

DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Integration of Nutrient and Water Recycling for Sustainable Algal Biorefineries

03/25/2015

ALGAE TECHNOLOGY AREA

Presenters: (1) Sridhar Viamajala, The University of Toledo;
(2) Brent Peyton, Montana State University; (3) Matthew
Fields, Montana State University

Goal Statement

Develop the science and engineering for sustainable biomass production through use of:

- Wastewater and nutrients recycled from N- and P-rich post-conversion residues.
 - Minimizes inputs of water and synthetic fertilizers.
- High lipid-producing native alkaliphilic algae.
 - Cultures tolerant to high pH may outcompete unwanted algae in these harsh environments.
 - Alkaline solutions result in higher flux of ambient inorganic carbon for lipid production.
 - Significant savings accomplished by eliminating capital and energy costs associated with CO₂ distribution.
- Stimuli-sensitive hydrogel methods for harvesting and water recovery for reuse.
 - No use of contaminating chemicals (e.g. flocculants).

Quad Chart Overview

Timeline

- Project start : 2/1/2013
- Project end : 12/31/15
- Percent complete: 66%

Barriers

- Barriers addressed
 - Al-B. Algal Fuel Production
 - Feedstock development and nutrient supply
 - Harvest - Dewatering and water recycle

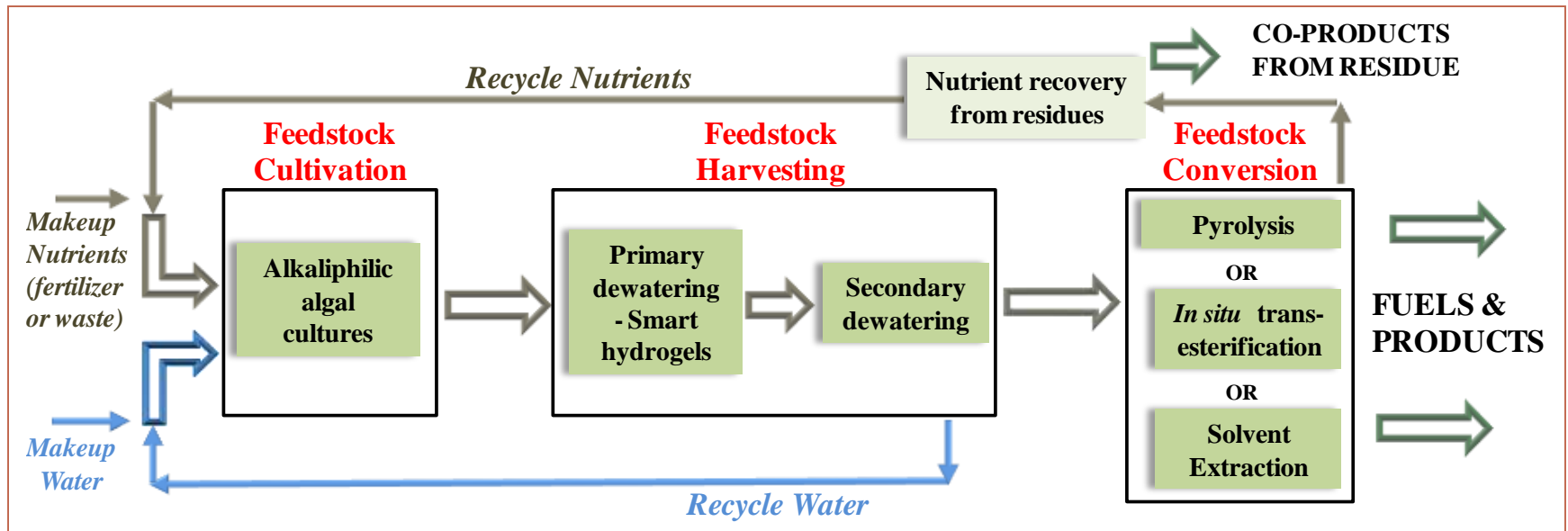
Budget

	Total Costs FY 10 – FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date)
DOE Funded	n/a	\$868,860	\$861,717	\$1,269,357
Project Cost Share (Comp.)*	n/a	\$201,377	\$213,137	\$335,579

Partners

- The University of Toledo – Lead (44%)
- Montana State University (44%)
- University of North Carolina (12%)
- AlgEvolve, Inc.
- Environmental Department, Logan City, Utah

Project Overview



PROJECT OBJECTIVES:

- Evaluate the effects of nutrient integration/recycle options on algae growth and lipid production.
- Develop low-cost water-recovery methods.
- Characterize the development, structure, and stability of microbial communities in algal systems.
- Perform economic and life cycle assessments (LCA) for sustainable algal biorefineries.

Approach

- Assess biomass productivity and nutrient utilization during growth on (1) wastewater and (2) nutrients recovered from post-conversion residues. *A.1.GN.1* and *A.1.ML.1* : 20 g/m²/d and 0.1% - Productivity and yield using wastewater (12/31/2014); *A.2.ML.1* : 20 g/m²/d and 0.1% - Productivity and yield using recycled nutrients (12/31/2015).
- Develop stimuli-responsive hydrogel-based techniques for recovering water and unused soluble nutrients. *B.2.ML.1* : 2% (20 g/L) biomass concentrations achieved after hydrogel dewatering (12/31/2015)
- Characterize bacterial and algal communities in lipid-producing alkaliphilic cultures grown in wastewater and on recycled nutrients. *C.3.ML.1* : At least one stable community characterized in wastewater and with recycled nutrients (12/31/2015).
- Assess economic and environmental impacts of the nutrient and water management alternatives using mass and energy balance data obtained from laboratory studies. *D.2.ML.1* : LCA model for integrated growth system (12/31/2015).
- Perform pilot scale growth on municipal wastewater with most productive and stable alkaliphilic strains. *D.2.ML.1* : Demonstration of algal growth at pilot scale (2000L) (12/31/2015).

Task A: Evaluation of nutrient recycling and integration options for algae growth

Subtasks:

- A.1 – Algae cultivation by nutrient integration with wastewater sources (*2/1/13 to 12/31/14*)
- A.2 – Algae cultivation by nutrient recycling from post-conversion residues (*1/1/2015 to 12/31/2015*)
 - Post extraction residues
 - Thermochemical residues

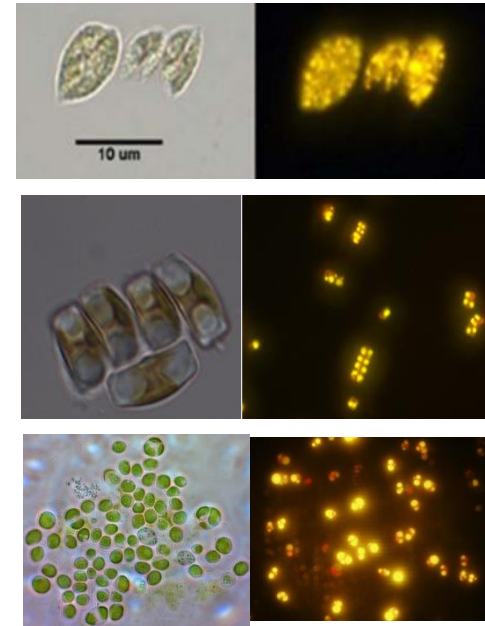
Milestones:

- 20 g/m²/day and 0.1% (1g/L) productivity and yield using wastewater (*12/31/2014*)
- 20 g/m²/day and 0.1% (1g/L) productivity and yield using recycled nutrients (*12/31/2015*)

Alkaliphilic algae

Context

- These alkaliphilic strains show high lipid productivity in well-defined media.
- High pH growth environments (i) increase driving force for CO₂ dissolution and achieve higher dissolved inorganic carbon concentrations, (ii) provide means of using low quality alkaline/saline water and (iii) may allow alkaliphilic algae to outcompete unwanted algae.
- Fatty acid profile is conducive for fuel production.



