U.S. DEPARTMENT OF ENERGY (DOE) BIOENERGY TECHNOLOGIES OFFICE (BETO) 2017 PROJECT PEER REVIEW

2.2.4.100 ENHANCED ANAEROBIC DIGESTION

March 7, 2017

WASTE TO ENERGY

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This presentation does not contain any proprietary, confidential, or otherwise restricted information

GOAL STATEMENT

- **Goal:** Transform negative-value or low-value sludge into high-energy-density renewable methane and fungible hydrocarbon precursors
  - Enhance anaerobic digestion of biosolids to produce biogas with ~90% methane content and hydrogen sulfide at non-detectable level (Task 1)
  - Develop a Comprehensive Waste Utilization System (CWUS) for production of hydrocarbon precursors from the anaerobic digestion of biosolids (Task 2)
- **Outcome:** Enable sustainable production of biogas that is considered as a cellulosic biofuel under new RFS2 (EPA, July 2014)
  - Biogas (600 BTU/scf) competes with conventional natural gas (1,000 BTU/scf)
  - Reduce greenhouse gas emissions relative to petroleum-derived fuels
  - Reduce U.S. dependence on foreign oil
  - Over 99% of RINs generated from biogas
- **Relevance:** Address DOE's goals of development of cost-competitive and sustainable biofuels by advancing efficient production strategies for drop-in biofuels

QUAD CHART OVERVIEW

Timeline

- Start Date: February 1, 2014
- End Date: September 30,2017
- Percent complete: 83%

Budget

	Total Costs FY 12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	$250 k	$750 k	$750k	$500 k
Project Cost Share (Comp.)*	N/A	N/A	N/A	N/A

*If there are multiple cost-share partners, separate rows should be used.

Barriers

- Task 1:
  - Cleanup/Separation (Bt-H)
  - Product Acceptability and Performance (Bt-K)
  - Overall Integration and Scale-Up (Ft-J)
  - Cost of Production (Im-E)
- Task 2:
  - Biomass and Feedstock Recalcitrance (Bt-B)
  - Pretreatment Processing and Selectivity (Bt-D)
  - Biological Conversion Process Integration (Bt-J)
  - Cost of Production (Im-E)

Partners

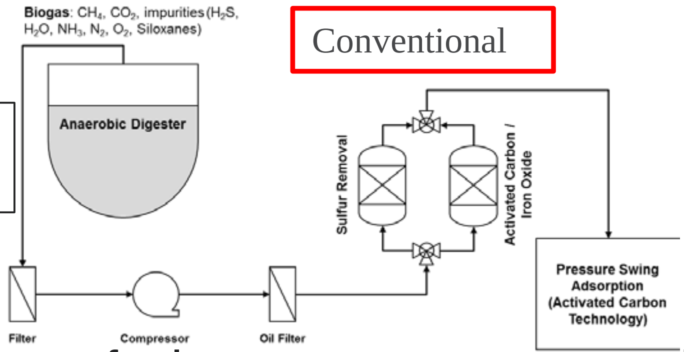
- University of Illinois at Urbana Champaign (8%, FY16)
- Roeslein Alternative Energy (10%, FY17)

1 - PROJECT OVERVIEW

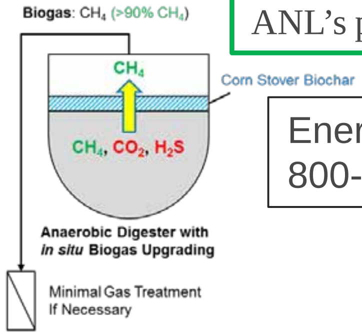
- Development and deployment of a novel AD process to produce pipeline quality methane (Task 1)(FY14-FY17)

Energy density:
600 BTU/scf

Conventional

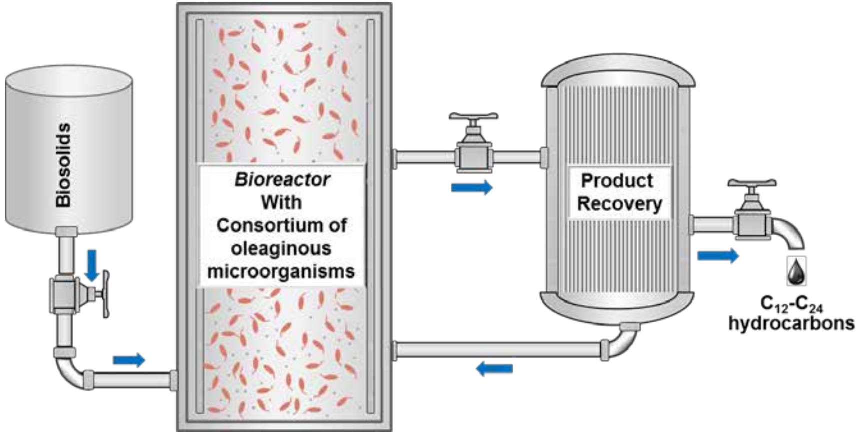


ANL's process



Energy density:
800-1,000 BTU/scf

- Development of a low-cost process to produce hydrocarbon fuels (Task 2)(FY15-FY16)



2 – TECHNICAL APPROACH- TASK 1

ANL's Patented Process (US 8,247,009)
Accelerated weathering
Ca/Mg rich rock sequester CO2 into carbonates

→ Bench-scale Testing
Serum bottles (FY14-15)

→ *Biochemical Model TEA*

Pilot-scale testing
14 liter Bioflo Fermenter (FY15-16)

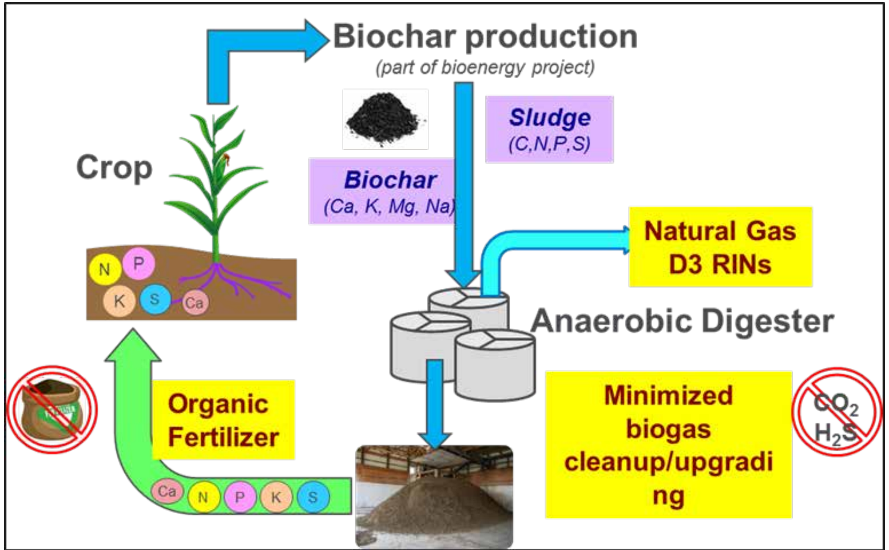
→ *Biochemical Model TEA*

Field-scale technology demonstration
Test at the field-scale digester (FY17)

