DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Major Nutrient Recycling for Sustained Algal Production

3/25/2015

Algae Technology Area Review

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Goal Statement

The grand objective of the proposed work is to develop a system for the processing of algal biomass that will enable the reuse of major nutrients thus reducing the operational requirement for external nutrients.

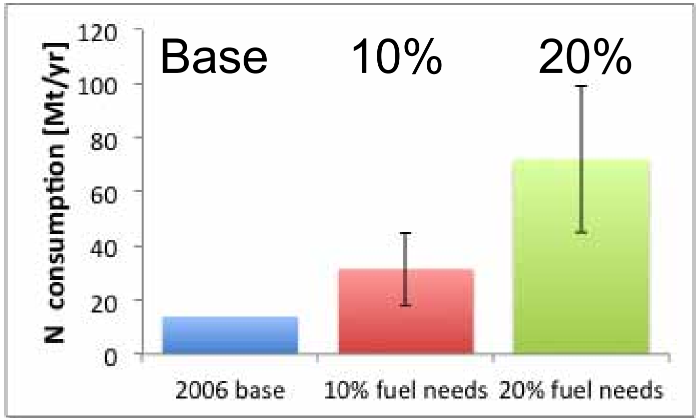
Aft-J. Resource Recapture and Recycle: "Residual materials remaining after preprocessing and/or residual processing may contain valuable nitrogen, phosphorus, other minor nutrients, and carbon that can displace the need for fresh fertilizer inputs in upstream cultivation. The recapture of these resources from harvest and logistics process waste streams may pose separation challenges, and the recovered materials may not be in biologically available chemical forms. In closed-loop systems, the potential for buildup of inhibitory compounds also exists."

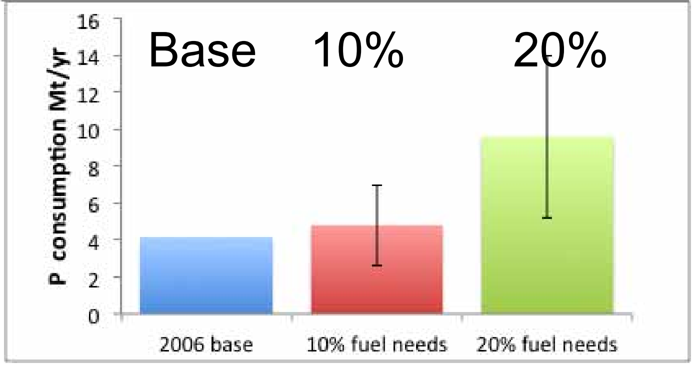
Outcomes: Nutrient recycling technologies, for use in the algal production industry, that will overcome one of the economic barriers to fuel production, and ultimately enhance the energy security of the United States.

Biomass at energy-consumption relevant scales exceeds current nutrient production

"The Achilles Heel of Algae Biofuels: Peak Phosphate," Forbes, Feb. 2012.

- To meet 10% of liquid fuel needs (roughly 30 BGY)
  - Algal biomass: 200 – 500 Mt/yr.
  - Nitrogen: 18 – 45 Mt/yr
    - Compare 14 Mt/yr in 2006
    - Haber-Bosch process requires energy.
  - Phosphorous: 2.4 – 6 Mt/yr
    - Compare 4.1 Mt/yr in 2006
    - P is mined resource.
    - Recent concerns over 'peak phosphate:'
- N/P is the biggest single energy input into the system, accounting for ~30-40% of the total





Pate, Klise, Wu, *Applied Energy*, 88:3377-3388 (2011).
Liu, *et al.*, *Bioresource Technology*, 148:163-171 (2013).

1. Project Overview: A partnership between national lab, university and industry

- Laboratory to pilot/field scale
- Sandia National Labs
  – Project Lead
  – Biochemistry
  – Precipitation Science
- Texas AgriLife (TAMU):
  – biomass production
  – pilot scale field trials
  – Marine species
    - *Nannochloropsis salina*
    - *Phaeodactylum* tricornutum
      – (NAABB strains)
- OpenAlgae
  – Non denaturing electro-lysis
  – Non denaturing TAG extraction
  – Non denaturing DAG extraction
    - Converted phospholipids

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Quad Chart Overview

Timeline

- Project start date: 3/15/2013
- Project end date:3/14/2016
- Percent complete: 66%

Barriers

- Barriers addressed
  - **Aft-J. Resource Recapture and Recycle**

Budget

	Total Costs FY 10 –FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	0	$203,565	$624,087	$1,317,474
Project Cost Share (Comp.)* TAMU	0	$28,129	$51,930	$75,731
Project Cost Share (Comp.)* Open Algae	0	$68,768	$126,956	$185,144

