Reactor Microbiomes for Bioenergy Generation

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Biomass to biochemicals: biorefinery platforms

Sugar platform
*(enzymes-yeast)*

Syngas platform
*(thermochemical)*

Syngas: H₂ + CO + CO₂

Carboxylate platform
*(open microbial communities = reactor microbiome)*

Acetate: CH₃COOH (C2)
Propionate: CH₃CH₂COOH (C3)
n-Butyrate: CH₃CH₂CH₂COOH (C4)
n-Caproate: CH₃CH₂CH₂CH₂CH₂COOH (C6)
Advantages of reactor microbiomes

1. Food web - microbial products removed
2. Metabolic flexibility - complex waste changes
3. Open cultures - no need to sterilize waste
4. Oxygen removal - dissolved O$_2$ not a problem
5. Microbial hydrolysis - no need to buy enzymes

Disadvantage

1. Limited end product spectrum

Plant material = lignocellulosic material
Carboxylate platform

Hydrolysis

Primary Fermentation

(a) NADH + H⁺ + H⁺ → H₂ + NAD⁺
(b) NADH + H⁺ → NAD⁺ + H⁺

H₂
CO₂

Acetate
Propionate
Lactate
n-Butyrate
Ethanol

Pyruvate

Secondary Fermentation

(c) H₂ + CO₂ → Acetate
(d) H₂ + CO₂ → CH₄

H₂ or Ethanol

Acetate
Propionate
n-Butyrate
n-Caproate

Ethanol
Propanol
n-Butanol
n-Hexanol

Shape the community

Agler, Angenent et al. (2011)
Trends in Biotechnology

Solid Polymers
Polysaccharides

Monomers & Oligomers
Sugars

Oxidation Products
Pyruvate

Intermediates
Short-chain carboxylates, Ethanol

Endproducts
Various
Reactor microbiomes for bioenergy

1. Are microbiomes chaotic or robust?
   --- Methane in anaerobic digesters ---

2. Can we generate soluble products?
   --- n-Caproate (with in-line extraction) ---
Methane

Hydrolysis

Primary Fermentation

Secondary Fermentation

Agler, Angenent et al. (2011) Trends in Biotechnology
Time series of nine full-scale granular upflow digesters treating brewery wastewater

Upflow anaerobic digesters

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

Angenent et al. (2004) Nature Immunology

Work performed by Dr. Jeff Werner (now at SUNY Cortland)
Sequencing and operating data

OTU Table  

Phylogeny

Operating data

Samples  →  Evolutionary Distance  ←  Time

Variables

9
Bacterial 16S rRNA genes (V1-V2) sequenced with bar-coded 454 pyrosequencing

- >420,000 non-chimeric reads, 112 samples
- Picked ~5,000 operational taxonomic units (OTUs) at 97% similarity
Sequencing and operating data

- Aligned OTUs to Greengenes core set with PyNAST
- Approximate maximum likelihood tree built with FastTree
- Also assigned consensus taxonomy to each OTU using naïve Bayesian classifier and the Greengenes database
Sequencing and operating data

- Daily measurements of SCOD, TCOD, F:M ratio, pH, total VFAs, temperature, Fe(II) supplements, gas production, methane content
- Concurrently with biological sample, measured methanogenic activity
Predictive OTUs (145) selected using machine learning
97% accuracy for predicting facility location with synthrophs

Relative abundance

Under/Over-representation

Community is not unpredictable and chaotic

1. *n*–caproate is a 6 carbon salt

2. At low pH environments in *n*-caproic or *n*-hexanoic acid

3. Uses
   - Pharmaceutical Industry
   - Animal Feed
   - Biofuels precursor (C11 after ketonization and dehydrogenation)

4. $2100$ per ton
*Clostridium kluyveri* metabolism (chain elongation pathway of \textit{n}-butyrate to \textit{n}-caproate = 2 carbons)

Need ethanol to push reducing equivalent
Corn Ethanol Industry (50 billion liter/year)

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

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

$n$-Caproate production and specificity is similar to methane production, but value is 20x higher

**Bioreactor:** 30 degrees C; pH 5.5; ethanol beer (15% etoh); pertraction = extraction
Shaping reactor microbiomes with enhanced function

![Graphs illustrating microbial community dynamics and diversity metrics](image)

- **Production rate (gCOD L⁻¹ d⁻¹)**
  - Acetate
  - n-Butyrate
  - n-Caproate
  - n-Caprylate
  - Propionate
  - n-Valerate
  - n-Heptanoate

- **Relative Abundance**
  - Ruminococcaceae (f)
  - Bacteria – OTU 1 (k)
  - Bacteria – OTU 2 (k)
  - Clostridium kluvyeri (s)
  - Sporoanaerobacter acetigenes (s)

- **Phylogenetic Diversity**
  - Day 64
  - Day 50
  - Day 78
  - Day 92
  - Day 106
  - Day 120

- **Gini Coefficient**
  - Day 0

**a-b. Bacterial Community Dynamics**

**c. Unweighted UniFrac Principal Coordinate**
How did we shape this microbiome

- Temperature: 30°C
- Continuous and selective extraction (pertraction) of \( n \)-caproate (max. solubility is 1%: much lower than \( n \)-butyrate)
- pH of 5.5
- Feeding ethanol as a reduced compound
Conclusions

1. Microbiomes are robust and predictable

2. Product extraction is important to pull food web to desired product
Thank you!

- Angenent Lab (Cornell University)
- USDA-NIFA
• Questions??