NREL Transforming ENERGY

DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

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

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

- ~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
2-Noike et al. 1985. Biotech Bioeng, 27:1482-1489
3-Singh et al. 1982. Agricultural Wastes 4:267-272
4-Matsakas et al. 2014. Biotech. Biofuels. 7:4-12
5-Kim et al. 2008. Biores. Technol. 99:5165–5176

Quad Chart Overview

Timeline

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

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 18-Project End Date)
DOE Funded	N/A	N/A	$500K	$1.5M
Project Cost Share*	N/A	N/A	N/A	N/A

- **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

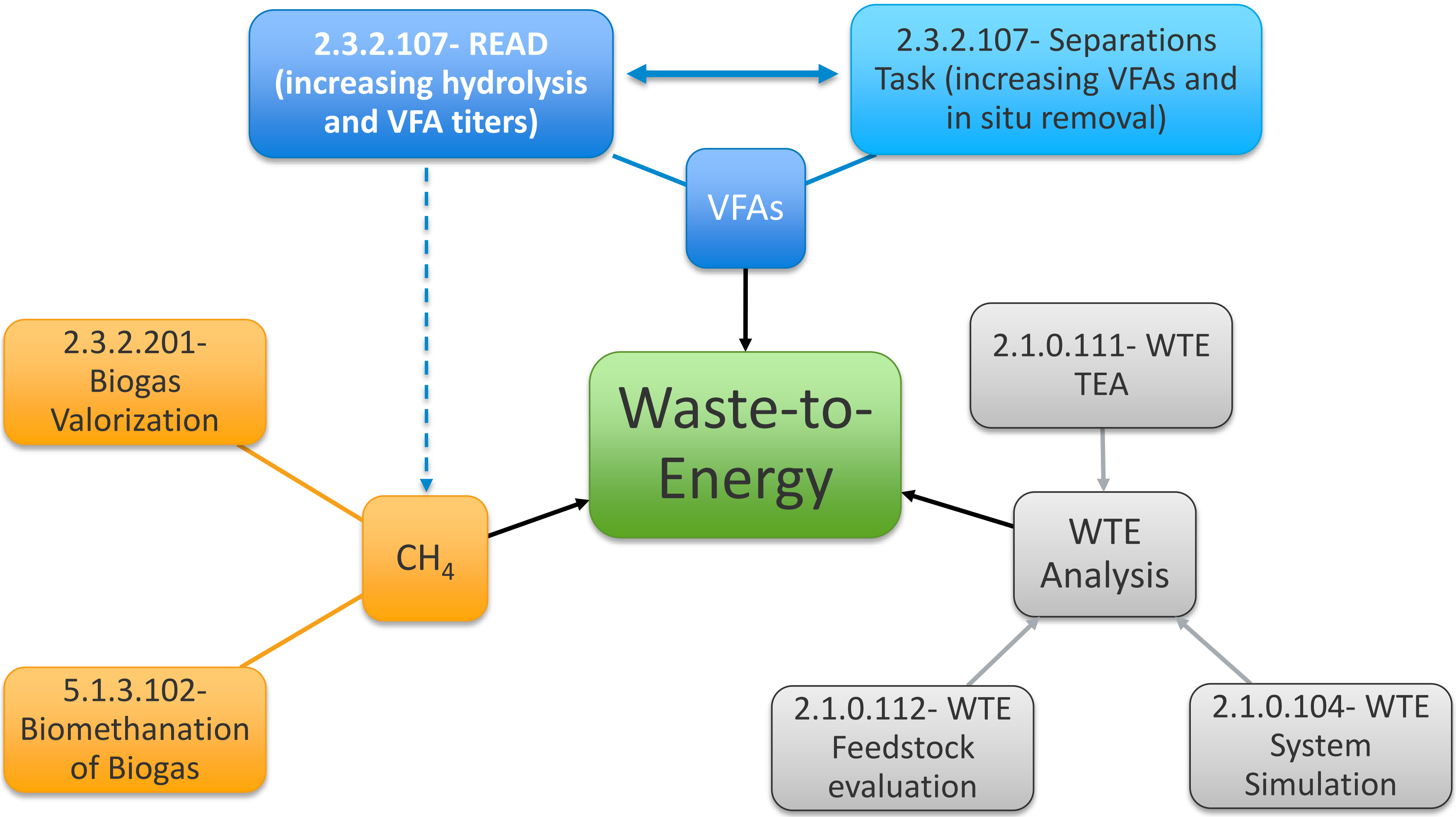
Objective

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

End of Project Goal

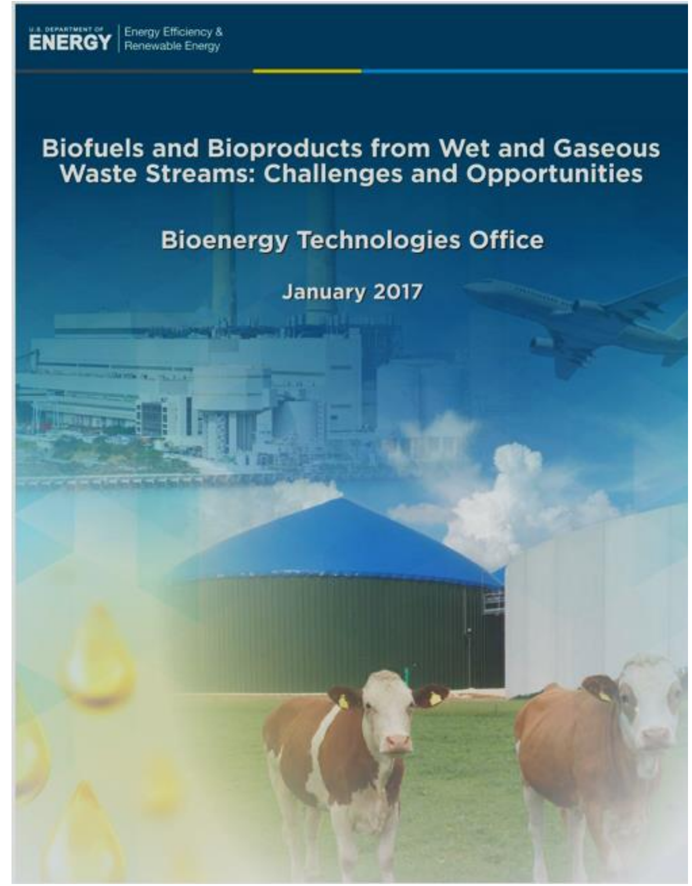
VFA titers > 50 g/L during continuous anaerobic digestion at pH 5.0 or lower with >50% conversion of wet waste

