Reverse Engineering
Anaerobic Digestion

Conversion- Waste-to-Energy
March 2019

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National Renewable Energy Laboratory
Goal Statement

- **GOAL** - Improve rate/extent of lignocellulosic wet waste conversion during AD
  - Faster conversion/higher VFA yields from wet waste feedstocks
  - Low pH, high temp to facilitate reduced methane and VFA extraction for separations task

- **OUTCOME** - Faster, more complete anaerobic digestion of wet waste, increased product (VFA) titers, reduced waste volume, decreased digester footprint
  - Greater extent of lignocellulose conversion for higher yields
  - Faster conversion rate to reduce footprint/increase throughput
  - Increased feedstock range and utilization
  - Applicable to CH$_4$ and/or VFA AD operations

- **RELEVANCE**
  - VFAs inhibit cellulose hydrolysis - Rate limiting step
  - Overall AD efficiencies are low (~40-60%), cellulose content is high (25-60%) in many wet waste feedstocks, and cellulose conversion rates are often poor (~20-40%)
    - Impacts yield and D3 RIN credits
  - Achieving this at low pH will feed into VFA separations work
  - Applicable to existing infrastructure of commercial AD units
## Relevance

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Dry Tons/year(^1)</th>
<th>Approximate cellulose content</th>
<th>Estimated cellulose conversion by AD</th>
<th>Unconverted cellulose (MM dry tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sewage Sludge</td>
<td>14.82</td>
<td>20(^2)</td>
<td>30%</td>
<td>2.1</td>
</tr>
<tr>
<td>Manures</td>
<td>41.00</td>
<td>25(^3)</td>
<td>30%</td>
<td>7.2</td>
</tr>
<tr>
<td>Food Waste</td>
<td>15.30</td>
<td>15(^4)</td>
<td>30%</td>
<td>1.6</td>
</tr>
<tr>
<td>DDGS</td>
<td>44.00</td>
<td>16(^5)</td>
<td>30%</td>
<td>4.9</td>
</tr>
<tr>
<td>115.12 MM dry tons</td>
<td></td>
<td></td>
<td></td>
<td>15.8 MM dry tons</td>
</tr>
</tbody>
</table>

- ~14% of LC-containing wet waste feedstocks end up as unutilized cellulose
- ~7% would remain as hemicellulose
- Disproportionately low estimate of yield since much of the remaining feedstock is non-convertible

1-DOE Wet and Gaseous Waste Report  
3-Singh et al. 1982. Agricultural Wastes 4:267-272  
4-Matsakas et al. 2014. Biotech. Biofuels. 7:4-12  
Quad Chart Overview

Timeline
- 15 November 2017
- 30 September 2020
- ~30% complete

<table>
<thead>
<tr>
<th>Total Costs Pre FY17**</th>
<th>FY 17 Costs</th>
<th>FY 18 Costs</th>
<th>Total Planned Funding (FY 18-Project End Date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOE Funded</td>
<td>N/A</td>
<td>$500K</td>
<td>$1.5M</td>
</tr>
<tr>
<td>Project Cost Share*</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Partners: (unfunded)
- JBS USA, MWRD (Denver Waste Water)
- Miller-Coors, New Belgium Brewing
- Leprino Foods, USDA-ARS (citrus)

Barriers addressed
Ct-I. Development of processes capable of processing high moisture feedstocks in addition to conventional Anaerobic Digestion
- convert higher fractions of waste
- ID organisms/consortia that produce organic acids
Ot-B. Cost of Production
- waste streams require greater conversion efficiency
- convert waste (an expense) into products
Ct-B. Efficient Preprocessing and Pretreatment
- optimize preprocessing w/ downstream processes

Objective
Increased conversion rate/extent of wet-waste lignocellulosic fractions to enhanced production of VFAs during non-methanogenic anaerobic digestion

End of Project Goal
VFA titers > 50 g/L during continuous anaerobic digestion at pH 5.0 or lower with >50% conversion of wet waste
Task Integration

2.3.2.107- READ (increasing hydrolysis and VFA titers)

2.3.2.107- Separations Task (increasing VFAs and in situ removal)