Partners

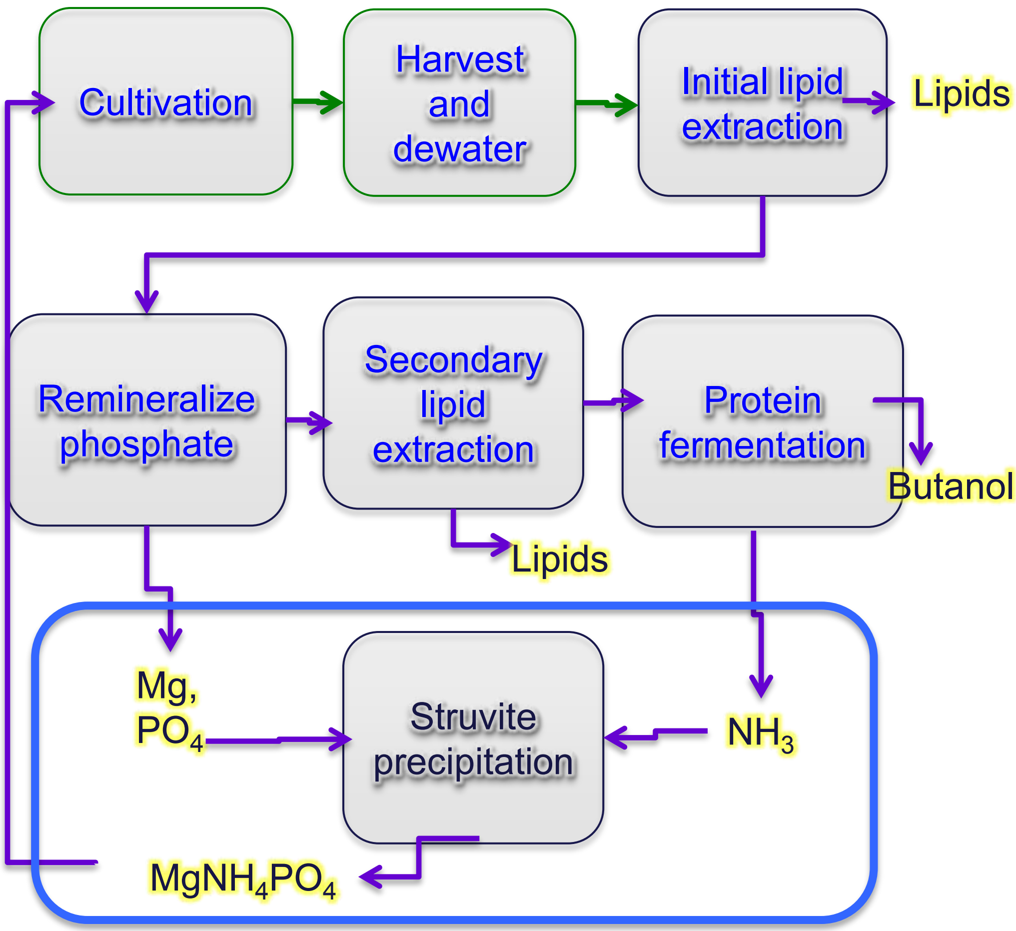
Texas Agrilife (TAMU)
OpenAlgae LLC

1 - Project Overview

The grand objective of the proposed work is to develop a system for the processing of algal biomass that will enable the reuse of major nutrients thus reducing the operational requirement for external nutrients. To meet this overall objective, the team will:

1. Develop a process to liberate nitrogen and phosphorous present in algal biomass.
2. Convert phospholipids to DAG, remineralized phosphate, and recover both.
3. Remineralize nitrogen and phosphorus and demonstrate a recovery process.
4. Demonstrate the ability of recycled nutrients to support algal growth.
5. Operate the growth process at a lower overall N:P ratio to favor lipid production and minimize nitrogen cycling.

2 – Approach (Technical)



- Non denaturing method of neutral lipid extraction
- Remineralization of cellular phosphate by mild pH and enzymatic treatment
- Conversion of cellular nitrogen by protein fermentation
- Return of recycled nutrients to pond for subsequent biomass production.

Critical Success factors

Technical

- Quantitative recovery and recycling of nutrients
  - Must return the maximal amount of biologically accessible nutrients to the pond
  - Nutrients must not contain inhibitors.

Market/Business

- Reduction of energy and material costs
- Determine the most cost effective means to the desired result
  - Must develop and evolve methods to require lowest level of inputs and highest level of return
  - Linking fuel generation with nutrient recycle
  - Creating integrated 1-pot methods
- Creation of savings over potential nutrient costs

Potential challenges

- Various algal strains may require substantially different lysis and or incubation conditions for fast and efficient nutrient remineralization.
  - We will target our approach for two distinct algal species, *Nannochloropsis salina* and *Phaeodactylum tricornutum*, that have been identified as preferred by the National Alliance for Advanced Biofuels and Bioproducts (NAABB), and are actively co-cultured in open raceway environments.
- Some phosphate and or nitrogen pools within the cells may appear to be recalcitrant
  - Pre-processing of biomass
  - Biochemical analysis to identify recalcitrant pools
  - Targeted enzymatic digestion if necessary
- Off target complexes may be formed during the capture process
  - Recycle nutrients in aqueous solution
  - Adjust the chemistry of the process

Management structure reflects task structure

Principal investigator:
Todd Lane
Sandia National Labs

CoPI Anthony Siccardi
Texas Agrilife
Algal growth trials
biomass production

CoPI Peter Kipp
OpenAlgae
Neutral lipid extraction
Phospholipid conversion

SNL Technical Lead
Ryan Davis
Nutrient Liberation

SNL Technical Lead
John Hewson
Nutrient Capture

Meetings

- Monthly telecon with PI, Co-Pi's Team leads, and members
- Annual face to face meetings
- Other meetings on *ad hoc* basis

Quarterly reports/tracking of milestones

- Data flows through the PI
- PI tracks milestones and generates all reports
- Synthesis of results into publication and solutions tracked and mediated by PI

- Decision making is through consensus of PI, CoPIs, and technical leads
- PI, CoPIs and technical leads are responsible for achieving task milestones
- PI retains ultimate decision-making authority, and responsibility for milestones and deliverables

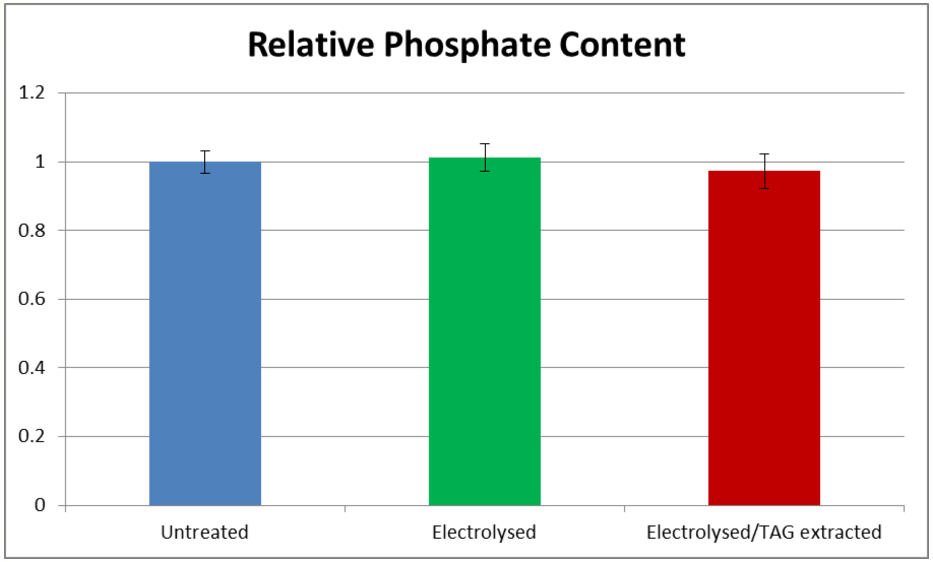
Go/No-Go milestones: Phase 1 ending 10/14