→ *Technology Deployment*

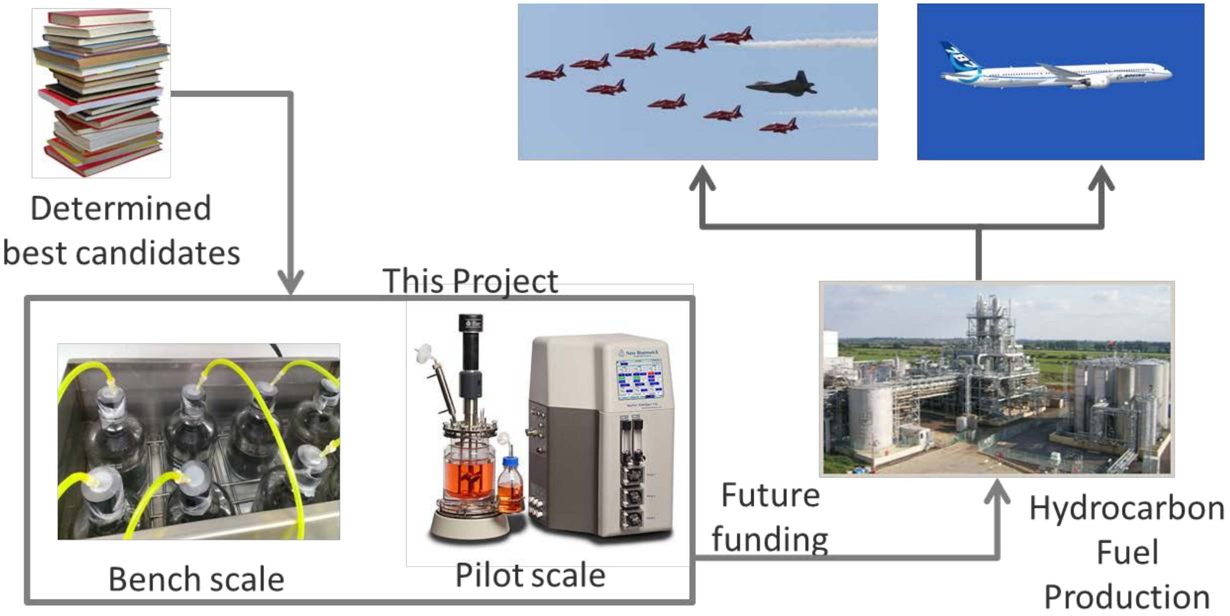
Metrics:

- Target:
  - Produce pipeline quality biogas (>90% CH_4) Go/No-go Decision Points:
    - FY15 – >90% CH_4 biogas (3/31/2015)(bench-scale)
    - FY16 – >90% CH_4 biogas (3/31/2015)(pilot-scale)



ANL's Patent Pending Process (U.S. Serial No. 14/540,393)

2 – TECHNICAL APPROACH- TASK 2



(FY15) (FY17)

Metrics:

- Target:
  - Demonstrate hydrocarbon precursors production with the CWUS process
- Go/No-go Decision Points:
  - FY16 – Produce 30 g/L weight with 50% (w/w) lipid content

2 – APPROACH (MANAGEMENT) (FY15-16)

Use of milestones for monitoring progress:

- Product titer and yield
- Feedstock resource assessment
- Technoeconmic assessment of the process from bench- and pilot-scale
- Meeting with project partners

Critical success factors:

- Cost effectiveness of pipeline quality methane production
- Identification of technology users
- Engagement with WWTPs and digester technology users

Potential challenges:

- Acceptance of new technology by utilities
- Cost effectiveness of CWUS

3 – TECHNICAL ACCOMPLISHMENTS/RESULTS- TASK 1

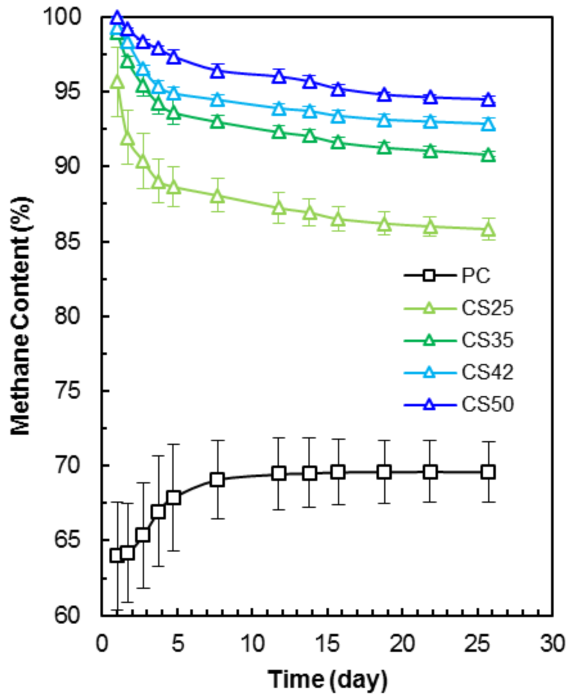
- Completed resource assessment at WWTPs and determined challenges and opportunities towards energy-neutral WWTPs (FY 15)
- Conducted bench-scale experiments (FY15)
  - Three different biochar types (corn stover, oak and pine)
  - Two different operating temperature (mesophilic and thermophilic)
  - Different biochar/organic loading rates
  - One stage and two-stage digesters
  - Batch and semi-continuous operating mode

