# Applying Multi-Omics Tools to Guide New Treatment Processes: Anaerobic Conversion of Industrial Wastes to Valuable Chemicals

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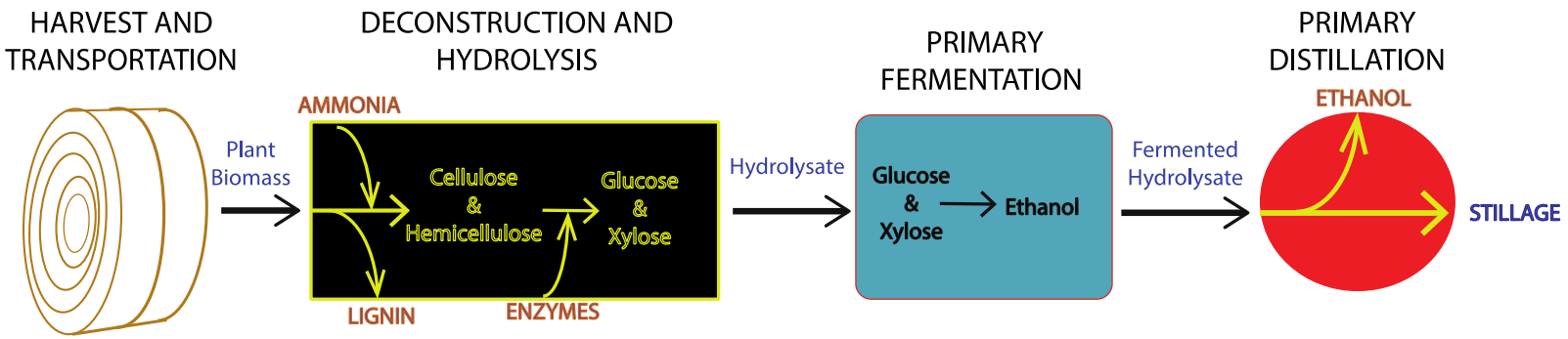
The University of Vermont

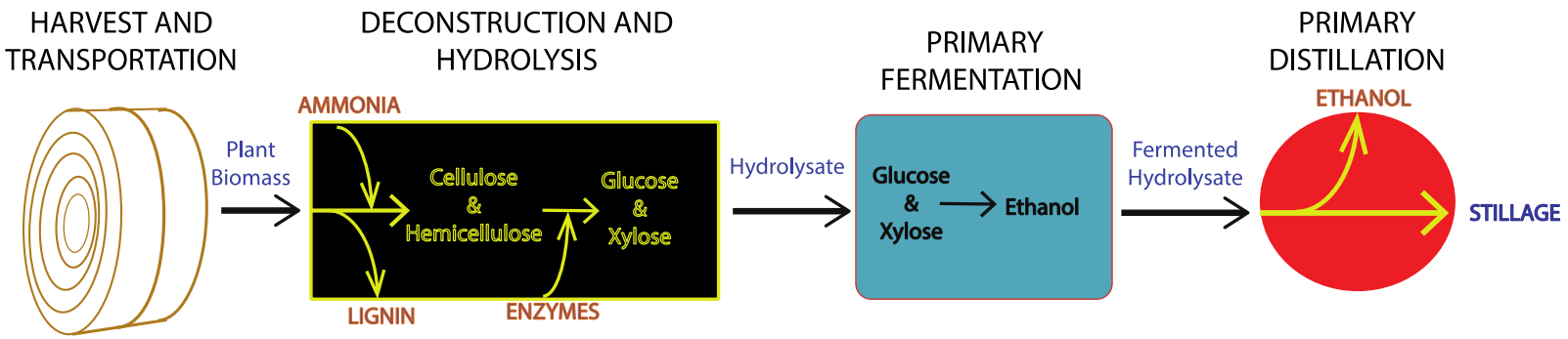
# The quest for lignocellulosic transportation fuels