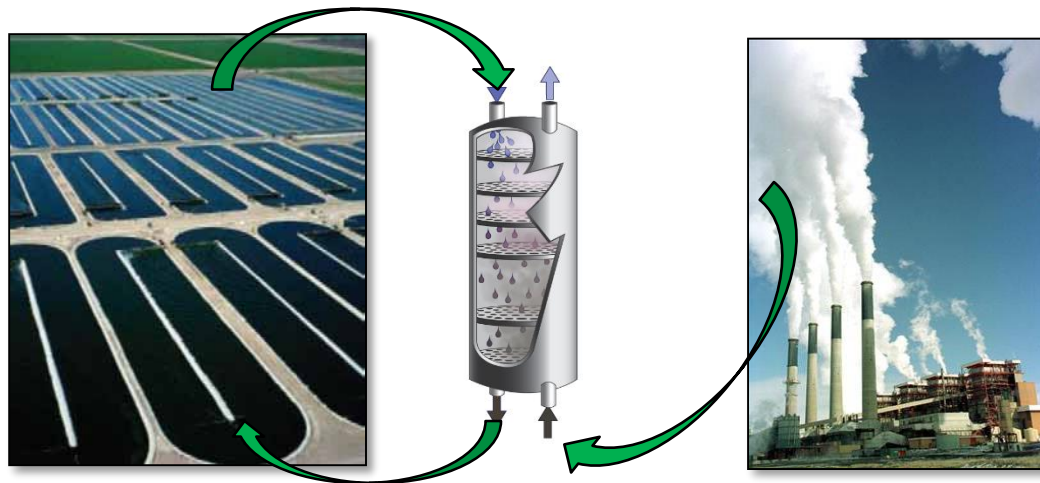
Strain	Organism	FAME yield (w/w)
WC-1	<i>Scenedesmus sp.</i>	26%
RGd-1	Diatom	46%
SLA-04	<i>Chlorella sp.</i>	42%

Fatty acid profile

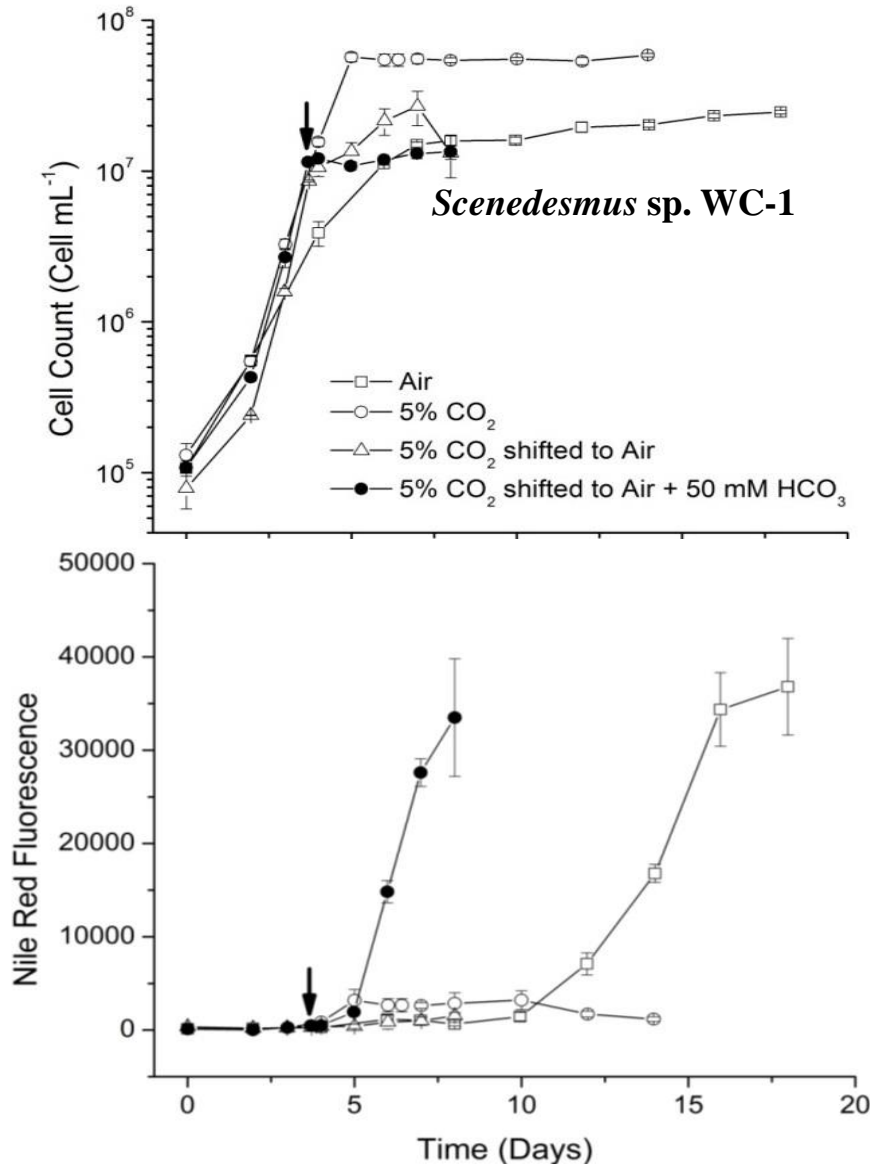


Bicarbonate as an inorganic carbon substrate

- Algal biofuel farms can use carbon dioxide captured from flue gas
 - May not be economically practical due to
 - Cost associated with transport and storage of CO₂
 - Low mass transfer efficiency (loss of CO₂ back into atmosphere)
- Bicarbonate can be produced by sorbing CO₂ in alkaline solutions (e.g. Na₂CO₃)
 - $Na_2CO_3 + CO_2 + H_2O \rightarrow 2 NaHCO_3$
 - Solutions (and solids) can be readily transported, stored or regenerated



“Bicarbonate trigger”



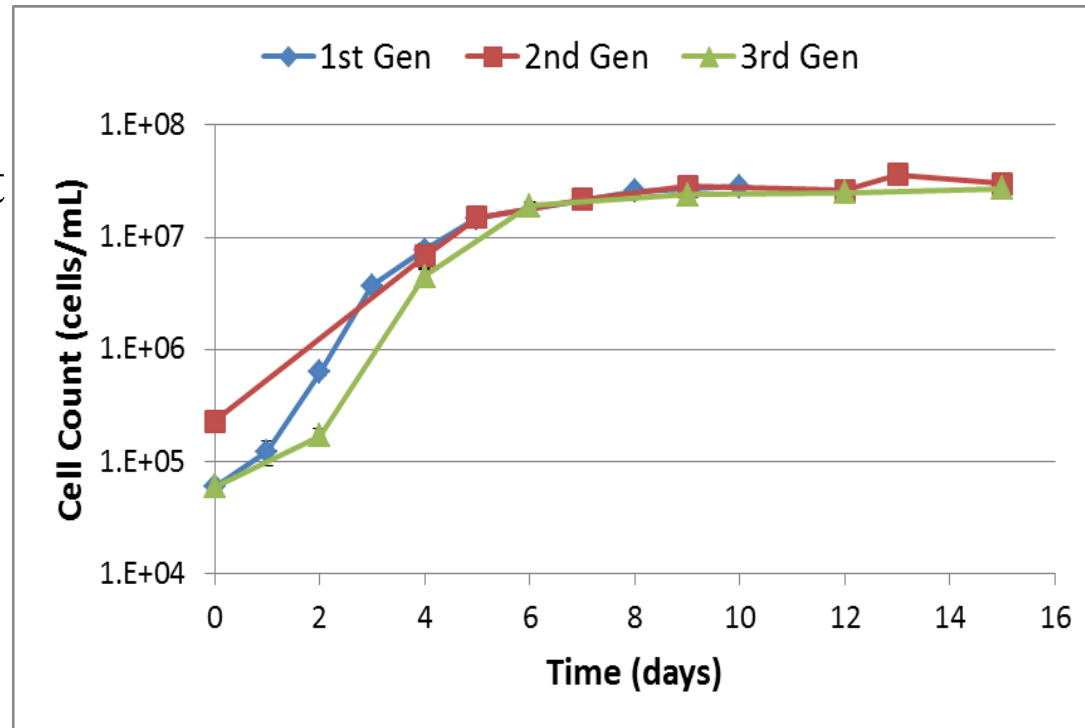
- Addition of HCO₃⁻ induces predictable TAG accumulation.
- Confirmed on over 20 Chlorophyte and diatom strains – freshwater and marine.
 - However, bicarb trigger does not always work
 - more fundamental work to be done

Gardner R, et al. 2012. Journal of Applied Phycology. 24(5): 1311-1320
Gardner R, et al. 2013. Biotechnology and Bioengineering 110(1): 87-96

Laboratory-scale cultivation on municipal wastewater (MWW)