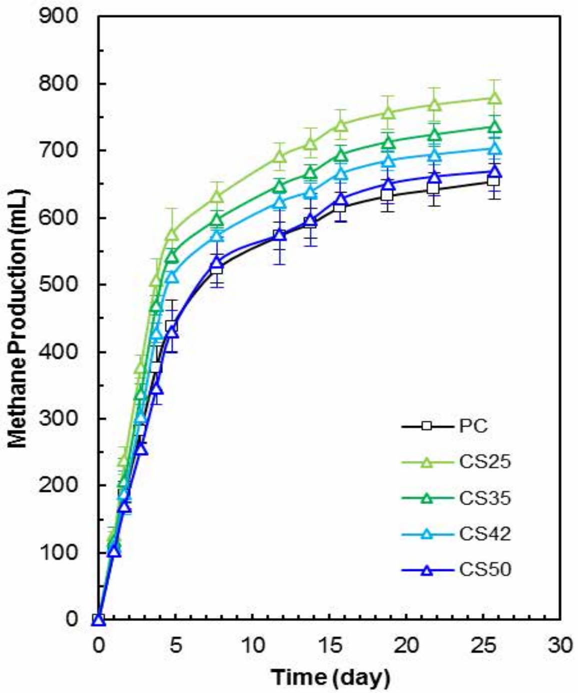
❖Milestone: Produced a gas composition with at least >90% CH_4 (batch mode)**– Achieved**

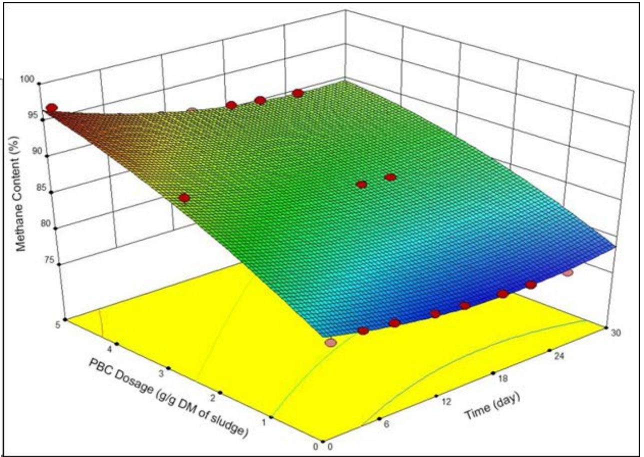
- Conducted pilot-scale experiments (FY16)
  - Scaled up the process up to 14 liters.
  - Determined impacts of biochar addition on digester microbial community structure and composition
  - Developed the mathematical model to understand the complexities in the digester environment
  - Completed techno-economic assessment of the process

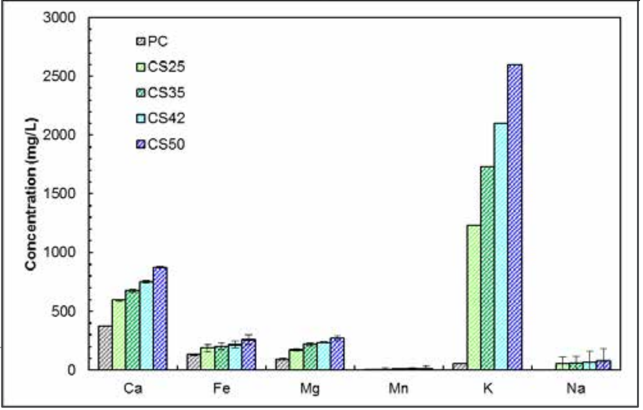
❖ Milestone: Produced a gas composition with >90% CH_4 (semicontinuos)**– Achieved**