image courtesy of USDOE

# The Lignocellulosic Biorefinery



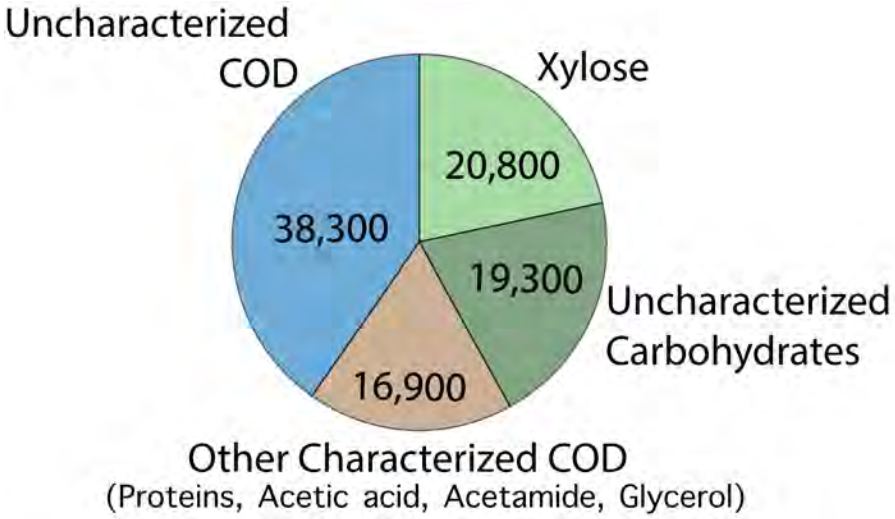
# Stillage: Lignocellulosic leftovers



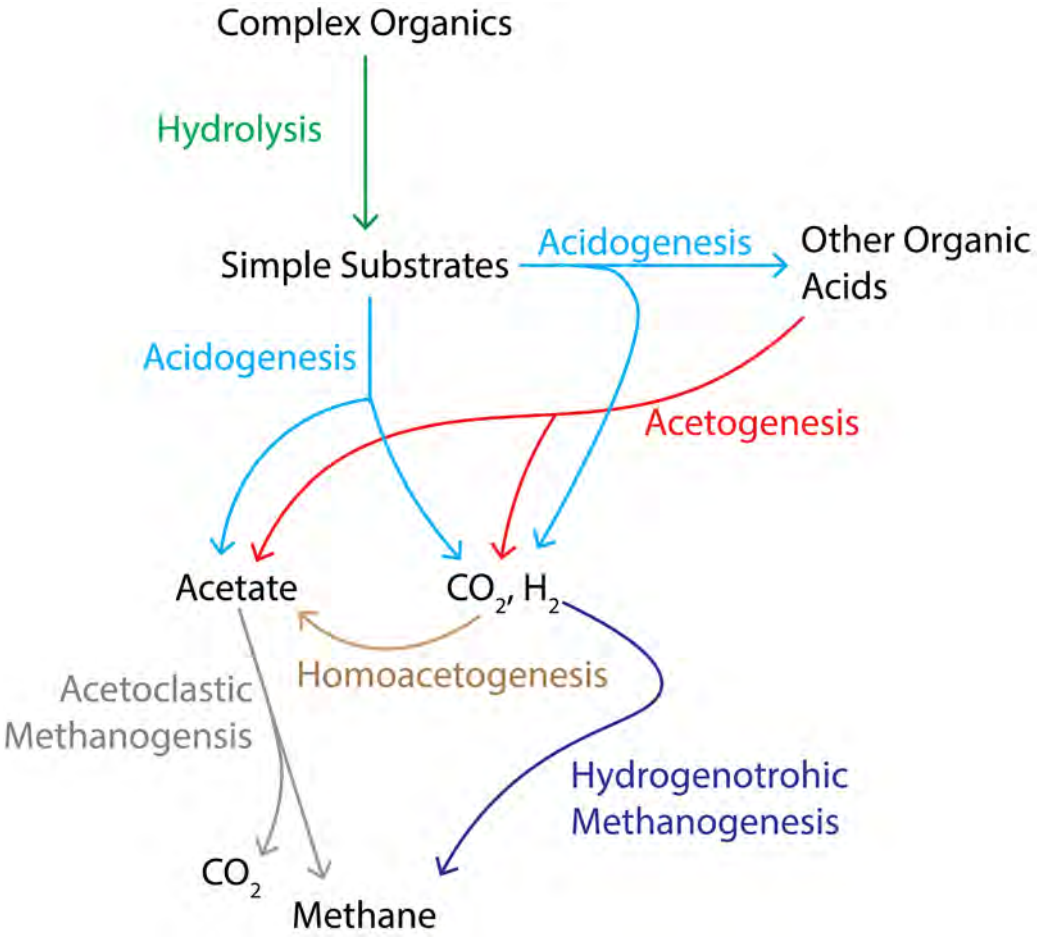

Stillage contains half of the chemical energy of the plant biomass

# Stillage: Lignocellulosic leftovers

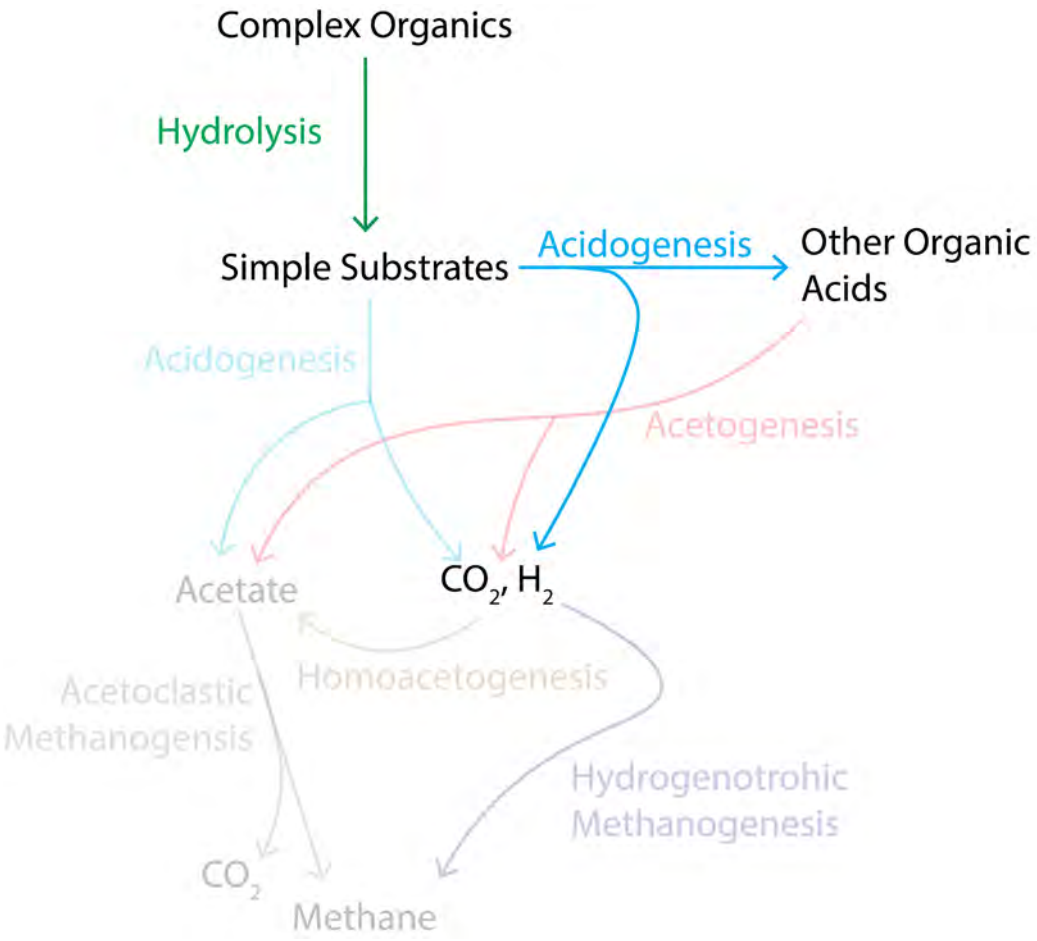
**Stillage (aka Conversion Residue)**
**sCOD = 95,000 mg $L^{-1}$**



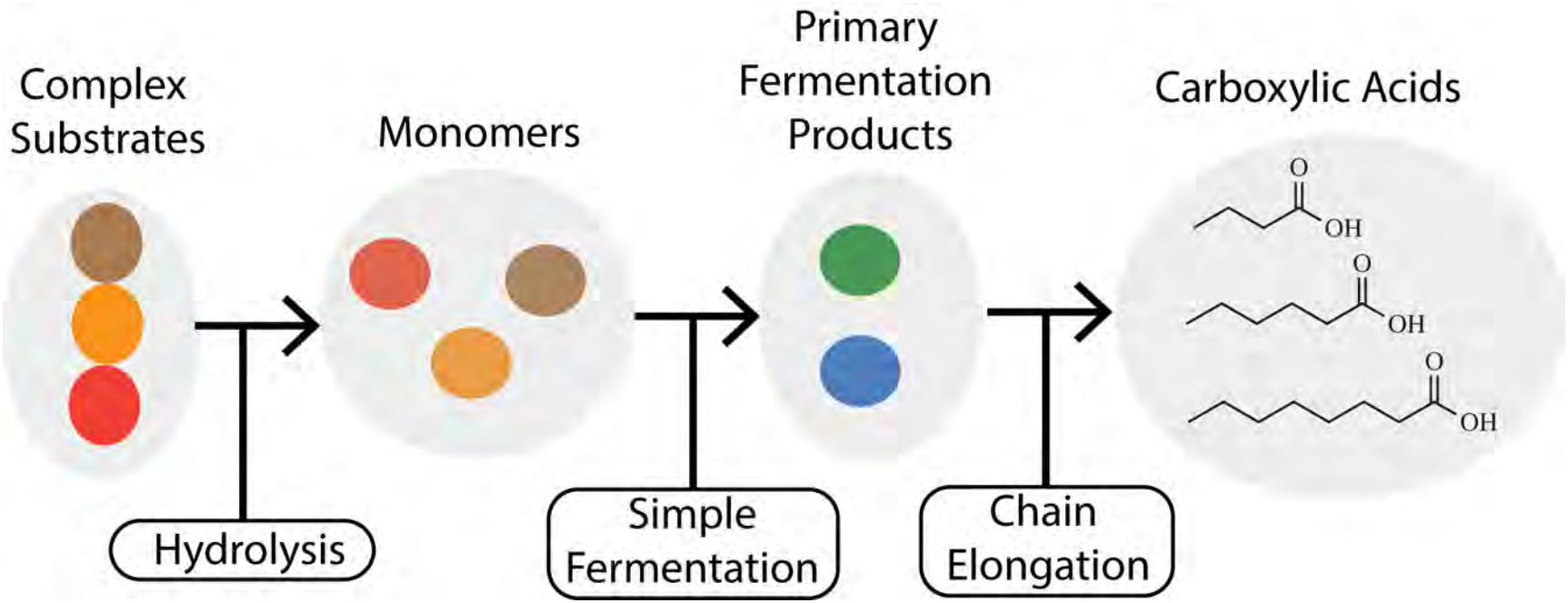
# Conventional Anaerobic Digestion



# “Carboxylate Platform” or “Chain Elongation”



# An overview of the carboxylate platform



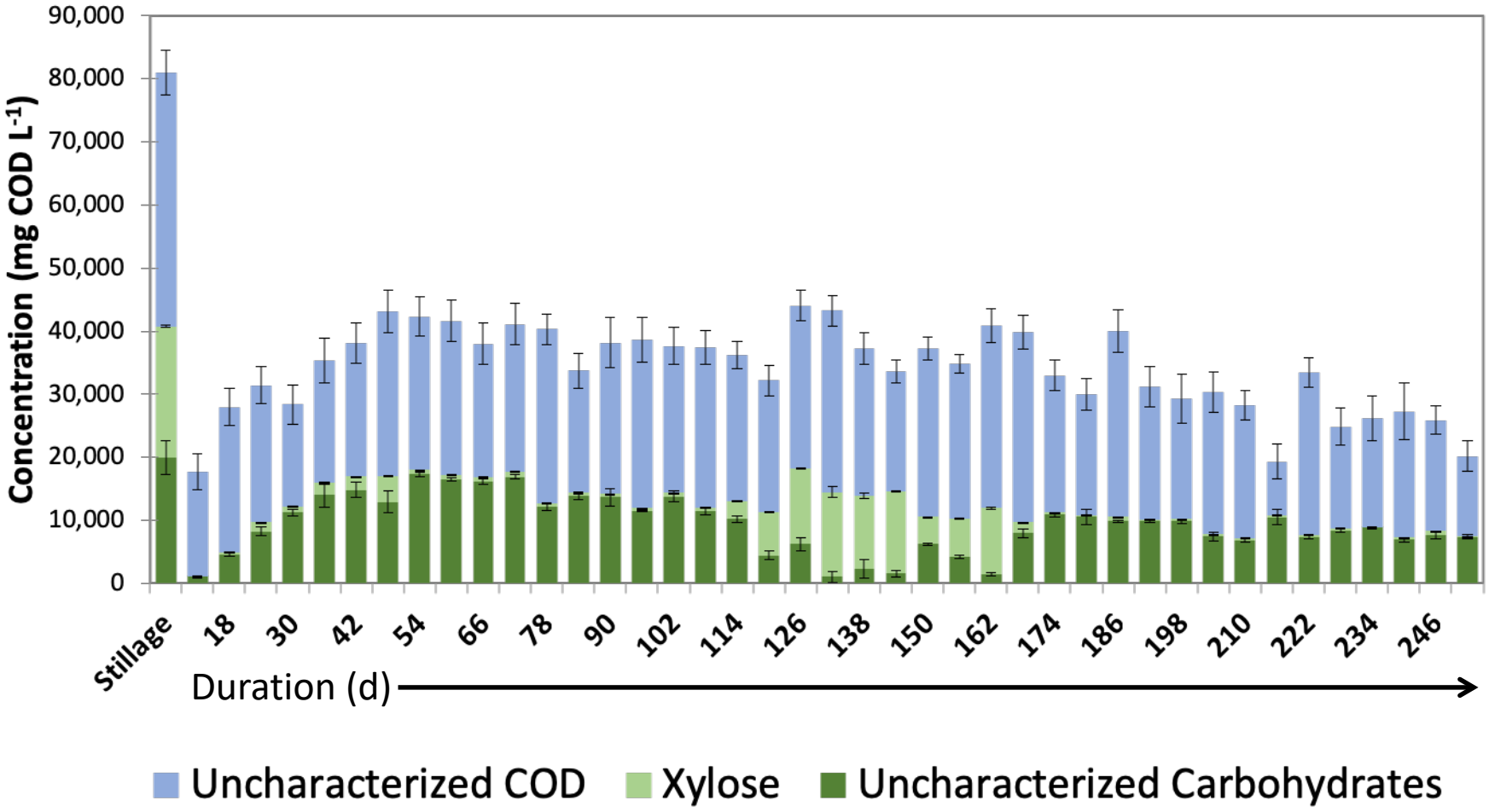
# Why medium chain fatty acids?

