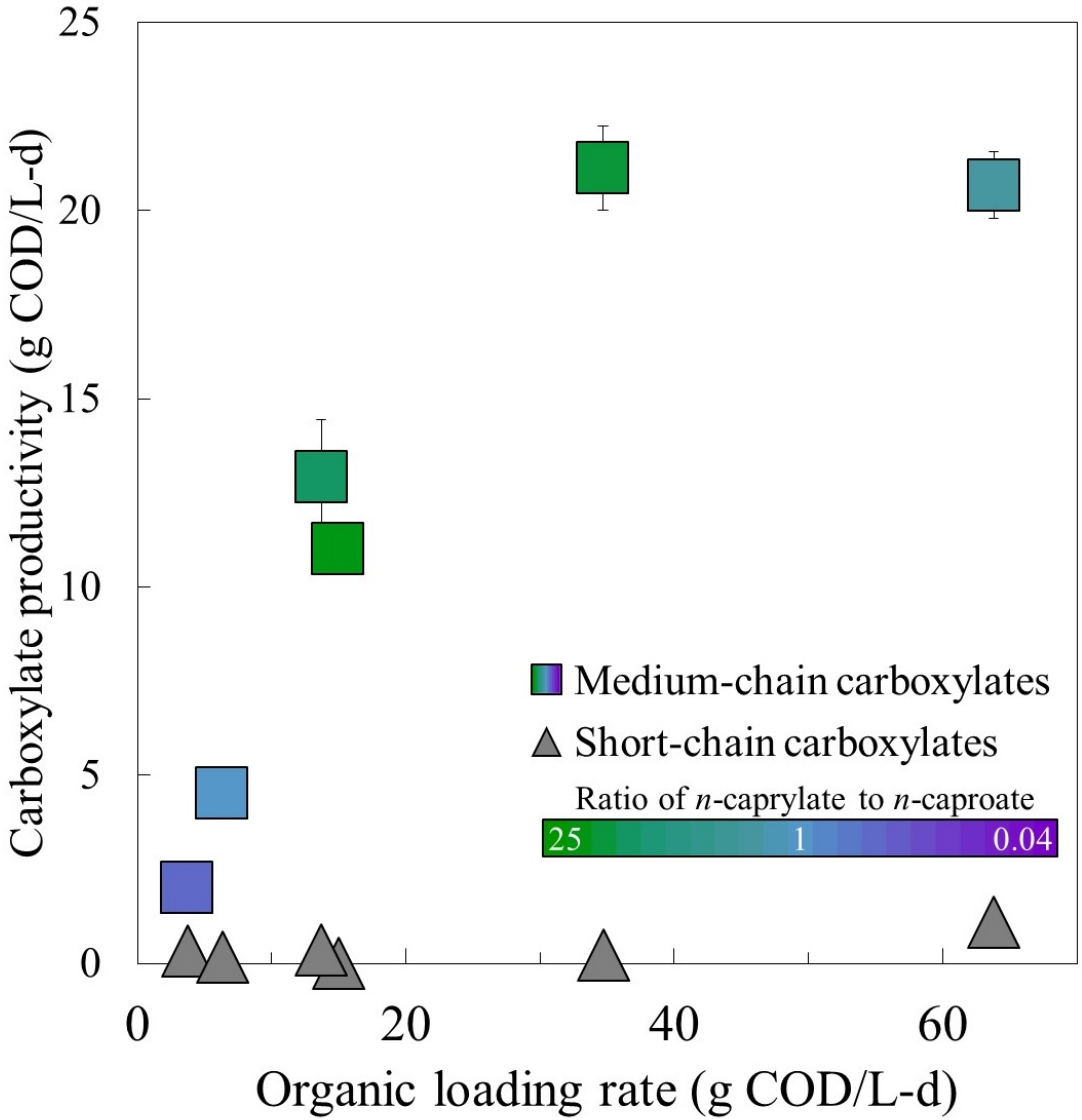
Shaping reactor microbiomes with enhanced function