TASK 1-FY 15 RESULTS





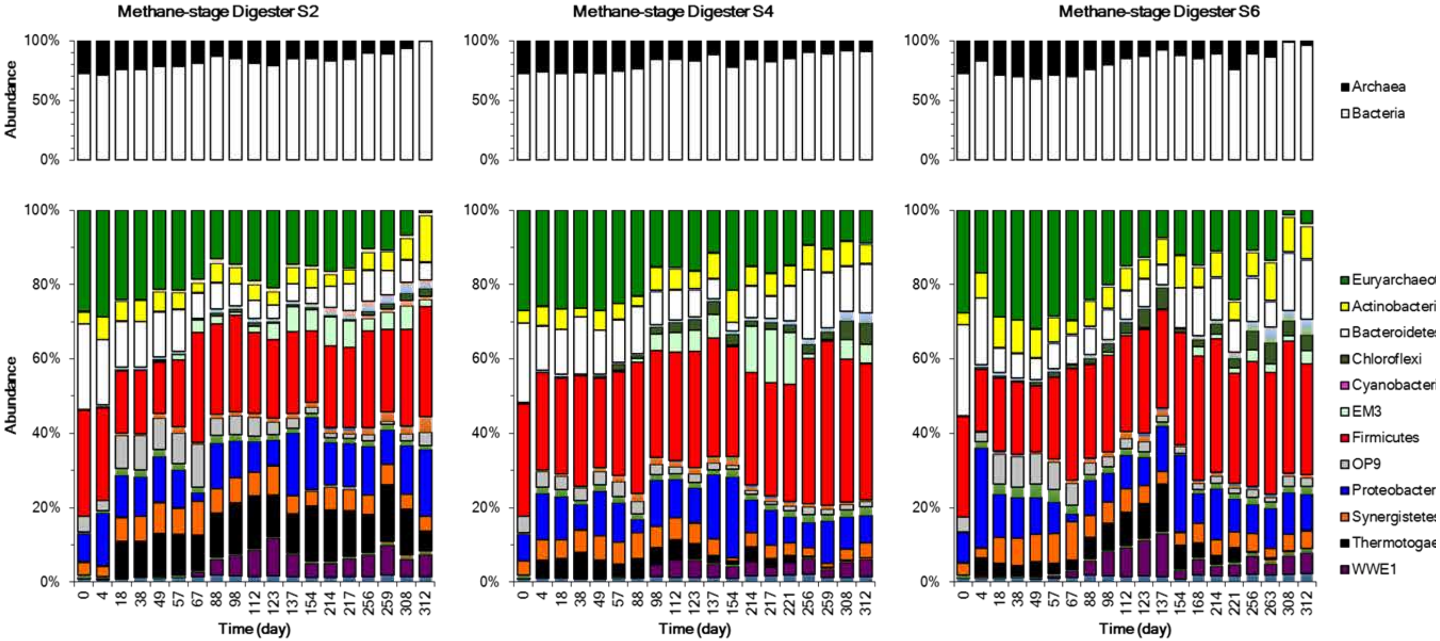


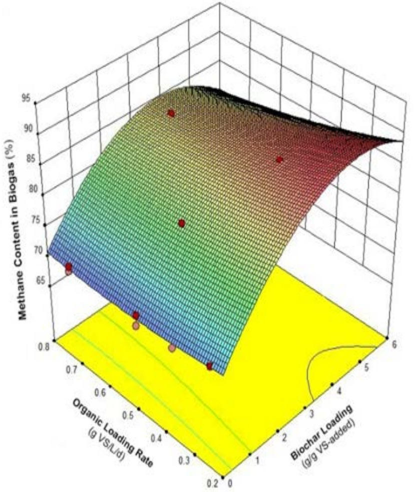


Digester operations in 0.5 liter serum bottles

TASK 1- FY 16 RESULTS 1/2

Data from 0.5 liter semi-continuous operations



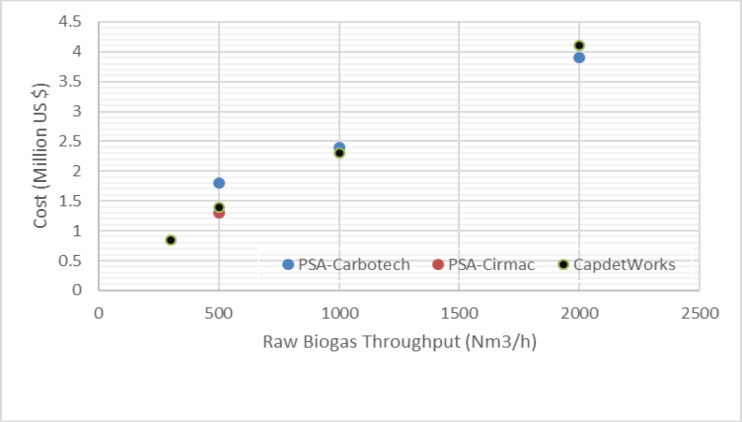


Reactor	Response	Equation	R^2
S4	Methane content in biogas	$CH_4\% = 74.60 + 9.07 \cdot BCL - 12.51 \cdot OLR - 1.91 \cdot BCL \cdot OLR - 1.00 \cdot BCL^2 + 9.99 \cdot OLR^2$	0.993
	Biogas production rate	$P_{Biogas} = 77.02 + 2.66 \cdot BCL + 350.25 \cdot OLR$	0.869
	Methane production rate	$P_{CH_4} = -18.77 + 33.98 \cdot BCL + 577.61 \cdot OLR - 4.45 \cdot BCL \cdot OLR - 4.90 \cdot BCL^2 - 326.52 \cdot OLR^2$	0.905
S6	Methane content in biogas	$CH_4\% = 71.80 + 1.15 \cdot BCL - 0.934 \cdot OLR$	0.751
	Biogas production rate	$P_{Biogas} = 75.27 + 1.57 \cdot BCL + 353.10 \cdot OLR$	0.892
	Methane production rate	$P_{CH_4} = -24.66 + 13.18 \cdot BCL + 609.72 \cdot OLR - 1.89 \cdot BCL \cdot OLR - 1.99 \cdot BCL^2 - 363.42 \cdot OLR^2$	0.917

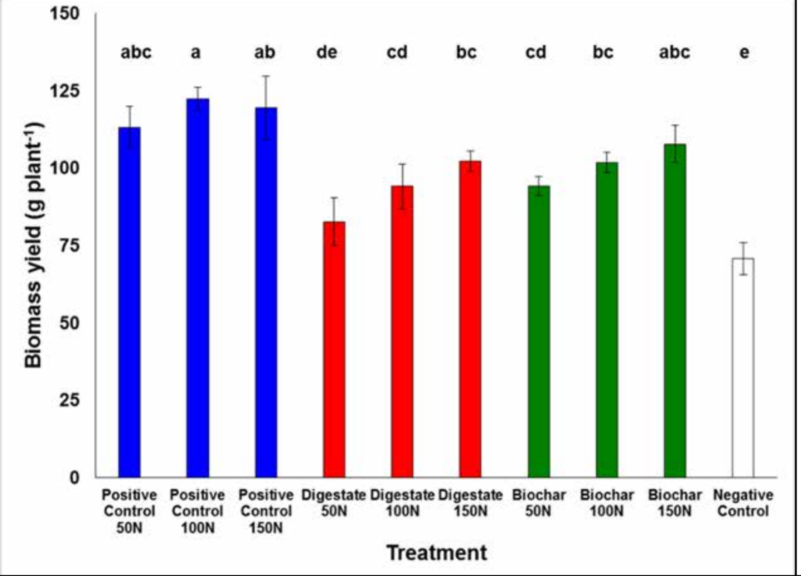
TASK 1- FY 16 RESULTS 2/2

Phase	B1 HRT (day)	B2 HRT (day)	OLR (g VS/L/d)	Biochar (g/d)	Min. – Max. Methane (%)	Average Methane content (%) at steady state
1	15	30	1	0	63–70	68
2	10	20	1	0	65–69	67
3	10	20	2	0	63–67	65
4	10	20	2	14	68–76	70
5	10	20	1.5	14	61–72	68
6	10	20	1.5	21	70–87	77
7	10	20	1.5	25	72–87	81
8	5	15	3	21	80–93	90
9	3	15	5	0	67–76	68.5
10	3	15	5	21	70–90	81

Data from pilot-scale studies (14 liter digesters)



Biogas upgrading equipment costs by technology and manufacturer

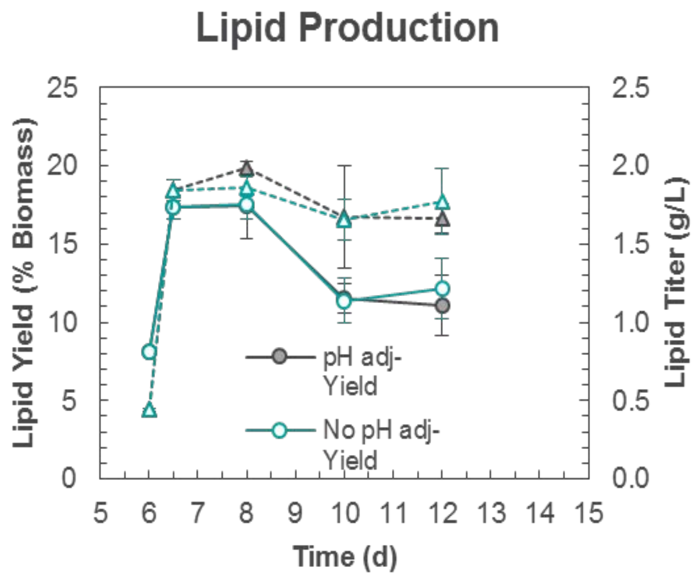


No Nitrogen Urea

Root growth of switchgrass in green house

3 – TECHNICAL RESULTS- TASK 2 (FY 15- FY16)