Strain WC-1 Growth in PC Effluent

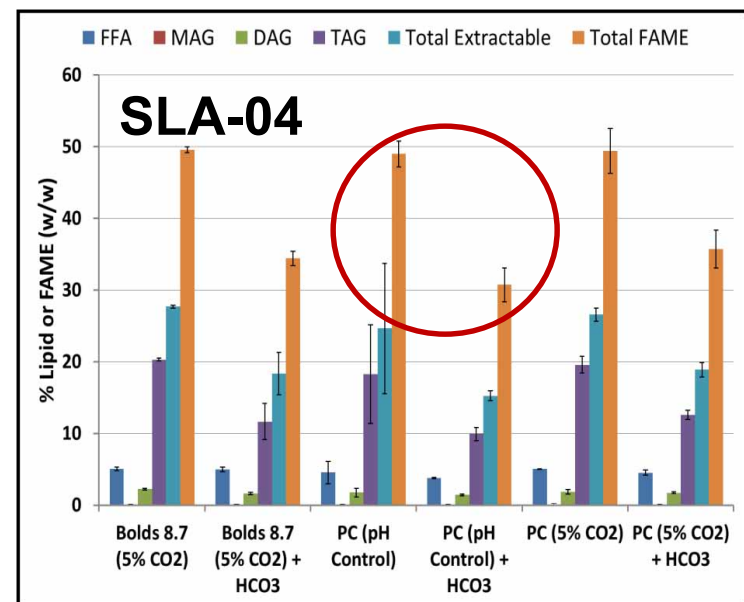
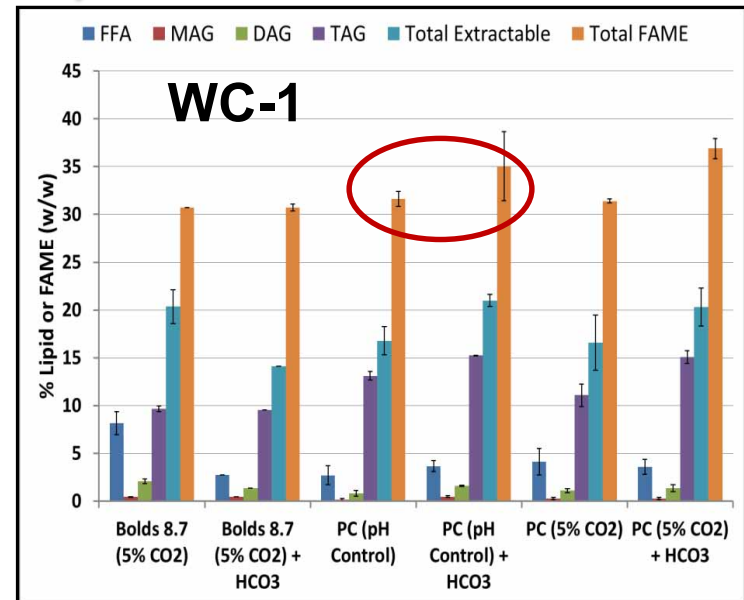
- Experiments were focused on growth in primary clarifier effluent
- WC-1 growth very similar between generations
 - Reached stationary growth ~6 days after inoculation
 - No additions (N, P etc.) necessary



Laboratory-scale cultivation on municipal wastewater (MWW) (cont.)

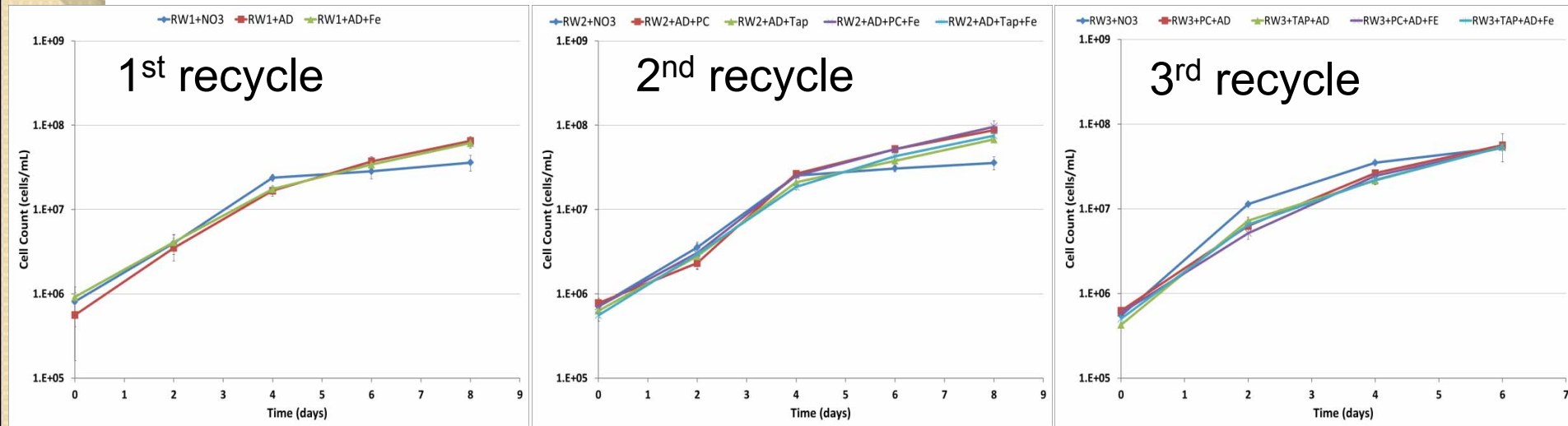
Lipid Productivity and Biofuel Potential

- High lipid content was achieved in PC effluent – 30-50% (w/w) FAME
 - Similar lipid content as in Bold's cultures
- Addition of bicarbonate increased FAME and TAG content for WC-1, but not for SLA-04
 - Multiple forms of nitrogen present in wastewater may complicate the timing of bicarbonate addition



Laboratory-scale cultivation on municipal wastewater (MWW) (cont.)

SLA-04 Growth in Recycled Harvest Water



- Recycling harvest water appears to have no inhibitory effects on growth
 - Cells reached roughly 6×10^7 cells/mL
 - Inorganic-N was depleted after 6-8 days of growth
 - Biomass production remains consistent over multiple harvest water re-use cycles

Biomass productivity calculations

Strain	Condition	μ_{\max} (day ⁻¹)	T _d (days)	CDW (g/L)	P _{vol} (g/L/day)	P _{area} (g/m ² /day)
WC-1	Bold's 8.7 (5% CO ₂)	1.69 ± 0.01	0.41 ± 0.00	2.23 ± 0.00	1.07	45.6
	Bold's 8.7 (5% CO ₂) + HCO ₃	1.65 ± 0.15	0.42 ± 0.04	1.18 ± 0.31	0.54	23.1
	PC (5% CO ₂)	1.50 ± 0.05	0.46 ± 0.02	1.52 ± 0.16	0.71	30.4
	PC (5% CO ₂) + HCO ₃	1.49 ± 0.27	0.47 ± 0.08	1.05 ± 0.33	0.48	20.4
	PC (pH Control)	1.56 ± 0.07	0.44 ± 0.02	0.75 ± 0.01	0.33	13.9
	PC (pH Control) + HCO ₃	1.62 ± 0.07	0.43 ± 0.02	1.29 ± 0.35	0.60	25.5
SLA-04	Bold's 8.7 (5% CO ₂)	2.07 ± 0.43	0.34 ± 0.07	1.68 ± 0.01	0.79	33.9
	Bold's 8.7 (5% CO ₂) + HCO ₃	1.65 ± 0.32	0.42 ± 0.08	1.28 ± 0.09	0.59	25.3
	PC (5% CO ₂)	2.22 ± 0.22	0.31 ± 0.03	1.46 ± 0.05	0.68	29.1
	PC (5% CO ₂) + HCO ₃	2.28 ± 0.02	0.30 ± 0.00	1.32 ± 0.03	0.61	26.1
	PC (pH Control)	1.55 ± 0.07	0.45 ± 0.02	1.36 ± 0.04	0.63	27.0
	PC (pH Control) + HCO ₃	1.77 ± 0.04	0.39 ± 0.01	1.19 ± 0.05	0.55	23.4

Tubular reactors of volume, V = 1.5 L;

Reactor dimensions = 7 cm (dia) × 50 cm (height)

Illuminated surface area, A = 0.035 m²

GNG
0.1% (1 g/L)

GNG
20 g/m²/day

Summary of Task A

- Indoor cultivation studies show alkaliphilic strains show high productivity on municipal wastewater
- We demonstrated successful accomplishment of our Go/No-go milestone “*20 g/m²/day and 0.1% productivity and yield using wastewater*”

Task B: Water recovery through use of stimuli-sensitive hydrogels

Subtasks:

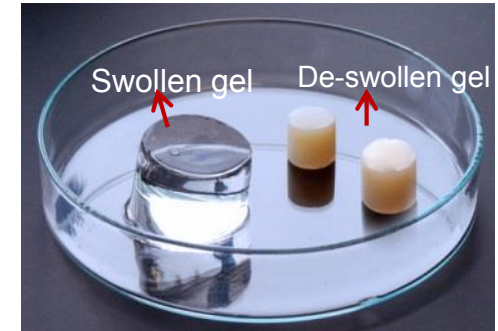
- B.1 – Gel synthesis and characterization (*2/1/13 to 12/31/14*)
 - Temperature-sensitive gels
 - pH-sensitive gels
- B.2 – Dewatering algal slurries using hydrogels (*1/1/2015 to 12/31/2015*)

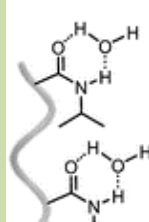
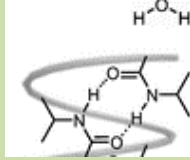
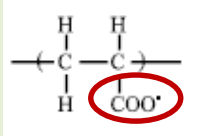
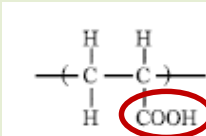
Milestone:

- 2% (20 g/L) biomass concentrations achieved after hydrogel dewatering (*12/31/2015*)