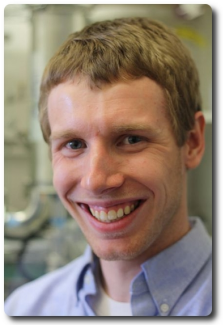


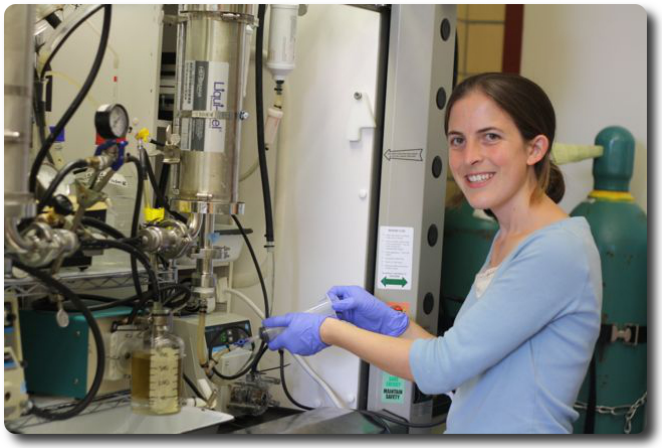


With ethanol and acetate (20:1 in syngas fermentation effluent) we produced mainly *n*-caprylic acid



Leo Kucek now at Applied Technologies, Inc.





Catherine Spirito

Kucek, L. A., C. M. Spirito and L. T. Angenent (2016). "High *n*-caprylate productivities and specificities from dilute ethanol and acetate: chain elongation with microbiomes to upgrade products from syngas fermentation." Energy & Environmental Science. DOI: 10.1039/C6EE01487A.

Answer 3

Reactor microbiome can produce a chemical (= caproate; caprylate) at high specificities

Outline – 4 questions

1. Is the reactor microbiome of AD stable and predictable?
2. Why does AD produce methane at such high specificities?
3. What else can we produce with microbiomes at high specificities?
4. What parameters did we use to shape the reactor microbiome?

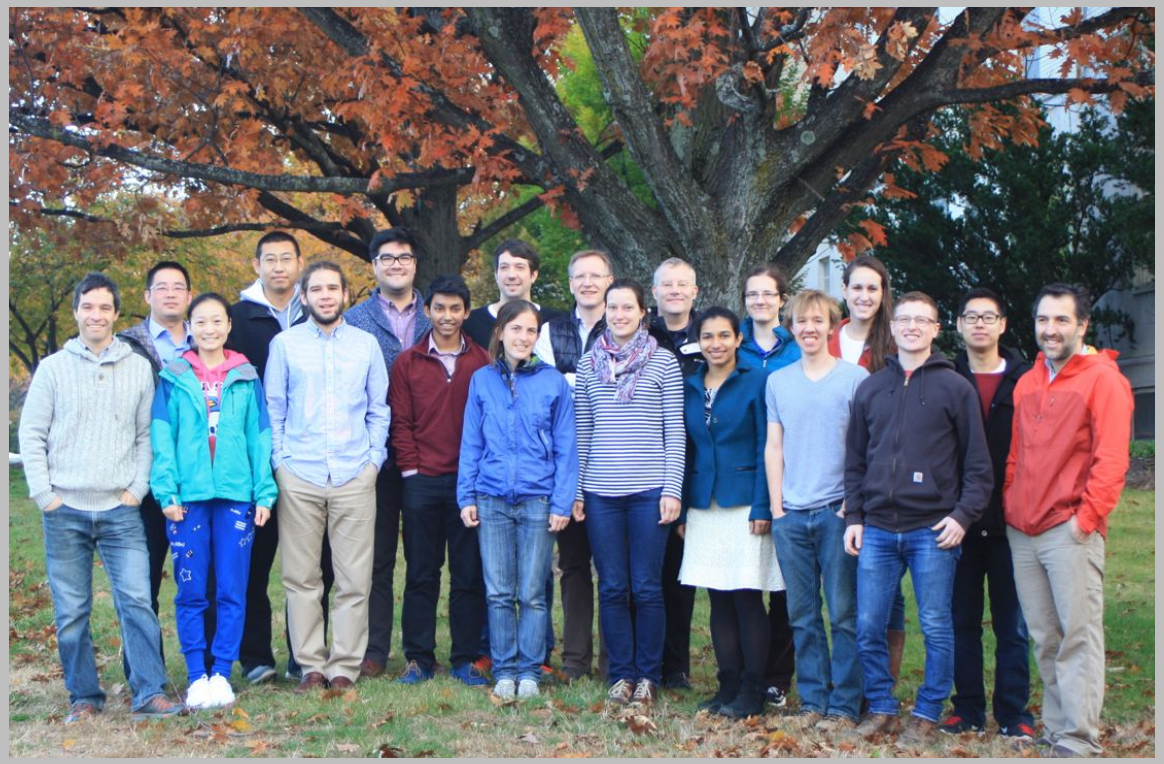
Answer 4

- Temperature: 30˚C (not at 55˚C)
- pH of 5.5 (to prevent acetoclastic methanogens)
- Continuous and selective extraction (pulling) of medium-chain carboxylic acids
- Feeding ethanol as a reduced compound (pushing)
- Maintaining high enough H_2 partial pressures
- Product toxicity (to select for chain elongating bacteria)