Task Integration



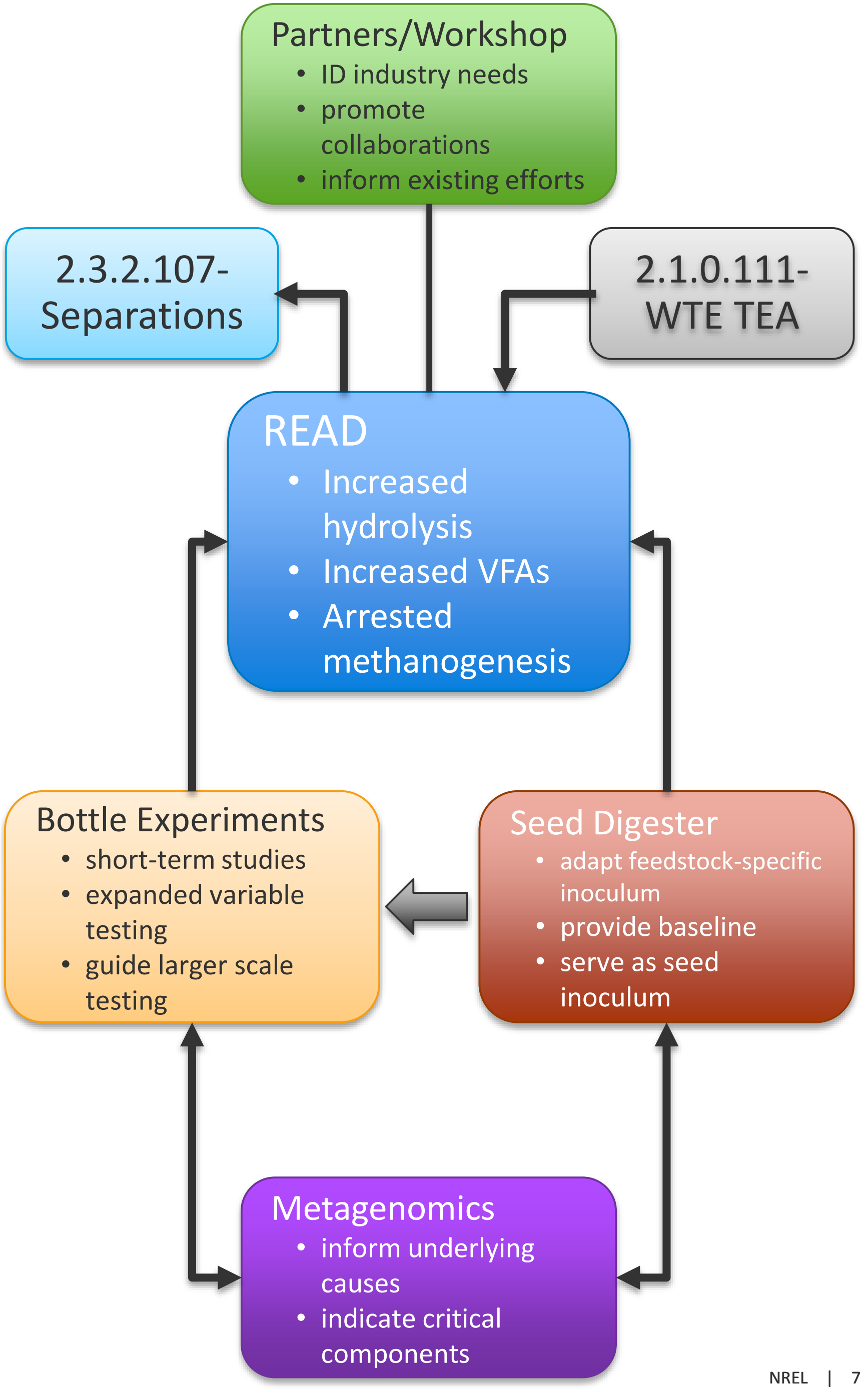
1 - Project Overview

- FY17 lab-call- improved conversion, decreased methane, increased VFAs, reutilization of CO_2 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 CH_4 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



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)