Stimuli-responsive hydrogels

- Hydrogels that absorb and release water in response to an external stimulus

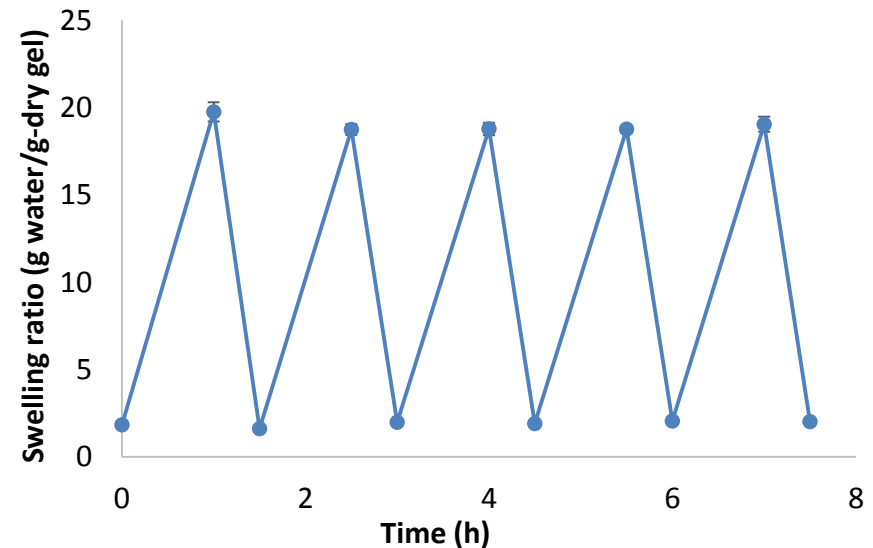
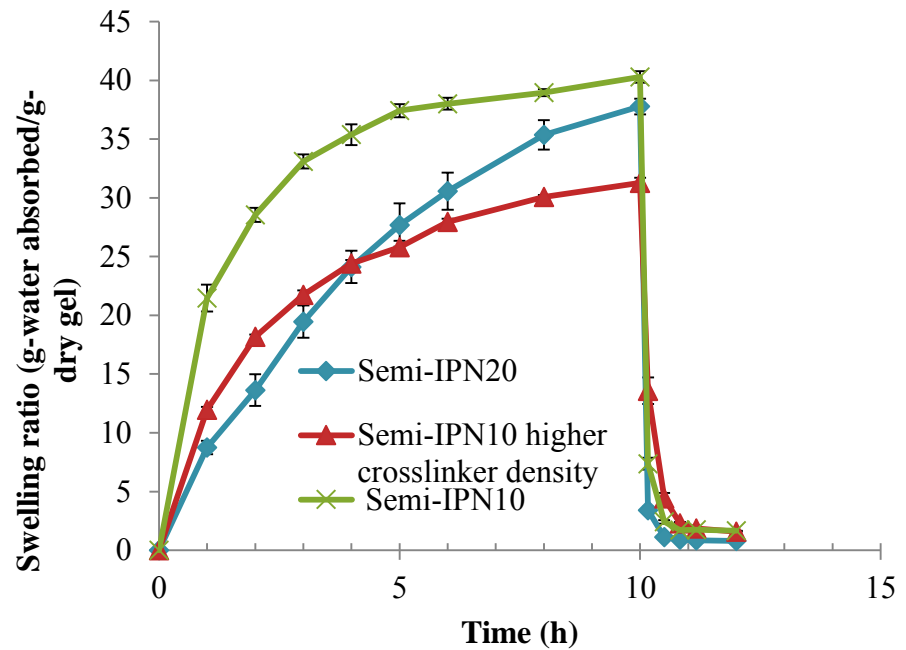


Stimulus type	Water absorption	Water release
Temperature	T < 30°C 	> 33°C 
pH sensitive	high pH (>7) 	low pH (<5) 

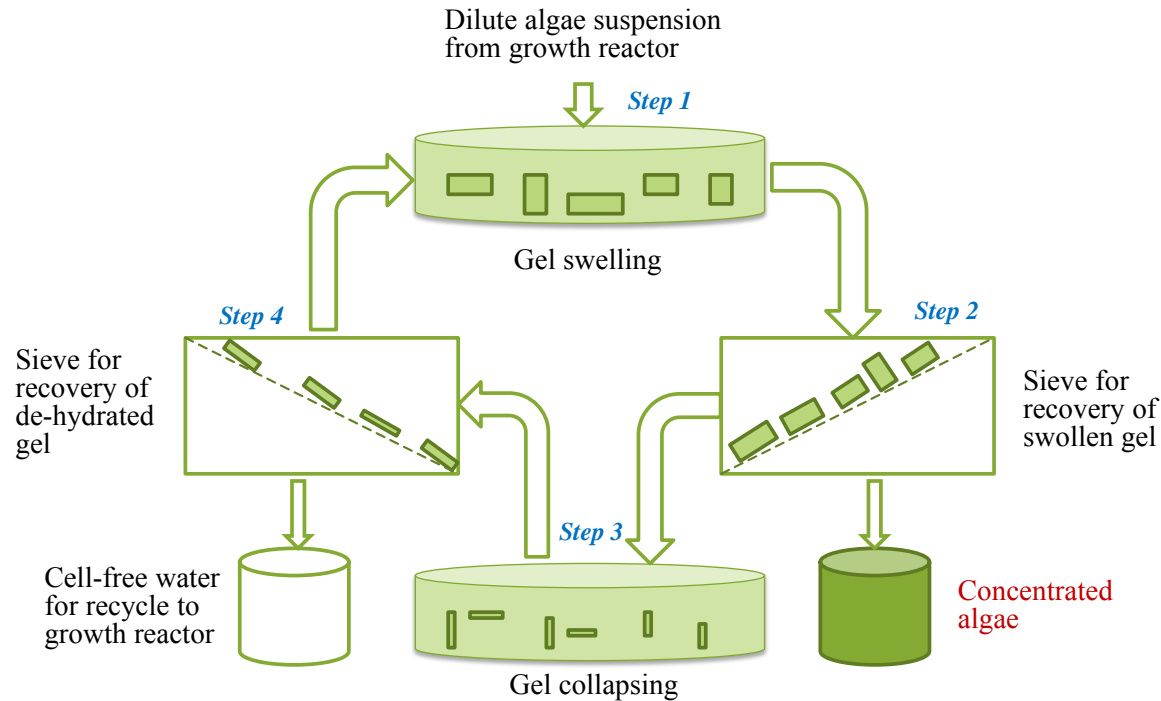
- Examples –
 - N-isopropyl acrylamide (pNIPAAm) is a temperature-sensitive hydrogel
 - Poly acrylic acid (PAA) is a pH-sensitive hydrogel

Improvements in performance of temperature-sensitive gels

- Semi-IPN 10 gels (10% PVA + 90% p-NIPAAm) showed rapid swelling and deswelling
 - Swelling ratio of 20 was achieved in 1h
 - Deswelling time = 15 min
- Gels retain performance over multiple cycles



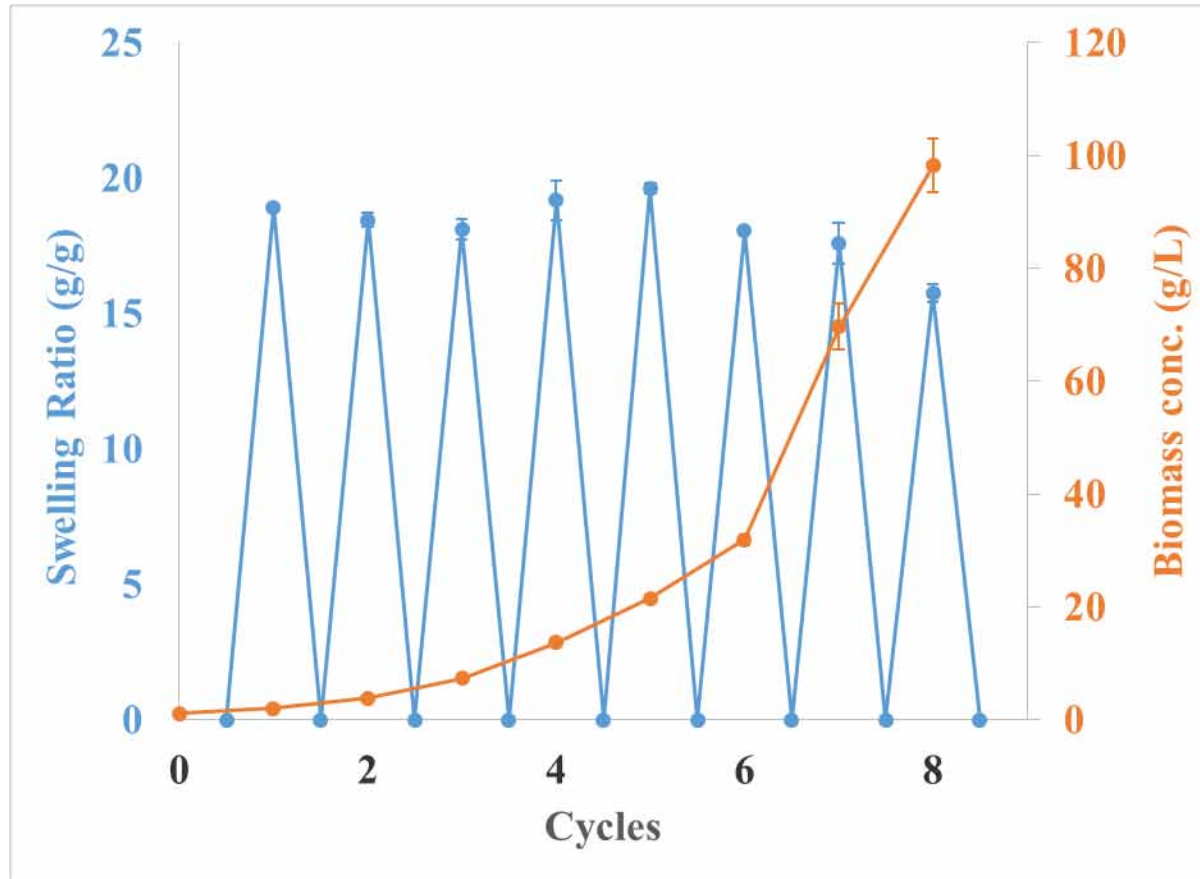
Hydrogel dewatering method



Key process parameters:

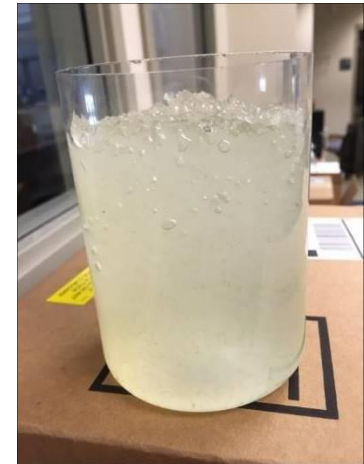
- Swelling and de-swelling rates in culture medium
- Water uptake per gram of de-hydrated gel
 - Swelling ratio
- Operating conditions
 - Swelling and de-swelling period
 - Culture-swollen gel volume ratio

Algae harvesting by cyclic hydrogel use



- Concentration of algae was ~ 100 g/L after 8 cycles.
- No significant difference in swelling ratios across cycles.
- Algae was not absorbed into hydrogels

Algae harvesting using Semi-IPN gels



Shows the variations in the culture volumes and colors when the concentrations were increased from 1.08 – 7.38 g/L.

Shows algae-free clear gels were recovered after harvesting process



Dewatering experiment in progress

Algae harvesting using Semi-IPN gels

- High gel loading used in experiment to reduce duration of swelling cycle
- Reduces processing time by a factor of ~ 5

Hydrogel	Swollen Gel to Culture Volume Ratio	Initial Concentration (g/L)	Final Concentration (g/L)	Time Required (hour)
PNIPAAm	1:2	1.12	7.37	9
PNIPAAm-PVA Semi-IPN	2:1	1.08	7.38	1.75

Conclusions for Task B

- With the hydrogel dewatering method, concentrations of up to ~100 g/L can be achieved.
- Gels can be re-used over multiple cycles without loss of gel functionality
 - High mechanical strength and elasticity
- Overall processing time could be 3-4 h – comparable with residence times of other conventional processes
- The energy costs associated with the hydrogel-dewatering could be minimized by integration with low-grade waste heat

Task C: Structure/function relationships in algal system microbial communities

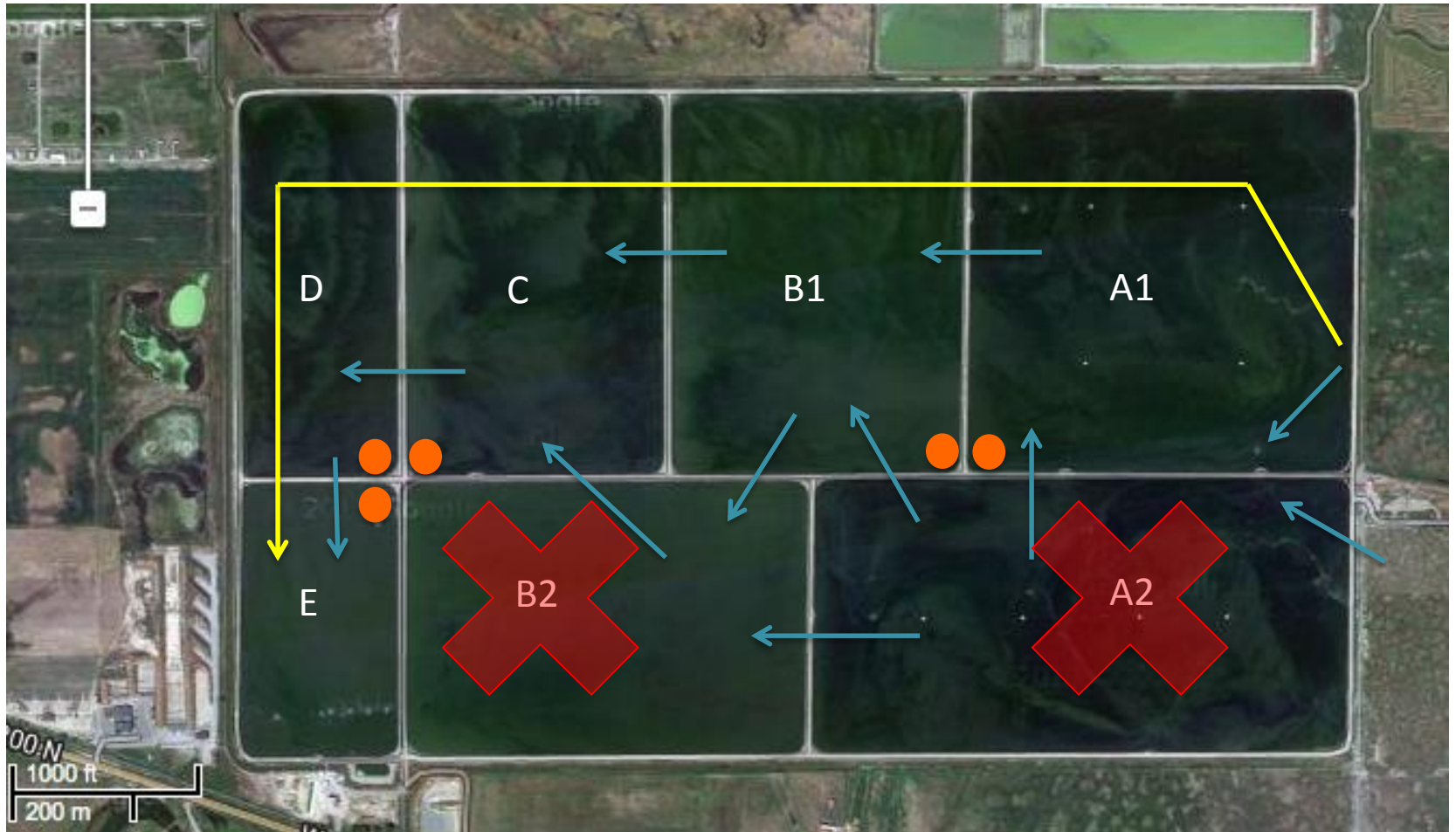
Subtasks:

- C.1 – Establish molecular methods under well-defined conditions. *(1/1/13 to 12/31/13)*
- C.2 – Microbial community characterization during growth on waste stream nutrients *(1/1/2014 to 12/31/2014)*
- C.3 – Microbial community characterization during growth on recycled nutrients *(1/1/2015 to 12/31/2015)*

Milestones:

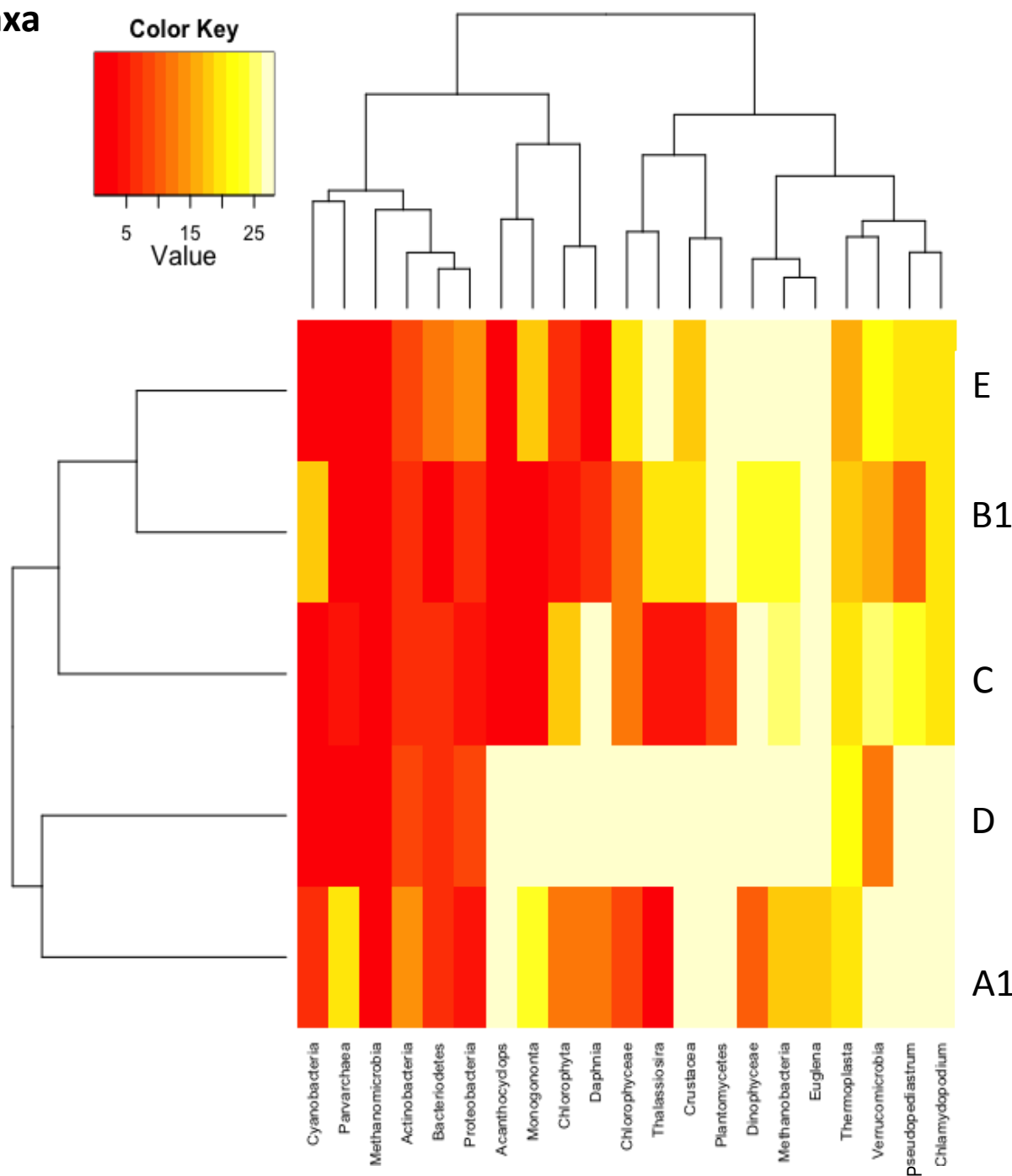
- At least one stable community characterized in wastewater and with recycled nutrients *(12/31/2015)*