- Hexanoic (C6) and octanoic acid (C8) are particularly valuable
- Currently derived as a byproduct of palm refining
- Many industrial applications
- Relatively easy to recover
- Precursor to transportation fuels

# Can we use the carboxylate platform to produce MCFA from stillage?

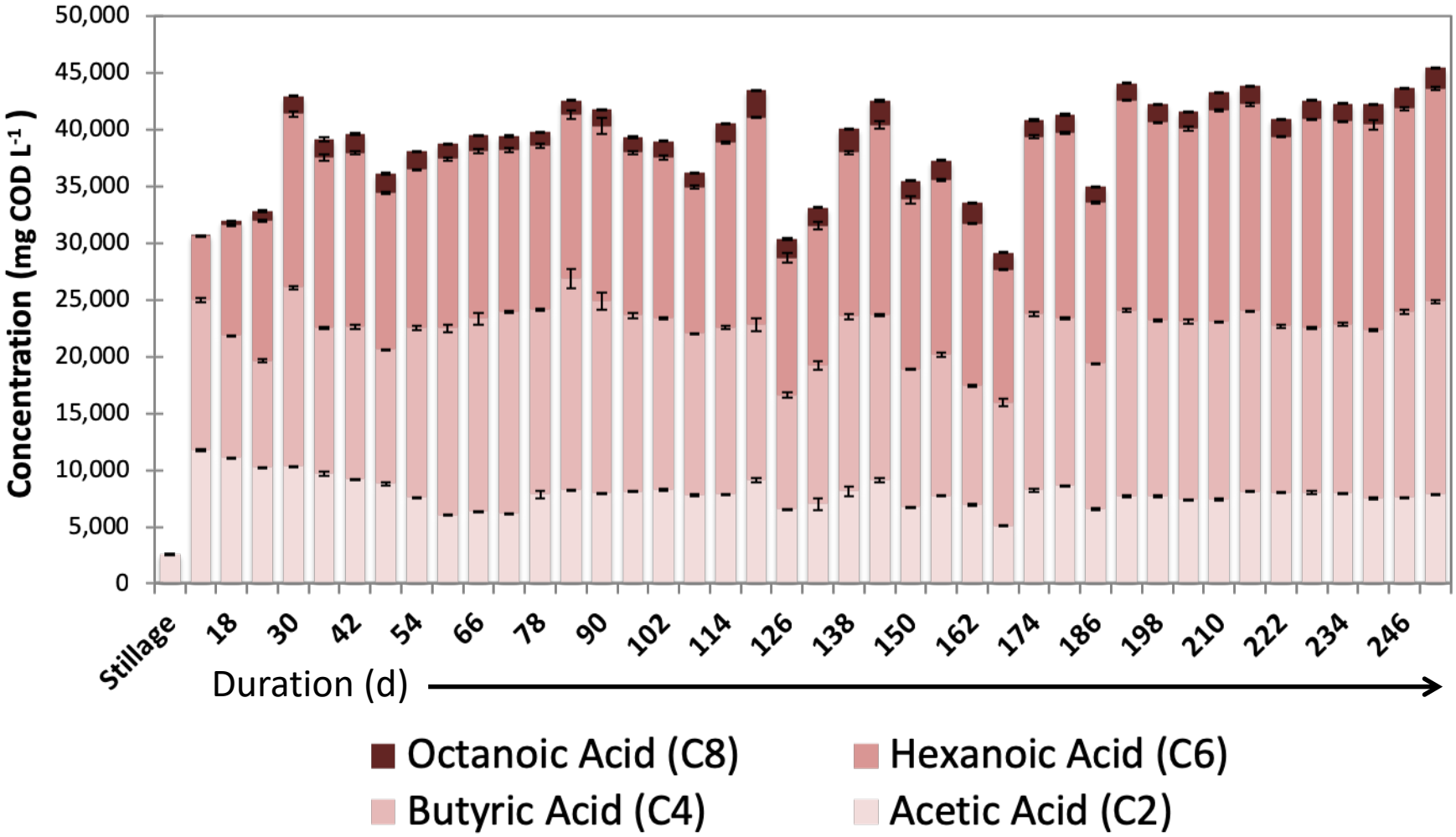
- Seed: Acid Digester Sludge
- Feed: Switchgrass Stillage
- SRT: 6d
- Temp: 35 deg C
- pH: 5.5
- VS: ~ 10 g/L
- Vented to atmosphere

# Xylose and uncharacterized carbohydrates transformed




*Adapted from Scarborough, M, et. al. 2018. Biotech for Biofuels.*

# A mixture of carboxylates produced

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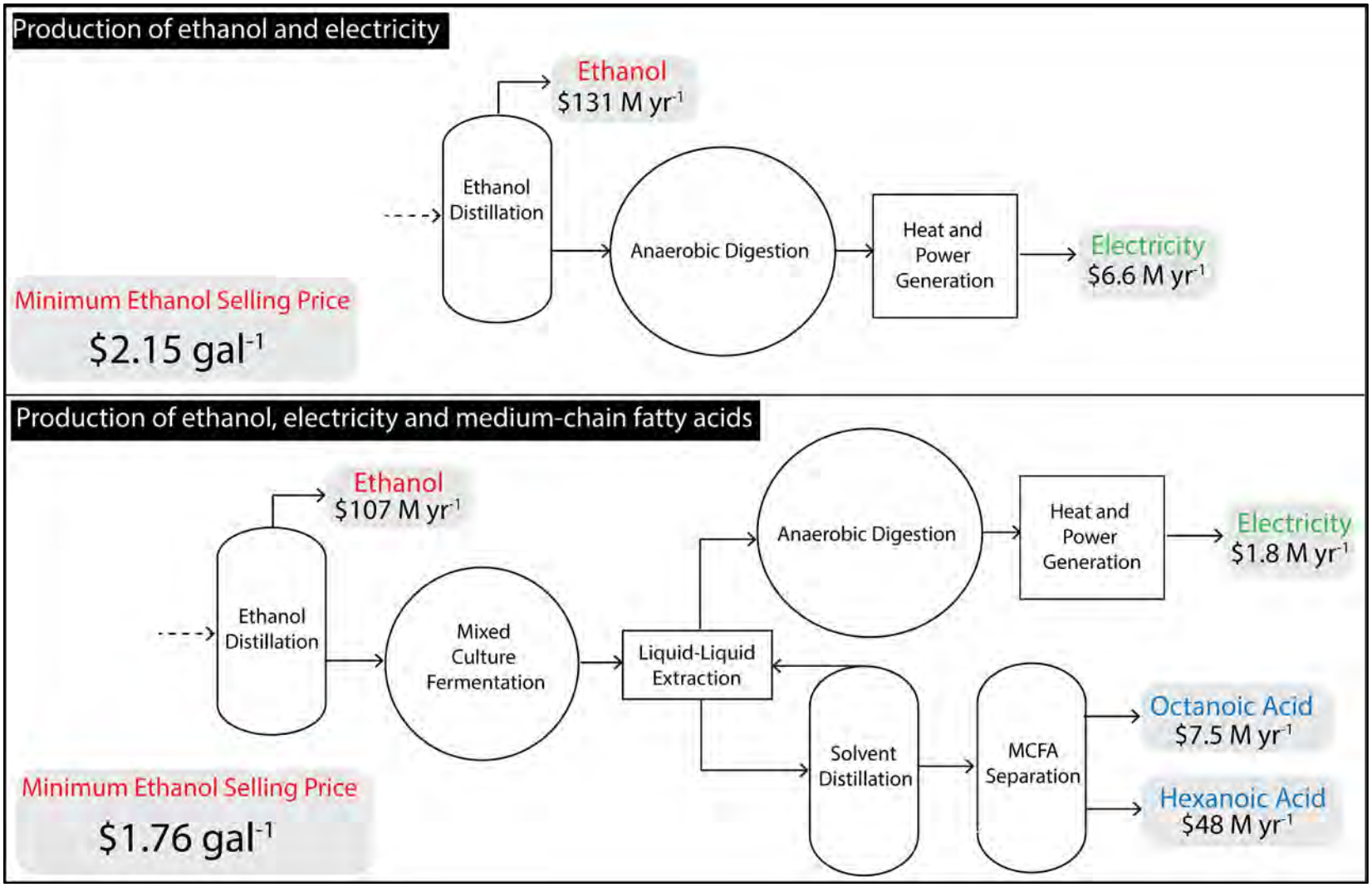
# The economics are promising