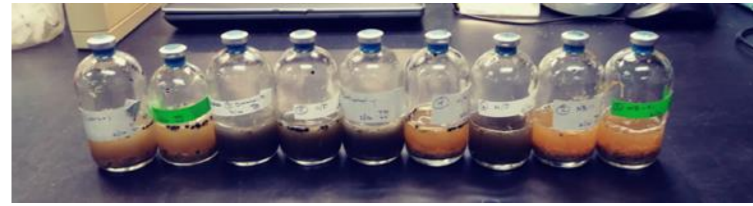
Critical Success Factors

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

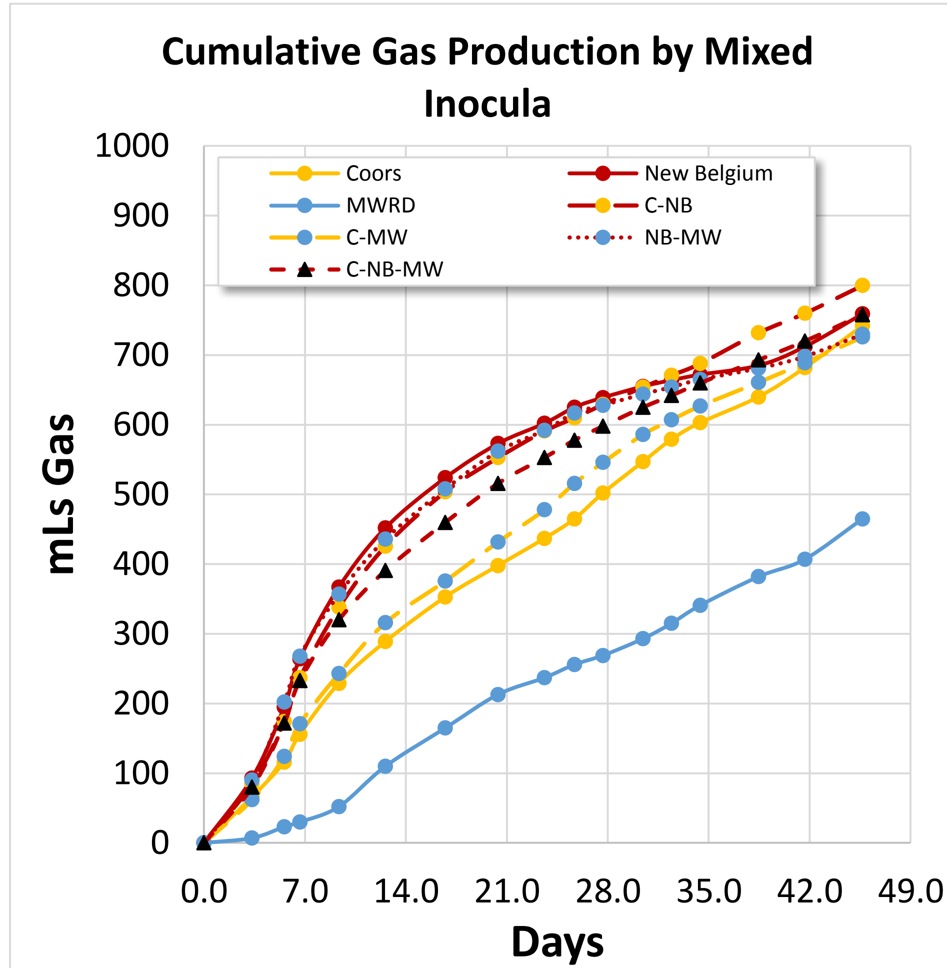
- 3-year project plan started November 2017
  - FY18Q1 MS- Developed working relationships with local AD operators/wet waste generators and established feedstock choice
  - FY18Q2 MS- Established digester operations and analyses for VFAs/metagenomics
  - FY18Q3 MS- baseline productivities and product 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

Bottle Digestions- Inocula Screening



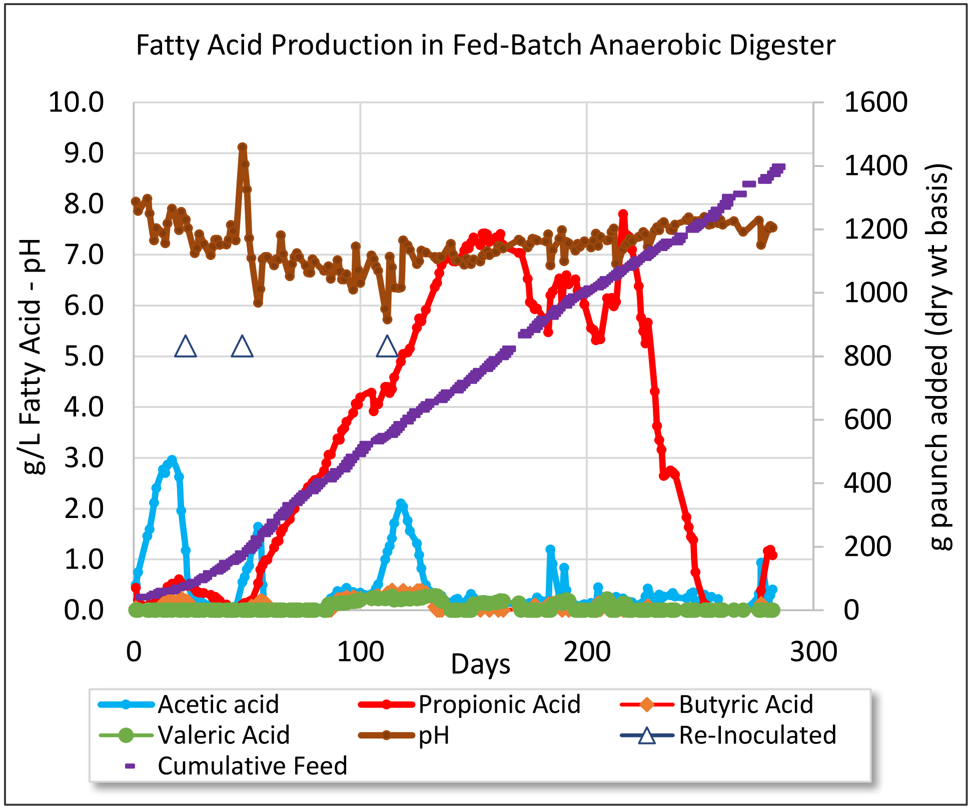
- Hypotheses-
  - Mixed inocula may be synergistic
- Background-
  - rumen fiber + 3 seed consortia
- Results
  - New Belgium most effective (biogas)
  - MWRD was least effective, but steady
  - Mixed inocula tended to rise to the most effective subpopulation
- Outcome
  - Different rates due to different mechanisms of populations adapted to different feedstocks
  - No detrimental effects w/ mixing so synergy is possible



