Integration of Nutrient and Water Recycling for Sustainable Algal Biorefineries

05/23/2013
BETO 2013 PEER REVIEW

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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 inorganic carbon for lipid production.
  - Significant savings accomplished by eliminating capital and energy costs associated with CO_2 distribution.
- Stimuli-sensitive hydrogel methods for harvesting and water recovery for reuse.
  - Low-energy .
  - No use of contaminating chemicals (e.g. flocculants).

Quad Chart Overview

Timeline

- Project start : 2/1/2013
- Project end : 12/31/15
- Percent complete: 5%

Barriers

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

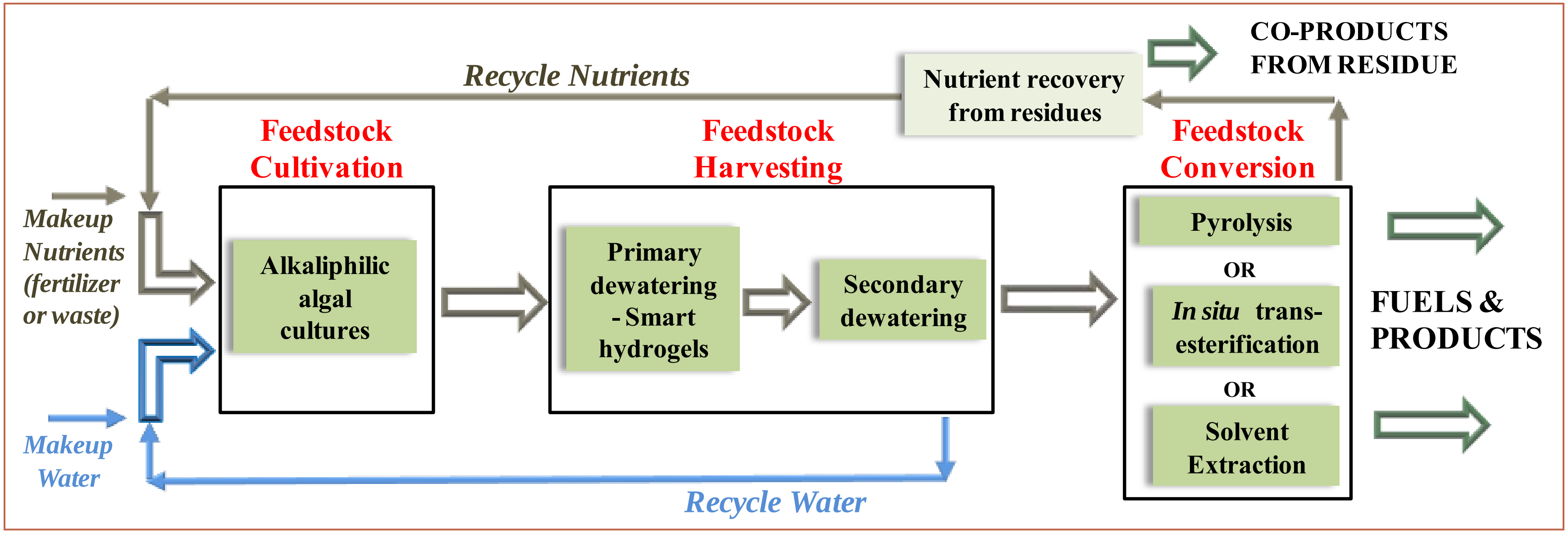
Budget

- Total project funding
  - DOE share: $2,999,934
  - Contractor share: $750,092
- FY12 Funding - n/a
- FY13 Funding - $651,645
- ARRA Funding – n/a

Partners

- Montana State University
- University of North Carolina
- AlgEvolve, Inc.
- Environmental Department, Logan City, Utah
- U Toledo is lead and partners are subcontractors

Project Overview



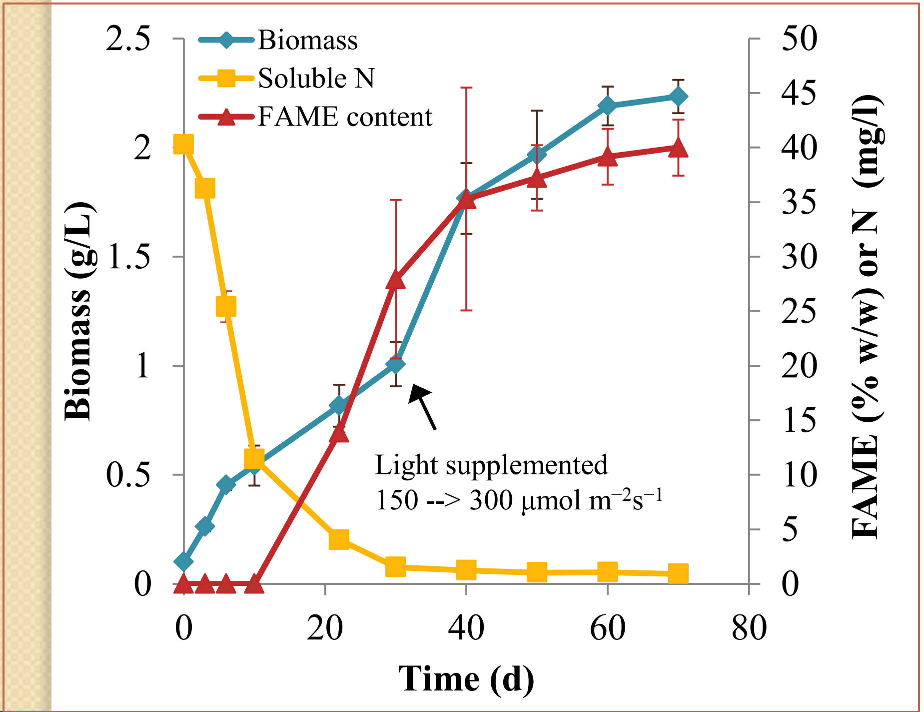
PROJECT OBJECTIVES:

- 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.

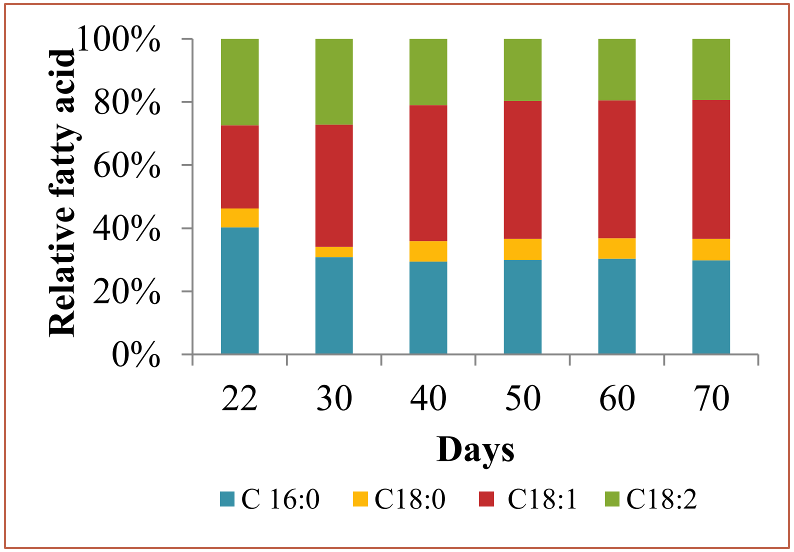
Overview – Alkaliphilic algae

Strain	Organism	FAME yield (w/w)	FAME productivity (gal/acre/yr)
WC-1	*Scenedesmus sp.*	26%	6750
RGd-1	*Phaeodactylum sp.*	46%	4200
SLA-04	*Chlorella sp.*	42%	3200

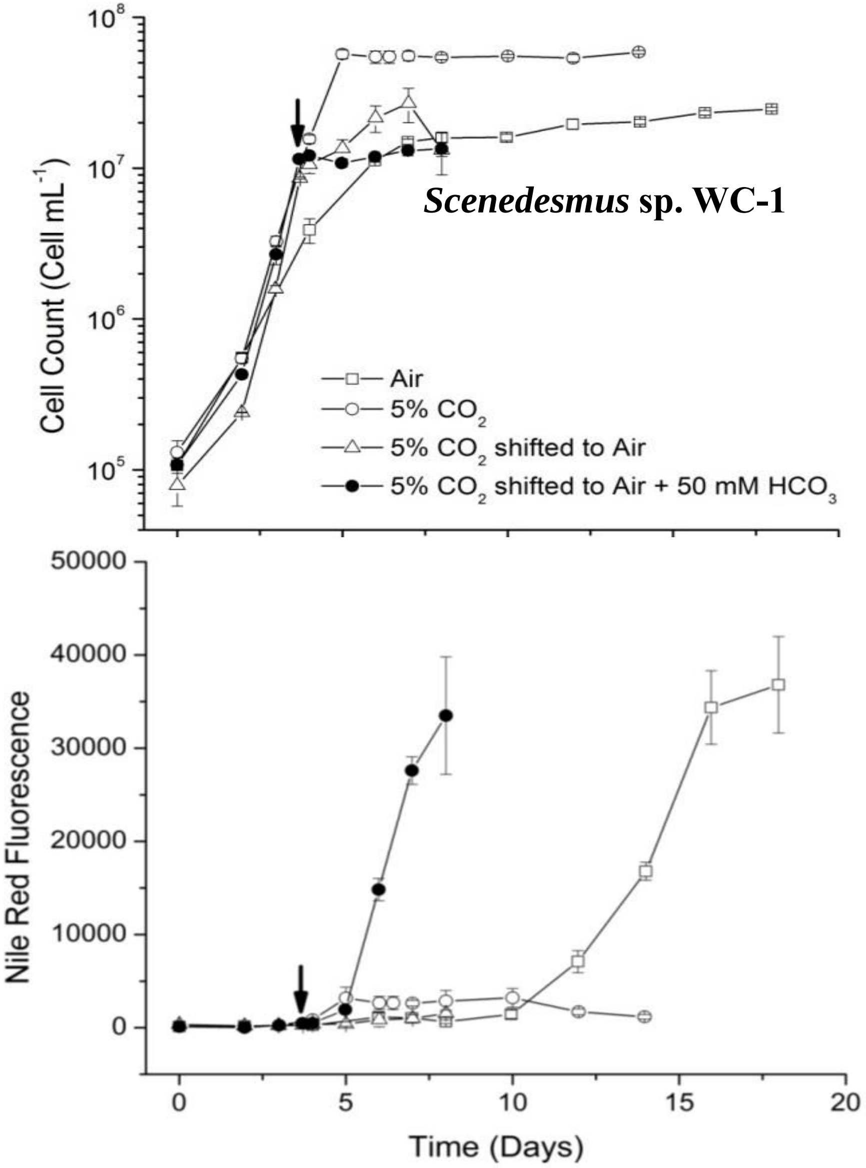
- All alkaliphilic strains show high lipid productivity.
- Fatty acid profile is conducive for fuel production.