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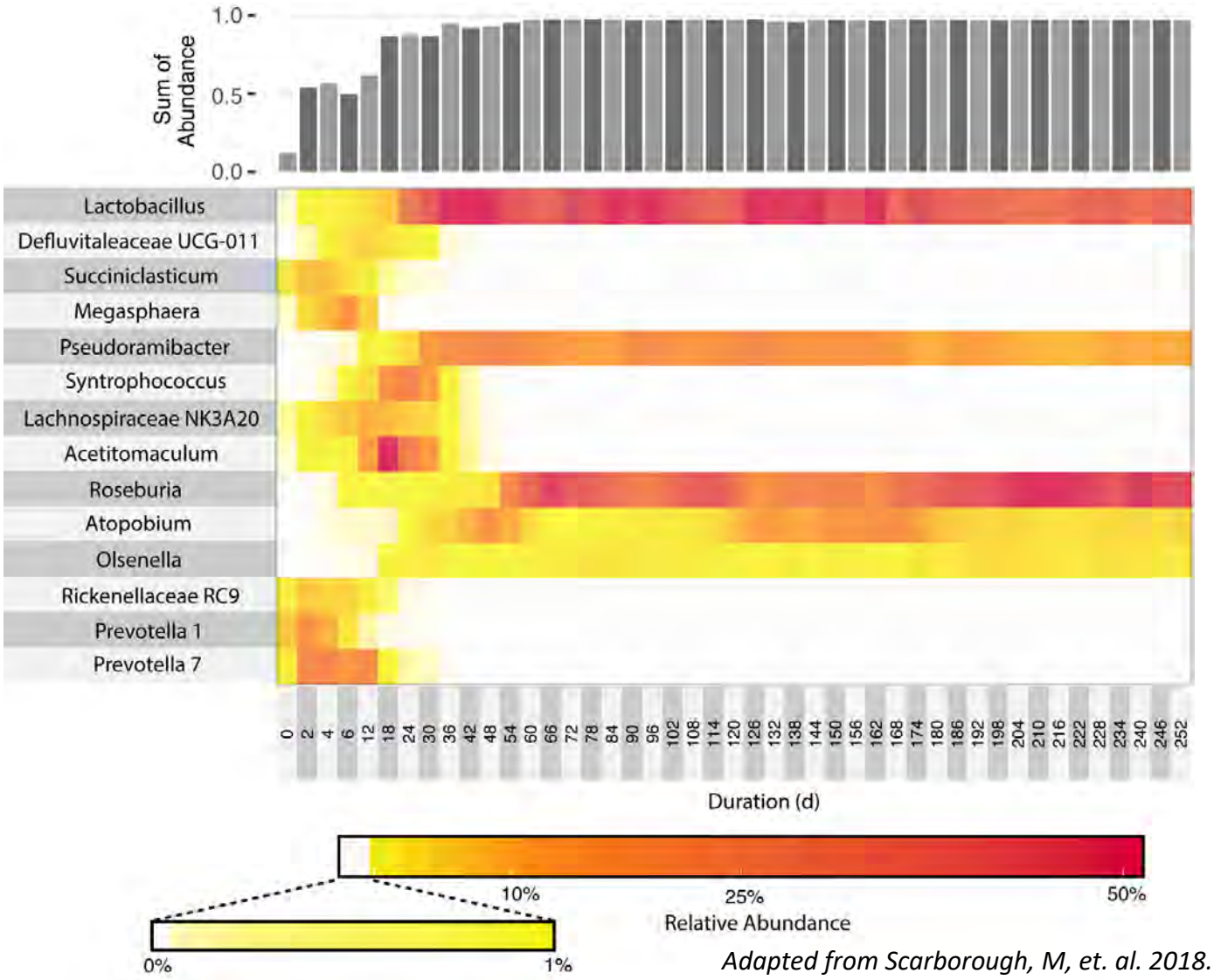
# A stable microbiome emerged

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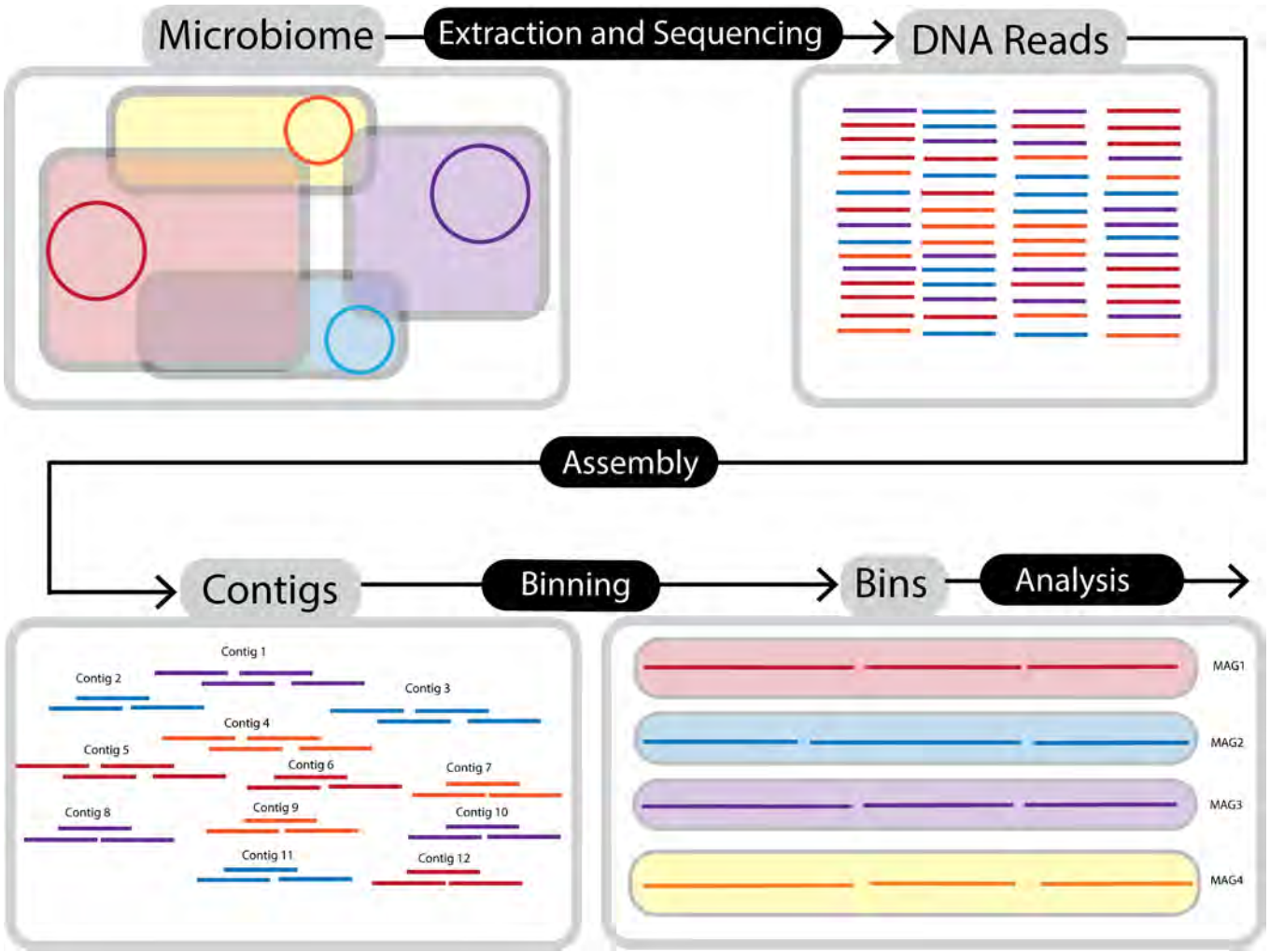
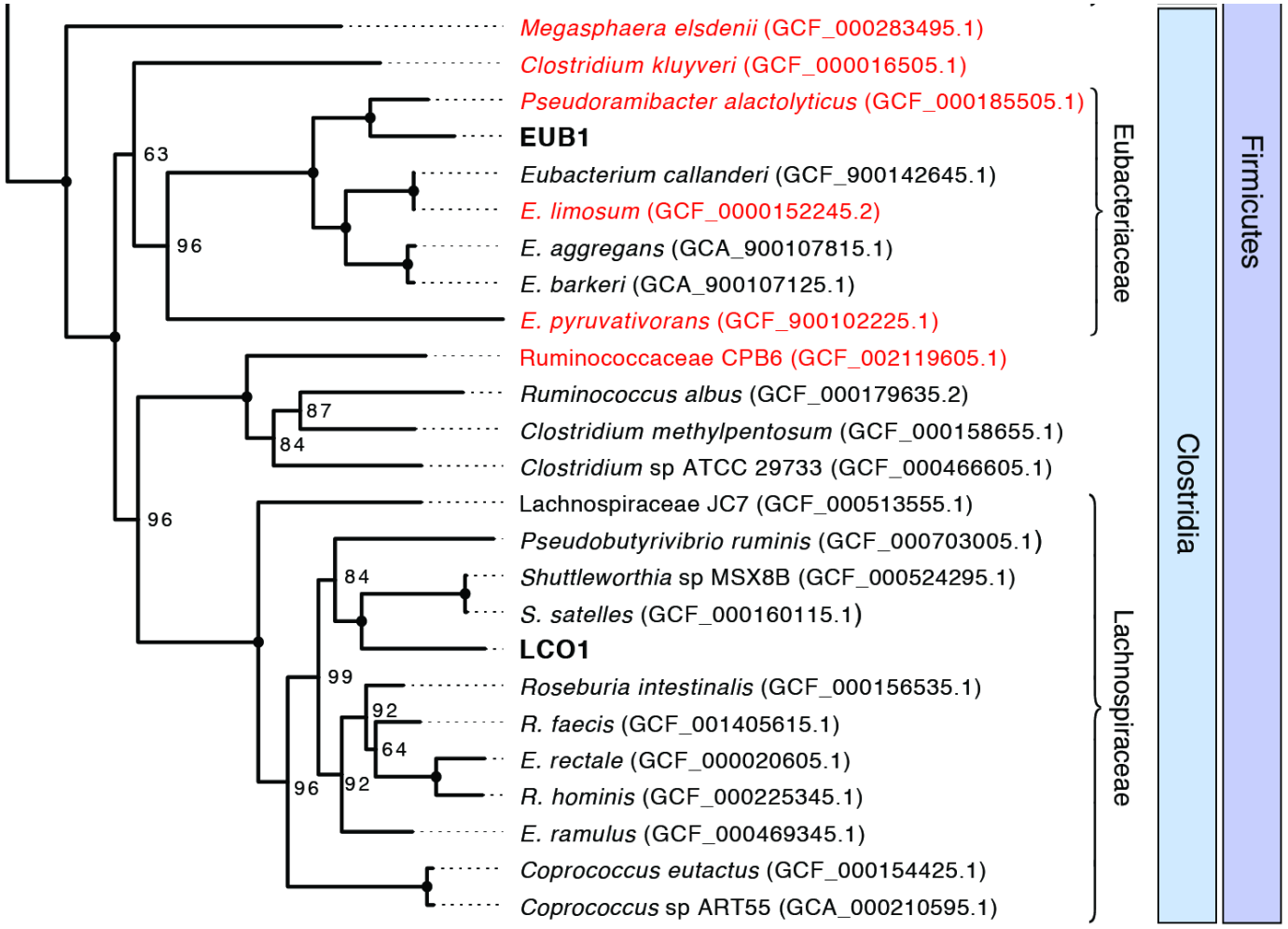
# Uncovering the metagenomic potential



