Microbial Ecology of Engineered Anaerobic Bioreactor Systems

Lutgarde Raskin
Dept. of Civil and Environmental Engineering
University of Michigan
“It is important to better understand microbial communities to improve the operation of anaerobic digesters and optimize biogas production…”

• We need to go beyond this statement…

• We need to truly link microbial ecology and environmental biotechnology in AD research: Perform hypothesis driven research to improve anaerobic digester design and operation, “Engineer” microbial communities to accomplish certain function

• Of course, this is not a new message…

• Two types of journal papers deliver this message:
  1. Concept and review papers: Microbial ecology and environmental biotechnology are closely linked disciplines
  2. Research papers: Use of ecological theory applied to microbial systems (not just environmental biotechnology)
1. Microbial ecology and environmental biotechnology are closely linked disciplines

- Theoretical ecology for engineering biology (Curtis et al., 2003, *ES&T*)
  “Are we standing on the threshold of a renaissance in designing biological systems?”

- A vista for microbial ecology and environmental biotechnology (Rittmann et al., 2006, *ES&T*)
  “Together, [microbial ecology and environmental biotechnology] offer much promise for helping society deal with some of its greatest challenges…”

- Microbial resource management (MRM): the road to go for environmental biotechnology (Verstraete et al., 2007, *Eng Life Sci*)
  “The purpose of MRM is to control and steer the capabilities associated with complex microbial communities. MRM has been developed to solve practical problems through the use of microorganisms” (Read et al., 2011, *Appl Microbiol Biotechnol*).
2. Use of ecological theory applied to microbial systems (not just environmental biotechnology systems)

Example papers:

- The role of ecological theory in microbial ecology (Prosser et al., 2007, *Nature Reviews Microbiology*)

- Resistance, resilience, and redundancy in microbial communities, (Allison and Martiny, 2008, *PNAS*)

- Initial community evenness favours functionality under selective stress (Wittebolle et al., 2009, *Nature*)

- Environmental conditions and community evenness determine the outcome of biological invasion (De Roy et al., 2013, *Nature Communications*)
Perform hypothesis driven research to improve anaerobic digester design and operation

- With rapidly changing molecular tools, it is sometimes difficult to focus on hypothesis driven research
Rapidly changing molecular methods

We have used for some time:
• “Conventional” molecular biology tools, most are targeting 16S ribosomal RNA (genes) and functional genes
  ✓ Hybridization methods, including FISH and microarrays
  ✓ Clone libraries and Sanger sequencing
  ✓ Fingerprinting methods (e.g., DGGE, T-RFLP)
  ✓ Quantitative PCR

We now have:
• High throughput (next generation) DNA sequencing methods
  ✓ 16S ribosomal RNA genes and functional genes
  ✓ Metagenomics and metatranscriptomics
• Metaproteomics, single cell genomics
Rapidly changing molecular methods

Microbial Community

Structural Assessment
- DNA Fingerprinting
  - DGGE
  - T-RFLP
- FISH
- Phylochips

Functional Assessment
- 16S rRNA gene Sequencing
  - Clone Library and Sanger Sequencing
  - Pyrosequencing
    - Illumina
    - Ion Torrent
- qPCR
- Metagenomics
- Metatranscriptomics
- RT-qPCR

Next Gen Sequencing
Pyrosequencing of 16S rRNA gene is growing exponentially

Publications of 16S rRNA gene pyrosequencing

Publications of pyrosequencing in AD
Bench-top sequencing is becoming accessible

454 GS Junior (pyrosequencing)  
Ion Torrent PGM  
MiSeq Personal Sequencer (Illumina)

---

<table>
<thead>
<tr>
<th>Platform</th>
<th>List price</th>
<th>Approximate cost per run</th>
<th>Minimum throughput (read length)</th>
<th>Run time</th>
<th>Cost/Mb</th>
<th>Mb/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>454 GS Junior</td>
<td>$108,000</td>
<td>$1,100</td>
<td>35 Mb (400 bases)</td>
<td>8 h</td>
<td>$31</td>
<td>4.4</td>
</tr>
<tr>
<td>Ion Torrent PGM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(314 chip)</td>
<td>$80,490a,b</td>
<td>$225c</td>
<td>10 Mb (100 bases)</td>
<td>3 h</td>
<td>$22.5</td>
<td>3.3</td>
</tr>
<tr>
<td>(316 chip)</td>
<td>$425</td>
<td></td>
<td>100 Mb (100 bases)</td>
<td>3 h</td>
<td>$4.25</td>
<td>33.3</td>
</tr>
<tr>
<td>(318 chip)</td>
<td>$625</td>
<td></td>
<td>1,000 Mb (100 bases)</td>
<td>3 h</td>
<td>$0.63</td>
<td>333.3</td>
</tr>
<tr>
<td>MiSeq</td>
<td>$125,000</td>
<td>$750</td>
<td>1,500 Mb (2 x 150 bases)</td>
<td>27 h</td>
<td>$0.5</td>
<td>55.5</td>
</tr>
</tbody>
</table>

Loman et al., 2012, *Nature Biotechnology*
Which sequencing depth is necessary to characterize a microbial community?