3 – Technical Accomplishments/ Progress/Results

Seed Digester

- 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% CH_4, 35% CO_2, 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

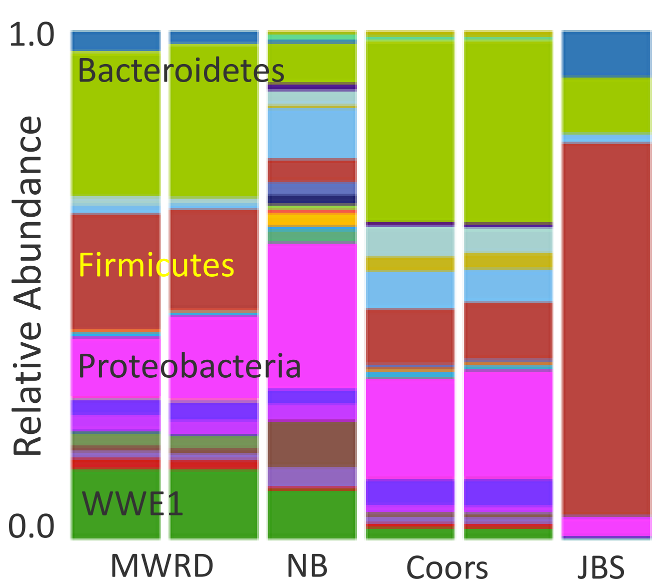


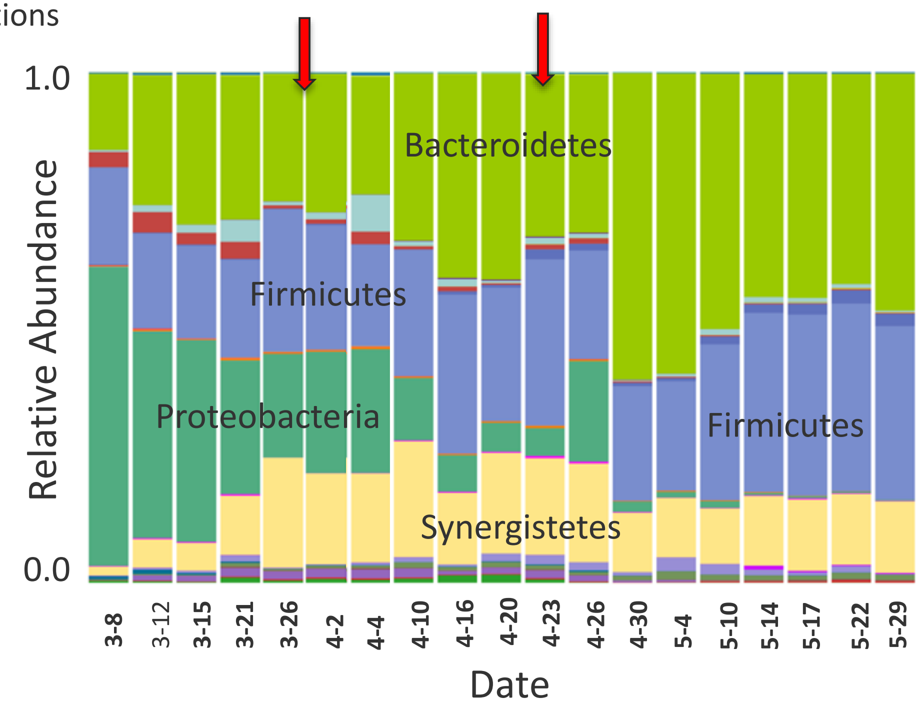
3 – Technical Accomplishments/ Progress/Results

Metagenomic Analysis

"What exactly do we have here?"