But, we used the underlying thermodynamic advantage of the most reduced product

Acknowledgements

- Angenent Lab



- Collaborators:
  - Korneel Rabaey, Ghent
  - Falk Harnisch, Helmholtz-Leipzig
- Funding sources:

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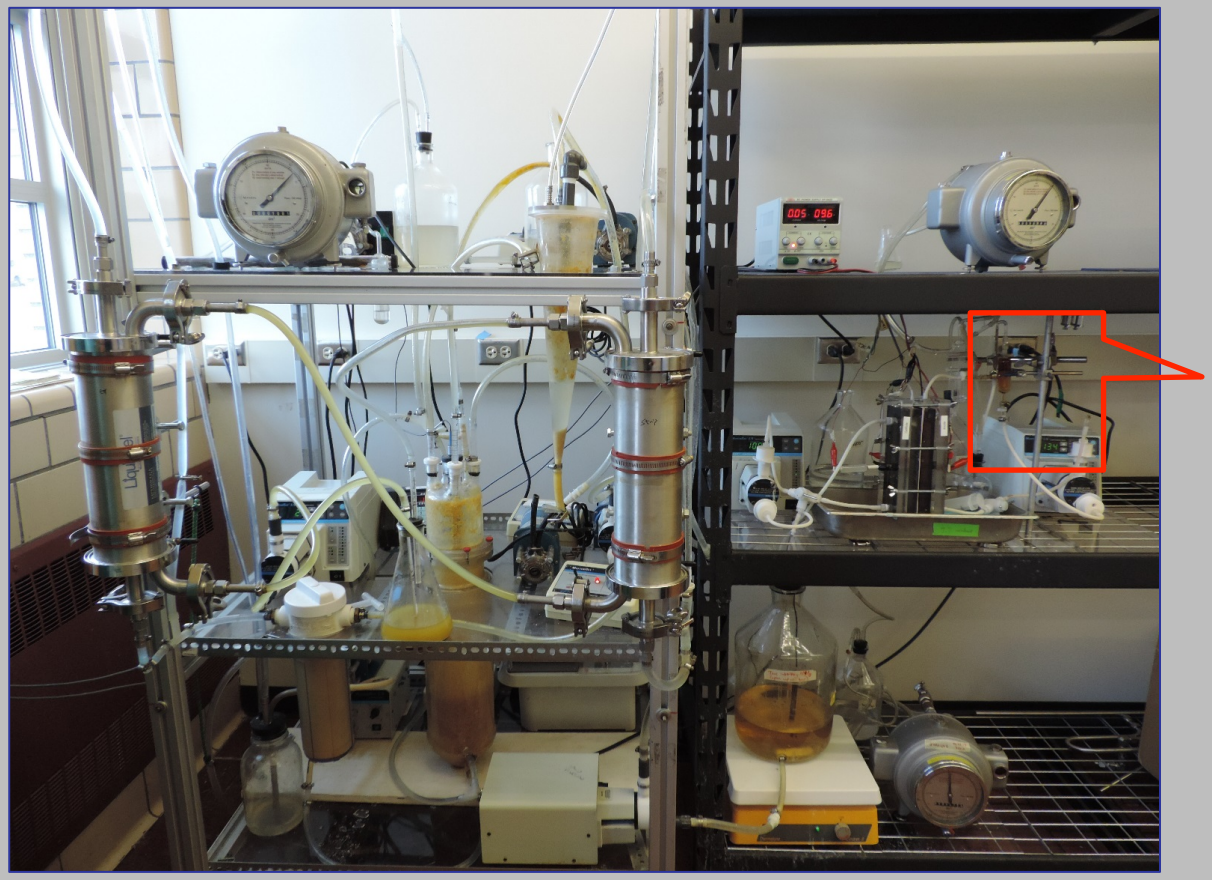
- Microbial oil production – questions?