SLA-04



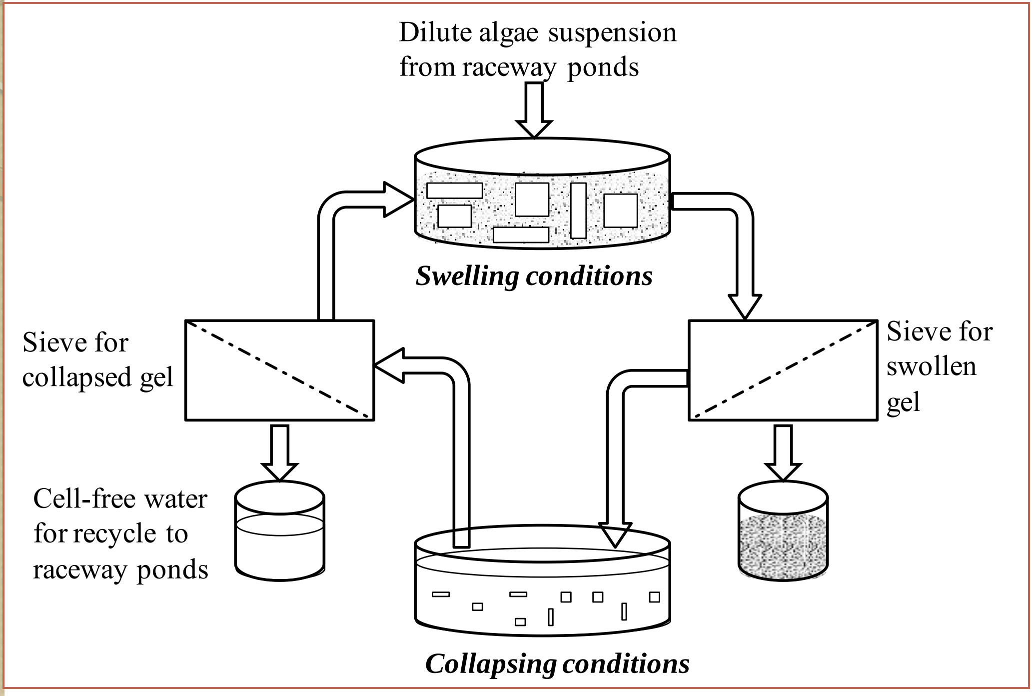
Overview – "bicarbonate trigger"



- Addition of HCO_3^- induces predictable TAG accumulation.
- Confirmed on over 20 Chlorophyte and diatom strains – freshwater and marine.
- Carbonates are cheap and environmentally sustainable – can be easily regenerated by addition of CO_2.
- If appropriately integrated, the HCO_3^-may be derived from anaerobically digested animal waste that typically contains 7-10g/L of carbonates (in addition to high N, P and organic C).

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

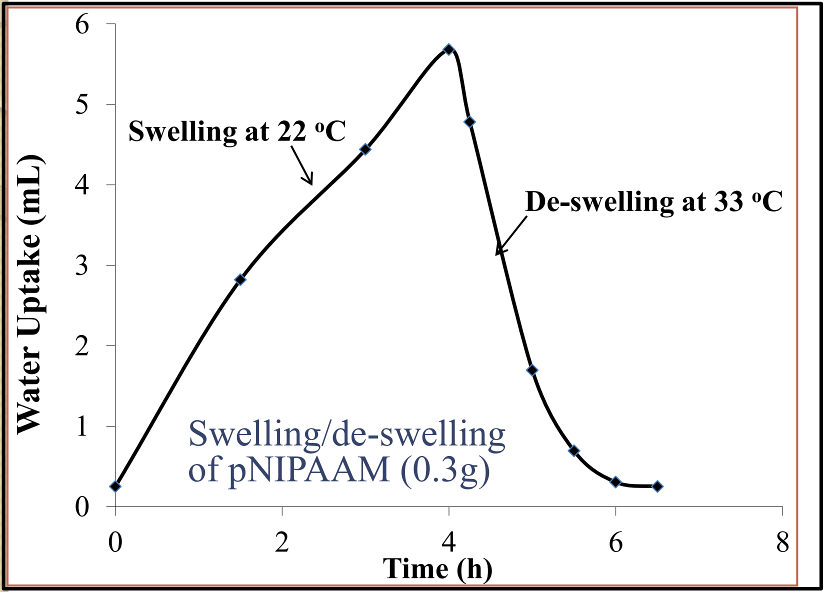
Overview – "smart" hydrogels

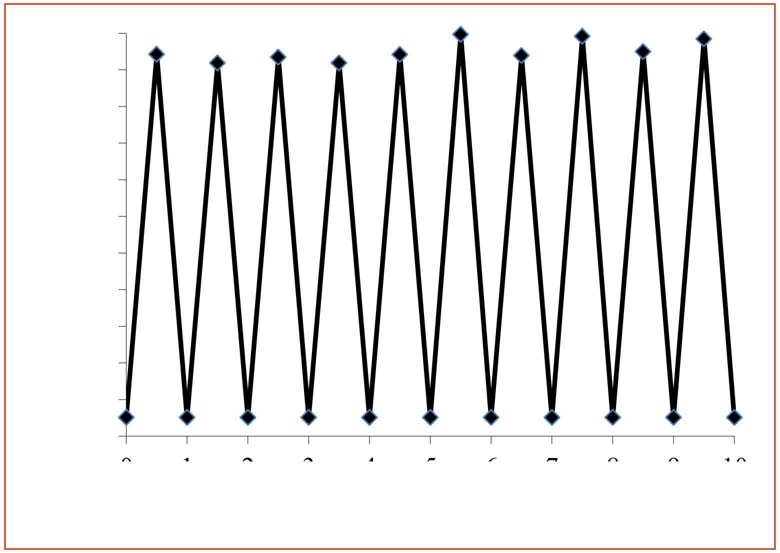


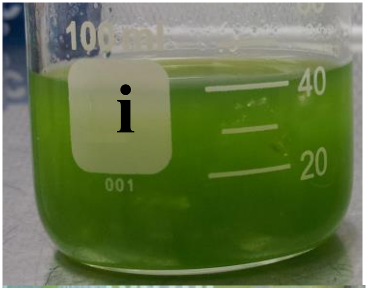
- Superabsorbent materials capable of reversibly "soaking-up" large quantities of culture media.
- Can be responsive to various kinds of stimuli (swell and shrink).

- Temperature-responsive – *e.g.* poly-N-isopropyl acrylamide (pNIPAAM) swells at room temperature and shrinks at T >32 °C.
- pH-responsive – *e.g.* poly-acrylic acid (PAAc) cross-linked with polycaprolactone and hydroxymethylmethacrylate (AC-PCL-HEMA) swells at pH>10 and collapses at pH<7. pH-swing can be accomplished through manipulation of CO_2 partial pressure.

Overview – “smart” hydrogels (cont.)