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





3 – Technical Accomplishments/ Progress/Results

Population adaptation of the NREL seed anaerobic digester

- 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

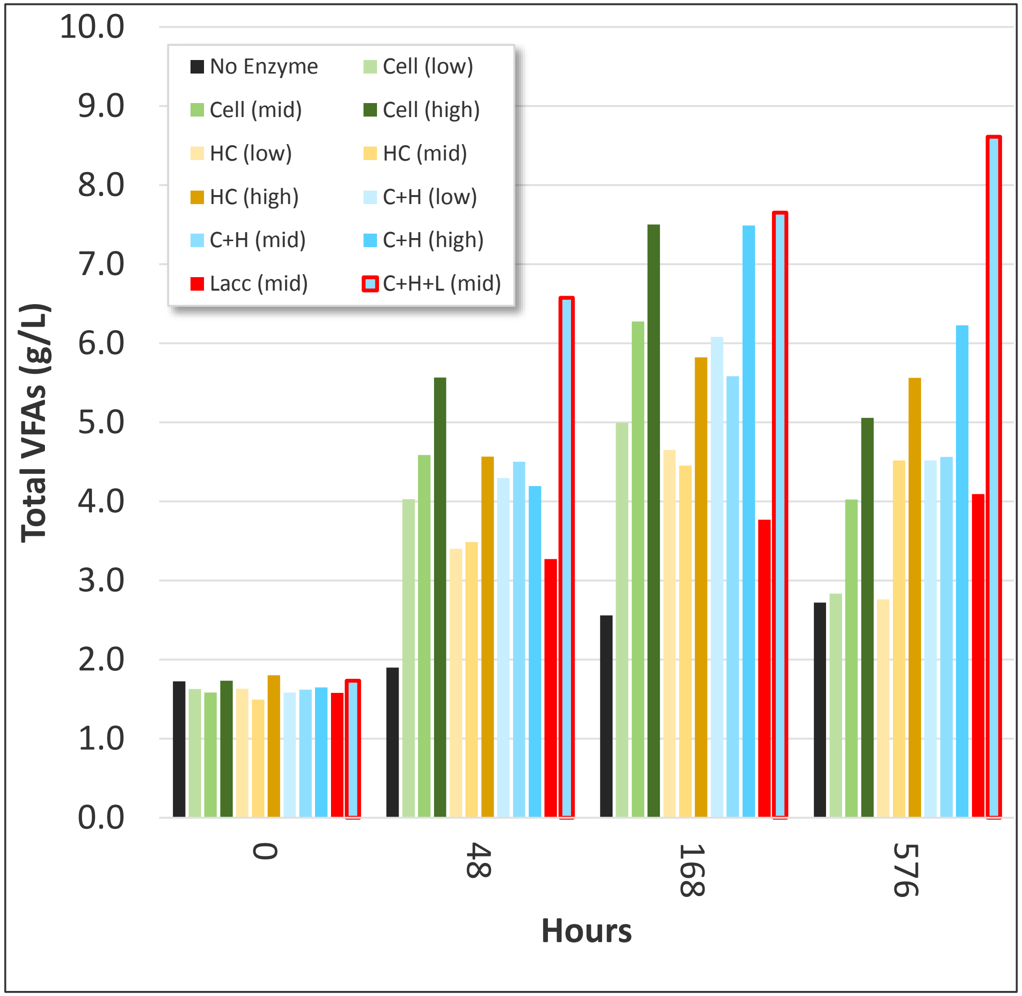
Taxon	Typical function	MWRD	Coors	New Belgium	JBS	Seed Digester
Bacteroidetes	**Hydrolysis**, **Cellulase**, VFAs	29.94	35.81	7.8	11.15	46.41
Firmicutes	Acetate, **Cellulase**	19.81	11.28	4.57	73.46	33.98
Synergistetes	amino acid catabolism, VFAs	2.86	1.26	3.31	0.01	14.02
Fibrobacteres	**Cellulase**, VFAs	0.06	0	0.04	0	2.45
Unclassified	Unknown	1.28	1.36	3.9	0	1.04
Chloroflexi	Acetate	1.21	5.01	2.92	0	0.31
Actinobacteria		2.55	0	0.67	9.11	0.24
Verrucomicrobia		1.88	0.91	0.57	0	0.23
Proteobacteria	VFAs	16.57	21.24	28.81	3.86	0.2
Euryarchaeota	methanogens	1.08	6.46	9.99	1.76	0.15
Tenericutes		2.44	0	0	0	0.09
Spirochaetes		3.67	5.17	2.9	0.14	0.08
Thermotogae		0.97	0.67	9.07	0	0.03
WWE1	Acetate	13.68	2.02	9.63	0	0.01
AC1		0	1.18	0.66	0	0
Acidobacteria		0.02	0.59	1.16	0	0
Chlorobi		0	0.83	1.4	0	0
Crenarchaeota		0	3.19	0.54	0	0
GN04		0	0.02	2.47	0	0
KSB3		0	0.18	1.97	0	0
OP8		0	0.08	2.41	0	0
Planctomycetes		0.12	0.32	2.47	0	0

Demonstrates that feedstock selects consortia, even if it requires large shifts
Consortia dynamics are fluid and adaptable

3 – Technical Accomplishments/ Progress/Results

Bottle Digestions- Enzyme Augmentation

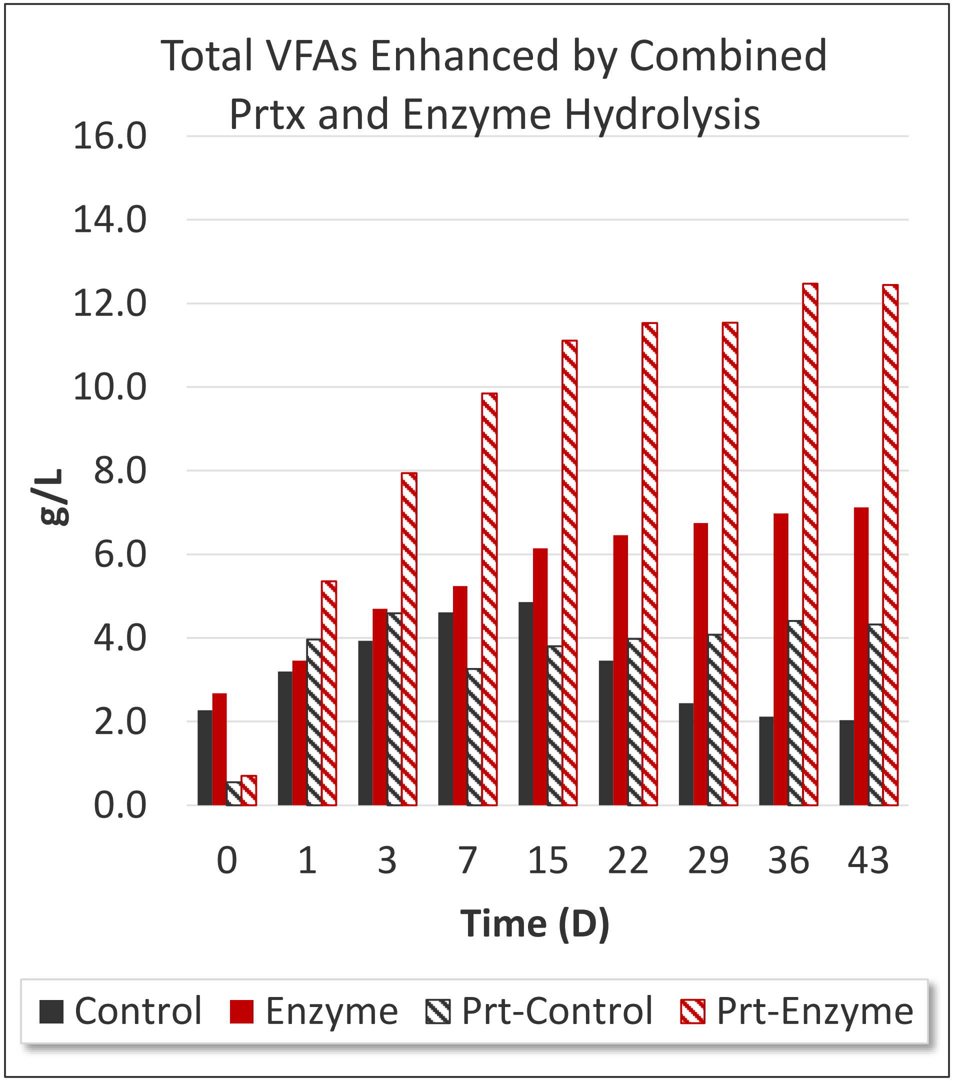
- Hypothesis-
  - Biomass-active enzymes can increase hydrolysis and low pH will arrest CH_4
- Background
  - CTec2/HTec2/Laccase at pH 4.5
  - Measured 7 VFAs at 9 timepoints
- Results
  - No biogas produced
  - Cellulase (C) increases VFA production, Hemicellulase helps, some H/C synergy
  - Laccase 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