Logan Wastewater Treatment Plant (WWTP)

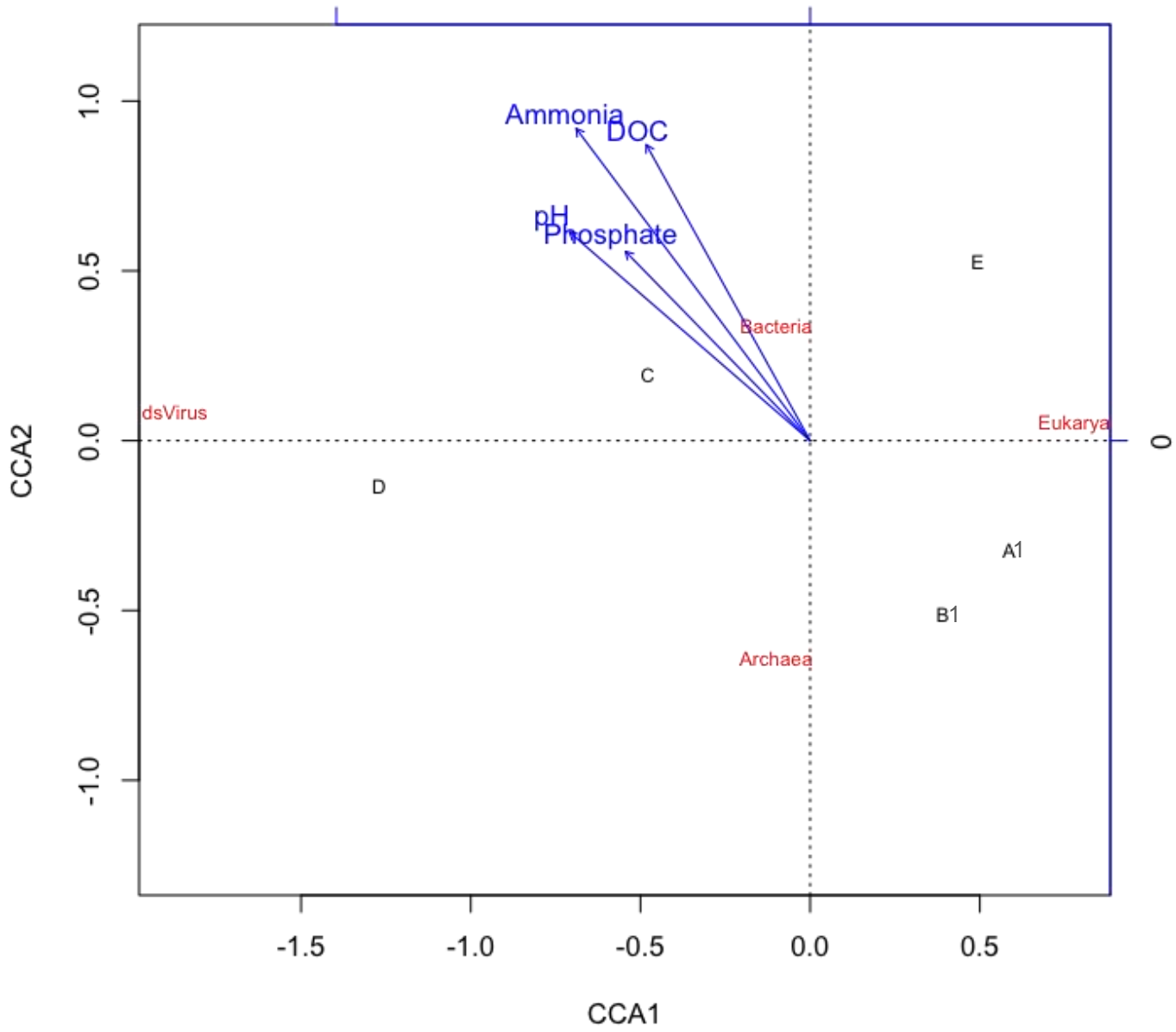


Logan WWTP heat map of taxa in each lagoon at a finer resolution

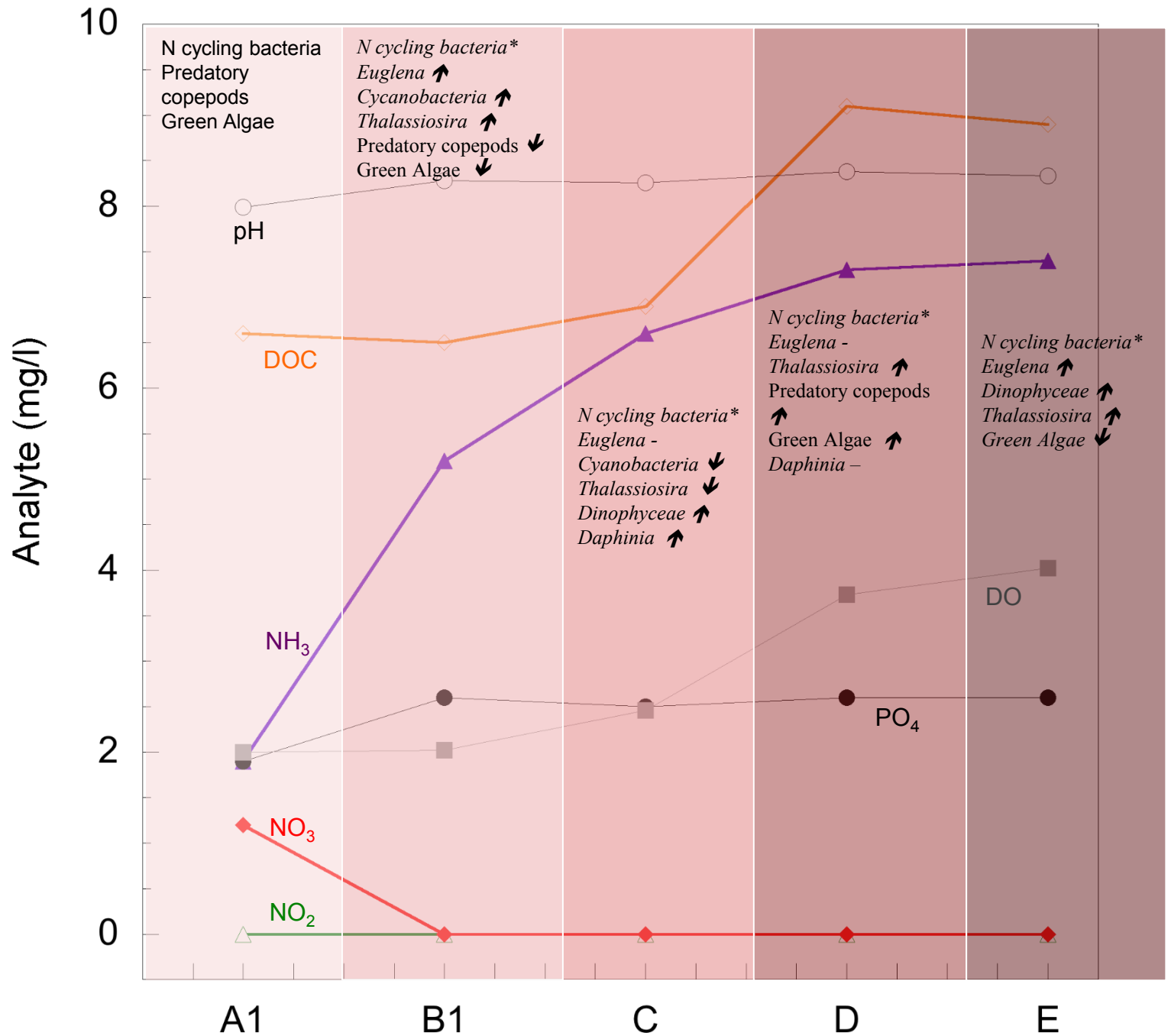
When ds viruses are not included in the analyses, ponds A and D are more similar while ponds B and E are more similar. The similarity in ponds A and D appeared to be driven by *Bacteroidetes*, *Acanthocytops*, *Crustacea*, *Plantomyces*, *Pseudopediastrum*, and *Chlamydomodium*. Pond C was unique in terms of more *Daphnia*, *Dinophyceae*, *Euglena*, and *Chlorophyta*. Ponds B and E were more similar with respect to *Thalassiosira*, *Crustacea*, *Plantomyces*, *Dinophyceae*, *Euglena*, *Thermoplasma*, and *Verrucomicrobia*.