“before”
(~1.5 g-cells/L)

“after”
(~80 g-cells/L)

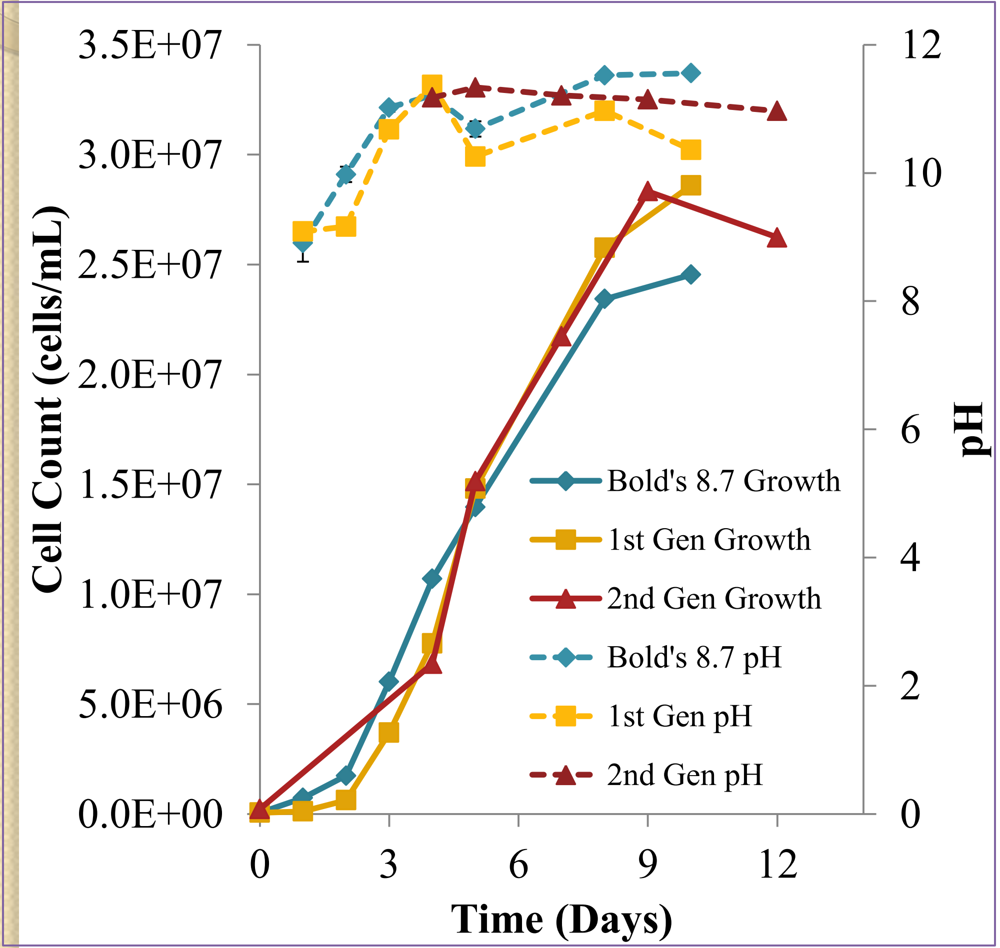
- Rapid uptake and release of water. Gels absorb up to 20x their mass in 4h. Cells don’t penetrate through gel pores.
- Gels can be reused over multiple cycles without loss in performance. We have reused gels over a period of >6 months.
- Concentrations of (at least) 8% (w/v) can be achieved . Cell loss <1%.

1 - Approach

- Assess biomass productivity and nutrient utilization during growth on (1) municipal wastewater and anaerobically-digested dairy manure (2) nutrients recovered from post-conversion residues. *A.1.GN.1* and *A.1.ML.1* : 20 g/m^2/d and 0.1% - Productivity and yield using wastewater; *A.2.ML.1* : 20 g/m^2/d and 0.1% - Productivity and yield using recycled nutrients.
- Develop stimuli-responsive hydrogel-based techniques for recovering water and unused soluble nutrients. *B.2.ML.1* : Demonstrate recovery and re-use of at least 90% of culture media.
- 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.
- 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* : Perform LCA (net carbon footprint and water demand) for growth systems integrated with wastewater and recycled nutrients.
- Perform pilot scale growth on municipal wastewater with most productive and stable alkaliphilic strains. *D.2.ML.1* : Demonstrate productive and stable growth on wastewater at 2000L scales.

2 - Technical Accomplishments/Results

Growth on Municipal Wastewater



Experimental conditions

- Media: Primary clarifier effluent from the Bozeman Wastewater Plant. Particles allowed to settle and “clear” supernatant used for growth.
- Strain: WC-1 (*Scenedesmus* sp.)

Results

- Behaves consistently between generations.
- No measurable difference in growth relative to sterile Bold’s medium.
- pH increases and stays high (pH >10) during growth.

3 – Relevance

Relevance to BETO performance milestones:

- 2014 Q4: Demonstrate productivity of $20g/m^2/d$ at a non-integrated PDU scale
  - Lab and scale-up studies (2000L) will demonstrate these targets.
- 2018 Q4: Demonstrate algal productivity of $25g/m^2/d$ with integrated nutrient and water recycling.
  - Results from our nutrient and water recycling studies will directly contribute to this milestone.

Relevance to BETO Feedstock Supply Technical Barriers

- Ft-A. Feedstock availability and cost:
  - System stability and long-term productivity – (i) high-pH tolerant cultures may outcompete unwanted algal contaminants in these harsh environments; (ii) aqueous alkaline solution equilibrium results in higher dissolved inorganic carbon for algal growth and lipid production .
  - System Predictability – Bicarbonate trigger allows for predictable lipid accumulation and recovery.
  - Lower cost – (i) Low-quality nutrients and water use; (ii) suitable for open pond cultivation due to lesser risk of detrimental contamination; (iii) cost savings due to elimination of CO_2 delivery and storage costs.

3 – Relevance (cont.)

- Ft-B. Sustainable production
  - Integration of low quality nutrients and water allows opening new areas of non-competitive water use.
  - Nutrient recycling would further lower the environmental burden
  - Energy savings (and thereby greenhouse gas emissions) from avoidance of CO_2 sparging systems.
- Ft-D. Sustainable harvesting
  - Low-energy and low-capital hydrogel based harvesting can be used as a preliminary step.
  - No use of contaminating chemicals (*e.g.* flocculants) allows water re-use
  - Suitable for shear sensitive cultures unlike other methods such as cross-flow filtration.
  - Can increase solids concentrations to ranges where other traditional methods (*e.g.* belt press) can be easily used.
- Ft-G. Feedstock quality and monitoring
  - Bicarbonate accelerates TAG accumulation.
  - Scale-up to 2000L outdoor cultures (summer 2015) will yield more accurate large-scale feedstock quality estimates.
  - Ongoing method development to accurately and easily quantify TAGs.

4 - Critical Success Factors

The project objectives, when complete, will advance the development of lipid production from algae feedstocks by using alkaliphilic algae.

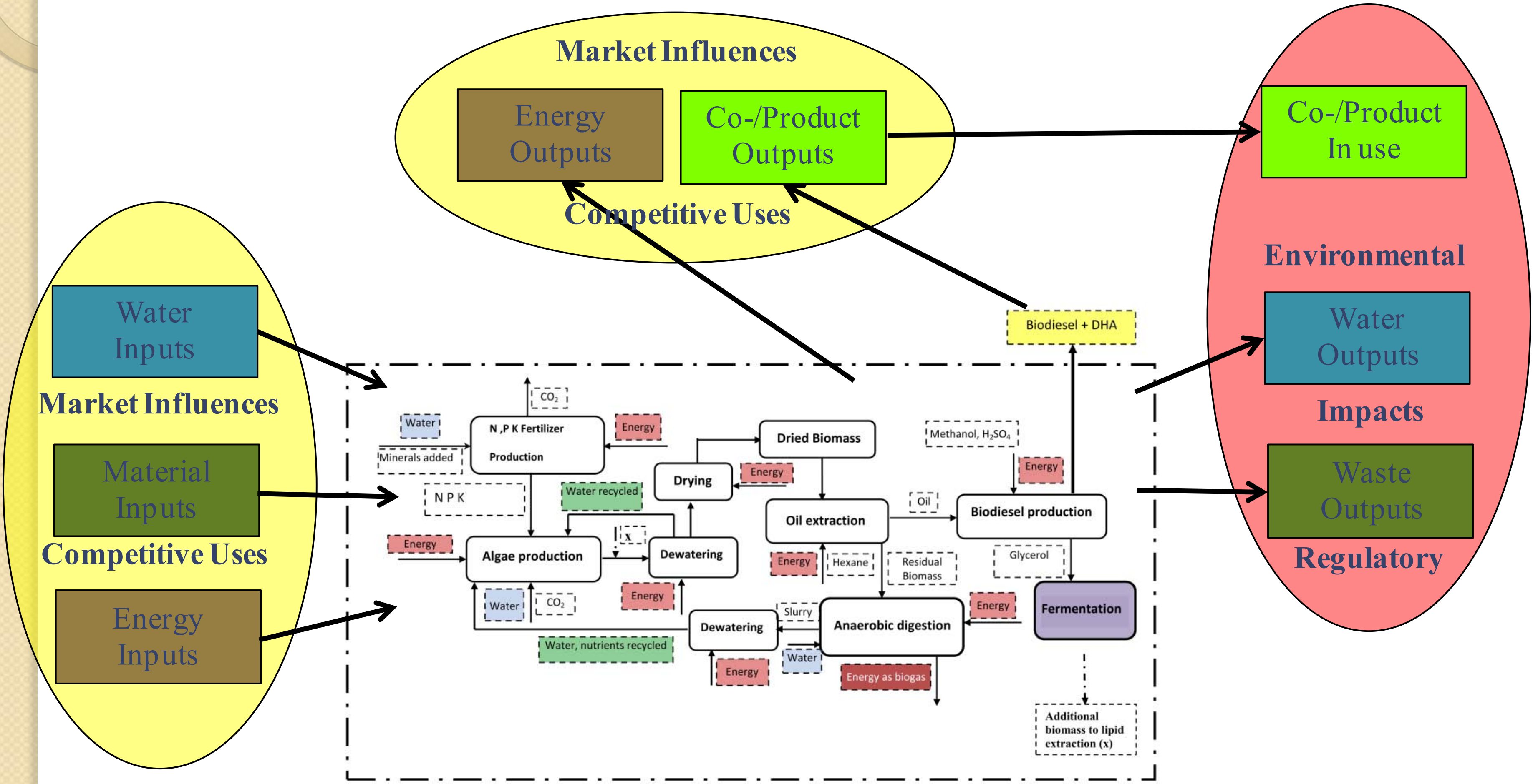
- Develop and characterize organisms/communities adapted to municipal and farm waste water and nutrients (often considered " very low value").
- We must show that high pH conditions (harsh to non-alkaliphilic algal species) help maintain the stability and lipid productivity of algal species without genetic modification.
- It is critical that outdoor tests quantify rates and total yields of TAG and biodiesel potential (as FAMES). Some laboratory grown algae do not grow well in larger systems with "contaminating" organisms and undefined nutrients.
- We have signed NDA's with a number of companies on the bicarbonate work (jointly developed with DOD AFOSR) and are in active license discussions with a large company in this area.
- We are looking into licensing strains WC-1 and RGd-1.
- We are also in discussions with companies on licensing the hydrogel method
- ***Window of opportunity:***
  - Estimated - 3 to 10 years. Companies are trying well characterized strains now, but will be looking for improved strains/conditions for increased pond stability, increased CO_2 uptake, use of lower quality water sources and high lipid productivity.