- Analytical Issues –solved
- Getting a representative sample for analysis- solved
- Limited carbon availability to pure strains- solved
- Low C/N ratio in sludge samples- pretreatment and addition of other waste



Suggested C/N ratio for lipid production>100
Obtained C/N ratio from experiments: 6-20

- G/NG criteria of 30 g/L and 50% lipids by weight was not met unless the medium supplemented with glucose
- Informed BETO office that oleaginous conversion for sludge is not promising
- Task 2 was terminated on July 7, 2016 since sludge and other wastes were not amenable to these organisms which require glucose and yeast supplementation to produce 30 g/L of biomass.

FUTURE WORK FOR TASK: 1 (FY 2017)
COMMERCIALIZATION PLAN

- Scale up the process to field-scale digesters (Roeslein Alternative Energy's Farm)
- Determine commercial viability of the additive process by adding value of
  - pipeline quality biogas production
  - improved performance of the biogas cleaning system
  - utilization of digestate as a fertilizer and soil conditioner for the growth of energy crops
- Conduct a comprehensive market analysis of biochar and organic fertilizer to determine their market size, potential growth and price.
- Identify the AD technology companies that might adopt this additive technology to improve their portfolio returns.
- Develop approaches to commercialize process and incorporation of biochar production and loading system into the operation of ADs that handle a variety of organic wastes.

2 – APPROACH (MANAGEMENT) (FY17)

Field-scale Study:

- Coordinate activities on a weekly basis or as needed
- Responsible from progress on meeting scheduling, milestone, and financial requirements
- Assess road-blocks, technical challenges, and resource allocations needs
- Organize technical meetings
  - » Technical team meetings: Face-to-face as needed, teleconferences via blue jeans
- Responsible for data storage
  - » Set up a password protected share point to facilitate rapid and complete information delivery.
  - » All data will be stored in cloud storage “box”

TASK 1- FY 17 ACHIEVEMENTS

- Conducted a study on the availability of biochar in close proximity to the field site that this process could utilize.
- Characterized and tested biochar samples to determine suitable biochar source for the field scale testing.
- Evaluate biochar providing companies in terms of their biochar price and delivery method to the site (on going).
- Obtain the permit from DNR for utilization of biochar in the field-scale digester.
- Design and install new digesters equipped with
    - biochar loading system,
    - level sensors in the digesters,
    - liquid transfer pumps,
    - effluent disposal system, and
    - connected to biogas clean up and upgrading system and the flare (ongoing).

4 – RELEVANCE

Barriers addressed

Task 1:

- Bt-H: Cleanup CO_2 and H_2S from Biogas
- Bt-K: Pipeline quality methane production (90% CH_4)
- Ft-J: Scaling up process from bench- to full-scale digesters

The outcome of this project:

- Supports BETO's work to develop renewable and cost-competitive biofuels from non-food biomass feedstocks (waste)
- Contributes to fulfilling BETO's goal of developing commercially viable technologies for converting feedstocks via biological routes into energy-dense, fungible, transportation fuels and chemicals.
- BETO actively pursues R&D in the emerging area of Waste-to Energy
- Over 99% of RINs generated from biogas (Since July, 2014)
- Conversion of biosolids, manure and food waste to biogas would be
  - capable of displacing the equivalent of 2.5 Billion gallons of gasoline equivalent per year
  - reduce US dependence on foreign oil, increasing energy security, and mitigating climate change.
- Informed BETO office that oleaginous conversion for sludge is not promising

SUMMARY

- Developed and scale up a novel process using biochar for producing renewable methane at pipeline quality (>90% CH_4)
- A new paradigm of efficient and economical renewable methane production for the AD industry
  - Both methane production and *in situ* sequestration of carbon dioxide and hydrogen sulfide take place in the same reactor
  - Facilitated CO_2 sequestration by up to 86.3% and H_2S removal (< 5ppb), and boosted average CH_4 content in biogas by up to 30.1%
  - Enhanced AD performance
    - Methane yield, biomethanation rate and maximum methane production rate increased by up to 7.0%, 8% and 28%, respectively.
    - Increased alkalinity and mitigated ammonia inhibition, hence providing sustainable process stability
  - Increased fertilizer value of digestate
    - K, Ca, Fe and Mg in the biochar-amended digesters increased by 2051-4435%, 122-273%, 60-134%, 43-95%, and 82-183%, respectively.
- Engaged with WWTPs and digester technology users
- Two companies have keen interests in licensing technology

ACKNOWLEDGEMENT

- BETO Technology Managers: David Babson, Beau Hoffman and Mark Philbrick
- Chicago area wastewater treatment plants: MWRD's Stickney WRP, DuPage County Greene WWTP
- Roeslein Alternative Energy
- ANL Team:

Yanwen Shen

Lionel Mojekwu

Robin Schoene

CJ Guron

ADDITIONAL SLIDES

RESPONSES TO PREVIOUS REVIEWERS' COMMENTS

- **Reviewers' Comments:**

1. The reactor seems to be a novel design with positive results. The capture of CO2 and sulfur in the reactor (in-situ) is very interesting, plus the prcess produces >90% methane. What are the next steps for methane as far as converting to liquid hydrocarbons? Or is the objective to produce lipids (Task 2)?

2. This is an interesting project that utilizes biochar in an innovative manner and provides the potential to create clean biogas cheaply in a commercial environment. The approach of working with industrial facilities from the beginning and incorporating techno-economic analyses between each scale-up are excellent.

3. The project has produced very promising results with biochar to improve performance of anaerobic digestion of biosolids. Techno-economic analysis will be key for determining if this leads to improved economics. The development of a process for biosolid conversion to lipid intermediates is early stage, but an interesting approach to valorizing these waste biosolids as a feedstock.

4. I am not certain of the connectivity between Task 1 (biogas production) and Task 2 (lipid production). Enhanced anaerobic digestion will improve the conversion of waste products into more valuable materials, however, biogas is more expensive and contains more impurities as compared to natural gas.