Logan WWTP CCA of water chemistry and taxa (Eukarya, Bacteria, Achaea, ds Virus)



September 2013



Summary Based Upon November 2013 Sample

- *Bacteria* diversity is complex and many populations are increasing and decreasing.

However, overall *Bacteroidetes*, *Actinobacteria*, *Planctomycetes*, and *Verrucomicrobia* predominated. Oscillations between *Planctomycetes* and *Verrucomicrobia* most likely linked to N cycling.

- *Archaea* diversity was more stable but noticeable changes in *Parvarchaea*, *Methanomicrobia*, and *Methanobacteria*
- *Cyanobacteria* diversity was low
- Diversity between green algae (*Chlorophyta*, *Chlorophyceae*, *Pseudopediastrum*, *Chlamydomodium*) and the diatom, *Thalassiosira*, fluctuated
- Diversity between predatory copepods and dinoflagellates fluctuated
- Increase in dsViral diversity corresponded to decline in green algal diversity
- Lipid levels were low. Extreme diversity and competition might make it difficult for lipid-accumulating algae/diatoms to establish in open, wastewater environment

Task D: Process economics and LCA for integrated algal biorefineries

Subtasks:

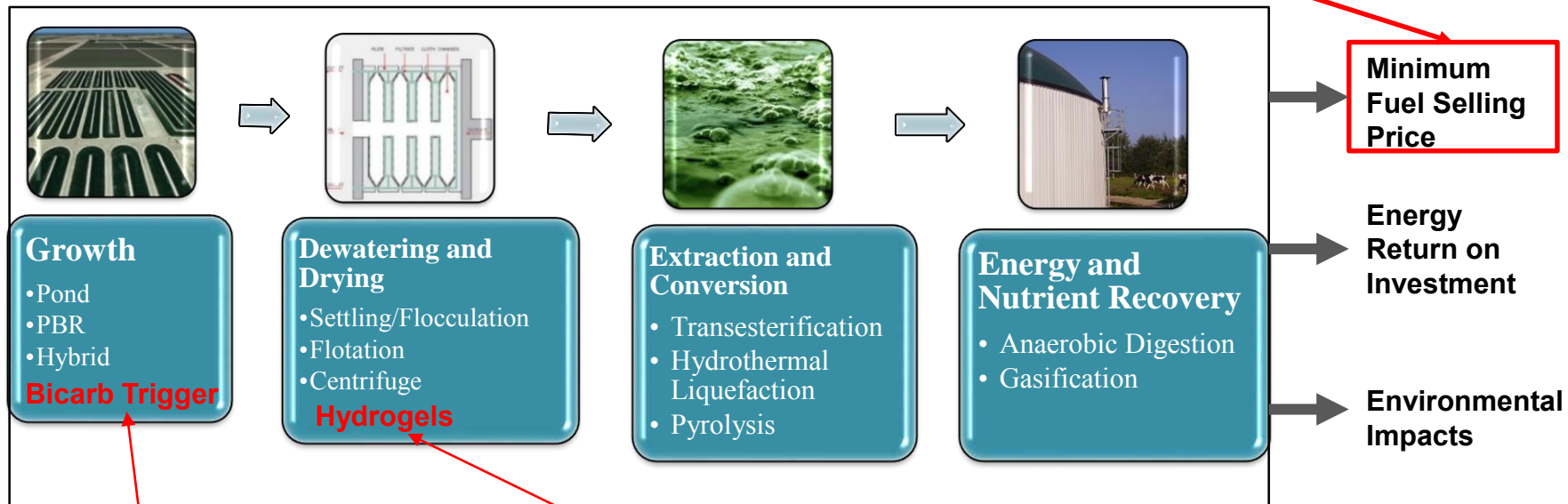
- D.1 – Process economic modeling (*1/1/13 to 12/31/15*)
- D.2 – LCA of algal biorefineries with nutrient integration and recycling (*1/1/13 to 12/31/15*)

Milestones:

- D.2.ML.1 : LCA model for integrated growth system (*12/31/2015*)

Approach

Economic and Financial Criteria (UNC)
Cost of Capital (Loan Guarantees, Project Financing)
Accelerated Depreciation
Tax Incentives



MSU microbial growth enhancement trials

UT results from testing of stimuli-sensitive hydrogels

Performance Analysis

Baseline

13.2 g/m²/d
growth
productivity,
25% lipid
content

Case 2

Replace
dissolved air
flotation with
hydrogels

Case 2, AD

Include anaerobic
digestion for
energy and nutrient
recovery

Case 2, AD, Bicarb

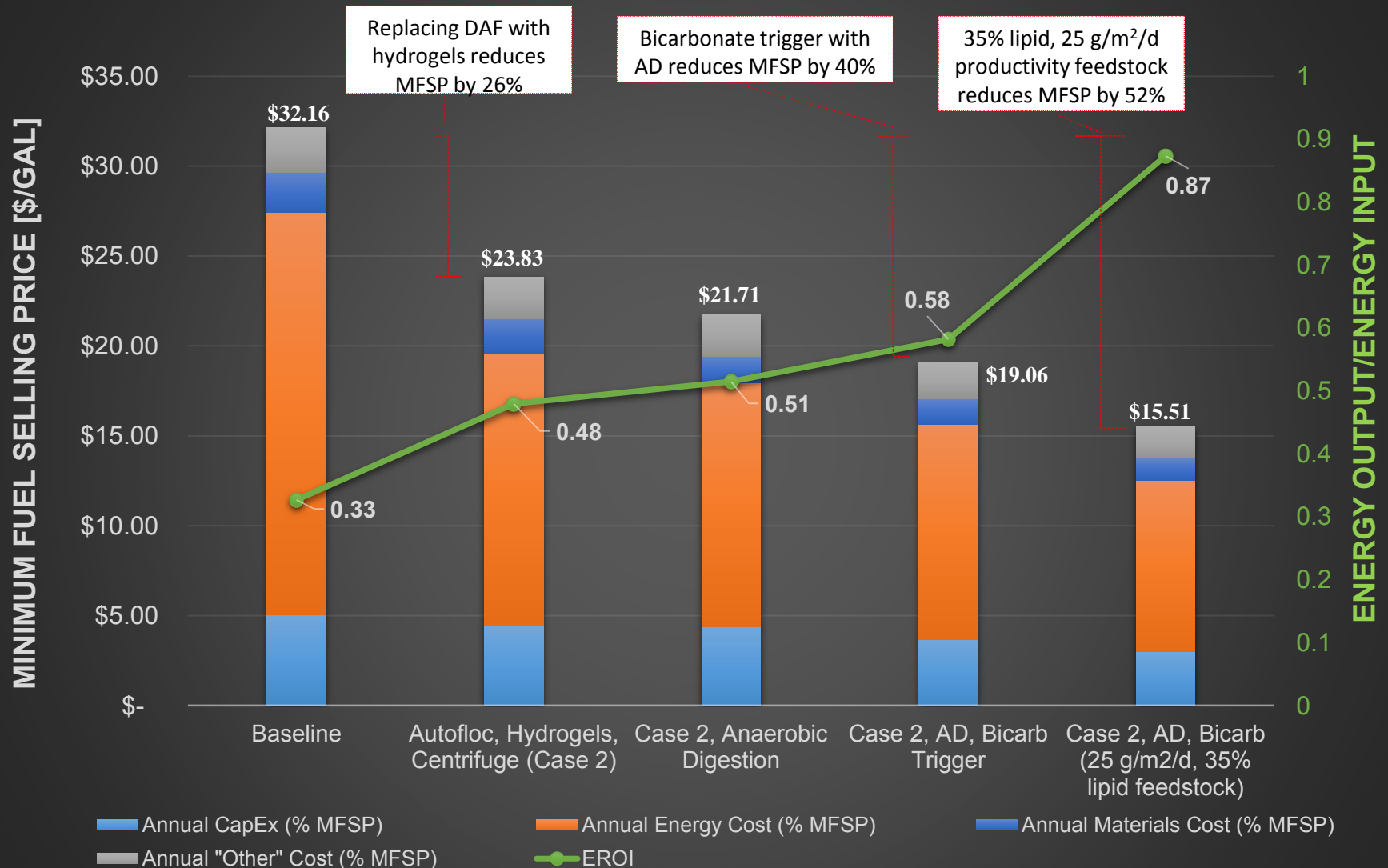
Addition of
bicarbonate
triggering

Case 2, AD, Bicarb (25 g/m²/d, 35% Lipid)