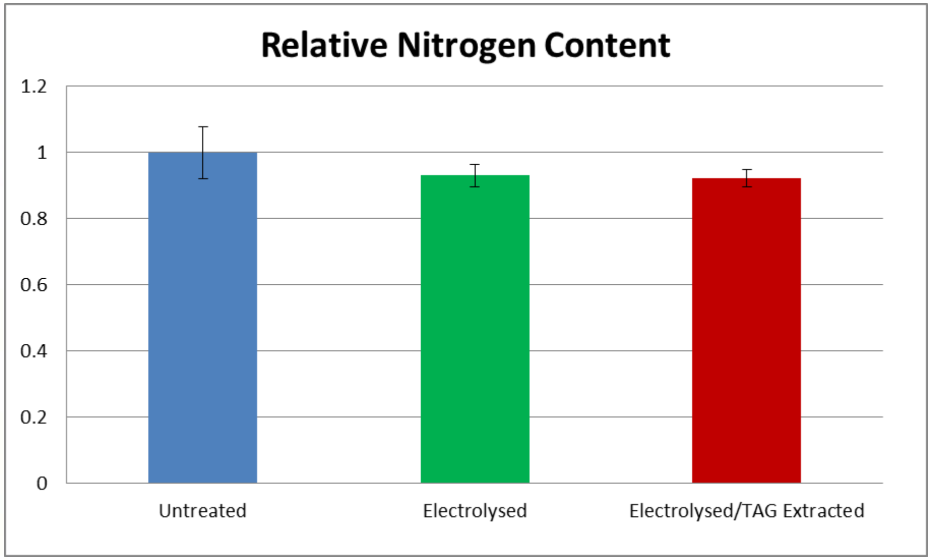
- The residual biomass obtained after TAG extraction must contain at least 40% of the original phosphorous and nitrogen content of the untreated biomass.
- The recaptured nutrients must contain at least 20% of the original phosphorous and nitrogen content of the untreated biomass.
- The expected final product of the mineralization (struvite) must provide a suitable nutrient activity when provided to culture media as a nutrient for algal growth.
- The rate of separation of the remineralized nutrients from the solution (likely through precipitation) must not lead to activities of these nutrients that adversely impact the kinetics.

3 – Technical Accomplishments/ Progress/Results

- TAG extracted biomass (OA process) retains ~90% of N & P
- Phosphate can be remineralized, in soluble form, from non denatured *N. salina* biomass by enyzmatic digest or mild pH treatment
  - 50-70% yield
  - The OA lysis and neutral lipid extraction process has little impact on down stream Pi remineralization processes
- Nitrogen can be remineralized in soluble form from *N. salina* biomass by protein fermentation.
  - 57 % yield
  - The OA lysis and neutral lipid extraction process has little impact on down stream protein fermentation processes
- Soluble, remineralized phosphate can provide 100% of phosphate required for growth of *N. salina* or *P. tricornutum*
- Crude struvite can provide 100% of phosphate and large fraction of nitrogen for the growth for the growth of *N. salina* and *P. tricornutum* at laboratory scale
- Crude struvite can provide 100% of phosphate and large fraction of nitrogen for the growth for the growth of *N. salina* in pilot scale outdoor raceways.

Open Algae oil extraction process Preserves N & P in Biomass





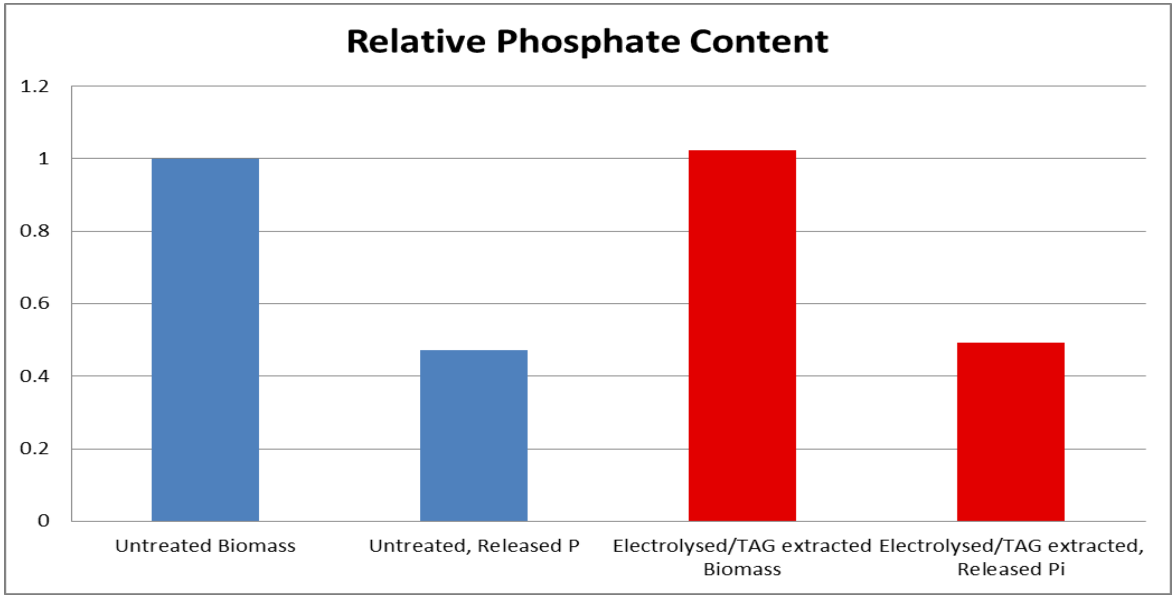
- **G/N-G Milestone:** Retain at least 40% of cellular phosphate and nitrogen after TAG extraction by Open Algae