- **PI Response to Reviewers' Comments**

1. Tasks 1 and 2 are separate product pathways built from the same feedstocks: biogas and hydrocarbon fuels. Task 1 - Biogas now qualifies as a D3 cellulosic biofuel under RFS2. Task 1 reduces cost to meet transportation fuel specifications from biogas. This will help industry to meet cellulosic biofuels mandates. Task 2 maximizes market penetration.

RESPONSES TO PREVIOUS REVIEWERS' COMMENTS

BETO's primary target is hydrocarbon replacements for existing liquid fuels. Task 2 opens a new pathway to produce liquid hydrocarbon biofuels that meet RFS2 D3 (cellulosic biofuel) mandates. Task 1 is more short term and Task 2 is more long term. Both enable BETO to advance waste-to-energy. Regarding biogas quality, there are commercial technologies to meet CNG and LNG purity levels. This project brings the costs down significantly. Regarding costs for natural gas, fossil-based natural gas does not meet RFS2 mandates and cannot address this market. Therefore, conventional natural gas does not compete with biogas. This project reduces costs for biogas production and upgrading and is expected to out-compete existing biogas processes.

2. The biochar industry is emerging in the U.S., associated with the fast development of biomass electric power plants. Woody biomass is the main feedstock for the bio-electric power plants in the U.S., with a net power generation of 43.1 billion kWh in 2014. There are 135 facilities (as of April 21, 2015) utilizing forest wood, wood waste and logging and mill residues as the feedstock (Biomass Magazine, 2015). Assuming that a 10-MW-capacity plant consumes 10 BDT/hour (BDT = bone dry ton) (Mayhead, 2010) and that gasification or pyrolysis process produces 10-20% biochar on the feedstock dry weight basis (Brewer, et al., 2012), the bio-electric power plants in the U.S. could generate 4.3 to 8.6 million tons of ash annually, which can be used as a substitute or replacement of biochar.

3. The scope of this project includes development and deployment of new processes to produce either renewable natural gas via biogas or hydrocarbons via fatty acid intermediates. Fatty acids are captured as an intermediate in the digestion process. Conversion of methane to liquid fuels and chemicals has significant potential, but is beyond the scope of this phase of the project. We are considering partners for downstream methane conversion in future project phases.

PUBLICATIONS, PATENTS, PRESENTATIONS, AWARDS, AND COMMERCIALIZATION

- Patent Application: Method For Generating Methane From A Carbonaceous Feedstock, U.S. Serial No. 14/540,393 (pending)
- *Journal Papers*

Published:

1. Shen Y, Linville JL, Ignacio-de Leon PAA, Schoene RP, Urgun-Demirtas M (2016) Towards a sustainable paradigm of waste-to-energy process: Enhanced anaerobic digestion of sludge with woody biochar, *Journal of Cleaner Production*, Vol. 135:1054–1064
2. Shen Y, Linville JL, Urgun-Demirtas M, Mintz MM, Snyder SW. (2015) Review of enhanced biogas production and utilization at full-scale wastewater treatment plants (WWTPs) in the United States: Towards energy-neutral WWTPs, *Renewable and Sustainable Energy Reviews*, Vol. 50: 346–362
3. Shen Y, Linville JL, Urgun-Demirtas M, Schoene RP, Snyder SW. (2015) Producing pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover biochar with in-situ CO2 removal, Applied Energy, *Vol.158: 300-309*.

Under review:

1. Evaluating Biogas Cleaning Options Using a Novel Design and Costing Platform – Model Development and Validation
2. Yearlong semi-continuous operation of thermophilic two-stage anaerobic digesters amended with biochar for enhanced biomethane production

PUBLICATIONS, PATENTS, PRESENTATIONS, AWARDS, AND COMMERCIALIZATION

– *Conference Papers and Invited Talks*

1. Urgun-Demirtas M and Shen Y (2017) Application of a simultaneous biogas production and upgrading process for renewable methane production using municipal sludge in laboratory- and pilot-scale digesters. AIChE Midwest Regional Conference, Feb 28-Mar 1, Chicago.
2. Linville JL, Schoene R and Snyder SW (2016). Integrated waste to energy and nutrient production system (IWENPS) for renewable methane production and fertilizer-grade digestate recovery. 38th Symposium on Biotechnology for Fuels and Chemicals organized by Society for Industrial Microbiology and Biotechnology, April 25 - 28, 2016, Baltimore, MD
3. Urgun-Demirtas M, Shen Y, Linville JL, Snyder SW. Bringing It All Back to Nature: A New Process to Produce Renewable Natural Gas and High Fertilizer Value Products. Residuals and Biosolids 2016 Water Environment Federation Conference on April 3–6 in Milwaukee, WI.
4. Urgun-Demirtas M. Enhanced Anaerobic Digestion and Hydrocarbon Precursor Production from Sewage Sludge (invited talk), *Bioenergy 2015,* BETO Office of EERE-DOE, June 23–24, 2015, Washington DC
5. Urgun-Demirtas M, Snyder SW., Shen Y, Linville JL, *New* Opportunities and Pathways to Produce Renewable Fuels from Waste Streams at *Bioenergy 2015,* BETO Office of EERE-DOE, June 23–24, 2015, Washington DC (poster presentation)
6. Shen Y, Linville JL, Urgun-Demirtas M, Mintz MM, Snyder SW. Current Biogas Production and Utilization at U.S. Wastewater Treatment Plants: It's All About Co-Digestion, Water and Energy 2015 Conference: Opportunities for Energy and Resource Recovery in the Changing World organized by Water Environment Federation in Washington DC (June 8-10) (poster presentation)
7. Urgun-Demirtas (invited speaker) Enhanced Anaerobic Digestion and Hydrocarbon Precursor Production. Hydrogen, Hydrocarbons, and Bioproduct Precursors from Wastewaters. Organized by BETO and Fuel Cell Technologies Offices of DOE, March 18-19, 2015, Washington, DC

REVENUE FROM BIOGAS AND NUTRIENTS

Exhibit 2: Potential Production and Value of Products and Co-Products for 2,647 Dairy Anaerobic Digesters

