RECOVERY AND UTILIZATION OF VOLATILE FATTY ACIDS FROM ORGANIC WASTE FOR BIODIESEL PRODUCTION AND MICROBIAL INACTIVATION

Shashwat Vajpeyi, Kartik Chandran*
Columbia University

FSM3
Hanoi, Vietnam, January 21st, 2015
Lack of adequate sanitation is a global challenge

Is it possible to link sanitation with higher value chain biofuels and commodity chemicals?

Often limited by access to reliable energy inputs and chemicals
Fecal sludge to biodiesel

• Biodiesel

• Lipids

• Lipids in feces
• Biodiesel process agnostic to ‘waste’ stream?
Our approach

Commodity chemicals, lipids, biodiesel

Channel through fermentation platform

Domestic and Food waste

Faecal sludge

Municipal solid waste

Animal by-product waste
Complex organic polymers
Sugars, amino acids
VFA
Acetic acid
Methane

Hydrolysis
Acidogenesis
Acetogenesis
Methanogenesis

Anaerobic Digestion

HRT > 10 d
Complex organic polymers

Sugars, amino acids

VFA

HRT ~ 2 d

Acetic acid

Anaerobic Fermentation

Hydrolysis

Acidogenesis

Acetogenesis

- Fermentation is more advantageous than just anaerobic digestion
- Fermentation can be incorporated into existing digestion processes
Overview of our process

Organic waste

Anaerobic fermentation to produce volatile fatty acids (VFA)

Convert VFA to lipids

Harvest and extract lipids

Convert lipids to biodiesel
Conversion of VFA to Lipids

- Different COD sources
  - VFA from food waste fermentation
  - Synthetic VFA
  - Glucose
- Different initial VFA concentrations

Lipid content of *C. albidus*

6:1:3 acetate, propionate, butyrate. 2 day HRT

- Different initial N concentrations
  - Excess N: COD:N = 5:1
  - Limiting N: COD:N = 25:1, 50:1, 125:1, 250:1
  - Stoichiometric COD:N supply = 33:1
Effect of feedstock composition

Process can handle variability in influent feedstock
Major fatty acids accumulated are palmitic (C16:0), oleic (C18:1), and linoleic acid (C18:2)
Similar to soybean oil and jatropha oil, which are used as feedstock for biodiesel production in the US and the EU
Genome of *C. albidus* sequenced.
3 million reads, 1 G bases, 30x genome coverage

Assembly of library reads into contiguous sequences (contigs).
Consensus length 25 MB, 915 contigs, N50 = 83 kB

Allows understanding of mechanisms and metabolic pathways for lipid accumulation.
Can be used to increase lipid accumulation even further
## Economic analysis

### Cost of biodiesel production

<table>
<thead>
<tr>
<th>Carbon source cost</th>
<th>$30/ton</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Much lower if sludge comes in pre-fermented, as in Kumasi, GH)</td>
<td></td>
</tr>
<tr>
<td>Lipid yield from <em>C. albidus</em> (kg lipid/ton VFA)</td>
<td>40.96</td>
</tr>
<tr>
<td>(lowest observed value during our studies)</td>
<td></td>
</tr>
<tr>
<td>Lipid cost ($/lb)</td>
<td>0.33</td>
</tr>
<tr>
<td>Gross cost ($/L biodiesel)</td>
<td>0.71</td>
</tr>
<tr>
<td>Gross cost ($/Kg biodiesel)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Not competing with biodiesel industry, rather making sanitation enterprise energy neutral or energy positive
Microbial inactivation using VFA

Ascaris sp.

High VFA conc. (60 g/L)
Low pH (4.75)

Salmonella sp.

High temperature (49°C)
Low pH (5.5)

E. coli

High temperature (55°C)
Reduction after 7 days

What is the impact of exposure to VFA under extant conditions of anaerobic fermentation?
Novel and flexible platform to convert a variety of organic ‘waste’ streams to biodiesel or other lipid based commodity chemicals.

Not reliant upon inherent lipid content - other organic classes can be converted to lipids.