5. Future Work (FY 13 and 14)

- ***Task 1:*** Evaluation of nutrient recycling and integration options for algae growth
  - We will evaluate the potential for wastewater nutrients (municipal wastewater and dairy manure) for algal growth. Algal growth kinetics will be measured to determine the effects of N, P, inorganic carbon and pH value. Samples will be used to evaluate the evolution of microbial ecology.
- ***Task 2***: Water recovery through use of stimuli-sensitive hydrogels
  - We will quantify the swelling kinetics of pH-sensitive AC-PCL-HEMA hydrogels using CO_2 to affect the pH.
  - We have also synthesized PVA/pNIPAAM semi-IPN hydrogels with faster response and higher swelling than pNIPAAM. We will fully characterize the kinetics and mechanical properties of these gels.
- ***Task 3:*** Structure/function relationships in algal system microbial communities
  - Phylogenetic diversity will be characterized via SSU rRNA gene-based cloning methods. Ecological analyses will be done to determine relationships between communities and improve the understanding of important trends in the microbial populations associated with lipid producing algae.

5. Future Work (cont.)

- ***Task 5***: We will evaluate the socio-economic and environmental impact outside the system boundary as shown



Summary

- **Relevance of objectives**

Cultivation barriers addressed

Key Barrier	Proposed approach
Long-term culture stability due to frequent contamination	Alkaline growth conditions to prevent contamination from common mesophilic bacteria, viruses and other algae
Inorganic carbon limitations for growth	Alkaline media to facilitate high rates of CO_2 transfer from atmosphere into solution
Unpredictable and slow lipid accumulation	Use of bicarbonate "trigger" to stimulate rapid accumulation of lipids at predictable rates
Biomass productivity and lipid content	Our strains have low doubling times (8h – 3.5 d) and high lipid content (30-50%)

Harvesting barriers addressed

Key Barrier	Proposed approach
High energy consumption	Smart hydrogel-based approach is inherently low-energy.
Low process efficiency	Cell losses expected to be minimal with hydrogel and membrane methods.
Water re-use	Treatment needs for water are minimal since chemicals (flocculants) are not used.

Summary

- **Approach**
  - Focus on quantifying alkaliphilic algal growth on waste streams for scale-up studies.
  - Demonstrate productivities >20 g/m^2/d using wastewater and recycled nutrients
  - Demonstrate water reuse after harvesting with hydrogels
  - Characterize microbial communities that stabilize algal cultures
  - Develop models for predicting economic and environmental viability
- **Technical accomplishments**
  - Demonstrated growth of WC01 on municipal wastewater
  - Preliminary characterization of algal-bacterial communities
  - Developed TGA-based rapid lipid quantification method
  - pH- (AC-PCL-HEMA) and temperature- sensitive (PVA-pNIPAAM Semi-IPN) hydrogels synthesized

Summary

- **Success factors and challenges**
  - Alkaliphilic cultures maintain stability and lipid productivity while growing with other microbial populations in wastewaters
  - Bicarbonate trigger is successful in these “contaminated” systems
  - Diverse N and P sources are utilized
  - Hydrogel methods are able to recover water at low energy cost and with prolonged re-usability of the gels
- **Future work and Technology transfer**
  - Quantify growth on wastewater and recycled nutrients and ultimately demonstrate at pilot scale
  - Demonstrate feasibility of hydrogels as cost –effective water recovery media
  - Quantify microbial interactions and establish criteria for population stability
  - Develop economic and environmental assessments
  - License bicarbonate trigger and hydrogel dewatering technologies and strains

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 <u>will **not** be part of your Oral presentation</u>*
- *You may refer to them during the Q&A period if they are helpful to you in explaining certain points.*

Responses to Previous Reviewers' Comments

- If yours is an on-going project that was reviewed previously, address 1-3 significant questions/criticisms from the previous reviewers' comments

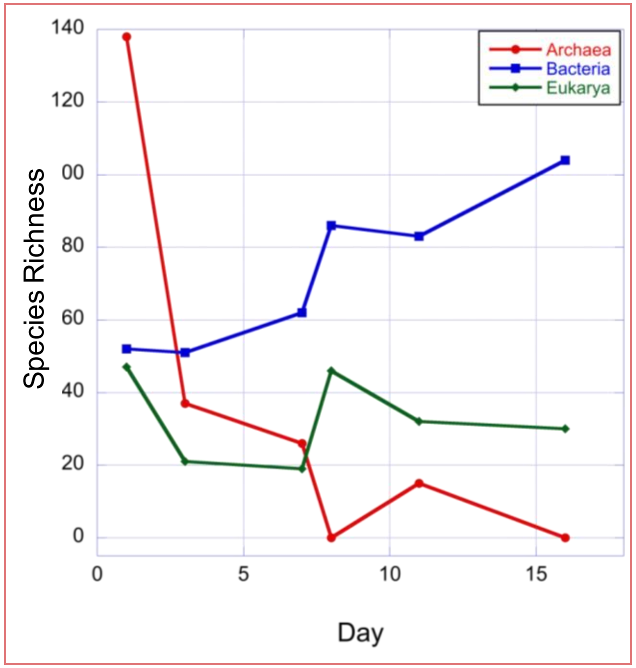
Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These additional slides will be included in the copy of your presentation that will be made available to the Reviewers.

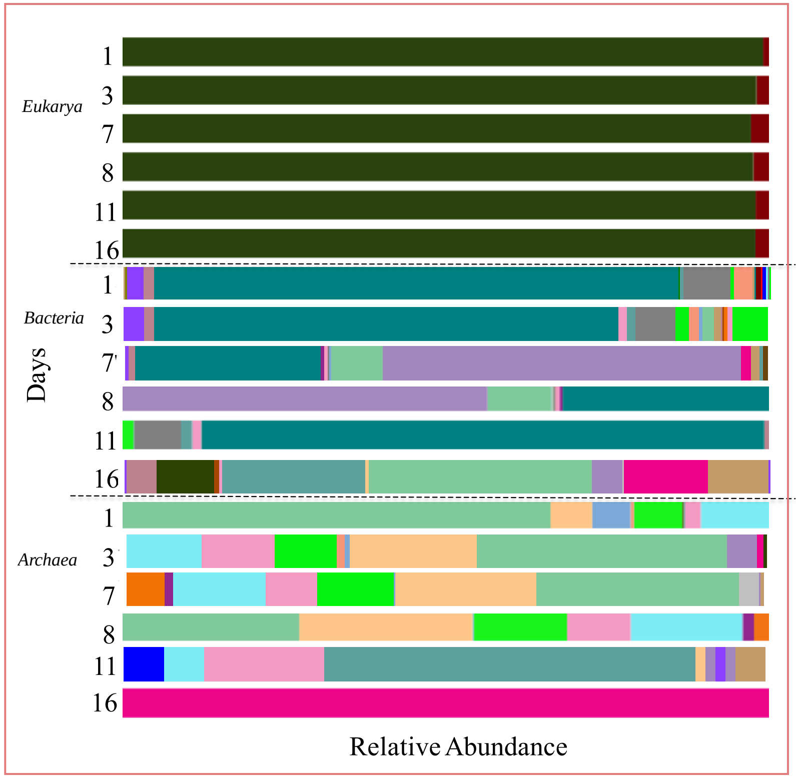
Publications and Presentations

- List any publications, presentations, or patents that have resulted from work on this project. Use at least 12 point font.

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.

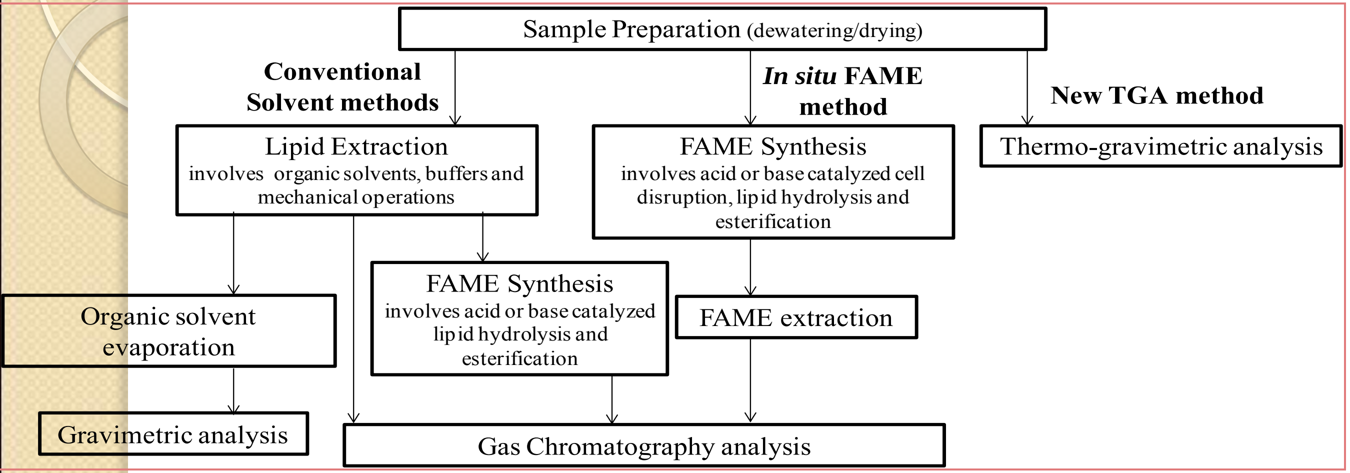
Characterization of Microbial Community Dynamics During Open Cultivation of *Chlorella vulgaris*





- During a 2-week cultivation of *Chlorella vulgaris* in an open, raceway pond, eukaryal sequences were dominated by *Chlorella*, bacterial genera increased, and archaeal genera declined.
- Predominating bacterial populations were dynamic and many of the original archaeal populations were lost (below detection levels).