Strain with higher
documented lipid
content and growth
productivity is
utilized

- **Minimum fuel selling price (MFSP):** the price per gallon of final biofuel product such that the project has a net present value (NPV) of zero
- **Energy return on investment (EROI):** the ratio of energy output in final product to energy inputs required to manufacture the product

Production System Performance: MFSP and EROI



5. Future Work (Year 3)

- Task A.2 – Algae cultivation by nutrient recycling from post-conversion residues (*1/1/2015 to 12/31/2015*)
 - Post extraction residues
 - Thermochemical residues
- Task B.2 – Dewatering algal slurries using hydrogels (*1/1/2015 to 12/31/2015*)
- Task C.3 – Microbial community characterization during growth on recycled nutrients (*1/1/2015 to 12/31/2015*)
- Task D – Continue to develop process economic and LCA models
- Task E – Pilot-scale evaluation of growth on municipal wastewater
 - E.1 Pilot-scale open pond evaluation
 - E.2 Pilot-scale photobioreactor evaluation

Additional Slides

(Not a template slide – for information purposes only)

- *The following slides are to be included in your submission for Peer Evaluation purposes, but will **not** be part of your oral presentation –*
- *You may refer to them during the Q&A period if they are helpful to you in explaining certain points.*

Publications and Presentations

Publications:

- Kesaano, M.; Gardner, R.D.; Moll, K.; Lauchnor, E.; Gerlach, R.; Peyton, B.M.; Sims, R.C. (2015): Dissolved inorganic carbon enhanced growth, nutrient uptake, and lipid accumulation in wastewater grown microalgal biofilms. *Bioresource Technology*. Accepted Dec 22, 2014, DOI: [10.1016/j.biortech.2014.12.082](https://doi.org/10.1016/j.biortech.2014.12.082)
- Mudiyansele, A.Y.; Yao, H.; Viamajala, S.; Varanasi, S.; Yamamoto, K (2014): Efficient Production of Alkanolamides from Microalgae. *Ind. Eng. Chem. Res.* Accepted Dec 22, 2014. **B:** 10.1021/ie503980g
- Abel, G. A.; Nguyen, K.O.; Viamajala, S.; Varanasi S.; Yamamoto K (2014): Cross-metathesis approach to produce precursors of nylon 12 and nylon 13 from microalgae. *RSC Advances* 4: 55622-55628 DOI: 10.1039/C4RA10980E
- Mudiyansele, A.Y.; Viamajala, S.; Varanasi S.; Yamamoto K (2014): Simple Ring-Closing Metathesis Approach for Synthesis of PA11, 12, and 13 Precursors from Oleic Acid. *ACS Sustainable Chemistry and Engineering*. 2 (12), pp 2831–2836. DOI: 10.1021/sc500599u
- Lohman, E.J.; Gardner, R.D.; Halverson, L.; Peyton, B.M.; Gerlach, R. (2014): Carbon Partitioning in Lipids Synthesized by *Chlamydomonas reinhardtii* when Cultured Under Three Unique Inorganic Carbon Regimes *Algal Research*. pp. 171-180. DOI: 10.1016/j.algal.2014.08.001
- Fields, M.W.; Hise, A.; Lohman, E.J.; Bell, T.; Gardner, R.D.; Corredor, L.; Moll, K.; Peyton, B.M.; Characklis, G.W.; Gerlach R. (2014): Sources and Re-sources: Importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. *Applied Microbiology and Biotechnology*. 98(11):4805-4816. DOI: [10.1007/s00253-014-5694-7](https://doi.org/10.1007/s00253-014-5694-7)
- Bernstein, H.C.; Kesaano, M.; Moll, K.; Smith, T.; Gerlach, R.; Carlson, R.P.; Miller, C.D.; Peyton, B.M.; Cooksey, K.E.; Gardner, R.D.; Sims, R.C. (2014): Direct measurement and characterization of active photosynthesis zones inside wastewater remediating and biofuel producing microalgal biofilms. *Bioresource Technology*. 156:206–215. DOI:[10.1016/j.biortech.2014.01.001](https://doi.org/10.1016/j.biortech.2014.01.001)
- Gardner, R.D.; Lohman, E.J.; Cooksey, K.E.; Gerlach, R.; Peyton, B.M. (2013): Cellular Cycling, Carbon Utilization, and Photosynthetic Oxygen Production during Bicarbonate-Induced Triacylglycerol Accumulation in a *Scenedesmus* sp. *Energies*, 6(11), 6060-6076; DOI:[10.3390/en6116060](https://doi.org/10.3390/en6116060)
- Lohman, E.J.; Gardner, R.D.; Halverson, L.; Macur, R.; Peyton, B.M.; Gerlach, R. (2013): An Efficient and Scalable Extraction and Quantification Method for Algal Derived Biofuel. *Journal of Microbiological Methods*. **94(3)**:235–244. DOI: [10.1016/j.mimet.2013.06.007](https://doi.org/10.1016/j.mimet.2013.06.007)

Publications and Presentations

Patents:

- Shao H, Vadlamani A, Viamajala S, Varanasi S, Relue P. 2013. Enzymatic digestion of microalgal biomass for lipid, sugar and protein recovery. US Serial No.: 61/877,497 filed September 13, 2013
- Yamamoto K., Viamajala S., Varanasi S., Nguyen K., Abel G., Mudiyansele A. Y., Cross Metathesis Approach to C12 and C13 Fatty-Chain Amino Esters from Oleic Acid Derivatives, (US Patent Pending) #1-56166/D2014-06
- Yamamoto K., Viamajala S., Varanasi S., Nguyen K., Mudiyansele A. Y Ring Closing Metathesis Approach To Produce Precursors Of Nylon 11, 12, And 13 From Oleic Acid (US Patent Pending) #1-56167/D2014-39
- Yamamoto K., Viamajala S., Varanasi S., Nguyen K., Mudiyansele A. Methods for Production of Fatty Acid Alkanolamides (FAAAs) from Microalgae Biomass. (US Patent Pending) #1-56670/D2017-07
- Maddi B.; Viamajala, S.; Varanasi, S. 2011. Thermal Fractionation of biomass of non-lignocellulosic origin for multiple high-quality biofuels (US Patent Pending) #13/294510. (Continuation in-part filed on Jan 3, 2015)

Presentations:

- Vadlamani A, Weir J, Zhao X, Viamajala S, Varanasi S. “Assessment of temperature- and pH-sensitive hydrogels for dewatering dilute algal suspensions” Platform presentation at the 2014 Annual AIChE conference, Atlanta GA. (November 16-21, 2014)
- Yapa AM, Viamajala S, Varanasi S, Yamamoto K. “A new route for producing nylon 11, 12 and 13 precursors from oleic acid” Platform presentation at the 2014 Annual AIChE conference, Atlanta GA. (November 16-21, 2014)
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- Vadlamani A, Viamajala S, Varanasi S. “Growth and Lipid Accumulation Kinetics of Alkaliphilic Microalgae during Phototrophic and Mixotrophic Conditions.” Poster presented at the 7th Annual Algae Biomass Summit, Orlando, FL (September 30 – October 3, 2013)
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- Lohman, E.J.; Gardner, R.D.; Pederson, T.; Cooksey, K.; Peyton, B.M.; Gerlach, R. Lipid Profiling, Carbon Partitioning, and Inorganic Carbon Optimization to Enhance Growth and Lipid Accumulation in Microalgae. Platform Presentation. Algae Biomass Summit 2013, Orlando, FL, September 30-October 03, 2013
- Gerlach, R.; Lohman, E.J.; Gardner, R.D.; Halverson, L.; Peyton, B.M. A High-Throughput, Efficient and Scalable Extraction and Quantification Method for Algal Derived Biofuel. Poster Presentation. Algae Biomass Summit 2013, Orlando, FL, September 30-October 03, 2013
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- Moll, K.M.; Gardner, R.D.; Newhouse, H.; Peyton, B.M. 454 Pyrosequencing: A Novel Approach for Determination of Unialgal Strains. Poster Presentation. Algae Biomass Summit 2013, Orlando, FL, September 30-October 03, 2013
- Lohman, E.J.; Gardner, R.D.; Halverson, L.; Peyton, B.M.; Gerlach, R. (2013): Lipid profiling of *Chlamydomonas reinhardtii* grown under three different inorganic carbon regimes. Platform Presentation. Montana Biofilm Meeting, Bozeman, MT, July 14-16, 2013

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Presentations (cont.):

- Lohman, E.J.; Gardner, R.D.; Halverson, L.; Peyton, B.M.; Gerlach, R. (2013): Lipid profiling of *Chlamydomonas reinhardtii* grown under three different inorganic carbon regimes. Poster Presentation. Montana Biofilm Meeting, Bozeman, MT, July 14-16, 2013
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