# Phylogenetic analysis revealed chain-elongating relatives

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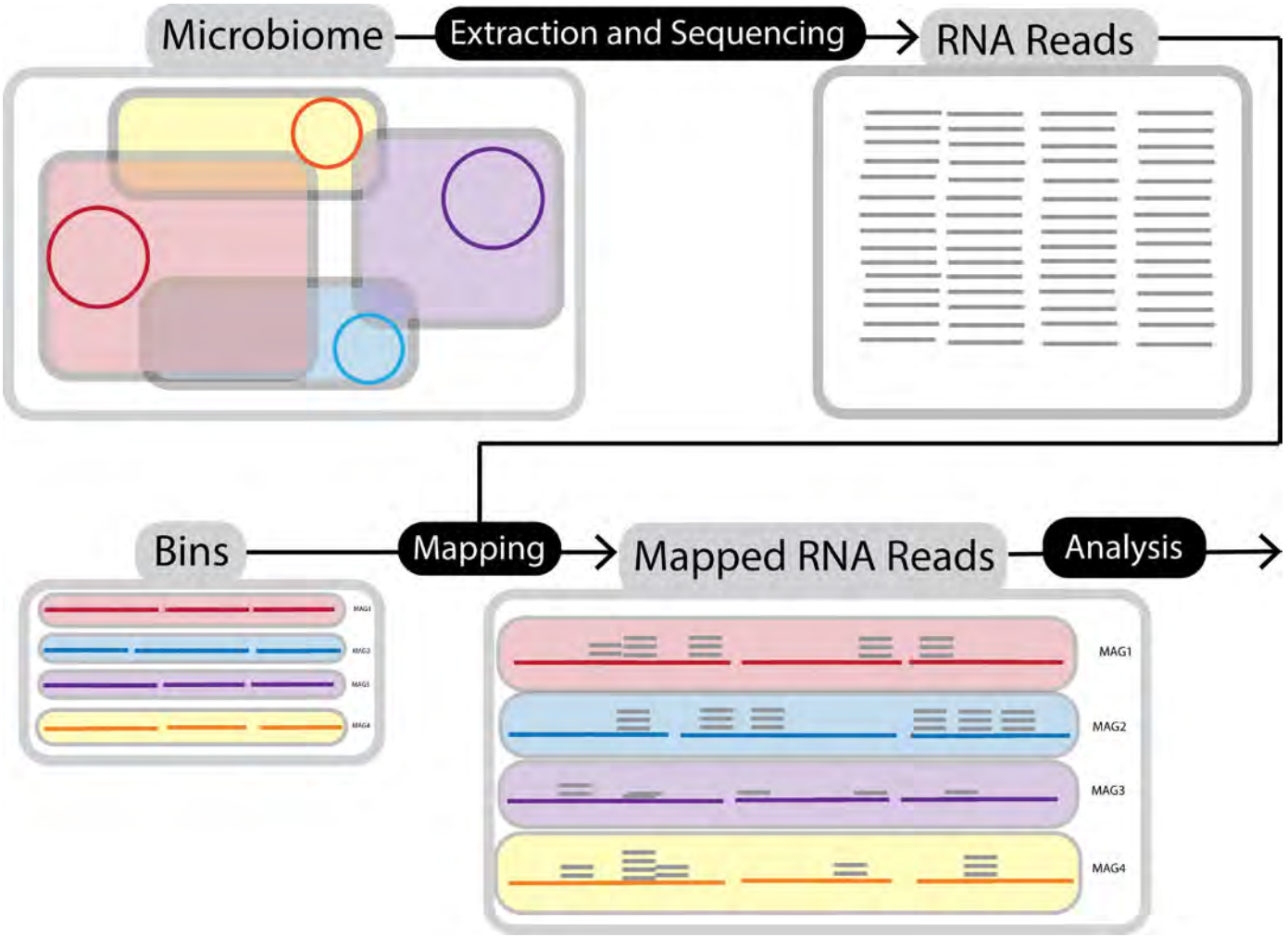
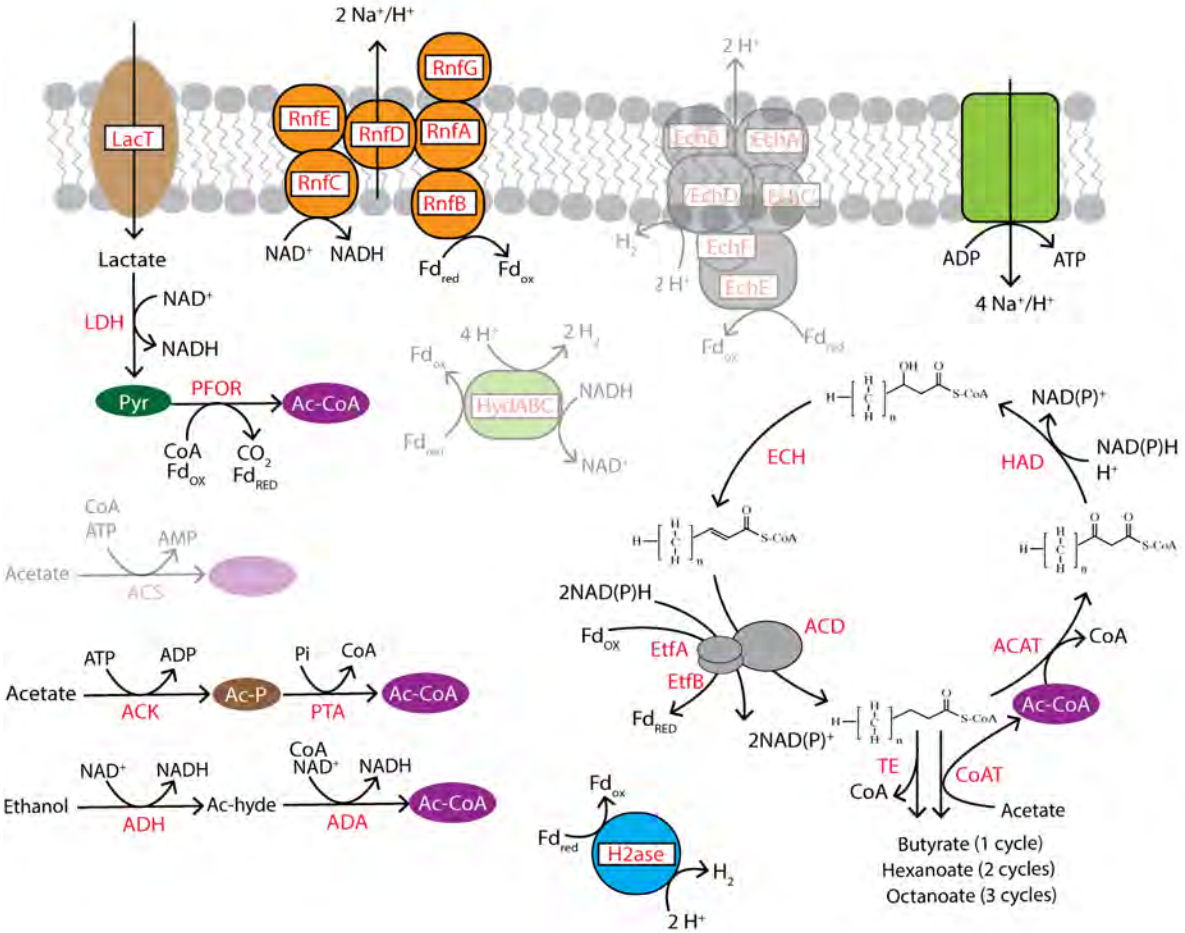
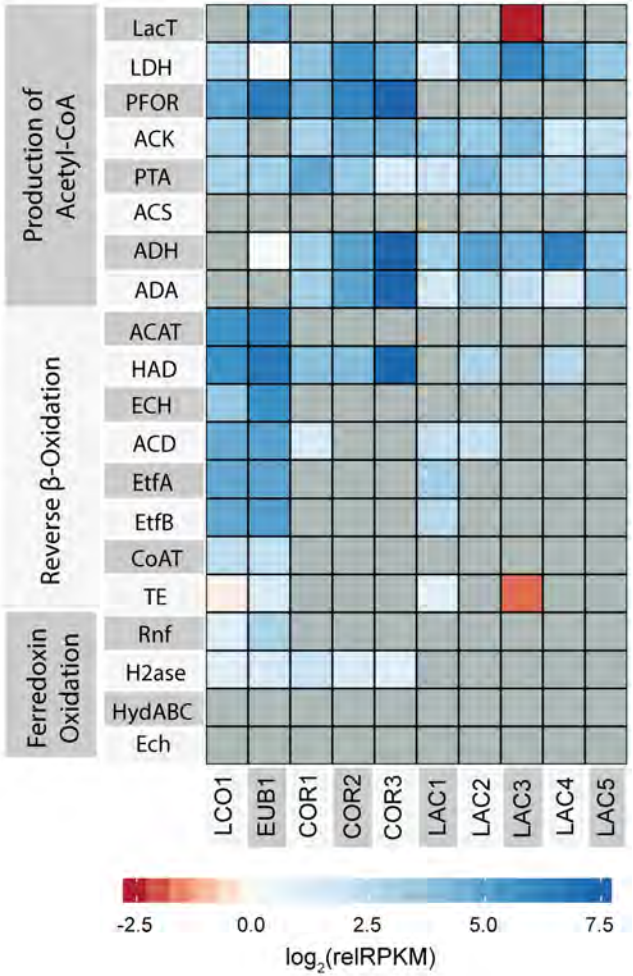
# Elucidating activity with metatranscriptomics