Result: retained ~90% of N & P after TAG extraction

Tested on untreated and TAG extracted, 3 Biological replicates N. salina

Total cellular phosphate and nitrogen assayed by standard methods (Oxisolv oxidation followed by colorimetric determination)

Phosphate extraction from TAG extracted Biomass

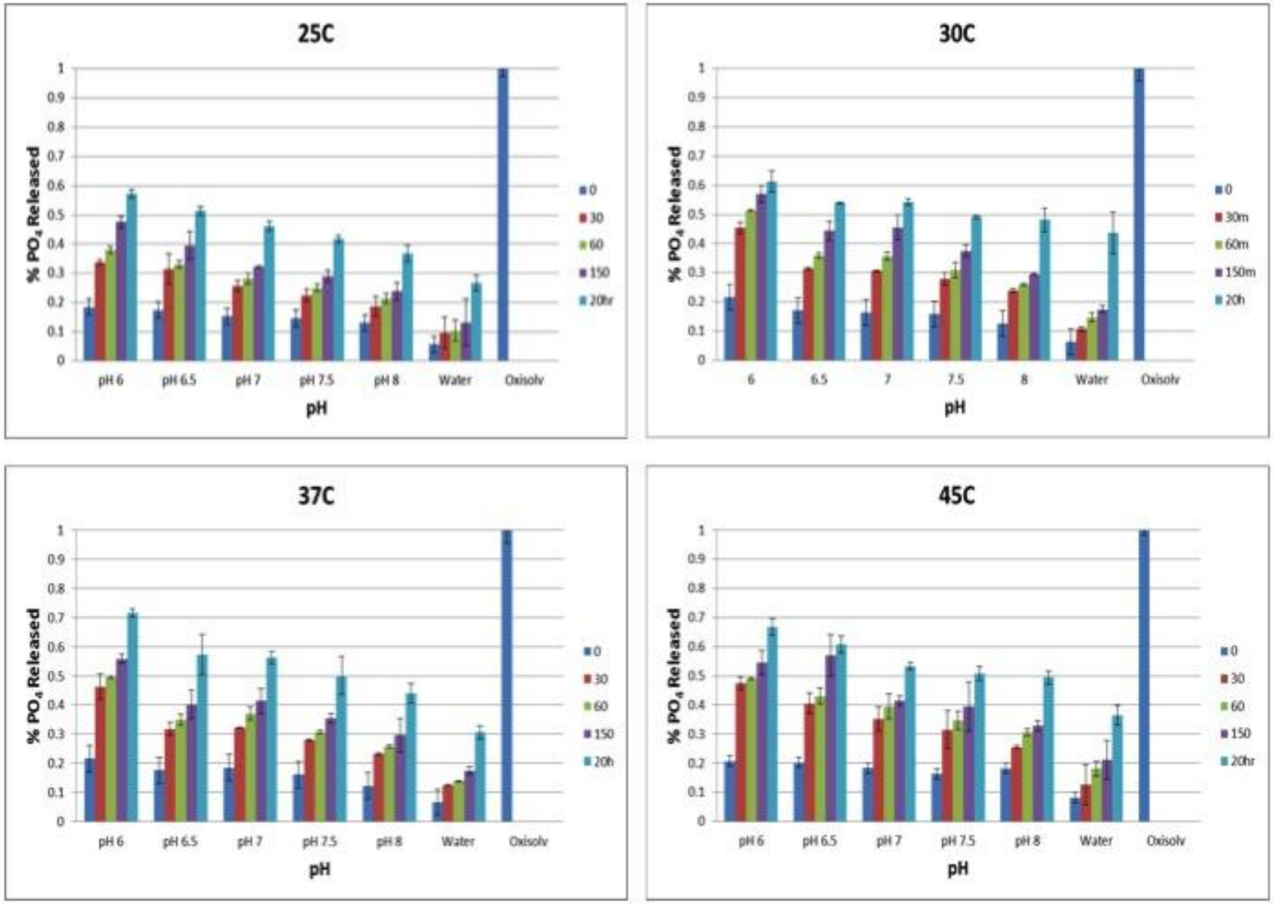


- **G/N-G Milestone:** Release at least 20% of Phosphate in TAG extracted biomass extraction (Open Algae TAG extraction process)

Result: released ~45-50% of P from TAG extracted biomass

Total cellular phosphate and nitrogen assayed by standard methods (Oxisolv oxidation followed by colorimetric determination)

Optimized reaction releases up to 70% of cellular P



Osmotically shocked native *N. salina*

Non lipid extracted

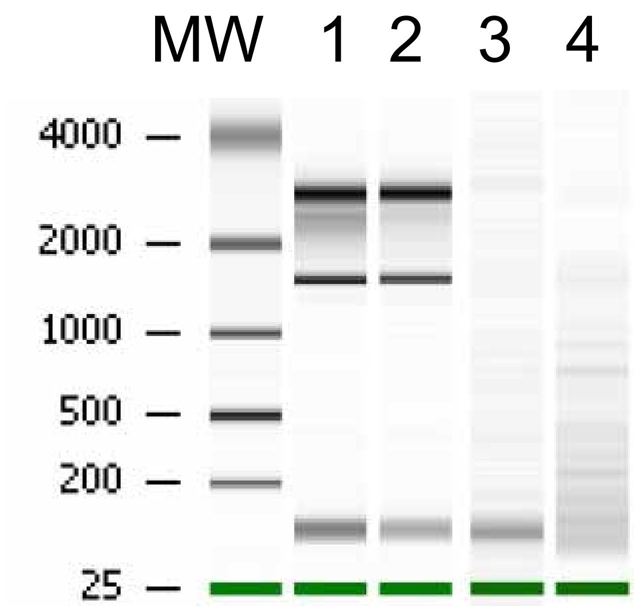
Liberating up
To 70% of total cellular phosphate under mild pH incubation conditions