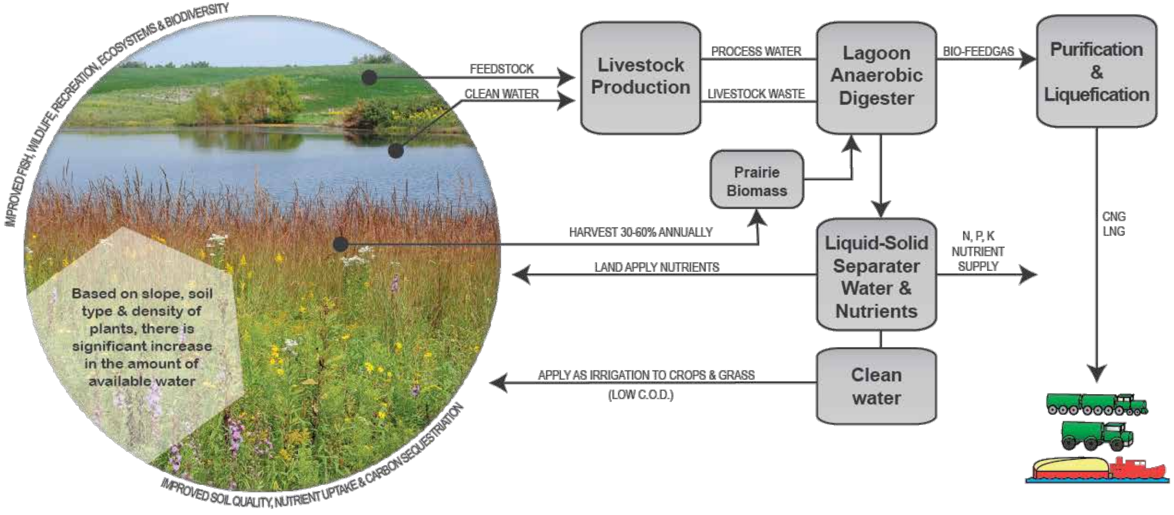
	Volume				Total Annual Dollar Value		
	Scenario 1: Low Valuation	Scenario 2: Mid Valuation	Scenario 3: High Valuation	Units	Scenario 1: Low Valuation	Scenario 2: Mid Valuation	Scenario 3: High Valuation
Inputs and Assumptions							
Number of Cows	3,974,143	3,974,143	3,974,143	Number	NA	NA	NA
Manure	108,792,165	108,792,165	108,792,165	Tons/year	NA	NA	NA
Organic Substrate/ Tipping Fees	19,849,474	19,849,474	21,197,583	Tons/year	$266,963,055	$574,997,350	$704,582,497
Outputs							
Electricity Production	11,701,222	11,701,222	12,062,917	MWh/year	$351,036,648	$894,270,196	$1,328,127,203
Co-Products							
Recovered Nitrogen (N)	331,163	331,163	341,678	Tons N/year	$311,510,905	$467,271,030	$964,216,106
Recovered Phosphorus (P)	108,782	108,782	112,287	Tons P/year	$162,302,945	$324,605,889	$418,831,327
Recovered Potassium (K)	-	-	-	Tons K/year	-	-	-
Nutrient Enriched Fiber	30,111,422	30,111,422	30,111,422	yd3/year	$180,668,532	$217,047,838	$231,255,721
GHG Offset Credits	34,327,120	34,327,120	35,177,415	MTCO2e/year	$34,327,120	$343,271,198	$879,435,367
RECs (Produced only when electricity is primary product produced)	11,701,222	11,701,222	12,062,917	RECs/year	$17,179,372	$34,358,745	$65,643,532
Subtotals							
Electricity + RECs					$368,216,021	$928,628,940	$1,393,770,735
Soil Amendments, Eco-System, and Other Products					$955,772,557	$1,927,193,305	$3,198,321,018
Total					**$1,323,988,577**	**$2,855,822,246**	**$4,592,091,753**

- National Market Value of Anaerobic Digester Products (2013) Prepared for Innovation Center for US Dairy

ROESLEIN ALTERNATIVE ENERGY

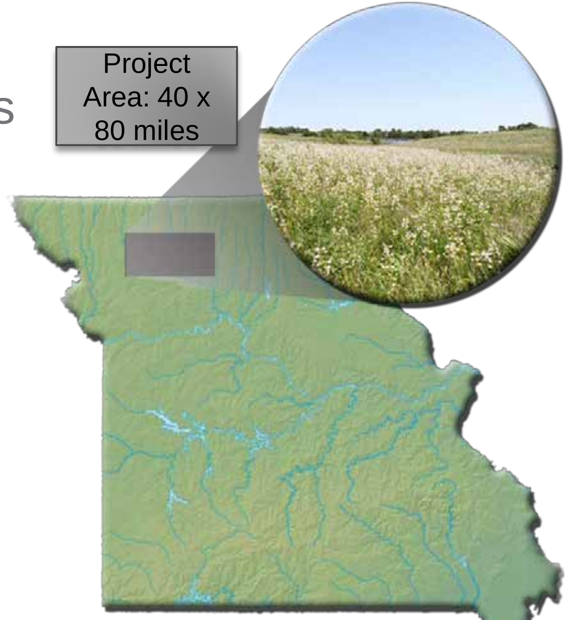
The Vision:

Providing a sustainable solution for industrial waste and livestock production by using the residue for bioenergy production & nutrient replenishment to support both the food production for the livestock and the natural prairie grasses that can supplement energy production and clean the water and air of both the livestock and wildlife, including fish.



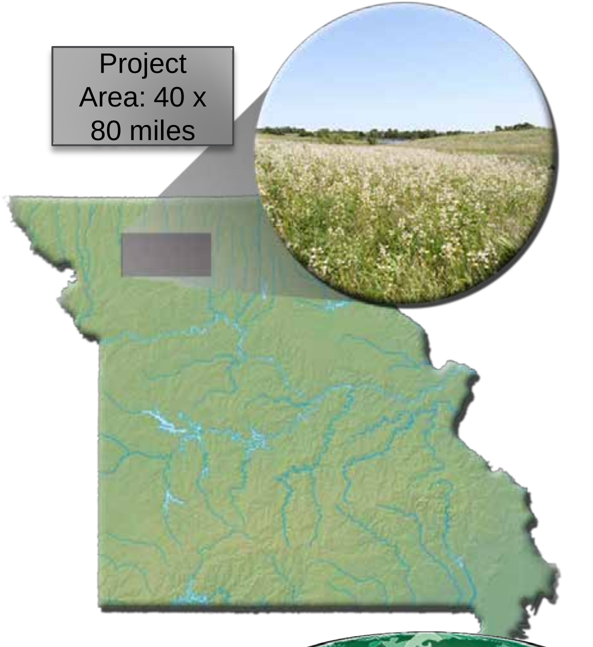
Project Overview – Horizon One Smithfield Hog Production