VFAs

Waste-to-Energy

CH₄

2.3.2.201- Biogas Valorization

2.1.0.111- WTE TEA

2.1.0.112- WTE Feedstock evaluation

2.1.0.104- WTE System Simulation

5.1.3.102- Biomethanation of Biogas

Biomethanation of Biogas
1 - Project Overview

• FY17 lab-call- improved conversion, decreased methane, increased VFAs, reutilization of CO₂ in anaerobic digestion
  – Leverage our experience in cellulase biochemistry and cellulolytic microbiology
• FY18, collaborations w/ local industries (JBS, MWRD, Miller-Coors, New Belgium)
  – Needs/interest, seed consortia, real-world feedstocks (JBS rumen/processing waste)
• Primary project goals include:
  – Increase rate/extent of hydrolysis w/ focus on lignocellulosic streams
    • Increased VFAs as measure of success
    • Diverse AD operations, including methanogenic
    • Expanded feedstock range/types
  – Coordination w/ Separations task
    • Decrease CH₄ with concurrent increase in VFA
    • Conditions that enhance in situ VFA extraction
2 – Approach (Management)

- Target milestones developed in conjunction with BETO under an AOP
  - Quarterly progress, annual, go/no go milestones
- Iterative interaction with WTE TEA task
- Held on-site mini-workshop with industry, academia, and DOE-BETO to gather feedback on needs and build collaborations
- PI/Task Lead- Steve Decker
  - Experimental design, set up, data analysis, project management
- Experimental (wet work)- Todd Vinzant, Todd Shollenberger
  - Experimental design, day-to-day operations, sampling, set up, analyses
- Experimental (metagenomics)- Venkat Subramanian
  - DNA purification, processing, JGI data analysis

- Partners/Workshop
  - ID industry needs
  - promote collaborations
  - inform existing efforts

- 2.3.2.107- Separations
  - Increased hydrolysis
  - Increased VFAs
  - Arrested methanogenesis

- READ
  - Increased hydrolysis
  - Increased VFAs
  - Arrested methanogenesis

- Bottle Experiments
  - short-term studies
  - expanded variable testing
  - guide larger scale testing

- Seed Digester
  - adapt feedstock-specific inoculum
  - provide baseline
  - serve as seed inoculum

- Metagenomics
  - inform underlying causes
  - indicate critical components

- 2.1.0.111- WTE TEA
2 – Approach (Technical)

• Intrinsic Challenges
  – AD is biologically/thermodynamically process-driven, *not well defined*
  – *Methanogenesis is the driver*, arresting it disrupts the system
  – Cellulose conversion by AD is inefficient and poorly investigated

• Technical Challenges
  – Enhancing persistent cellulose conversion to VFAs
  – Overcoming VFA inhibition to measure real VFA production
  – Measuring feedstock composition and conversion

• Integrated technical approaches
  – Adapt consortium to targeted feedstock
  – Augmentation-exogenous enzymes, hydrolytic microbes, feedstock pre-processing
  – Altered operational conditions to facilitate hydrolysis, arrest methanogenesis
  – End-point mimicry (*in situ* VFA removal)
  – Microbial tracking through metagenomic analysis

• There are several Key Critical Success Factors
## 2 – Approach (Technical)

### Critical Success Factors

<table>
<thead>
<tr>
<th>Target</th>
<th>Purpose</th>
<th>Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased hydrolysis</td>
<td>Improve yields, rates, titers</td>
<td>Consortium adaptation, feedstock preprocessing, enzyme and microbial augmentation</td>
</tr>
<tr>
<td>VFA sequestration</td>
<td>Remove product inhibition</td>
<td>S/L separation, L/L extraction, VFA adsorption</td>
</tr>
<tr>
<td>Arrested methanogenesis</td>
<td>Redirect C to VFAs</td>
<td>Low pH, thermophilic operation, VFA sequestration</td>
</tr>
<tr>
<td>Increased VFAs</td>
<td>Higher value product, enable separations</td>
<td>VFA sequestration, increase hydrolysis, arrest methanogenesis</td>
</tr>
<tr>
<td>TEA validation</td>
<td>Realistic research directions</td>
<td>Use TEA modeling to examine cost benefit for various approaches</td>
</tr>
<tr>
<td>Real world applicability</td>
<td>Ensure potential deployment</td>
<td>Industry input, technoeconomic analysis, test varied feedstocks</td>
</tr>
<tr>
<td>Baseline</td>
<td>Establish starting line</td>
<td>Adapted inoculum on rumen fiber, 37°C, methanogenic operation</td>
</tr>
<tr>
<td>Metagenomics</td>
<td>Inform on underlying causes, critical components</td>
<td>Consortia sampling and 16S RNA seq at JGI</td>
</tr>
<tr>
<td>Automated gas analysis</td>
<td>Expand variable numbers</td>
<td>BlueSens gas analysis - automated sampling - data collection</td>
</tr>
</tbody>
</table>