Higher temperature does speed the reaction somewhat

Identification of reactive cellular phosphate pools

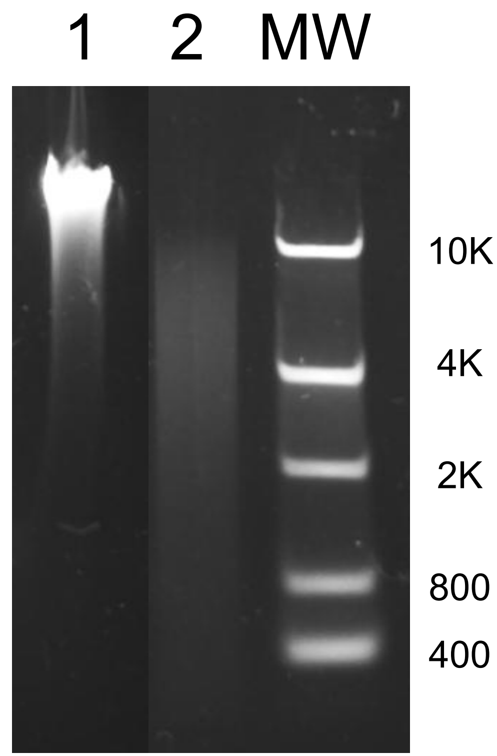
Geider & La Roche 2002 Eur. J. Phycol, 37:1-17

Biochemical fraction	% cell mass of fraction	gm P per gm DW of fraction
RNA	3-15	0.091
DNA	0.5-3	0.095
Phosphoglycerides	5-15	0.043
ATP	<0.1	0.18



1. *N. salina* RNA before digest
2. *N. salina* RNA before digest
3. *N. salina* RNA after digest
4. *N. salina* RNA after digest

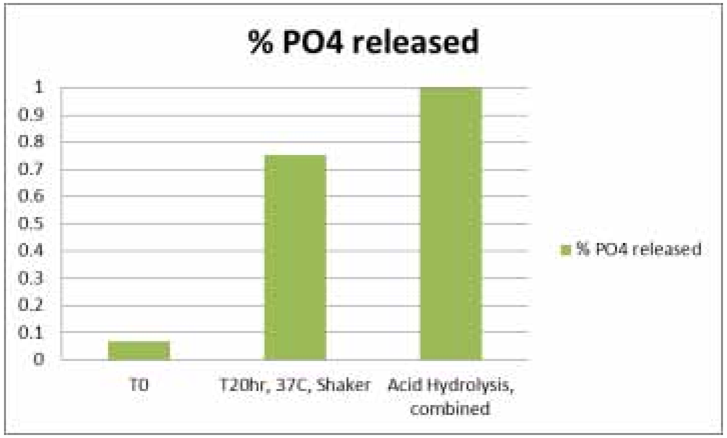
<u>Digest conditions:</u>
Resuspend biomass in water to 1% solids
Add MES pH6 to 50mM
Incubate at 37C, 20 hours

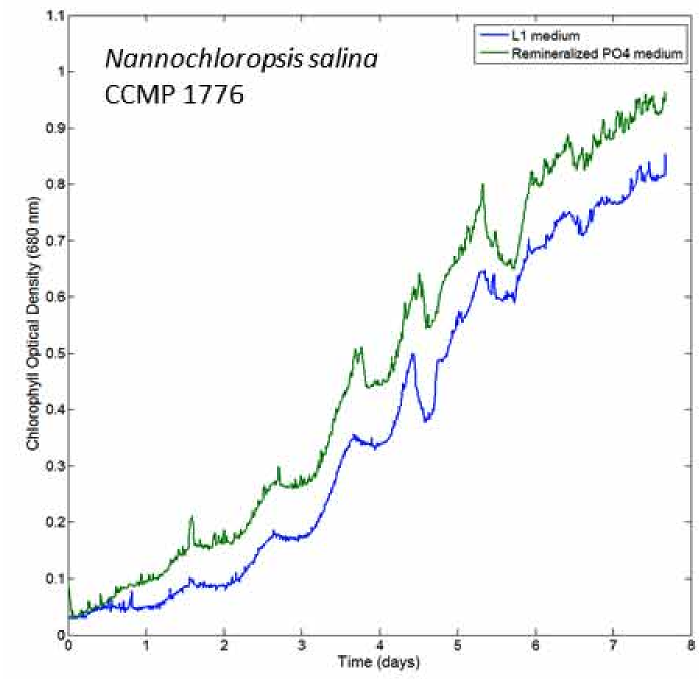


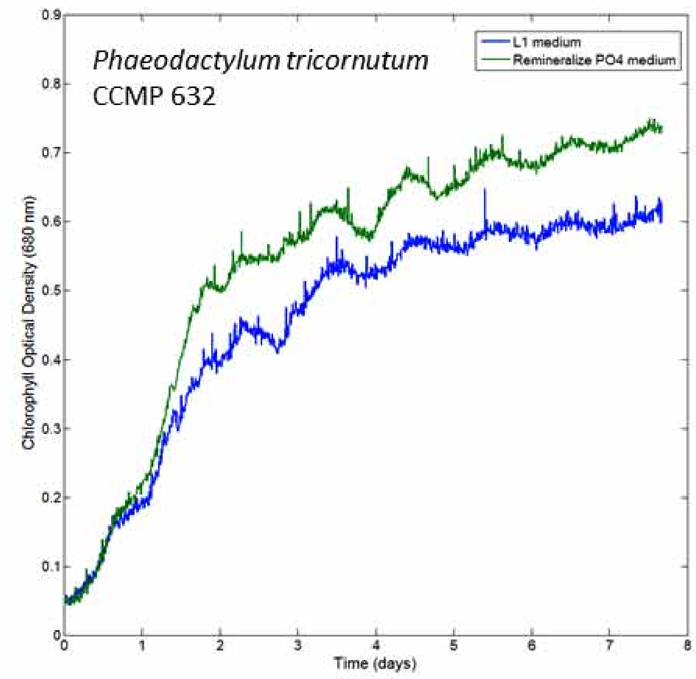
1. *N. salina* DNA before digest
2. *N. salina* DNA after digest--**25X loaded**