Rapid quantification of algal lipid content by thermogravimetric analysis (TGA)



Sample Preparation (dewatering/drying)

Conventional Solvent methods

***In situ* FAME method**

New TGA method

Lipid Extraction
involves organic solvents, buffers and mechanical operations

FAME Synthesis
involves acid or base catalyzed cell disruption, lipid hydrolysis and esterification

Thermo-gravimetric analysis

Organic solvent evaporation

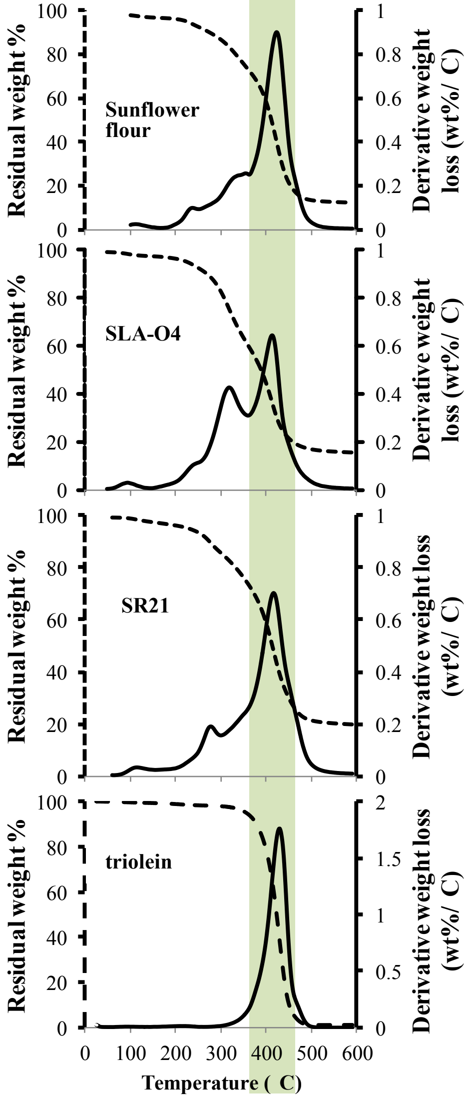
FAME Synthesis
involves acid or base catalyzed lipid hydrolysis and esterification

FAME extraction

Gravimetric analysis

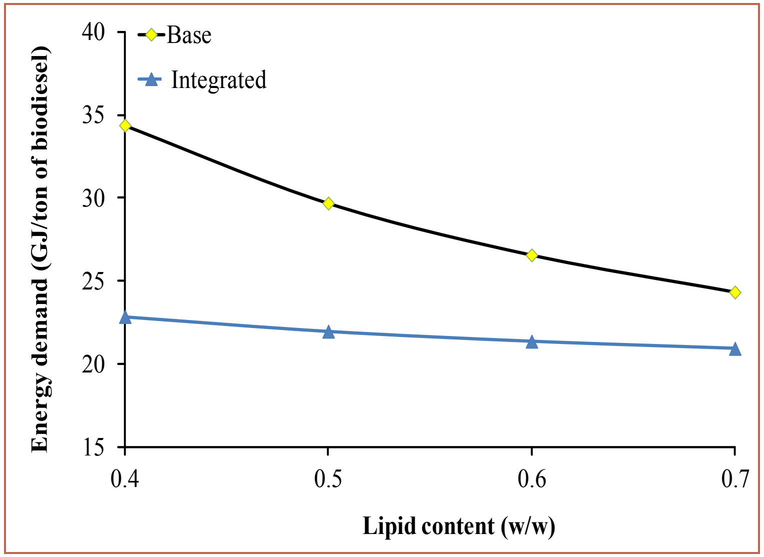
Gas Chromatography analysis

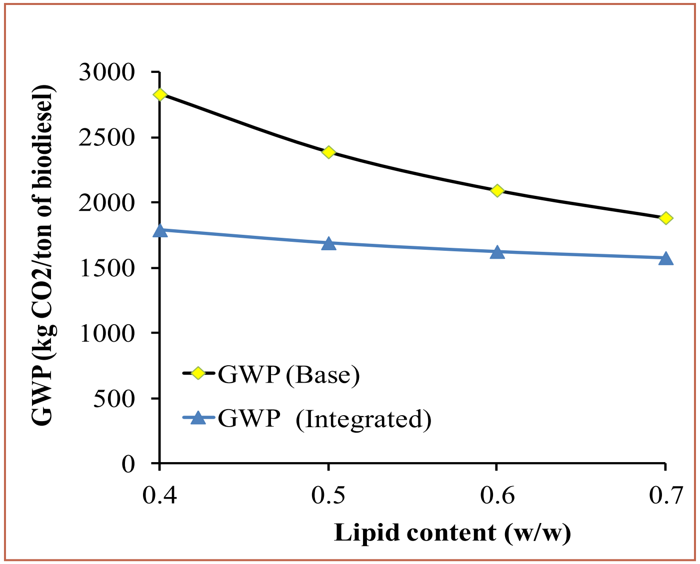
Feedstock	Triglyceride content, wt% dry-basis	
	TG method	*In situ* transesterification method
Soy flour	21.3 0.7	19.5 1.2
Sunflower seeds	51.0 0.05	49.1 4.3
Chlorella sp.	36.4 0.4	37.7 0.1
Schizochytrium sp.	42.3 0.1	43.7 0.2

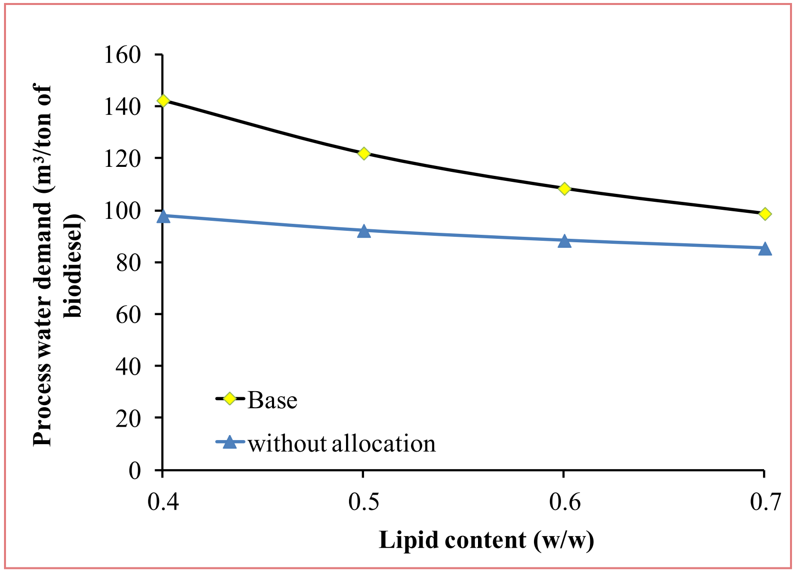


- TAGs volatilize over a narrow temperature interval of 370-450 °C.
- Simple and accurate quantification, no solvent, no GC. Heating in inert atmosphere
- Requires small sample mass (10 mg dry).
- Can be performed on wet pastes also by first drying samples in the TGA.