### 3-year project plan started November 2017
- FY18Q1 MS - Developed working relationships with local AD operators/wet waste generators and established feedstock choice
- FY18Q2 MS - Established digester operations and analyses for VFAs/metagenomics
- FY18Q3 MS - baseline productivities and product portfolios
- FY18 Annual MS - Metagenomic tracking of mixed inocula during adaptation to rumen fiber

### FY19
- Focus on increasing hydrolysis, including understanding extent of conversion and underlying causes of the enhanced conversion and VFA production
- Go/No-Go - Increased VFA production during anaerobic digestion
- VFA titers 20% higher than baseline using increased hydrolysis, adapted consortia, enzyme or microbial augmentation, VFA sequestration, and/or altered digester parameters
3 – Technical Accomplishments/Progress/Results

• Hypotheses-
  – Mixed inocula may be synergistic

• Background-
  – Rumen fiber + 3 seed consortia

• Results
  – New Belgium most effective (biogas)
  – MWRD was least effective, but steady
  – Mixed inocula tended to rise to the most effective subpopulation

• Outcome
  – Different rates due to different mechanisms of populations adapted to different feedstocks
  – No detrimental effects w/ mixing so synergy is possible

**Cumulative Gas Production by Mixed Inocula**

- Coors
- MWRD
- C-NB
- C-MW
- NB-MW
- C-NB-MW
• Hypothesis-
  – Adapting a range of inocula will generate a strong paunch-specific consortia

• Background-
  – Adapted a 5L CST fermenter
  – Rumen fiber feedstock/3 seed consortia

• Results
  – Stabilized system at ~8% solids, 37°C, pH 7.5 +/- 0.5, 4L working volume
  – ~60% CH₄, 35% CO₂, 50-60% conversion
  – Low VFAs after initial propionic acid
  – Reinoculation "blips" acetate due to transient shift in consortia

• Outcome
  – Hydrolyzers appear to have displaced rumen flora (↓propionic acid)
  – High conversion indicates good seed inocula for further experiments

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**Seed Digester**

**Fatty Acid Production in Fed-Batch Anaerobic Digester**

- Acetic acid
- Propionic Acid
- Butyric Acid
- Valeric Acid
- pH
- Re-Inoculated
- Cumulative Feed

![Graph showing fatty acid production over days with various lines representing different fatty acids and feedstock](image)
**3 – Technical Accomplishments/Progress/Results**

- **Hypothesis**
  - Adapted inoculum will be comprised of subpopulations from multiple seeds
  - Rumen fiber flora may persist in digester

- **Background**
  - 16S RNA seq to categorize populations
  - Rumen fiber and 3 seed consortia

- **Results**
  - Seed inocula had very different populations

- **Outcome**
  - Adapted digester population is different from any seed culture- no proteobacteria, increased firmicutes and synergistetes
  - Changing feedstock develops unique consortium
  - Very high hydrolyzer:methanogen ratio
  - Metagenomics provides insights into critical populations

**Metagenomic Analysis**

“What exactly do we have here?”

**Graphs**

- Relative Abundance bars for different organisms (Bacteroidetes, Firmicutes, Proteobacteria, WWE1) across different dates (MWRD, NB, Coors, JBS) for the period from 3-8 to 5-29.