Regrowth of biomass on remineralized phosphate

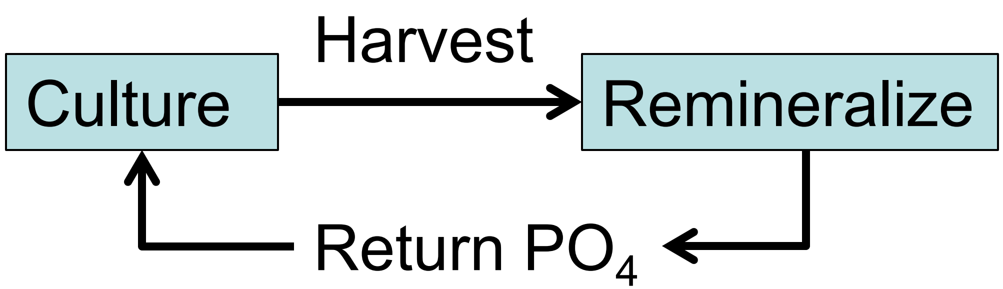
- ~50 gm of 20% solids. *N. salina*
- Diluted to 2% solids pH 6.5, 37°, 20hrs
- Liberated phosphate used to replace total phosphate in algal culture
- Growth of *P. tricornutum* and *N. salina* on soluble liberated phosphate

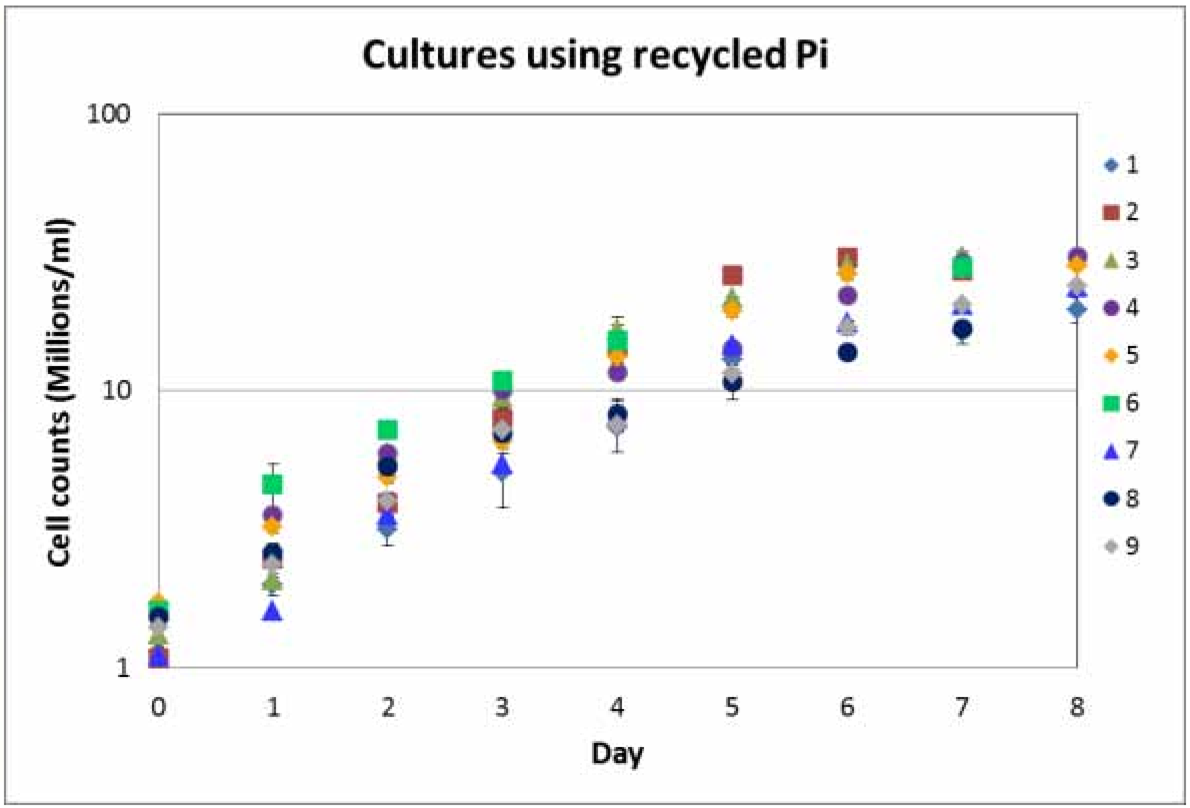






Repeated rounds phosphate remineralization and reuse in *N. salina* culture





After first round, recycled up to 66% of consumed phosphate

No difference in specific growth rates over the course of 8 rounds of recycle (9 culture rounds)