3 – Technical Accomplishments/ Progress/Results

Bottle Digestions-Enzyme Augmentation + Pretreatment

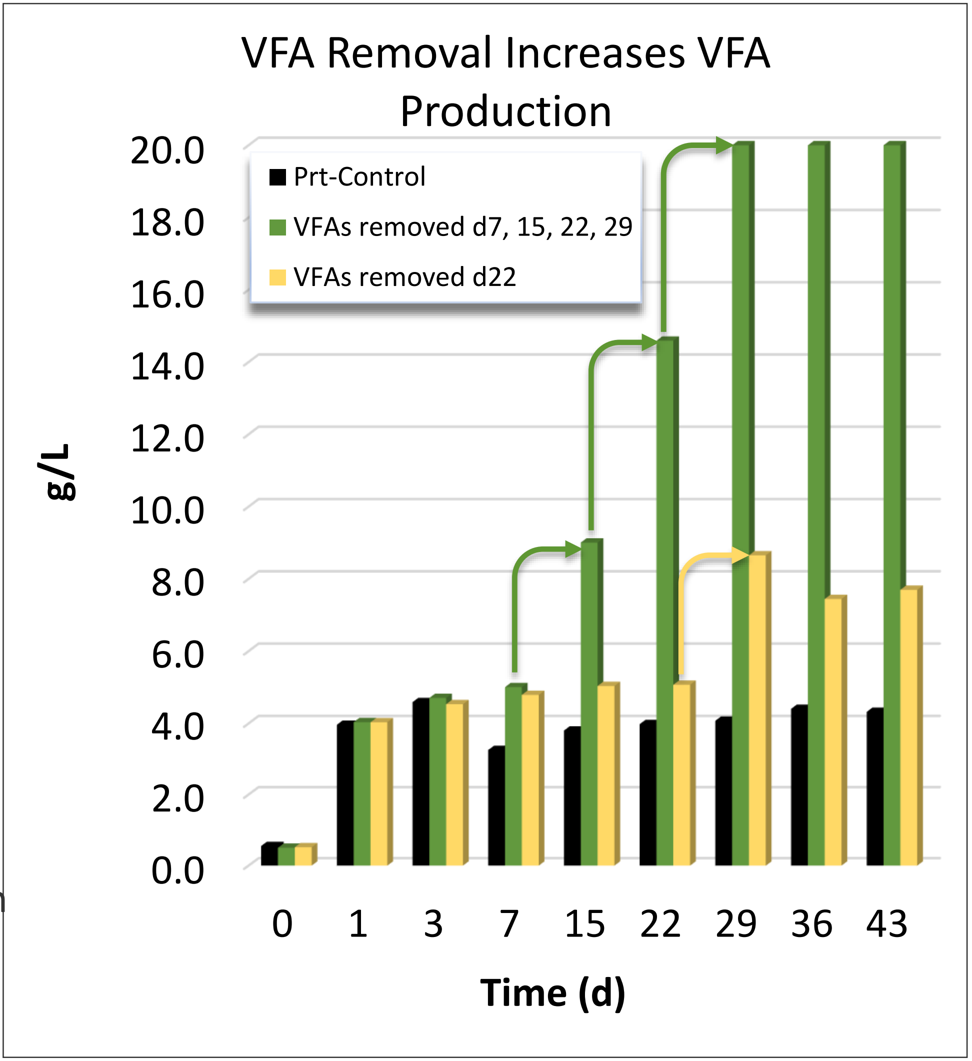
- Hypothesis-
  - Thermal pretreatment will increase enzymatic hydrolysis and enhance VFA production
- Background-
  - Pretreated rumen fiber 175°C for 15 min, NREL adapted seed inoculum, 37°C
  - Cellulase/hemicellulase 10 mg/g glucan
  - VFAs measured by HPLC
- Results
  - Enzyme augmentation increased 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 acido/acetogens



3 – Technical Accomplishments/ Progress/Results

Bottle Digestions- VFA Removal (Support of Separations Task)

- 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



4 – Relevance (programmatic)