- Date bar chart showing the relative abundance of different bacterial groups over time.
• Rumen fiber-adapted inocula derived from mixed seed and feedstock populations
• Track population shifts due to outside influences
• Identify potential detrimental shifts
• Track persistence of augmented strains
• Categorize critical or missing subpopulations

Demonstrates that feedstock selects consortia, even if it requires large shifts
Consortia dynamics are fluid and adaptable
Hypothesis-
- Biomass-active enzymes can increase hydrolysis and low pH will arrest CH₄

Background
- CTec2/HTec2/Laccase at pH 4.5
- Measured 7 VFAs at 9 timepoints

Results
- No biogas produced
- Cellulase (C) increases VFA production, Hemicellulase helps, some H/C synergy
- Laccase enhanced VFAs alone, w/ C+H
- Laccase continued increased VFAs perhaps due to exposing more cellulose

Outcome
- Test cellulase degraders
- Hydrolyzers appear inhibited by VFAs
- Develop TEA of enzyme addition
• **Hypothesis**-
  – Thermal pretreatment will increase enzymatic hydrolysis and enhance VFA production

• **Background**-
  – Pretreated rumen fiber 175°C for 15 min, NREL adapted seed inoculum, 37°C
  – Cellulase/hemicellulase 10 mg/g glucan
  – VFAs measured by HPLC

• **Results**
  – Enzyme augmentation increased VFA production
  – Pretreatment alone had minimal impact
  – Pretreatment + enzyme increased VFA production significantly

• **Outcome**
  – Combining pretreatment and enzymes increases conversion/VFA production
  – VFAs inhibit hydrolytic microbes before acidoacetogens

**Bottle Digestions-Enzyme Augmentation + Pretreatment**

The graph shows the total VFAs enhanced by combined pretreatment (Prt) and enzyme hydrolysis over time (D) from 0 to 43 days. The y-axis represents g/L of VFAs, and the x-axis represents time in days. The graph includes control, enzyme, pretreatment control, and pretreatment enzyme treatments.
3 – Technical Accomplishments/Progress/Results

- **Hypothesis**
  - Removal of VFAs increases total VFA production

- **Background**
  - Pretreated rumen fiber, NREL adapted seed inoculum, 37°C
  - VFAs were removed via S/L centrifugation

- **Results**
  - Removal of VFAs enhanced continued VFA production, 4X higher than control
  - VFA production ceased when left *in situ*
  - VFA production maxed out perhaps due to substrate depletion or consortia washout

- **Outcome**
  - VFA removal critical to relieve VFA inhibition
  - Investigate easier and more relevant means to remove VFAs

**VFA Removal Increases VFA Production**

- **Time (d)**
  - 0, 1, 3, 7, 15, 22, 29, 36, 43

- **g/L**

- **Bars**
  - **Prt-Control**
  - **VFAs removed d7, 15, 22, 29**
  - **VFAs removed d22**

**Bottle Digestions - VFA Removal (Support of Separations Task)**
4 – Relevance (programmatic)

• Improve rate/extent of lignocellulosic hydrolysis during AD of wet waste
• Most wet-waste contains lignocellulose
  – 25-35% sewage\(^1\), 40-60% MSW\(^1\), ~25% manure\(^2\)
  – Cellulose conversion is rate limiting step
  – 60-80% of the cellulose remains after AD - lost yield/ D3 RINs
• Enhanced hydrolysis applicable to varied wet waste operations
  – VFAs catalytically upgraded to biofuels
  – Applicable to methanogenic AD
  – Can utilize existing AD infrastructure
• Directly addresses 2017 Wet and Gaseous Waste report
  – Enzyme addition, consortia augmentation, feedstock pretreatment, understanding AD consortia dynamics

Leveraging Existing AD Infrastructure

<table>
<thead>
<tr>
<th>AD Units in US (from EPA/ABC)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AD-WWTP / total</td>
<td>1241/&gt;16,000</td>
</tr>
<tr>
<td>WWTP onsite biogas use</td>
<td>~860</td>
</tr>
<tr>
<td>WWTP biogas flared</td>
<td>~380</td>
</tr>
<tr>
<td>On-farm digesters</td>
<td>247</td>
</tr>
<tr>
<td>Stand alone digesters</td>
<td>38</td>
</tr>
<tr>
<td>Potential sites</td>
<td>~11,000</td>
</tr>
<tr>
<td>Existing digesters in Europe</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>

2016 U.S. Methane Emissions, By Source

- Coal Mining 8%
- Natural Gas and Petroleum Systems 31%
- Enteric Fermentation 26%
- Landfills 16%
- Other 9%
- Manure Management 10%
4 – Relevance (social, commercial)