Number of different OTUs

Next generation sequencing

Sanger sequencing

Number of sequences

Smith, Shimada, Raskin, *unpublished*
Pyrosequencing comparison of three “mock communities” each consisting of 33 known bacterial sequences

Accuracy of predicted community structure does not always depend on sequencing depth

Which sequencing depth is necessary to characterize a microbial community? Don’t forget the question...

Smith, Shimada, Raskin, *unpublished*
Perform hypothesis driven research to improve anaerobic digester design and operation

• With **rapidly changing molecular tools**, it is sometimes difficult to focus on hypothesis driven research

• Nevertheless, many excellent “survey” studies are being published

• Example: Werner et al., 2011, *PNAS*
Monthly, year long time series of nine full-scale granular upflow digesters treating brewery wastewater

Jeff Werner

Lars Angenent

Werner et al., 2011, PNAS
β diversity analysis of pyrosequencing data: Each bioreactor has a unique and stable microbiome

Werner et al., 2011, PNAS
Constrained redundancy analysis (RDA): Microbial community structure can be explained by function (performance) and environment (operating conditions).

Werner et al., 2011, PNAS
Predictive OTUs (145) selected using machine learning:
1. Facility location can be predicted with 97% accuracy
2. High percentage of syntrophs in set of predictive OTUs

Werner et al., 2011, PNAS
Time series revealed stable communities that were resilient to disturbances.
Time series revealed stable communities that were resilient to disturbances

Werner et al., 2011, PNAS
Goal: promote functional stability in anaerobic digesters, i.e., stable conversion of diverse substrates, under variable loading rates, and under stress conditions

Hypothesis: feeding pattern influences microbial community structure and dynamics, which influences functional stability

Strategy: operate bioreactor systems with variable feeding pattern (same overall loading rates) to promote functional stability

Example 1

Perform “microbial ecology based” hypothesis driven research to improve anaerobic digester design and operation
Repeated pulse feeding induces functional stability in anaerobic digestion

- Two anaerobic digesters for treatment of domestic wastewater
- Retention time of 20 days, average loading rate of 1 g COD L$^{-1}$ day$^{-1}$
- CSTR$_{\text{stable}}$ (fed every day) and CSTR$_{\text{dynamic}}$ (fed every two days)

- Short-term stress test (high loading rate):
  - CSTR$_{\text{dynamic}}$ was more tolerant to high organic loading rates = greater functional stability
  - Limited microbial community analyses, although somewhat greater bacterial community dynamics observed in CSTR$_{\text{dynamic}}$

- Perform similar experiments and include detailed microbial community analysis

- Two old case studies from my group suggest benefits of this approach
  
  De Vrieze, Verstraete, Boon, 2013, *Microbial Biotechnology*
Two old case studies suggest that functional stability in anaerobic digestion can be accomplished through "microbial ecology based" strategies.
Case Study 1

Anaerobic Migrating Blanket Reactor (AMBR)

Design and operate system to maintain high levels of syntrophic bacteria and methanogens

Biogas

Quantification of Methanogenic Populations (%16S rRNA) in Different Compartments

**Day 135**

%16S rRNA

- **Archaea**
- **Methanosarcina spp.**
- **Methanosaeta concilii**
- **Methanobacteriaceae**
- **Methanomicrobiales**
- **Methanococcae**

**Compartments**

1 2 3 4 5
Biomass Staging
%16S rRNA in different AMBR compartments

<table>
<thead>
<tr>
<th></th>
<th>Syntrophomonadaceae +Syntrophobacter spp.</th>
<th>Archaea</th>
<th>Aceticlastic methanogens</th>
<th>Hydrogenotrophic methanogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMBR 1</td>
<td>3.1</td>
<td>15.7</td>
<td>8.0</td>
<td>4.9</td>
</tr>
<tr>
<td>AMBR 2</td>
<td>4.3</td>
<td>29.7</td>
<td>19.3</td>
<td>8.0</td>
</tr>
<tr>
<td>AMBR 3</td>
<td>3.3</td>
<td>34.4</td>
<td>25.7</td>
<td>9.9</td>
</tr>
<tr>
<td>AMBR 4</td>
<td>3.4</td>
<td>24.9</td>
<td>16.8</td>
<td>6.5</td>
</tr>
<tr>
<td>AMBR 5</td>
<td>3.2</td>
<td>21.3</td>
<td>11.5</td>
<td>5.9</td>
</tr>
<tr>
<td>CSTR</td>
<td>2.0 – 3.0</td>
<td>4.5 - 8.3</td>
<td>1.5 - 3.6</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Case study 2:
Co-digestion of Municipal Solid Waste (MSW) and Sewage Sludge

- Operated two digesters
  - Digester 1: History of unstable performance
  - Digester 2: History of stable performance

