Funding sources:
Cornell University
David R. Atkinson Center for a Sustainable Future

arpa-e
Changing What’s Possible
nyserda
USDA
the angenentlab
Cornell University
Biological and Environmental Engineering
Biomass to bioenergy (biorefinery)

Sugar platform
(enzymes-yeast)

Syngas platform
(thermochemical)

Carboxylic acid platform
(nondefined mixed cultures)

Syngas: $H_2 + CO$

Acetate: $CH_3COOH$
Propionate: $CH_3CH_2COOH$
Butyrate: $CH_3CH_2CH_2COOH$
Advantages of microbiomes

1. Food web - microbial products removed
2. Metabolic flexibility - complex waste changes
3. Open cultures - no need to sterilize waste
4. Oxygen removal - waste stream contains $O_2$
5. Resistance to virus attack

Disadvantage

1. Only fermentation end products

Plant material = lignocellulosic material
What do we produce with the carboxylic acid platform?
(with nondefined mixed cultures)

1. Methane
2. Electric power or hydrogen
3. Caproate

= organization of this talk
1. Methane production in anaerobic digesters with a microbiome

Anaerobic food web

- **POLYMERS**
  - proteins, polysaccharides, lipids

- **MONOMERS & OLIGOMERS**
  - amino acids, sugars, fatty acids

- **INTERMEDIATES**
  - propionate, butyrate, alcohols

- **Fermenters**
- **Acetogens**
  - 30%

- **ACETATE**
  - 70%

- **Carboxylic acids**

- **Hydrogen-utilizing Methanogens**

- **Acetate-utilizing Methanogens**

\[ H_2 + CO_2 \]

\[ CH_4 + CO_2 \]
Upflow anaerobic sludge blanket (UASB) concept for soluble wastewaters

Anheuser-Busch offsets 10% of all boiler fuel with methane from biogas (9 digester facilities) - Bocher et al. (2008) J. Ind. Microbiol. Biotechnol.
Swine waste: a farm-based anaerobic digester in Iowa, USA; Crawford Swine Farm: 3,000 head

A 3,000-head swine farm in Iowa can generate ~ 1,800 kWh per day if methane is converted into electricity with a conventional generator.
2. Current generation in a microbial fuel cell with a microbiome

Sustainable wastewater treatment: waste to electricity
Known biological electron-transfer mechanisms from bacteria to electrode (carbon)

**Soluble electron mediators transfer**

**Direct electron transfer**

**Nanowire?**


SEM view of *Shewanella* sp. (picture by Dr. Miriam Rosenbaum)
Direct electron transfer for *Shewanella oneidensis*

Our approach: genetically modify *E. coli* to combine versatile sugar oxidation with direct electron transfer pathways (in collaboration with Bob Kranz at WUSTL).

Q = quinone  
MQ = menaquinone  
CymA = tetraheme cytochrome c on the cytoplasmic membrane  
Omc(X) are outer membrane cytochromes  
Mtr(X) are periplasmic decaheme cytochromes
Microbial fuel cells (MFCs) will not power your car!!

Chew chew the gastrobot:
Autonomous robot – just has to be fed with grapes.

Hydrogen fuel cell stacks

The stack on a fuel cell is made of graphite plates with a thin layer of a teflon-like substance between each plate. It is seen here between end plates held together with blue rods. Photo: Kettering PR & Communications.

http://www.kettering.edu/visitors/storypics/FC-stack.jpg
Classic microbial fuel cell (MFC)

- **Anode**
- **Cathode**
- **Platinum**
- **Ion exchange membrane**
- **Wastewater**
- **~ 0.5 V**
- **4OH⁻**

**Diagram Details**:
- **microorganisms**
- **e⁻ transfer**
- **air**
MFC with biocathode (bacteria use electrode as electron donor)
MFC with ferricyanide as a nonsustainable electron mediator for research

![Diagram of MFC with ferricyanide as a nonsustainable electron mediator](image-url)
Conventional H-type MFC
H-type MFCs

Excellent tool for research, however an H-type MFC typically:

• Requires mechanical mixing
• Has a small electrode surface area

= not practical for wastewater treatment
Electrodes made of granular activated carbon (GAC) 

Max. volumetric power: 29 W/m$^3$
Internal resistance: 17 Ohms

He et al. (2006) ES&T
Brewery wastewater treatment with a 6-L MFC (beer MFC)