# Two populations of chain elongators emerged




*Scarborough, M, et. al. 2018. mSystems.*

# Thermodynamics predict lactate favors MCFA production

## LCO1: Xylose Elongation

| Reaction | $\Delta G^{0'}$ per mol Xylose | mol ATP per mol Xylose |
|---|---|---|
| 3 $C_5H_{10}O_5$ → 3 **$C_4$**$H_7O_2^-$ + 3 $CO_2$ + 3 $H_2O$ + 3 $H^+$ | -264 | 3.00 |
| 3 $C_5H_{10}O_5$ → 1 **$C_6$**$H_{11}O_2^-$ +3 **$C_2$**$H_3O_2^-$ + 3 $CO_2$ + 4 $H^+$ + 2 $H_2$ | -248 | 2.83 |
| 3 $C_5H_{10}O_5$ → 1 **$C_8$**$H_{15}O_2^-$ + 2 **$C_2$**$H_3O_2^-$ + 3 $CO_2$ + 3 $H_2O$ + 3 $H^+$ | -265 | 3.00 |

## EUB1: Lactate Elongation

| Reaction | $\Delta G^{0'}$ per mol Lactate | mol ATP per mol Lactate |
|---|---|---|
| 2 $C_3H_5O_3^-$ + 1 $H^+$ → 1 **$C_4$**$H_7O_2^-$ + 2 $CO_2$ + 2 $H_2$ | -26 | 0.25 |
| 3 $C_3H_5O_3^-$ + 2 $H^+$ → 1 **$C_6$**$H_{11}O_2^-$ + 3 $CO_2$ + 2 $H_2$ + 1 $H_2O$ | -34 | 0.50 |
| 4 $C_3H_5O_3^-$ + 3 $H^+$ → 1 **$C_8$**$H_{15}O_2^-$ + 4 $CO_2$ + 2 $H_2$ + 2 $H_2O$ | -39 | 0.63 |

**ATP = Intracellular energy currency**

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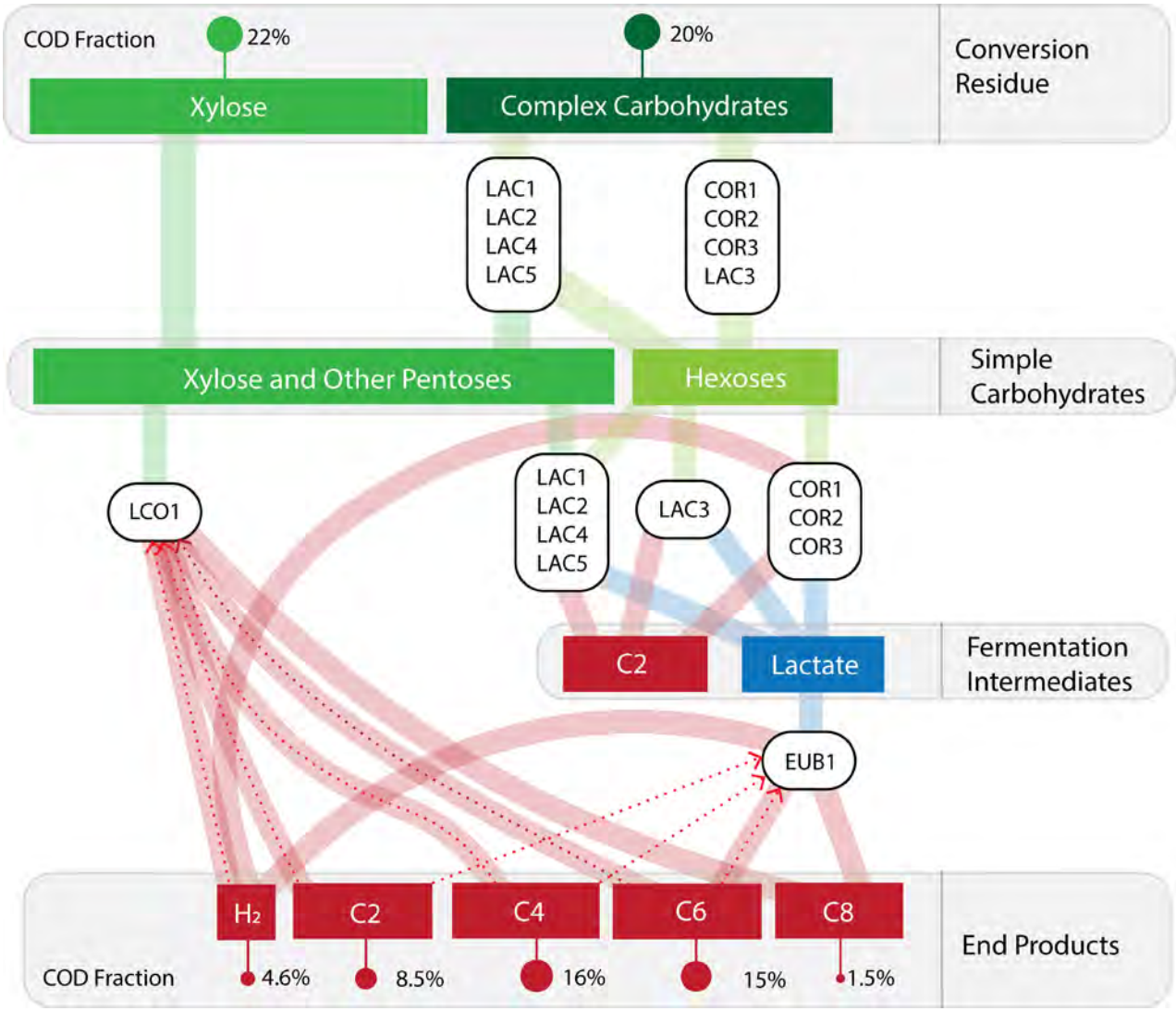
# Predicted roles within the microbiome

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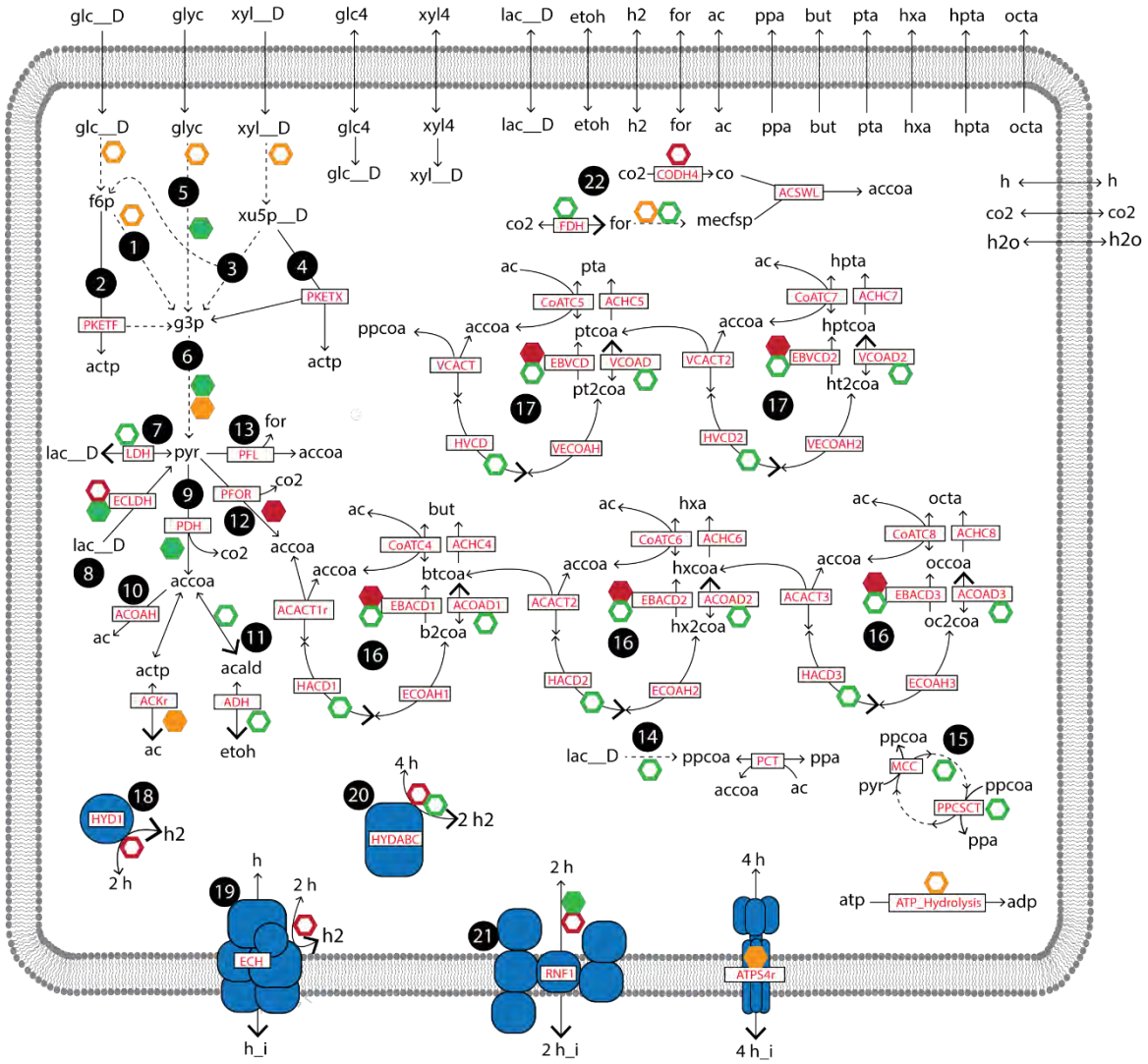
# Tying it all together with metabolic models