- For biodiesel as the preferred end point, reliance upon agricultural outputs is reduced or eliminated.
- Links sanitation practice with energy and chemical recovery.
- Microbial inactivation needs to be further characterized.
DISCUSSION

Contact information
Kartik Chandran
Columbia University
E-mail: kc2288@columbia.edu
Phone: (212) 854 9027
www.columbia.edu/~kc2288/
Microbial inactivation using VFA

Effect on cell viability of *E. coli* (cfu/mL)

- Different VFA sources:
  - Synthetic: Acetate, Propionate, Butyrate
  - Mixed Acids from anaerobic fermenter

- Physical conditions from pilot plant:
  - pH 8.0, Temp 25°C

- Different concentrations:
  - 0, 30, 300, 1000, 3000 mg/L

- Incubation time:
  - 12 days; reflective of pilot plant in the field
EFFECT OF SYNTHETIC VFA

- Impact of added VFA not statistically different from endogenous controls
- Ongoing studies with food waste fermentate
Cultures become more efficient in carbon uptake and storage (as lipids) with increasing N-limitation.
PREFERENTIAL CONVERSION OF VFA TO LIPIDS

- C. albidus exhibited higher preference for acetic acid before other VFAs *i.e.* propionic and butyric acid.

<table>
<thead>
<tr>
<th>VFA</th>
<th>Specific uptake rate (mg COD/g biomass/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>47.91±4.24</td>
</tr>
<tr>
<td>Propionate</td>
<td>3.97±1.54</td>
</tr>
<tr>
<td>Butyrate</td>
<td>7.42±3.01</td>
</tr>
</tbody>
</table>

- Specific uptake rate for acetate was higher than other VFA
The steady state biomass concentration was 1.02 g/L and the intracellular lipid content increased to 29.88%.

At this operational HRT of 3 days, the cells were able to assimilate all the influent carbon source.

Slow growth rate resulted in increase in the saturated fatty acid content.
## Effect of Different Carbon Sources and Concentrations

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>NH$_3$-N (mg/L)</th>
<th>Biomass (g/L)</th>
<th>$\mu m$ (h$^{-1}$)</th>
<th>Lipid content</th>
<th>$Y_{L/\Delta COD}$ (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure VFA</td>
<td>260</td>
<td>1.13</td>
<td>0.043</td>
<td>27.8%</td>
<td>52</td>
</tr>
<tr>
<td>VFA from fermenter</td>
<td>260</td>
<td>0.96</td>
<td>0.021</td>
<td>14.9%</td>
<td>31</td>
</tr>
<tr>
<td>Glucose</td>
<td>1300</td>
<td>5.14</td>
<td>0.095</td>
<td>43.3%</td>
<td>110</td>
</tr>
</tbody>
</table>

### Notes:
- **Intracellular lipid concentration and the cell growth rate decreased in case of fermenter VFAs.**
- **VFA concentration 6500 mg-COD/L**
  - Optimal
  - Substrate inhibition
- **Glucose**
  - $500/ton$
- **VFA**
  - $30/ton$

*Fei et al. 2011*
METABOLIC EFFECT OF NITROGEN CONCENTRATION

Key step in nitrogen assimilation, active at non-limiting nitrogen concentration.

High N: Higher metabolic activity and higher growth rate.

Low N: Low metabolic activity and lower growth rate.

Citric Acid Cycle

Isocitrate converted to Acetyl-CoA

Excess Acetyl co-A assimilated and stored as lipids.

Isocitrate is transported outside the mitochondria.
VFA consumption by *E. coli*

**Acetate**
- Acetate Initial
- Acetate Final

**Propionate**
- Propionate Initial
- Propionate Final

**Butyrate**
- Butyrate Initial
- Butyrate Final
At pH 8.0, >99.9% VFA exist as their conjugate base

**Water Chemistry**

<table>
<thead>
<tr>
<th>Acid</th>
<th>pKa</th>
<th>[A-]/[HA]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>4.75</td>
<td>1.78E+03</td>
</tr>
<tr>
<td>Propionate</td>
<td>4.88</td>
<td>1.32E+03</td>
</tr>
<tr>
<td>Butyrate</td>
<td>4.82</td>
<td>1.51E+03</td>
</tr>
</tbody>
</table>
EFFECT OF VFA FROM ANAEROBIC FERMENTATION OF FOOD WASTE

Impact of added VFA not statistically different from controls

Additional testing ongoing