- Hog Manure to renewable natural gas
- Projected 2 million finishing hogs per year
- Project Value > $100M
- Start of Construction, April 2014
- Completion – Q4 2018
- First renewable natural gas injected into natural gas pipeline system, July 2016 - Ruckman

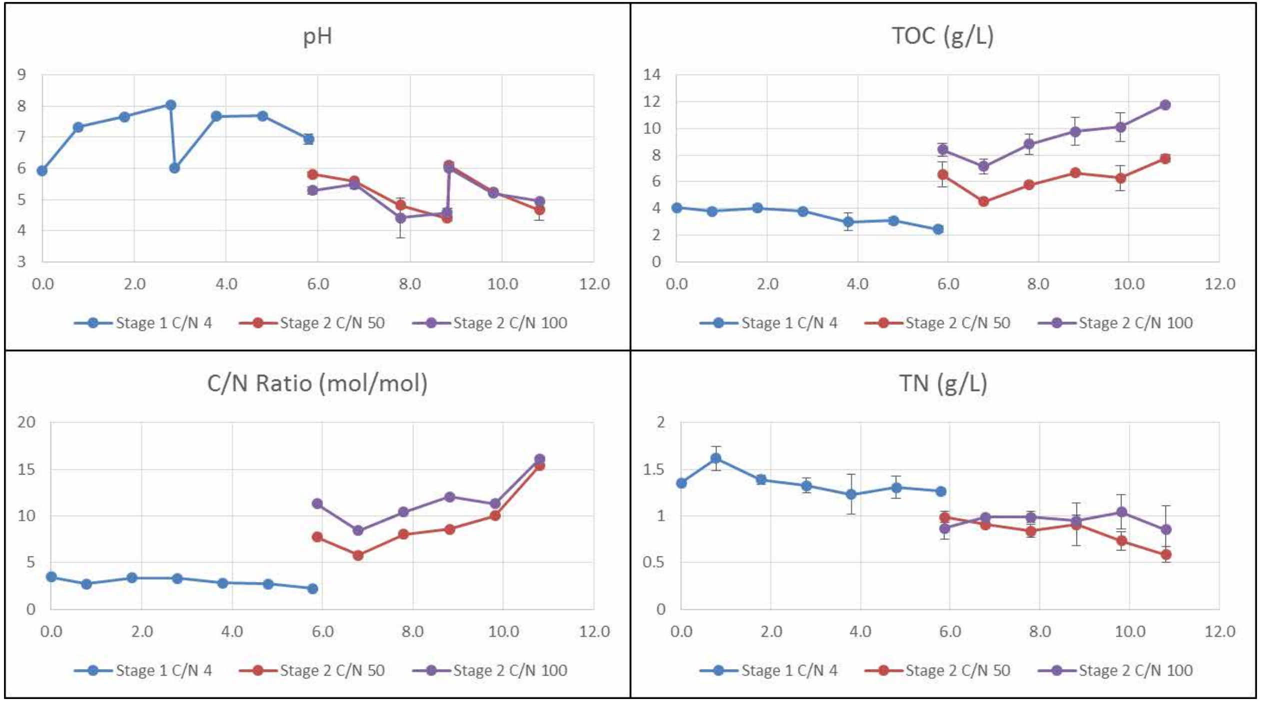


Project Overview – Horizon One Smithfield Hog Production

- Anaerobic Digestion
  - Covering 88 Existing Lagoons
  - Producing More Than 15 Million DGE per year, or **2.0 million MMBtu per year**
- Environmentally sustainable solutions
  - At hog barns, hog lagoons, and elimination of land application of hog manure
  - Improved nutrient management systems
  - Cleaner water



TASK 2-EXPERIMENTAL DATA



LIPID PRODUCTION DUE TO GLUCOSE/YEAST ADDITION, LITERATURE DATA DOES NOT EVALUATE CARBON AVAILABILITY SUFFICIENTLY

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Conversion of sewage sludge into lipids by *Lipomyces starkeyi* for biodiesel production

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2.3. Sludge medium

The ammonium nitrogen content (NH_4^+–N) of raw sewage sludge was determined and glucose as carbon-source was added to reach the desired C/N-ratio. To 50 g raw sewage sludge the required amount of glucose and distilled water were added to reach a final volume of 400 mL. Using H_3PO_4 the pH-value was adjusted to 5.0. This resulting sludge medium was sterilised, inoculated with 5 ml actively growing *L. starkeyi* cultivated as described above and incubated for 240 h.

Abstract

The potential of accumulation of lipids by *Lipomyces starkeyi* when grown on sewage sludge was assessed. On a synthetic medium, accumulation of lipids strongly depended on the C/N ratio. The highest content of lipids was measured at a C/N-ratio of 150 with 68% lipids of the dry matter while at a C/N-ratio of 60 only 40% were accumulated. Within a pH range from 5.0 to 7.5 the highest lipid accumulation was found at pH 5.0 while the highest yield per litre was pH 6.5. Although sewage sludge had no inhibitory effects on growth or accumulation on *L. starkeyi* when added to synthetic medium, there was no significant growth on untreated sewage sludge. However, pretreatment of sludge by alkaline or acid hydrolysis, thermal or ultrasonic treatment lead to accumulation of lipids by *L. starkeyi* with highest values of 1 g L^{-1} obtained with ultrasound pre-treatment. Based on the content of free fatty acids and phosphorus, lipids accumulated from sewage sludge could serve as a substrate for the production of biodiesel.

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Oil production by a consortium of oleaginous microorganisms grown on primary effluent wastewater

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General procedure for bioassays

Approximately 4 L of primary effluent wastewater were collected from Tuscaloosa Wastewater Treatment Plant as previously described. The primary effluent wastewater (400 mL) was added to nine 1 L baffled flasks. Six of the flasks were autoclaved at 121 °C for 15 min, and the remaining three flasks were utilized as negative controls. After autoclaving, the six flasks were allowed to cool to room temperature. All nine flasks were then kept overnight in the refrigerator. Before inoculation, glucose was added to each of the nine flasks to yield a concentration of 1 g L^{-1} glucose to increase the carbon in the wastewater to enhance consortium cell concentration. After each flask was well mixed by swirling each