- **Current AD operations**
  - high TRL, slow, inefficient
  - generate large volumes of waste
  - produce low value methane
  - inefficient cellulose conversion

- **Social/environmental drivers restrict WW disposal in landfills and methane production**
  - CA- zero food waste in landfills by 2025, 75% reduction in organics

- **Efficient conversion of cellulose to VFAs, not methane**
  - higher rates, greater extent of conversion, higher value products, smaller footprint, decreased capital
  - Higher cellulose conversion = increased D3 RIN credit

---

**Landfill use and potential application to MSW**

*Total MSW generation in the United States by type of waste, 2015*

- Total = 262 million tons
  - food waste: 15.1%
  - plastics: 13.1%
  - rubber, leather and textiles: 9.3%
  - wood: 6.2%
  - glass: 4.4%
  - paper: 25.9%
  - yard trimmings: 13.3%
  - other: 3.6%
5 – Future Work

• Increase cellulose hydrolysis rate and extent
  – Feedstock pre-processing: heat, mechanical, chemical
    • Hydrothermal autohydrolysis
    • Mechanical milling
  – Enzyme augmentation
    • Titer vs benefit for cellulase and laccase
    • Screen additional cellulases and laccases
  – Screen range of natural consortia sources for hydrolytic potential
    • High salt, high pH, low pH
    • Pursue subcontract for consortia supply
  – Digester operational parameters (temp/pH) to facilitate hydrolyzers/arrest methanogenesis
    • Increase thermophilic operation
    • Increase hydrolysis at low pH to facilitate VFA extraction
    • Evaluate adaptation to high pH hydrolysis for improved conversion
Future Work

- Increase VFA/decrease CH$_4$
  - Develop additional experimental VFA inhibition relief methods
    - Improve process now for separations system when it is deployed
    - Extraction, adsorption
  - Low pH/high temp digestion adaptation
  - Selective consortia screening for increased VFAs
  - Enhance syntrophy w/ CO$_2$-fixing microbes (C. ljungdahlii)

- Expand feedstock range/demonstrate broad utility or enhancements
  - Food waste
  - Working on agreements w/
    - USDA ARS (citrus waste)- acidic processing
    - Leprino Foods (dairy waste)- augmentation of digestion
  - Manure
    - High volume, moderate cellulose loading
  - MSW
    - Very large potential, high cellulose content
  - DDGS
    - Very large potential, moderate cellulose content
5 – Future Work

• Develop processing-relevant analytics
  – Feedstock and residuals composition to measure true extent
    • Not COD
  – Real-time VFA analysis- GC/HPLC/colorimetric
  – Automated biogas composition and quantity
    • Test variables for impact to biogas

• Develop more defined TEA analysis
  – Use improved analytics to generate TEA-targeted data
  – Tie actual data to models
  – Use results to prioritize focus
## 5 – Future Work

### Upcoming Milestones

<table>
<thead>
<tr>
<th>Increased VFA production</th>
<th>Using existing rumen fiber digestion as a baseline, demonstrate increased VFA titers of 20% or more through combined sequestration, increased hydrolysis, adapted consortia, enzyme or microbial augmentation, or altered digester parameters</th>
<th>6/30/2019 Go/No Go</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained enhanced conversion</td>
<td>Translate and maintain high digestibility/VFA production from 6/30/2019 Go/NoGo to additional feedstocks such as food waste, manure, MSW, or sewage.</td>
<td>9/30/2019 Annual</td>
</tr>
<tr>
<td>Increased VFA production</td>
<td>Demonstrate sustained (&gt;90 days continuous operation) VFA titers &gt;50 g/L using one or more of the following: consortia augmentation with acidogens and/or acetogens identified from QPM2, hydrothermal feedstock processing, higher solids loading, better natural consortium, thermophilic operation, decreased pH.</td>
<td>9/30/2020 Project End</td>
</tr>
</tbody>
</table>
Summary

– Overview
  • Anaerobic digestion holds potential for biofuels from existing/expanding waste streams
  • Limited by traditional use as waste treatment and efforts to increase methane
  • Hydrolysis of lignocellulosics is rate limiting and yield limiting step in most AD
  • Most WTE feedstocks have high lignocellulosic content