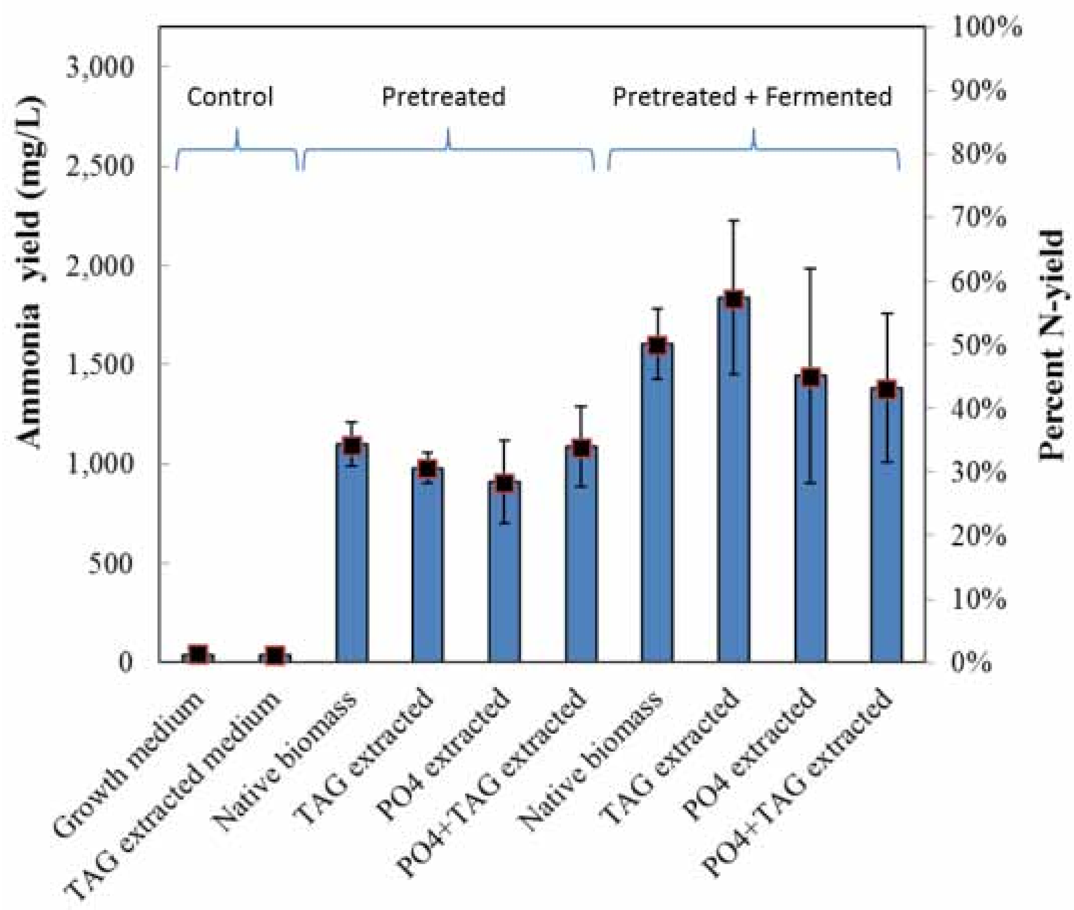
No evidence of accumulation of growth inhibitors through 8 recycles

Response to peer review 2013

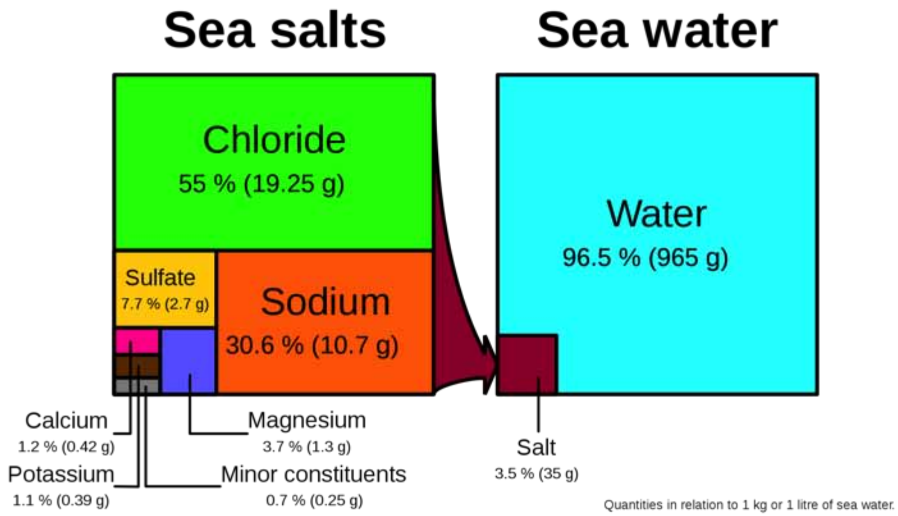
Nitrogen remineralization from *Nannochloropsis salina* biomass by biochemical conversion

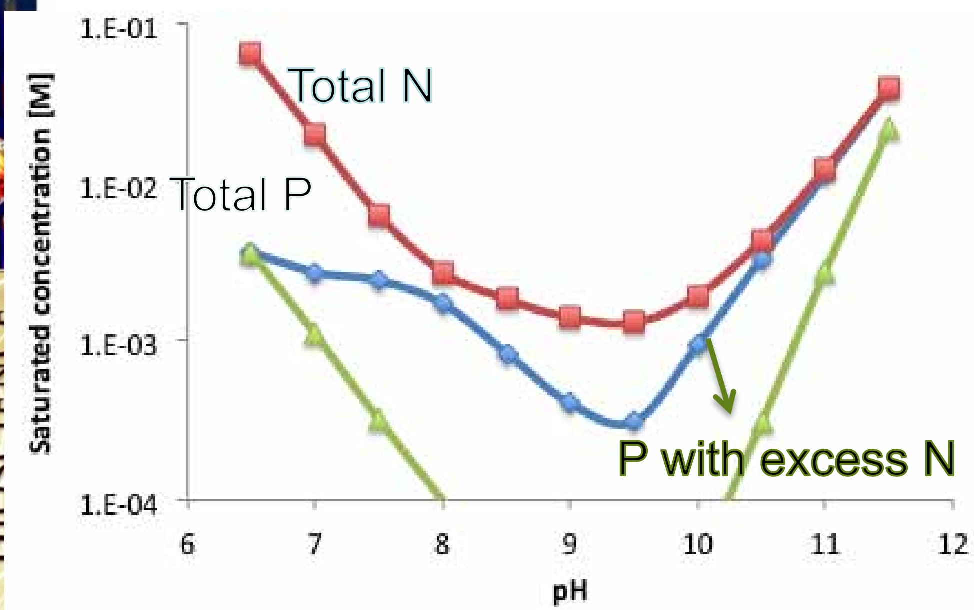
▯ **G/N-G Milestone:** Release at least 20% of nitrogen in TAG extracted biomass extraction (Open Algae TAG extraction process)