Overview – LCA of integrated biorefinery



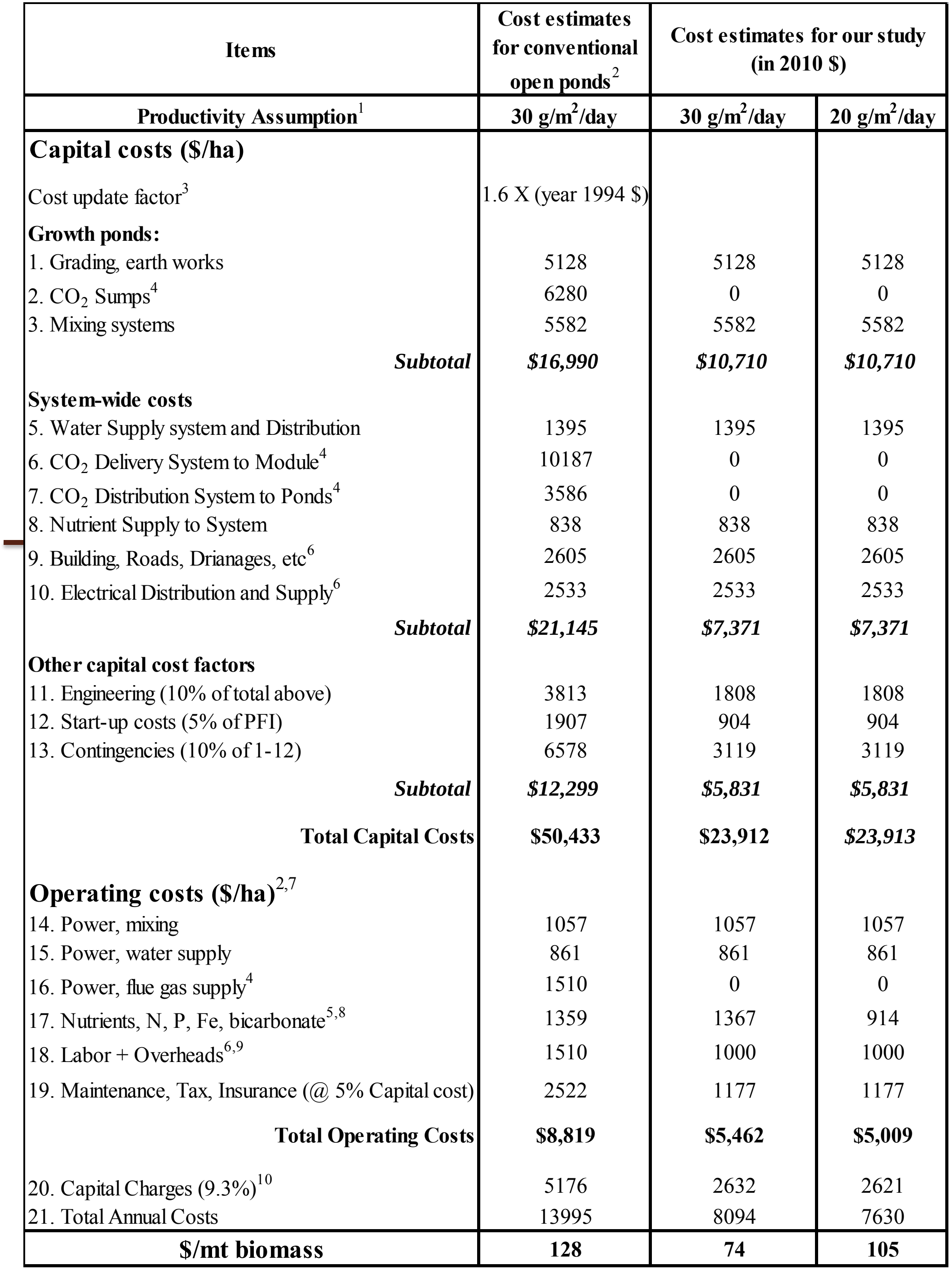




- <u>System definition</u>: (i) Nutrient use from digested animal waste, (ii) additional recycle of nutrients (iii) recycle of water.
- Integrated system has much higher sustainability.
- Environmental impact is insensitive to lipid content when >40% (w/w).

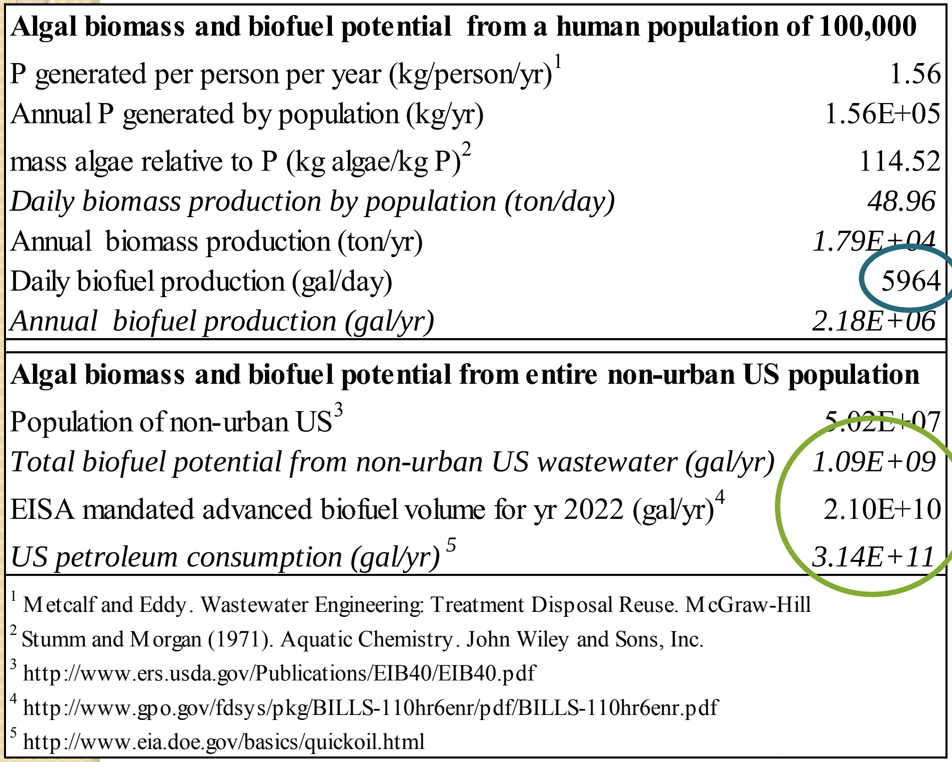
Chowdhury R et al., 2012. Bioresource Technology. 108:102-111

Comparison of raceway pond costs – mesophilic and alkaliphilic cultures



Items	Cost estimates for conventional open ponds[2]	Cost estimates for our study (in 2010 $)	
Productivity Assumption[1]	**30 g/m[2]/day**	**30 g/m[2]/day**	**20 g/m[2]/day**
Capital costs ($/ha)			
Cost update factor[3]	1.6 X (year 1994 $)		
Growth ponds:			
1. Grading, earth works	5128	5128	5128
2. CO_2 Sumps[4]	6280	0	0
3. Mixing systems	5582	5582	5582
Subtotal	***$16,990***	***$10,710***	***$10,710***
System-wide costs			
5. Water Supply system and Distribution	1395	1395	1395
6. CO_2 Delivery System to Module[4]	10187	0	0
7. CO_2 Distribution System to Ponds[4]	3586	0	0
8. Nutrient Supply to System	838	838	838
9. Building, Roads, Drianages, etc[6]	2605	2605	2605
10. Electrical Distribution and Supply[6]	2533	2533	2533
Subtotal	***$21,145***	***$7,371***	***$7,371***
Other capital cost factors			
11. Engineering (10% of total above)	3813	1808	1808
12. Start-up costs (5% of PFI)	1907	904	904
13. Contingencies (10% of 1-12)	6578	3119	3119
Subtotal	***$12,299***	***$5,831***	***$5,831***
Total Capital Costs	**$50,433**	**$23,912**	***$23,913***
Operating costs ($/ha)[2,7]			
14. Power, mixing	1057	1057	1057
15. Power, water supply	861	861	861
16. Power, flue gas supply[4]	1510	0	0
17. Nutrients, N, P, Fe, bicarbonate[5,8]	1359	1367	914
18. Labor + Overheads[6,9]	1510	1000	1000
19. Maintenance, Tax, Insurance (@ 5% Capital cost)	2522	1177	1177
Total Operating Costs	**$8,819**	**$5,462**	**$5,009**
20. Capital Charges (9.3%)[10]	5176	2632	2621
21. Total Annual Costs	13995	8094	7630
$/mt biomass	**128**	**74**	**105**

Biomass potential from animal and municipal wastewater



Algal biomass and biofuel potential from a human population of 100,000	
P generated per person per year (kg/person/yr)[1]	1.56
Annual P generated by population (kg/yr)	1.56E+05
mass algae relative to P (kg algae/kg P)[2]	114.52
Daily biomass production by population (ton/day)	*48.96*
Annual biomass production (ton/yr)	*1.79E+04*
Daily biofuel production (gal/day)	5964
Annual biofuel production (gal/yr)	*2.18E+06*

Algal biomass and biofuel potential from entire non-urban US population	
Population of non-urban US[3]	5.02E+07
Total biofuel potential from non-urban US wastewater (gal/yr)	*1.09E+09*
EISA mandated advanced biofuel volume for yr 2022 (gal/yr)[4]	2.10E+10
US petroleum consumption (gal/yr) [5]	*3.14E+11*

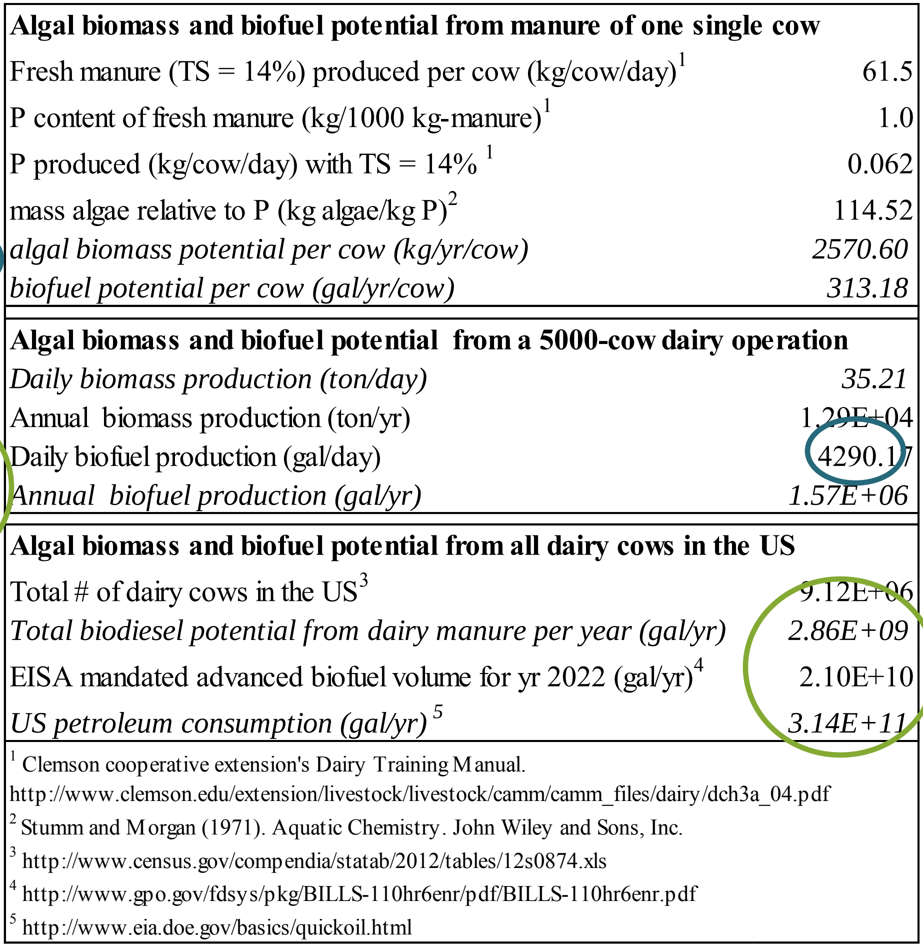
[1] Metcalf and Eddy. Wastewater Engineering: Treatment Disposal Reuse. McGraw-Hill