– Approach
  • Increased hydrolysis rate/extent increases productivity, efficiency, and decreases capital and footprint
  • VFA inhibition must be removed/sequestered to measure true production potential

– Technical Accomplishments/Progress/Results
  • Consortia adapted to specific feedstock
  • Augmented enzymes/microbes enhance conversion
  • Increased temperature or low pH shifts from biogas to VFA production

– Relevance
  • Directly addresses 2017 Wet Waste and Gaseous Feedstock Utilization report barriers
  • Increased social/environmental drivers are reducing biomass disposal options

– Future work
  • Further increase cellulose hydrolysis/VFA production (FY19Q2 MS)
  • Increase VFAs to feed into separations task (FY19 G/NG and EOP MS)
  • Expand range of feedstocks to demonstrate broad utility (FY19 annual MS)
Acknowledgements

• **Hydrolysis**
  – Todd Vinzant
  – Todd Shollenberger
  – Venkat Subramanian
  – Mike Himmel

• **Separations**
  – Eric Karp
  – Violeta Sanchez i Nogue
  – Patrick Saboe

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  – Justin Sluiter

• **Methane Upgrading**
  – Mike Guarnieri

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  – Mark Philbrick

• **JBS**
  – Mark Ritsema

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  – Mark Fischer

• **Denver MWRD**
  – Jim McQuarry
  – Quintin Schermerhorn

• **MillerCoors**
  – Larry Abernathy

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Publications, Patents, Presentations, Awards, and Commercialization

- February 2018- “Reverse Engineering Anaerobic Digestion” presented by Steve Decker at Swedish University of Agricultural Science, Uppsala, SE
- April 2018- Anaerobic Digestion mini-workshop at NREL, >35 attendees from industry, academia, national labs

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3 – Technical Accomplishments/Progress/Results

- **Hypothesis**
  - Addition of hydrolytic microbes can enhance conversion

- **Background**
  - *Clostridium thermocellum*- cellulase(+), 55°C
  - *C. ljungdahlii*- CO₂ fixation, 37°C
  - Seed inoculum 37°C

- **Results**
  - 55°C inhibited acetate → CH₄ leading to increased acetate
  - Increase in biogas w/ augmentation
  - @37°C, biogas leads to decreased acetate

- **Outcome**
  - Investigate thermophilic AD to inhibit methanogenesis and increase VFAs
  - Consortia biasing needs more investigation and TEA

### Bottle Digestions - Consortia Biasing

#### Clostridia Enhances Methanogenesis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time (h)</th>
<th>Gas (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed 37</td>
<td></td>
<td>1027</td>
</tr>
<tr>
<td>Seed+Ct 37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed+Cl 37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed+Ct+Cl 37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed+Ct 55</td>
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<tr>
<td>Seed+Cl 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed+Ct+Cl 55</td>
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</table>

#### ClostridiaEnhances Thermophilic VFA Production

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time (h)</th>
<th>Acetate (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed 37</td>
<td>46</td>
<td>1.0</td>
</tr>
<tr>
<td>Seed+Ct 37</td>
<td>214</td>
<td>3.0</td>
</tr>
<tr>
<td>Seed+Cl 37</td>
<td>330.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Seed+Ct+Cl 37</td>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>Seed 55</td>
<td>426</td>
<td>2.0</td>
</tr>
<tr>
<td>Seed+Ct 55</td>
<td>570.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Seed+Cl 55</td>
<td>739</td>
<td>6.0</td>
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<tr>
<td>Seed+Ct+Cl 55</td>
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<td>8.0</td>
</tr>
</tbody>
</table>

Note: All conditions are at 37°C and 55°C.
3 – Technical Accomplishments/Progress/Results

• Hypotheses-
  – Feedstock conversion varies by consortia
  – Consortia performance varies by feedstock
  – Feedstock blending/nutrients enhance

• Background-
  – Used non-adapted seed inocula
  – Rumen fiber, centrifuge cake, blend
  – Biogas production as a proxy for hydrolysis

• Results
  – Consortia varied in feedstock preference
  – MWRD best on blended mix
  – Yeast extract enhanced conversion but doesn’t serve as feedstock

• Outcome
  – Combined inocula may be synergistic

NB = New Belgium Brewing, MWRD = Denver Metro Wastewater Reclamation District