- Increased organic loading rate at end of experiment

History of unstable performance $\rightarrow$ better response to OLR increase

History of stable performance $\rightarrow$ worse response to OLR increase
History of unstable performance $\rightarrow$ better response to OLR increase

\[ \text{VFA (g/L)} \]

\[ \text{OLR increase} \]

\[ \text{acetate} \]

\[ \text{propionate} \]

\[ \text{butyrate} \]

\[ \alpha = 17 \text{ on day 92} \]

\[ \% 16S \text{ rRNA} \]

\[ \text{S. wolinii} \]

\[ \text{S. fumaroxidans} \]

\[ \text{S. pfennigii} \]

\[ \text{days from start up} \]

\[ \text{Smithella propionica} \]

\[ \text{Syntrophomonadaceae} \]

\[ D. \text{propionicus} \]
Case study 2: Co-digestion of Municipal Solid Waste (MSW) and Sewage Sludge

- Operated two digesters
  » Digester 1: History of unstable performance
  » Digester 2: History of stable performance

- Increased organic loading rate at end of experiment

→ Maintain high levels of syntrophs and methanogenic partners to improve stability during overload conditions
→ Operate system with variable loading rate to simulate “history of unstable performance”?

Two old case studies suggest that functional stability in anaerobic digestion can be accomplished through “microbial ecology based” strategies

• Design similar, but controlled experiments and include detailed microbial community and performance based analyses
Perform “microbial ecology based” hypothesis driven research to improve anaerobic digester design and operation

Example 2

• **Goal**: understand role of syntrophic acetate oxidizers in AD

• Willy Verstraete:  
  “Detour [through syntrophic acetate oxidation] is occurring whenever conditions are stressful”

• **Hypothesis**: ammonia stress induces shift in microbial community structure, resulting in an increase in syntrophic acetate oxidizers, which converged to a new stable microbial community structure better able to respond to future stress situation (Werner et al., unpublished).

• Related study from my group: Role of syntrophic acetate oxidizers in two phase digesters
Role of syntrophic acetate oxidizers in two-phase AD

Raw Sludge

\[ \text{Volatile Suspended Solids} \]

\[ \text{Fermenters} \]

\[ \text{Acid Phase Reactor} \]

Retention time = 1 – 3 days
pH = 5

\[ \text{H}_2\text{S}, \text{CO}_2 \]

\[ \text{VFA} \]

\[ \text{Syntrophs} \]

\[ \text{Methane Phase Reactors} \]

Retention time = 12+ days
pH = 7

\[ \text{CH}_4, \text{CO}_2 \]

\[ \text{Digested Solids} \]

\[ \text{Poster P0602} \ - \ Toshio Shimada, Adam Smith \]
Sampling locations of full-scale two-phase anaerobic digesters

2 Conventional mesophilic (CM)
6 Two-phase anaerobic digesters
  2 with thermophilic metanogenic phase (MP)
Hypothesis: syntrophic acetate oxidizers play an important role in two-phase digestion
Weighted Unifrac of pyrosequencing data set shows clustering of bacterial communities

**Thermophilic MP**

15.1%

**Mesophilic CM and MP**

43.4%

**Mesophilic AP**
Hydrogenotrophic methanogens predominate in all digesters

Relative Abundance of Methanogens

- unclassified Methanobacteriaceae
- unclassified Methanobacteriales
- Methanosphaera
- Methanobacterium
- Methanobrevibacter
- Methanobrevibacter
- Methanocorpusculum
- Methanospirillum
- Methanosaeta
- unclassified Methanomicrobiaceae
- unclassified Methanomicrobiales
- Methanomicrobacterium
- Methanothepobacter
Increased abundance of syntrophic bacteria in mesophilic methane phase relative to conventional digesters

Relative Abundance

1 sequence out of 54,000 bacterial sequences
Questions generated by pyrosequencing survey of two-phase digestion

• Are novel syntrophic acetate oxidizers involved?

• Does the AP seed the MP with hydrogenotrophic methanogens leading to their observed predominance?

• Are aceticlastic methanogens more active than hydrogenotrophic methanogens in the MP despite their low abundance?
Conclusions:
Perform hypothesis driven research to improve anaerobic digester design and operation

- With rapidly changing molecular tools, it is sometimes difficult to focus on hypothesis driven research

- “Survey” studies can provide excellent insights in AD assuming appropriate “ecological platforms” are used for analyses of microbial community structure and function

- Design controlled experiments to evaluate hypotheses that may lead to improved functional stability in anaerobic digesters
  - Variable feeding patterns
  - Syntrophic acetate oxidizers
Special thanks to current and former “anaerobic” graduate students and postdocs

Dandan Zheng
Matt Griffin
Katherine McMahon
Peter Stroot
Becky Daugherty
Sudini Padmasiri
Amily Zhang
Toshio Shimada
Tara Jackson
Tanna Borrell

Lars Angenent
Aurelio “Rocky” Briones
Krassimira Hristova
Adam Smith
Ameet Pinto
Xunchang Fei