[2] Stumm and Morgan (1971). Aquatic Chemistry. John Wiley and Sons, Inc.

[3] http://www.ers.usda.gov/Publications/EIB40/EIB40.pdf

[4] http://www.gpo.gov/fdsys/pkg/BILLS-110hr6enr/pdf/BILLS-110hr6enr.pdf

[5] http://www.eia.doe.gov/basics/quickoil.html



Algal biomass and biofuel potential from manure of one single cow	
Fresh manure (TS = 14%) produced per cow (kg/cow/day)[1]	61.5
P content of fresh manure (kg/1000 kg-manure)[1]	1.0
P produced (kg/cow/day) with TS = 14% [1]	0.062
mass algae relative to P (kg algae/kg P)[2]	114.52
algal biomass potential per cow (kg/yr/cow)	*2570.60*
biofuel potential per cow (gal/yr/cow)	*313.18*

Algal biomass and biofuel potential from a 5000-cow dairy operation	
Daily biomass production (ton/day)	*35.21*
Annual biomass production (ton/yr)	1.29E+04
Daily biofuel production (gal/day)	4290.17
Annual biofuel production (gal/yr)	*1.57E+06*

Algal biomass and biofuel potential from all dairy cows in the US	
Total # of dairy cows in the US[3]	9.12E+06
Total biodiesel potential from dairy manure per year (gal/yr)	*2.86E+09*
EISA mandated advanced biofuel volume for yr 2022 (gal/yr)[4]	2.10E+10
US petroleum consumption (gal/yr) [5]	*3.14E+11*

[1] Clemson cooperative extension's Dairy Training Manual. http://www.clemson.edu/extension/livestock/livestock/camm/camm_files/dairy/dch3a_04.pdf

[2] Stumm and Morgan (1971). Aquatic Chemistry. John Wiley and Sons, Inc.

[3] http://www.census.gov/compendia/statab/2012/tables/12s0874.xls

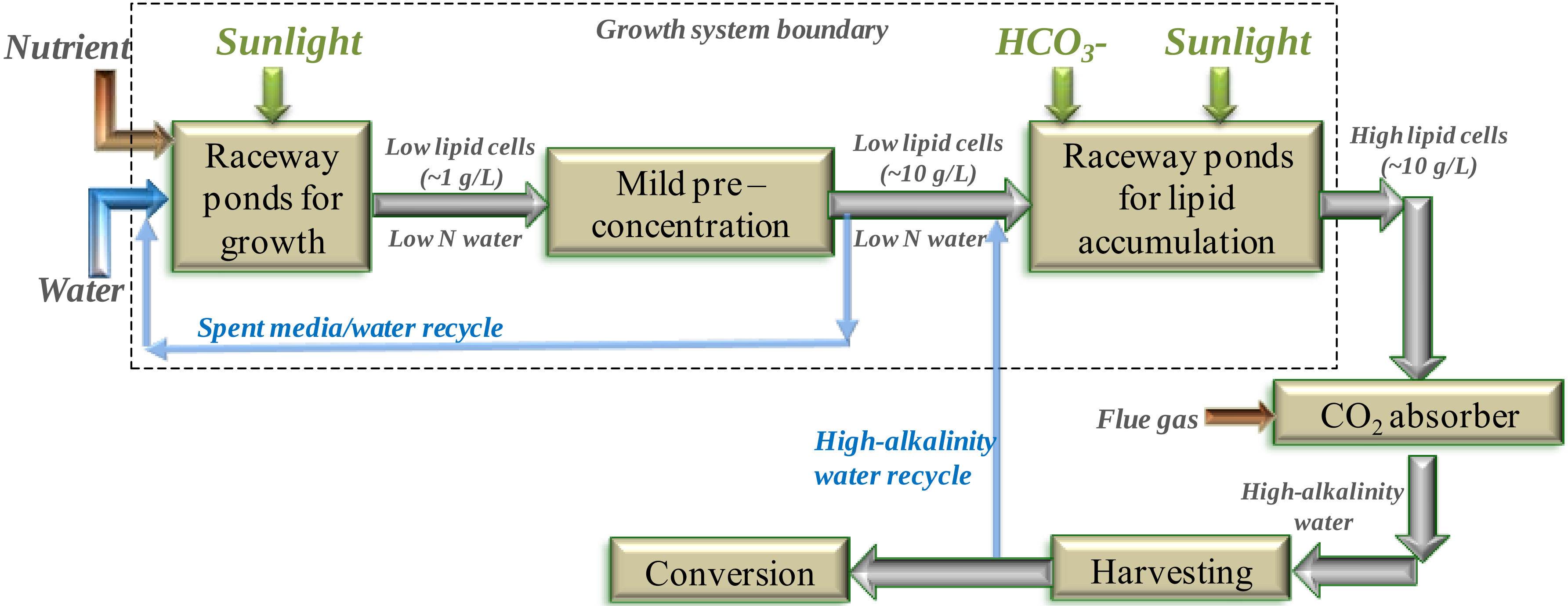
[4] http://www.gpo.gov/fdsys/pkg/BILLS-110hr6enr/pdf/BILLS-110hr6enr.pdf

[5] http://www.eia.doe.gov/basics/quickoil.html

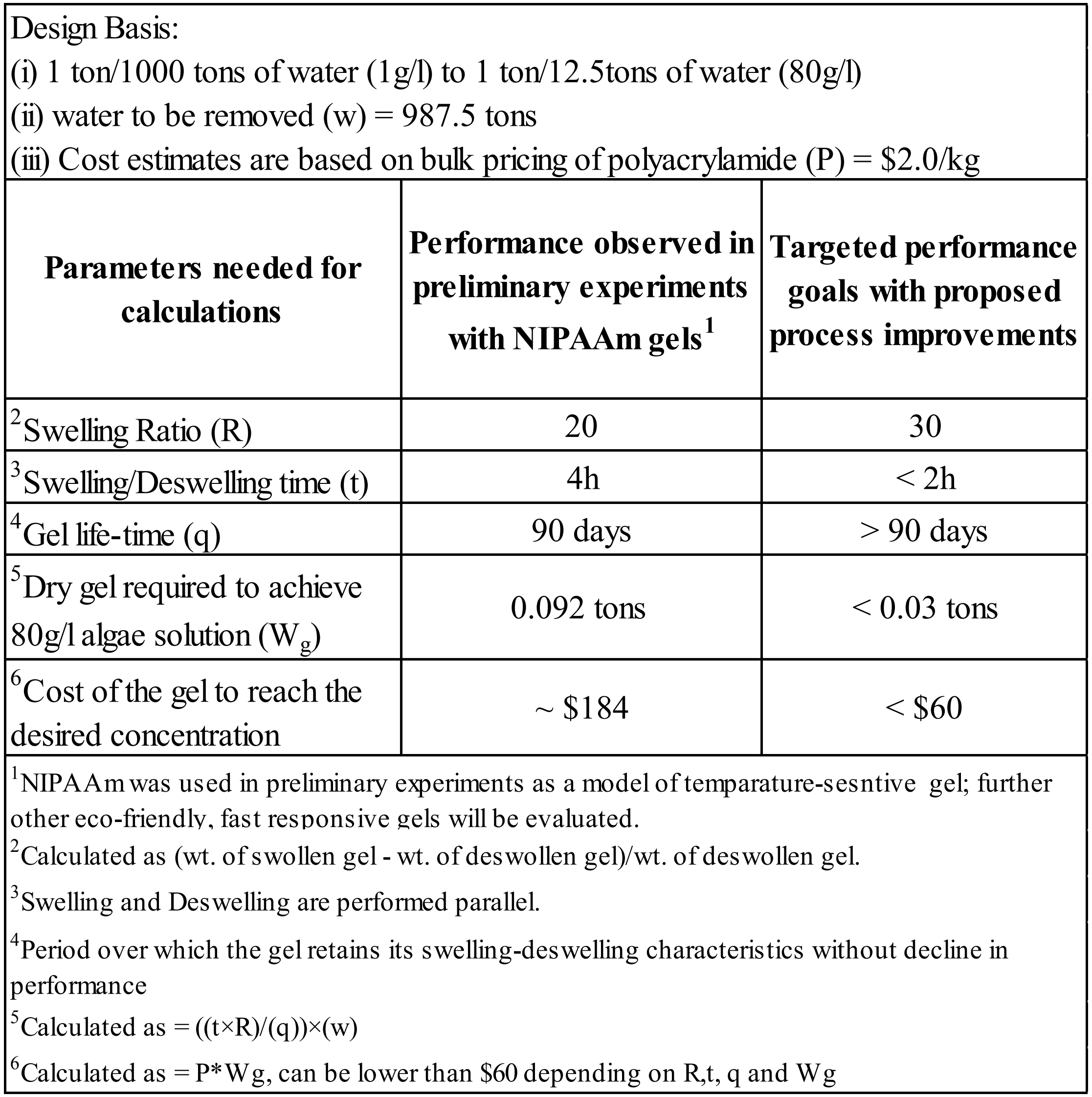
- P-limited growth assumed
- World mineral P reserves are expected to be depleted in 50 to 100 years†
- P used as fertilizer is not recovered – ends up in water bodies like rivers, lakes or oceans

†Cordell, D., J.-O. Drangert, et al. (2009). Global Environmental Change Journal 19(2): 292-305.