Jeff Fornero

Internal cathode tube
Scale up difficult: electrodes must be close. Modular systems?

\[ \sim 0.5 \text{ V} \quad \quad \sim 110 \text{V} \]

http://www.pall.com/images/pho_mgdmembrane2.gif
Hydrogen production through an electrochemically-assisted MFC: 8 moles of hydrogen gas out of 12 moles per mole of glucose

Biocatalysed electrolysis:

U = 0.6 V or less
Hydrogen with conventional anaerobic fermentation with mixed cultures: butyrate

A. $P_{H_2} < 60$ Pa:

\[
\text{glucose} \rightarrow 2 \text{ pyruvate} \rightarrow 2 \text{ acetate} \rightarrow 2 \text{ CO}_2 \\
2 \text{ NAD}^+ \rightarrow 2 \text{ NADH} \rightarrow 2 \text{ Fd}_{\text{ox}} \rightarrow 2 \text{ Fd}_{\text{red}} \\
2 \text{ H}_2 \rightarrow 2 \text{ H}^+ \\
\]

max 4 moles out of 12 moles

B. $P_{H_2} > 60$ Pa:

\[
\text{glucose} \rightarrow 2 \text{ pyruvate} \rightarrow 2 \text{ acetyl-CoA} \rightarrow \text{ butyrate} \rightarrow 2 \text{ CO}_2 \\
2 \text{ CoASH} \rightarrow 2 \text{ NADH} \rightarrow 2 \text{ Fd}_{\text{ox}} \rightarrow 2 \text{ Fd}_{\text{red}} \\
2 \text{ H}_2 \rightarrow 4 \text{ H}^+ \\
\]

max 2 moles

Angenent et al. (2004) *Trends in Biotechnology*
3. Caproate production with microbiomes

Butyrate in fermenters

- **POLYMERS**
  - proteins, polysaccharides, lipids

- **MONOMERS & OLIGOMERS**
  - amino acids, sugars, fatty acids

- **INTERMEDIATES**
  - propionate, butyrate, alcohols

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<th>H₂ + CO₂</th>
<th>ACETATE</th>
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Fermenters

**Carboxylic acids**

**Caproate**

**Ethanol**
Feedstock to oil with bacteria

Wasted biomass + undistilled ethanol  ->  Oil for biochemicals and biofuels

Corn kernel-to-ethanol fermentation beer (15% Etoh)  ->  Medium-chain carboxylic acid (MCCA) oil (>90% MCCAs)
Chain elongation with reactor microbiomes

Acetic acid (2 carbons)

\[ \text{H} - \text{C} - \text{C} - \text{OH} \]

\[ \text{H} - \text{O} \]

\[ \text{n-Butyric acid (4 carbons)} \]

\[ \text{H} - \text{C} - \text{C} - \text{C} - \text{OH} \]

\[ \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} \]

\[ \text{n-Caprylic acid (6 carbons)} \]

\[ \text{H} - \text{C} - \text{C} - \text{C} - \text{C} - \text{C} - \text{OH} \]

\[ \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} \]

Dilute ethanol (2 carbons)

\[ \text{H} - \text{C} - \text{C} - \text{C} - \text{C} - \text{OH} \]

\[ \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} \]

\[ \text{n-Caprylic acid (8 carbons)} \]

\[ \text{H} - \text{C} - \text{C} - \text{C} - \text{C} - \text{C} - \text{C} - \text{C} - \text{OH} \]

\[ \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} - \text{H} \]
Corn Ethanol Industry (50 billion L year\(^{-1}\))

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

Here, we fed corn kernel-to-ethanol fermentation beer (15% Etoh) to our bacteria to make oil – much easier to extract with lower energy tax

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

Corn-kernel-to-ethanol beer to MCCA oil
Caproate system

Biogas

Hollow Fiber Membranes

Filter

Reactor

Galvanostat

AEM

Anode

Cathode

Ref.

Gas

Extraction Solution

Fatty acid
Reactor setup
Syngas fermentation

60-liter two-stage syngas fermentation system

50 kg/h dry biomass input
slow pyrolysis unit
(together with Prof. Johannes Richter, H.; Martin, M. E.; Angenent, L. T., A two-stage continuous fermentation system for conversion of syngas into ethanol. Energies 2013, 6, (8), 3987-4000.)