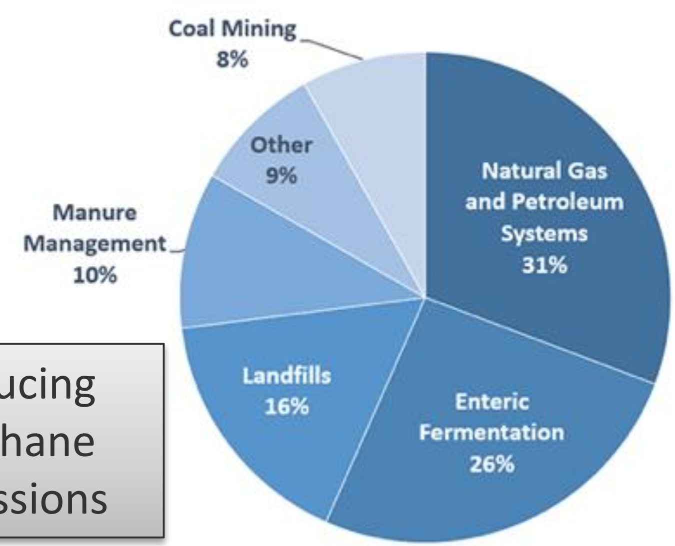
- Improve rate/extent of lignocellulosic hydrolysis during AD of wet waste
- Most wet-waste contains lignocellulose
  - 25-35% sewage[1], 40-60% MSW[1], ~25% manure[2]
  - Cellulose conversion is rate limiting step
  - 60-80% of the cellulose remains after AD- lost yield/ D3 RINs
- Enhanced hydrolysis 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

1-Noike et al. 1985. Biotechnology and Bioengineering, 27:1482-1489
2- Singh et al. 1982. Agricultural Wastes 4:267-272

Leveraging Existing AD Infrastructure

AD Units in US (from EPA/ABC)	
AD-WWTP / total	1241/>16,000
WWTP onsite biogas use	~860
WWTP biogas flared	~380
On-farm digesters	247
Stand alone digesters	38
Potential sites	~11,000
Existing digesters in Europe	>10,000

2016 U.S. Methane Emissions, By Source



U.S. Environmental Protection Agency (2018). Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2016

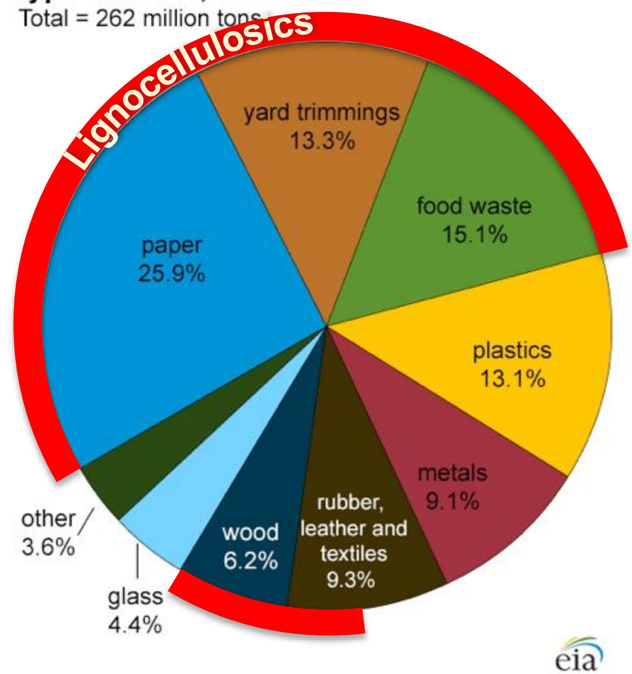
Reducing Methane Emissions

4 – Relevance (social, commercial)

Landfill use and potential application to MSW

- 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

Total MSW generation in the United States by type of waste, 2015
Total = 262 million tons



Source: U.S. Environmental Protection Agency, Advancing Sustainable Materials Management: 2015 Fact Sheet, July 2018

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

5 – 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*

Increased VFA production	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	6/30/2019 Go/No Go
Sustained enhanced conversion	Translate and maintain high digestibility/VFA production from 6/30/2019 Go/NoGo to additional feedstocks such as food waste, manure, MSW, or sewage.	9/30/2019 Annual
Increased VFA production	Demonstrate sustained (>90 days continuous operation) VFA titers >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.	9/30/2020 Project End

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
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  - Patrick Saboe
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- **Methane Upgrading**
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- **DOE-BETO**
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- **JBS**
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- **Denver MWRD**
  - Jim McQuarry
  - Quintin Schermerhorn
- **MillerCoors**
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Additional Slides

Responses to Previous Reviewers' Comments

- N/A

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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
- February 2019- “Waste-to-Energy Research at NREL: Methane and Beyond” presented by Steve Decker at the NAMI Environmental Conference for the Meat and Poultry Industry, Atlanta GA