Design of a growth system based on "bicarbonate trigger"



Cost estimation for pre-concentrating algae using stimuli-sensitive gels



Design Basis:
(i) 1 ton/1000 tons of water (1g/l) to 1 ton/12.5tons of water (80g/l)
(ii) water to be removed (w) = 987.5 tons
(iii) Cost estimates are based on bulk pricing of polyacrylamide (P) = \$2.0/kg

Parameters needed for calculations	**Performance observed in preliminary experiments with NIPAAm gels[1]**	**Targeted performance goals with proposed process improvements**
[2]Swelling Ratio (R)	20	30
[3]Swelling/Deswelling time (t)	4h	< 2h
[4]Gel life-time (q)	90 days	> 90 days
[5]Dry gel required to achieve 80g/l algae solution (W_g)	0.092 tons	< 0.03 tons
[6]Cost of the gel to reach the desired concentration	~ \$184	< \$60

[1] NIPAAm was used in preliminary experiments as a model of temparature-sesntive gel; further other eco-friendly, fast responsive gels will be evaluated.

[2] Calculated as (wt. of swollen gel - wt. of deswollen gel)/wt. of deswollen gel.

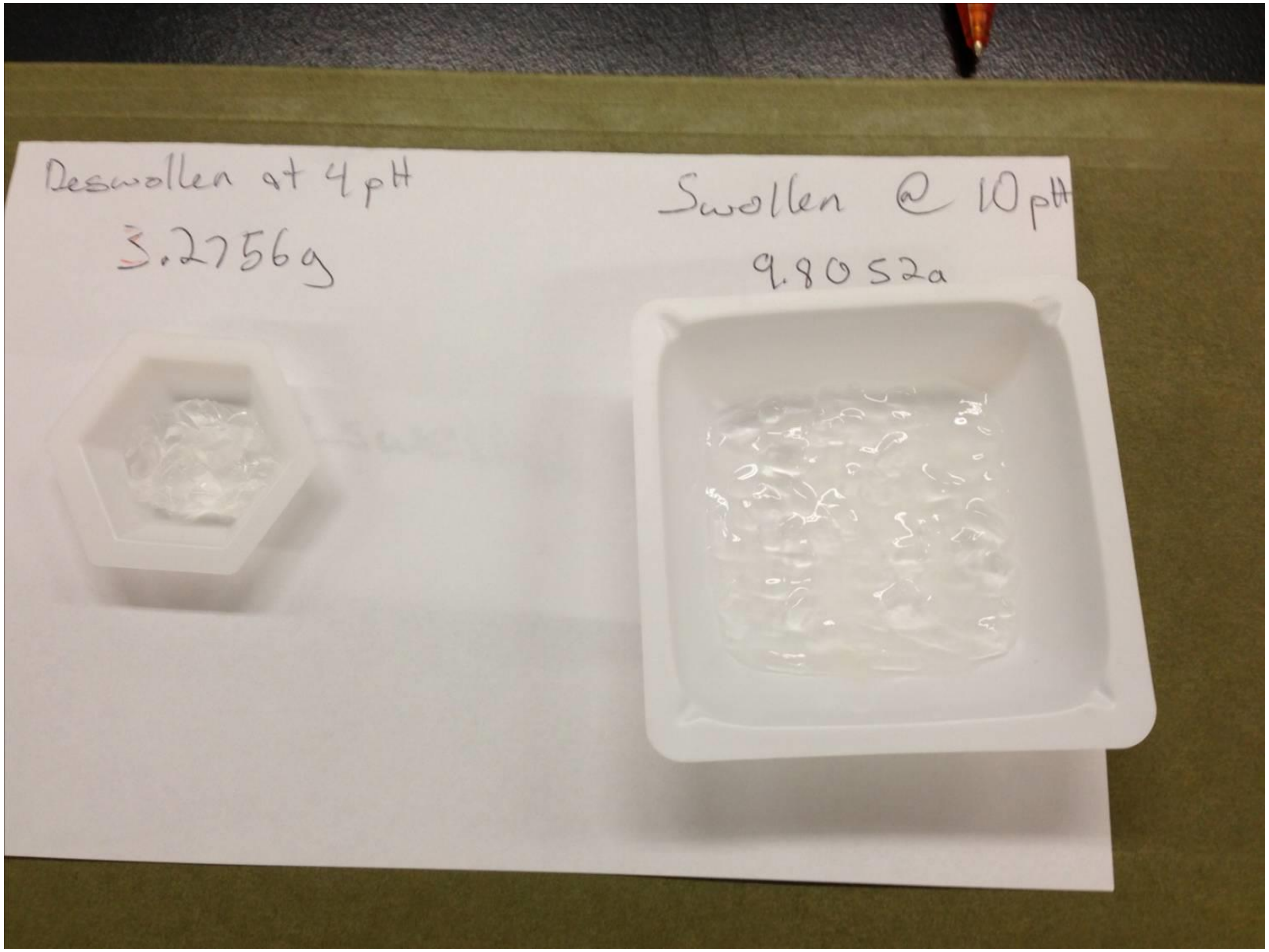
[3] Swelling and Deswelling are performed parallel.

[4] Period over which the gel retains its swelling-deswelling characteristics without decline in performance

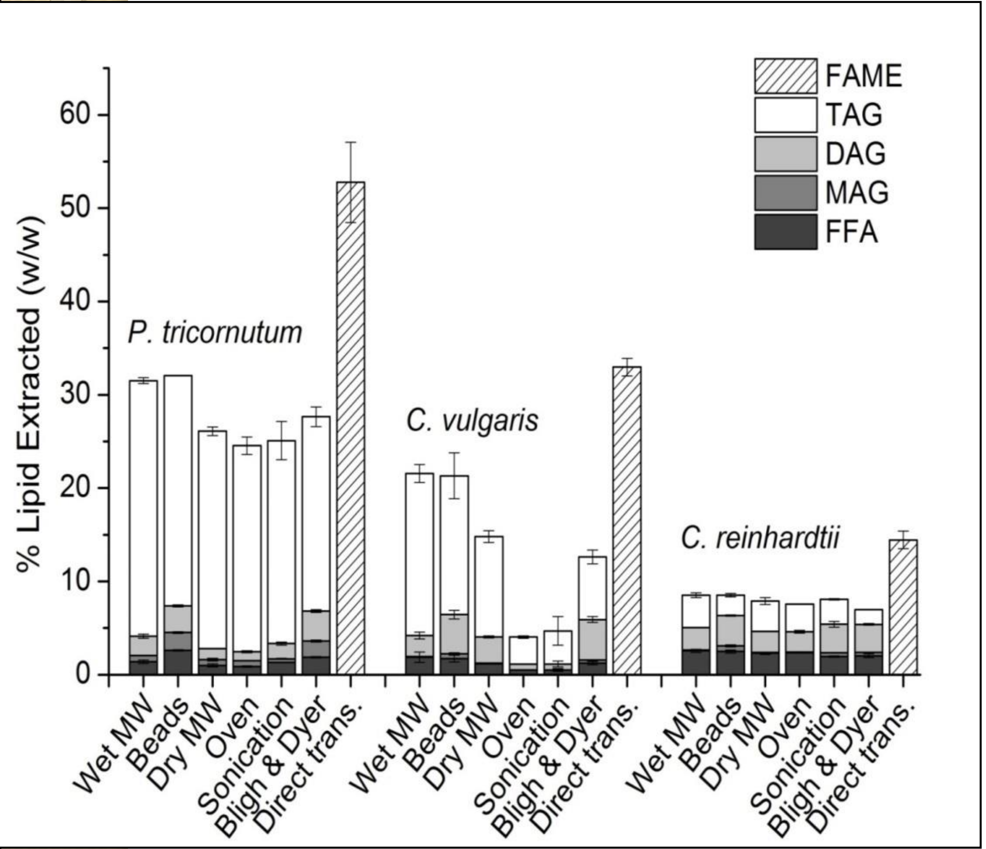
[5] Calculated as = ((t×R)/(q))×(w)

[6] Calculated as = P*Wg, can be lower than \$60 depending on R,t, q and Wg

pH-sensitive hydrogels



Lipid analysis by Microwave Extraction



- Elucidating Lipid Metabolism
  - Comparing FAME Profiles
  - Total FAME vs. Extractable FAME
  - Can help determine the originating lipid class, based on chain length
- Lipid Profile Analysis
  - Methods can be performed on wet (live) culture
  - Fast processing time
  - From harvest to GC data is less than 2 hours
  - Allows for multiple data points throughout experiment