Anaerobic Digestion:

- Introduction and reactor configurations (powerpoint)
- Fundamentals (blackboard and screen)
- Fundamentals and microbiology (powerpoint)
- Safety (powerpoint and blackboard)
• Fundamentals of anaerobic digestion: anaerobic food web
### Syntrophy: bacteria and methanogens (archaea)

<table>
<thead>
<tr>
<th>Reaction Type</th>
<th>Reaction Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntrophic propionic acid oxidation</td>
<td>$\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightleftharpoons \text{CH}_3\text{COOH} + 3\text{H}_2 + \text{CO}_2$</td>
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<tr>
<td>Syntrophic butyric acid oxidation</td>
<td>$\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightleftharpoons 2\text{CH}_3\text{COOH} + 2\text{H}_2$</td>
</tr>
<tr>
<td>Syntrophic acetic acid oxidation</td>
<td>$\text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \rightleftharpoons 4\text{H}_2 + 2\text{CO}_2$</td>
</tr>
<tr>
<td>Hydrogenotrophic methanogenesis</td>
<td>$4\text{H}_2 + \text{CO}_2 \rightleftharpoons \text{CH}_4 + 2\text{H}_2\text{O}$</td>
</tr>
<tr>
<td>Acetoclastic methanogenesis</td>
<td>$\text{CH}_3\text{COOH} \rightleftharpoons \text{CH}_4 + \text{CO}_2$</td>
</tr>
</tbody>
</table>

Propionate oxidation by acetogenic bacteria: $\Delta G_0 = +76.1 \text{ KJ}$

Only when the partial pressure of hydrogen is lowered by hydrogenotrophic methanogens to $< 10^{-4} \text{ atm}$ will this reaction become negative enough under biological conditions to occur.

Terminology: inter-species hydrogen transfer
Temperature is very important for anaerobic digestion

Figure 2.5. Influence of temperature on the rate of anaerobic digestion in the mesophilic range. After Henzen and Harremoes (1983)
Questions Common to Microbial Ecology of Engineered Systems:

1. What organisms are present: who is there?
2. In what quantities: how many?
3. What are they doing?
4. How to optimize the system?
Detection of Microbes:

1. **Culture** - <0.1% of viable bacteria in environment can be cultured.

2. **Specific tests** (e.g., antibodies, fluorescence in-situ hybridization [FISH]) - first need to know what you are looking for.

What Gene Sequence to Use to Relate All Life?
Ribosomal RNA (rRNA)

1. rRNA is ubiquitous.

2. Sufficiently highly conserved to relate all life.

3. Has resisted “lateral transfer” - tracks the genetic line of descent. The molecule is complex and consists of several compounds.

4. Abundant in all active cells.
Making Sense of Sequences: Molecular Phylogeny (Evolutionary Relationship)

1. Align sequences so that “homologous” residues are juxtaposed.

2. Count the number of differences between pairs of sequences; this is some measure of “evolutionary distance” that separates the organisms.

3. Calculate the “tree”, the relatedness map, that most accurately represents all the differences (this is not a theory, but based on counting differences).
The 3 Domains of Life: Pace et al.
Problems with Molecular Approach

- Requires significant material (> a few hundred bacterial cells).

- Contaminants in reagents, enzymes, etc. is a problem. (smaller problem for organism-specific primers.)

- General primers may not work with some rDNAs (biast).

- Clone/Sequence/Phylogenetic analyses are cumbersome.

- Information on rRNA **phylo**type may not reflect **phen**otype.
Alpha diversity