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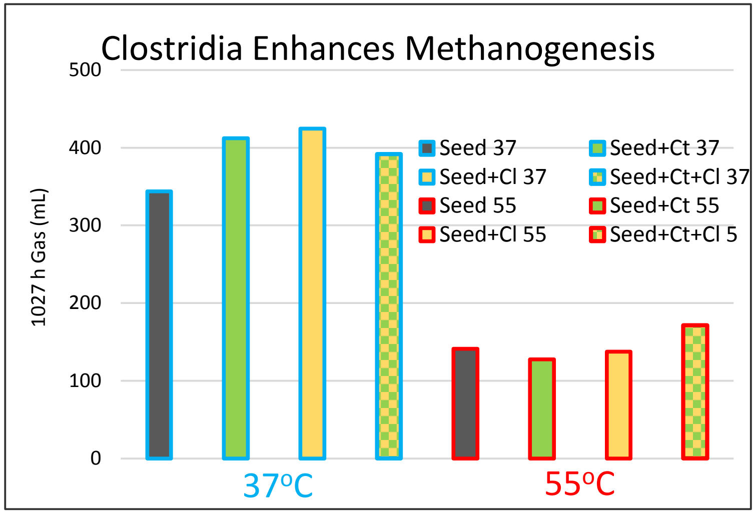
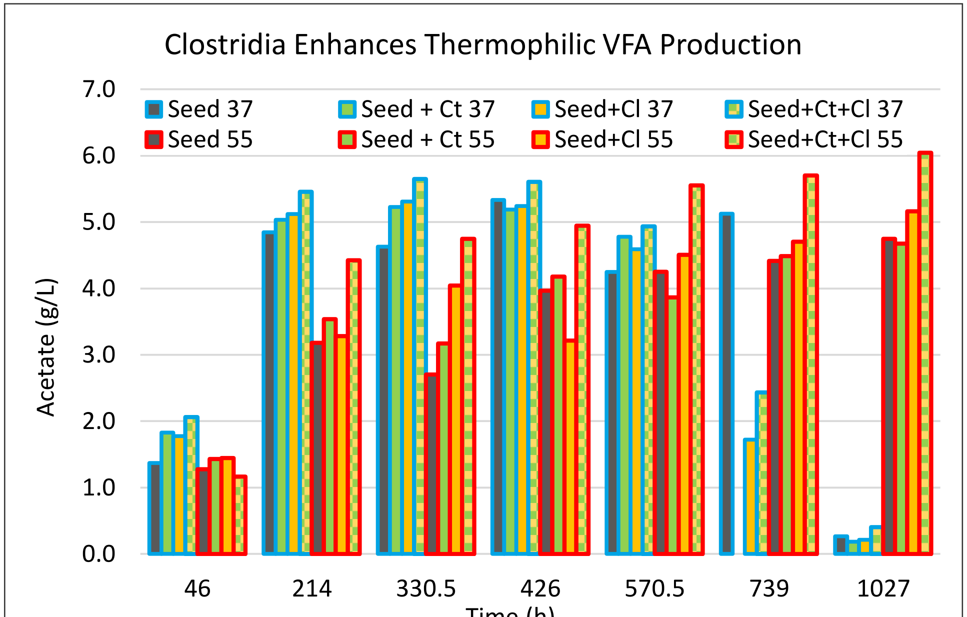
3 – Technical Accomplishments/ Progress/Results

Bottle Digestions- Consortia Biasing

- Hypothesis-
  - Addition of hydrolytic microbes can enhance conversion
- Background-
  - *Clostridium thermocellum*- cellulase(+), 55°C
  - *C. ljungdahlii*- CO_2 fixation, 37°C
  - Seed inoculum 37°C
- Results
  - 55°C inhibited acetate→CH_4 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

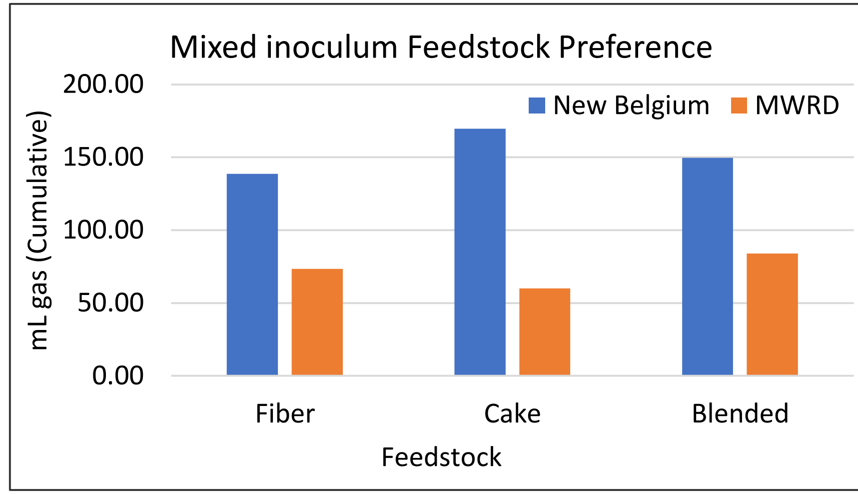


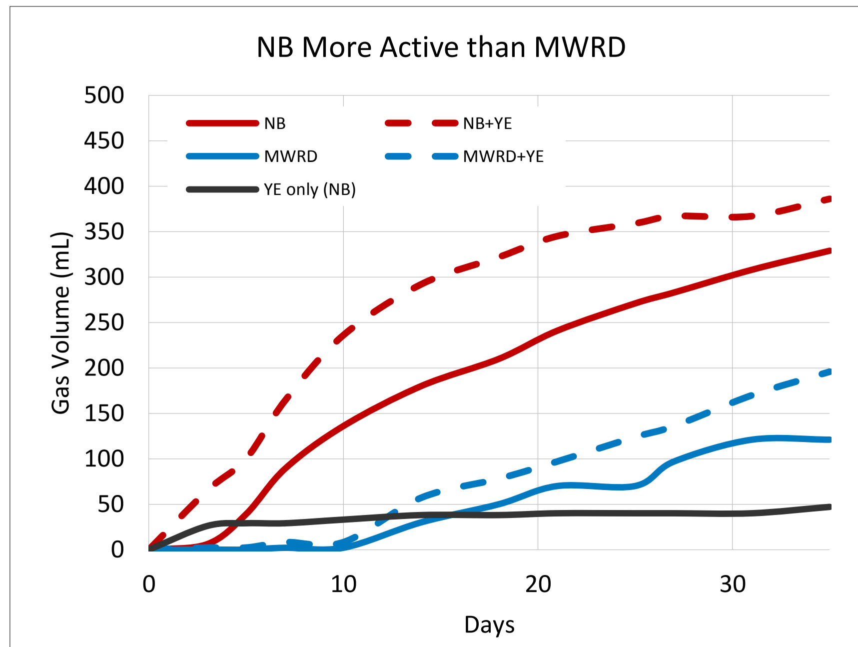


3 – Technical Accomplishments/ Progress/Results

Bottle Digestions- Feedstock, Inocula

- 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