*Scarborough, M, et. al. 2020. Under Review*

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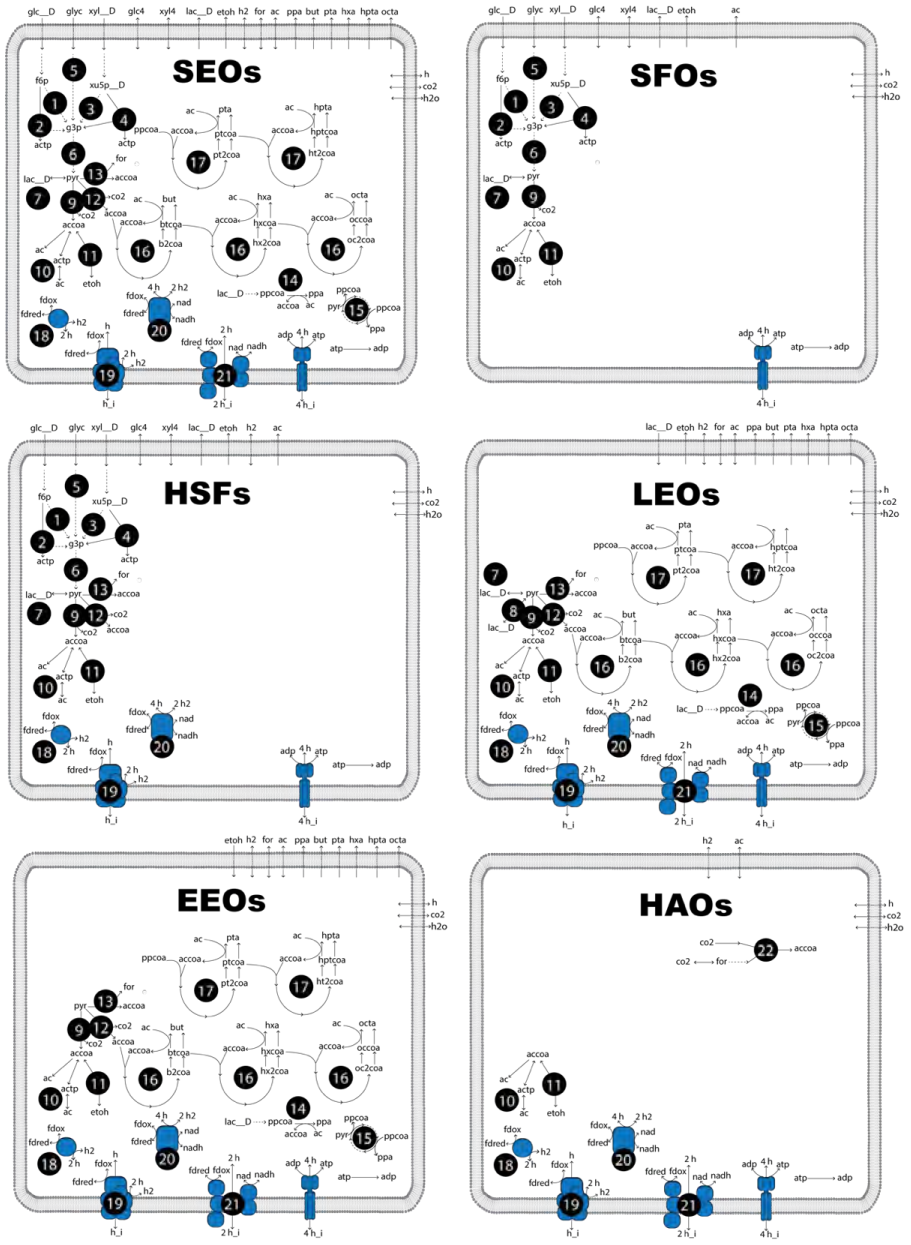


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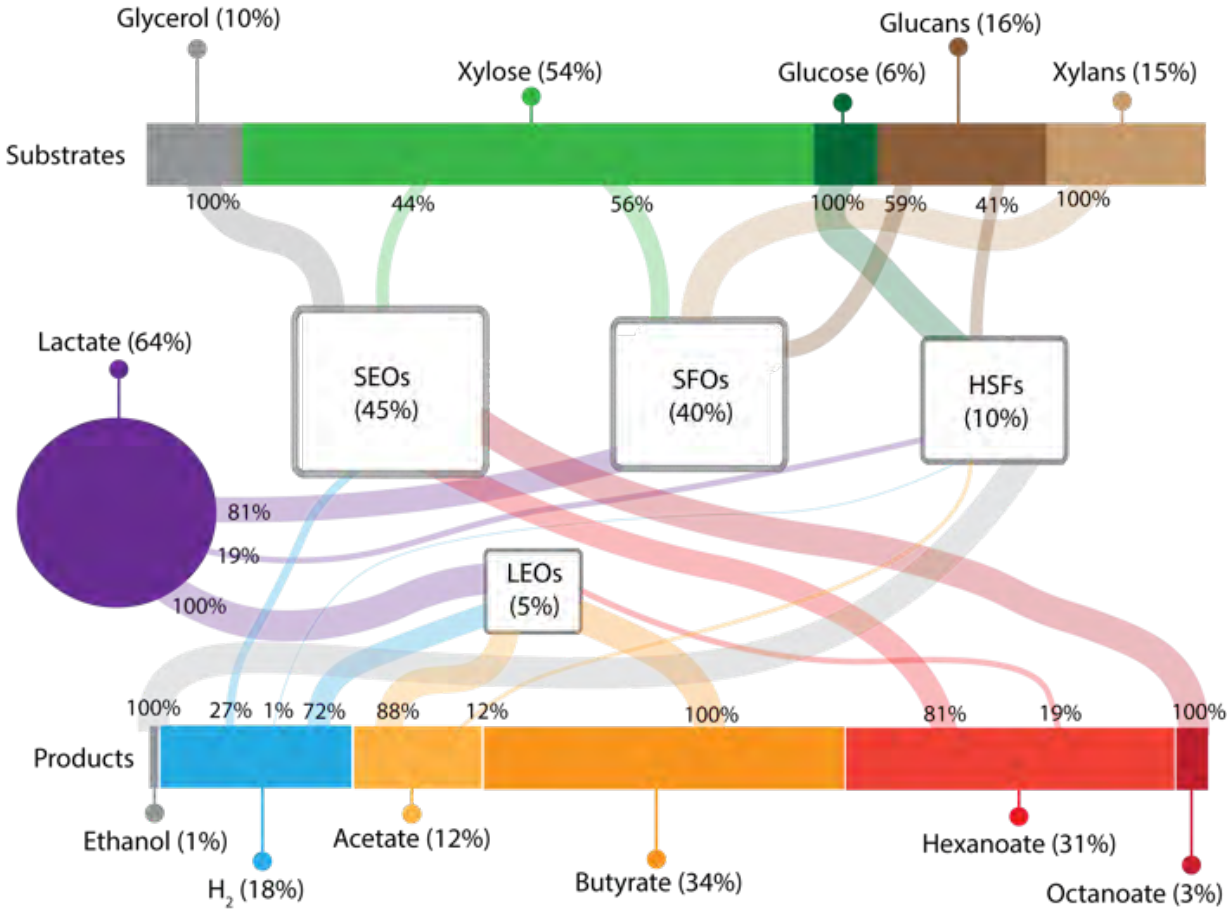
# Predicting bottlenecks in the reactor community