Bacterial phyla in UASBs

A  BP U1 Bacteria
   640 Sequences

B  BP U4 Bacteria
   482 Sequences

C  BP SC Bacteria
   611 Sequences
Phylogentic tree of Proteobacteria

Phylogenic Tree of Proteobacteria

- Clostridium amitophilum DQ278862
- Clostridium xylanivorans AF116920
- BP_U1_A4e09 (5 total- 1 U1, 2 U4, 1 SC)
- BP_U1_C4b05 (7 total- 6 U1, 1 U, 1 SC)
- BP_U1_C2b04 (2 total- 2 U1)
- BP_U1_A2e02 (1 total- 1 U1)
- BP_U4_C2c12 (2 total- 2 U4)
- BP_U4_B4e09 (30 total- 18 U1, 12 U4)
- BP_U1_B2b04 (5 total- 4 U1, 1 U4)
- Pelotomaculum isopelletaceum AA232785
- boyfriend degrading clone J AB091325
- BP_U4_C1e09 (7 total- 5 U4, 2 SC)
- BP_U1_B4c08 (45 total- 22 U1, 4 U4, 19 SC)
- Thermophilic propionate-oxidizing clone AB091325
- Pelotomaculum thermopropionicum NC_009454
- Pelotomaculum chitkii X91170
- Desulfotomaculum kuznetsovii AA036903
- Desulfotomaculum salinorum AA918123
- Sporotomaculum hydroxybenzoicum Y14845
- Sporotomaculum syntrophicum AB076610
- BP_SC_A2d09 (4 total- 4 SC)
- BP_SC_C3a09 (4 total- 2 U1, 12 SC)
- BP_SC_C2d09 (3 total- 1 U1, 2 SC)
- Dehalococccoides spp. AJ431247
- BP_SC_C3a08 (11 total- 2 U1, 4 U4, 5 SC)
- landfill leachate clone AJ853626
- BP_U1_C4b07 (5 total- 5 U1)
- BP_U1_C1a11 (2 total- 2 U4)
- Thermannaeovoribacter acidaminovorans AF071414
- benzene-degrading consortium clone AF323763
- Syntrophus buswelli X85131
- BP_U4_A4e05 (23 total- 130 U1, 104 U4)
- BP_U4_B4d04 (203 total- 60 U1, 143 U4)
- sulfate-reducing and methanogen populations sulfate-fed reactors clone EU104847
- BP_U4_A1e10 (192 total- 166 U1, 26 U4)
- BP_U4_C3e02 (17 total- 3 U1, 4 U4, 10 SC)
- BP_SC_C4a05 (13 total- 5 U1, 4 U4, 4 SC)
- BP_SC_C2d02 (1 total- 1 SC)
- BP_SC_C2b12 (23 total- 23 SC)
- BP_SC_C3g07 (1 total- 1 SC)
- polychlorinated dioxin dechlorinating microsm AB183649
- BP_SC_B2c12 (5 total- 5 SC)
- BP_SC_B2c06 (1 total- 1 SC)
- BP_SC_B2d02 (1 total- 1 SC)
- BP_SC_A4d07 (75 total- 75 SC)
- BP_U1_B2h01 (23 total- 6 U1, 4 U4, 13 SC)
- Syntrophus acetitrophicus NC_011145
- Syntrophus aceticophilus CP000252
- BP_SC_B3c04 (1 total- 1 SC)
- BP_SC_C2c04 (1 total- 1 SC)
- BP_U4_B3g07 (1 total- 1 U4)
- BP_U4_A4f01 (2 total- 1 U1, 1 SC)
- Desulfonomas alkaliphilus DQ309326
- Desulfonomas alkaliphilus YY46987
- BP_U4_B3e02 (3 total- 3 U4)
- BP_SC_C2g03 (3 total- 3 SC)
- BP_SC_B4e06 (23 total- 6 U1, 4 U4, 13 SC)
- root: Methanosphaera concilii

M5914
Time series of nine full-scale granular upflow digesters treating brewery wastewater

Sequencing with next-gen (no cloning)

- Bacterial 16S rRNA genes with bar-coded primers
- >420,000 non-chimeric reads, 112 samples
- Picked ~5,000 operational taxonomic units (OTUs) at 97% similarity
Sequencing preparation and computational analyses

- DNA extraction of samples selected
- Run duplicated PCR of extracted products (25 cycles)
- Confirm some bands with agarose gel
- Cleaning with MagBind
- PicoGreen
- Confirm bands in agarose gel
- Pool equimolar ratios and submit for sequencing (MiSeq)
- Computational analysis in Red Cloud
Beta diversity measures **between sample diversity**

- **Beta diversity measures** the variation in species composition between different samples.

- **UniFrac** is a method used to measure beta diversity.

- The diagram illustrates the concept with different groups (R1, R2, R3, R4) and arrows indicating the direction of diversity comparison.
β diversity: comparing between microbiome samples

Each bioreactor has a unique and stable microbiome