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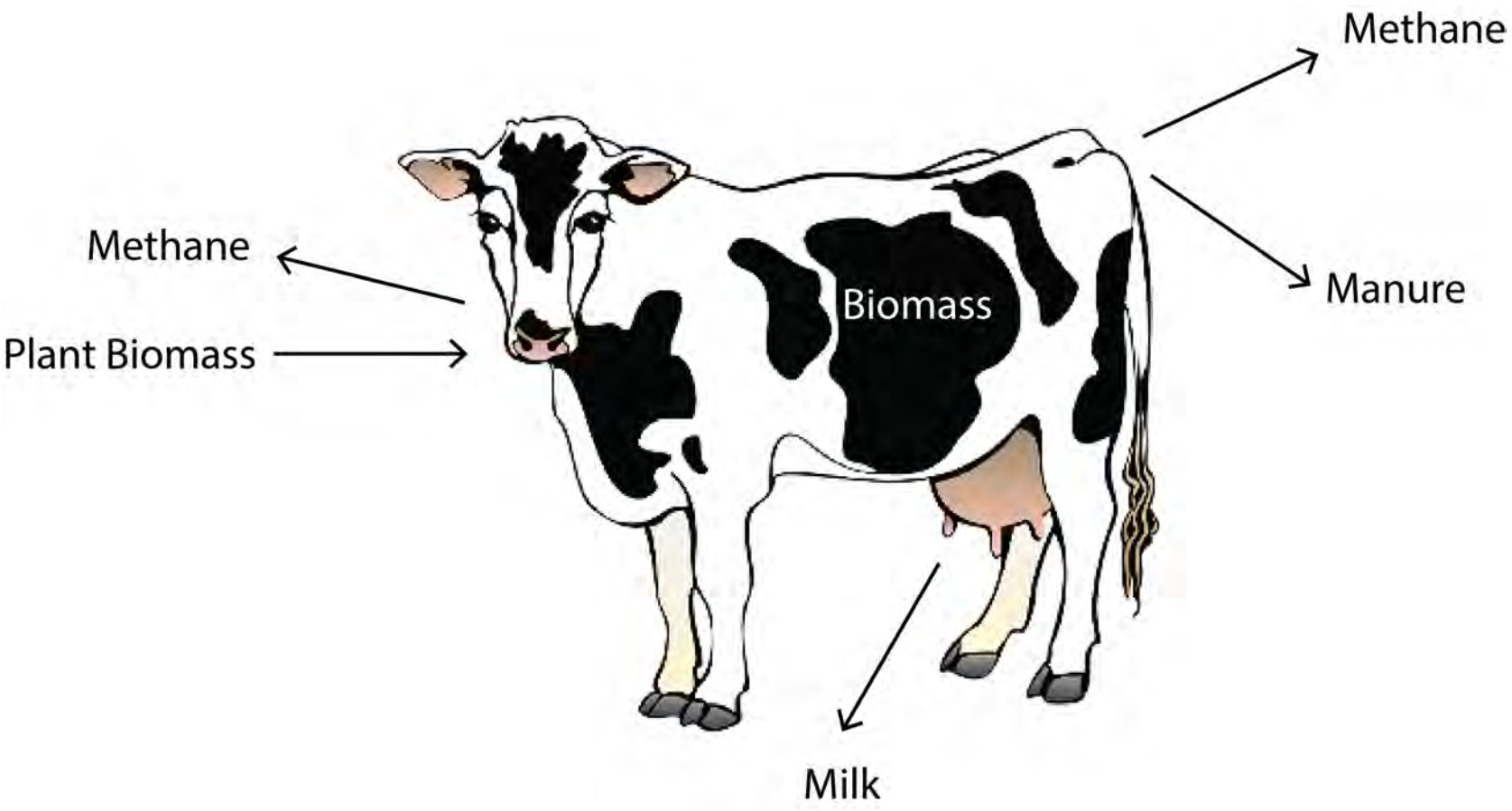
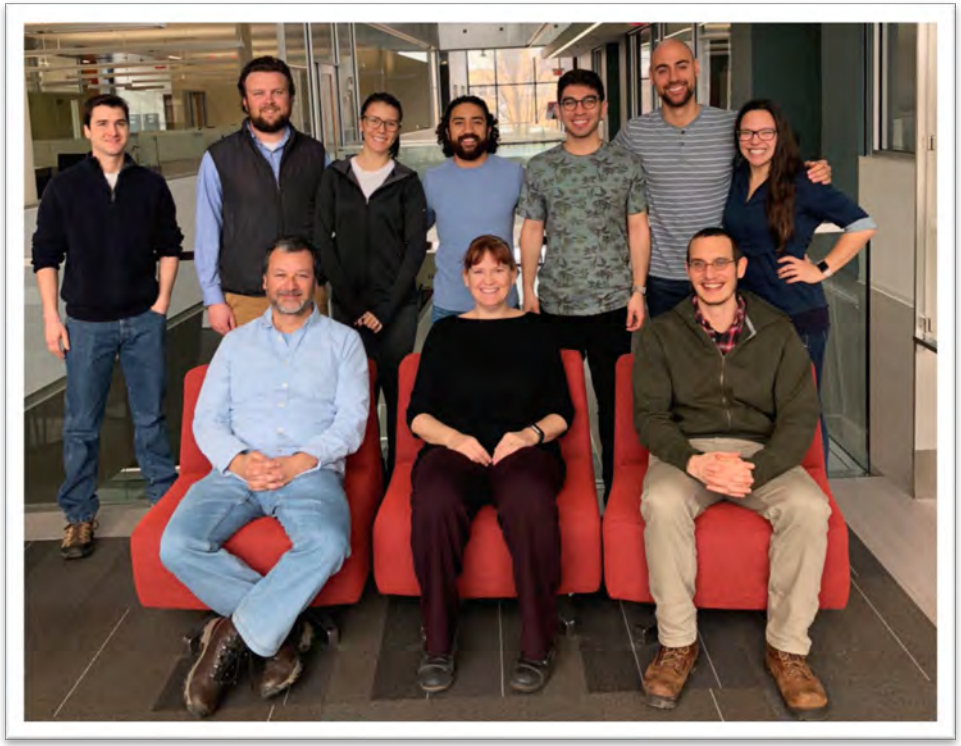
# Conclusions

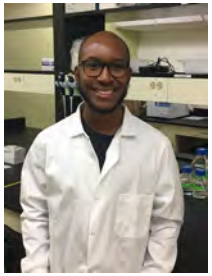
1. Novel anaerobic bioprocesses are emerging
2. MCFA production (aka, Carboxylate Platform, Chain Elongation) may be beneficial
3. Extraction of products is more difficult than biogas or biomethane
4. ”Chain Elongation” is performed by a specialized groups of microbes and supporting functional guilds
5. Metabolic models can help inform process design
6. Biogas and biomethane are still awesome!

# Future Work



# Acknowledgements



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# Thanks!

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# Metagenomics yielded 10 Draft Genomes

| Bin ID | Taxonomy | Completeness (%) | Contamination (%) | Genome size (bp) | # Scaffolds |
|---|---|---|---|---|---|
| **LCO1** | Lachnospiraceae; Shuttleworthia | 95.4 | 0.0 | 2,106,912 | 44 |
| **EUB1** | Eubacteriaceae; Pseudoramibacter | 97.8 | 0.2 | 2,002,609 | 35 |
| **COR1** | Coriobacteriaceae; Olsenella | 99.2 | 0.8 | 2,512,349 | 225 |
| **COR2** | Coriobacteriaceae; Olsenella | 100 | 1.6 | 2,422,853 | 155 |
| **COR3** | Coriobacteriaceae; Olsenella | 98.4 | 7.4 | 3,647,413 | 533 |
| **LAC1** | Lactobacillus | 99.5 | 1.1 | 2,633,889 | 18 |
| **LAC2** | Lactobacillus | 99.4 | 1.6 | 3,179,174 | 79 |
| **LAC3** | Lactobacillus | 99.2 | 1.4 | 2,704,063 | 174 |
| **LAC4** | Lactobacillus | 98.9 | 1.3 | 3,335,227 | 95 |
| **LAC5** | Lactobacillus | 80.1 | 0.8 | 1,487,044 | 181 |

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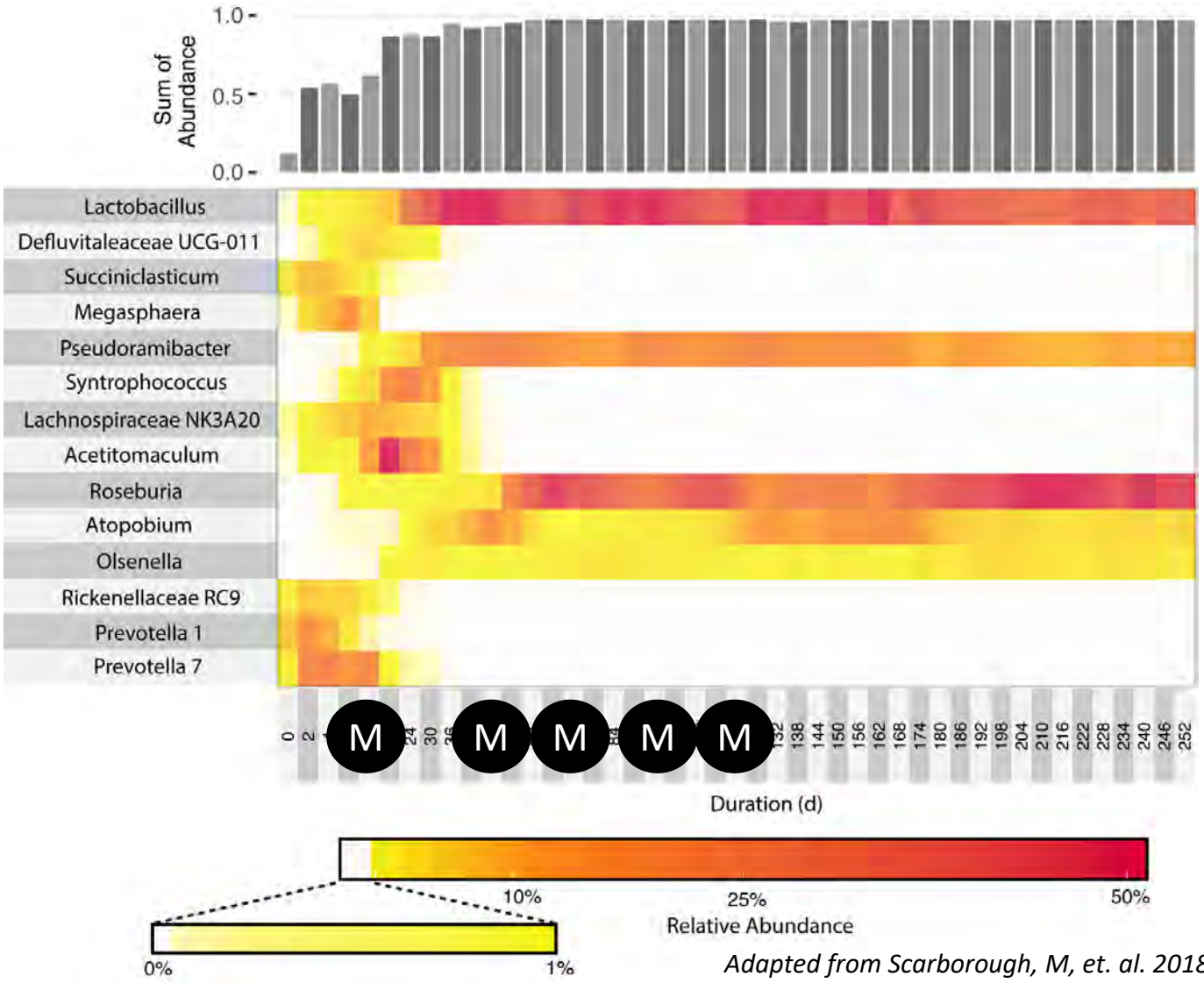
# A stable microbiome emerged




M

Metagenomics

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