- Dilute acid + enzymatic pre-treatment converts ~35% of the total N to ammonia
- Protein fermentation converts ~27% of total N to ammonia
- Extraction of TAG or PO_4 did not significantly alter the ability to remineralize biological N to ammonia
- **The N-remineralization yield was 57% (±14%) of theoretical**
- Concomitant fuel yield was 4.4 g/L mixed alcohols from starting 20 g/L protein



Struvite is a likely form of recycled P

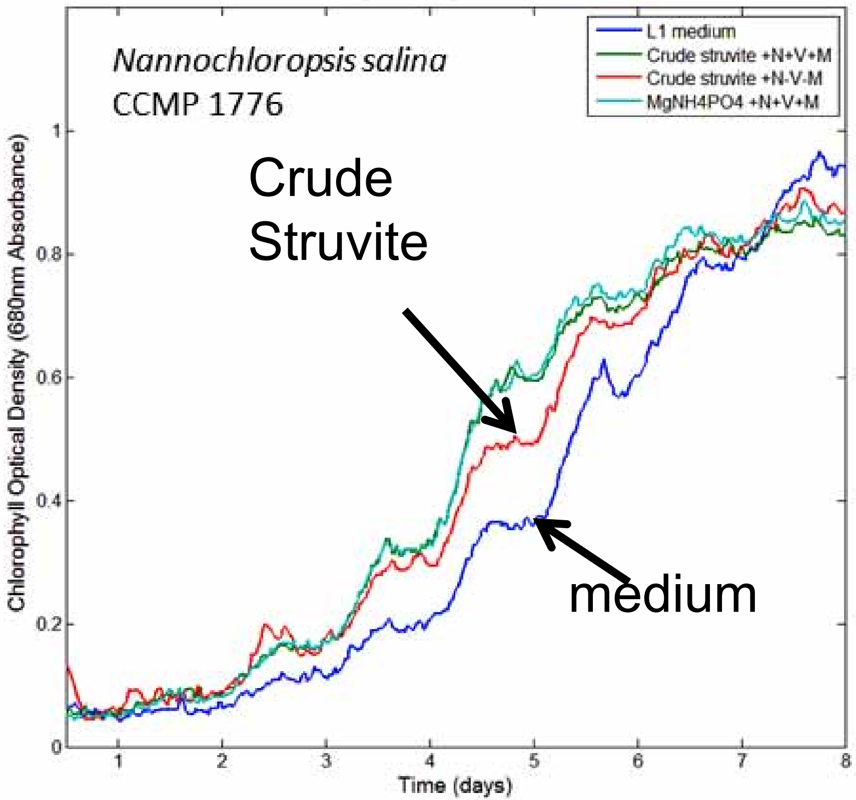


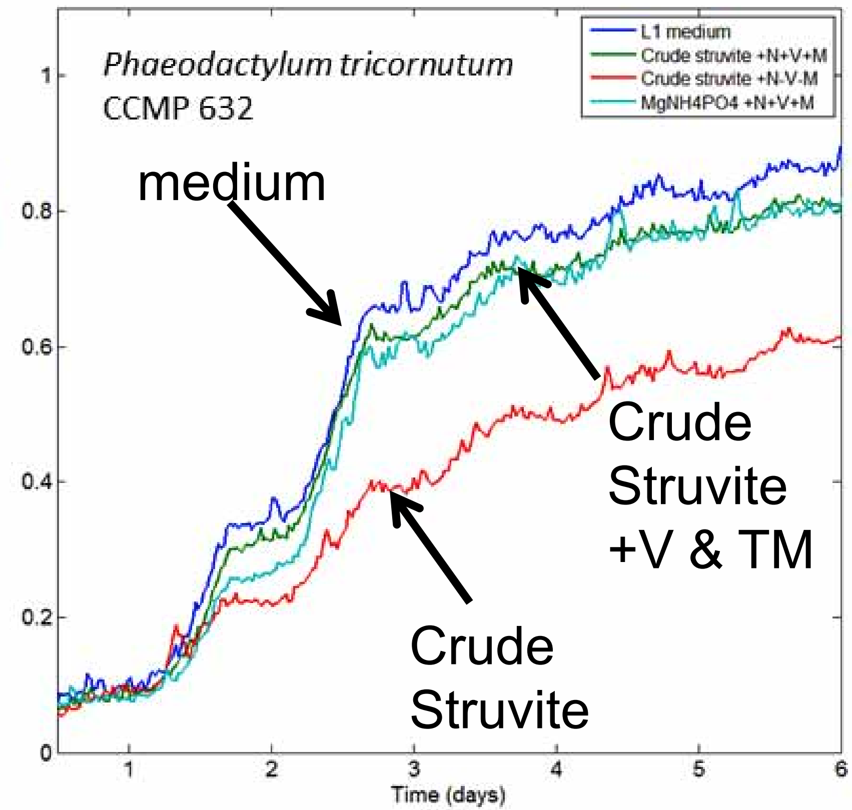


- 50mM in seawater
- If algae dewatered to 10% solids one can expect 5mM Mg to remain.
- Depending on excess NH4/NH3 used in precipitation, estimate 80-99% potential PO4 precipitation.
- Recovers 1:1 N:P
- Precipitates at accessible concentrations.
  - Experience in waste water treatment industry.
- Alternates include Ca and Mg phosphates.

The expected product, Struvite, can replace "new" nutrients in microalgal culture

- **G/N-G Milestone**: demonstrate that our expected final product can support algal growth





Multicultivator, sinusoidal 16/8 LD cycle, peak 1000μE, 21 to 24 C

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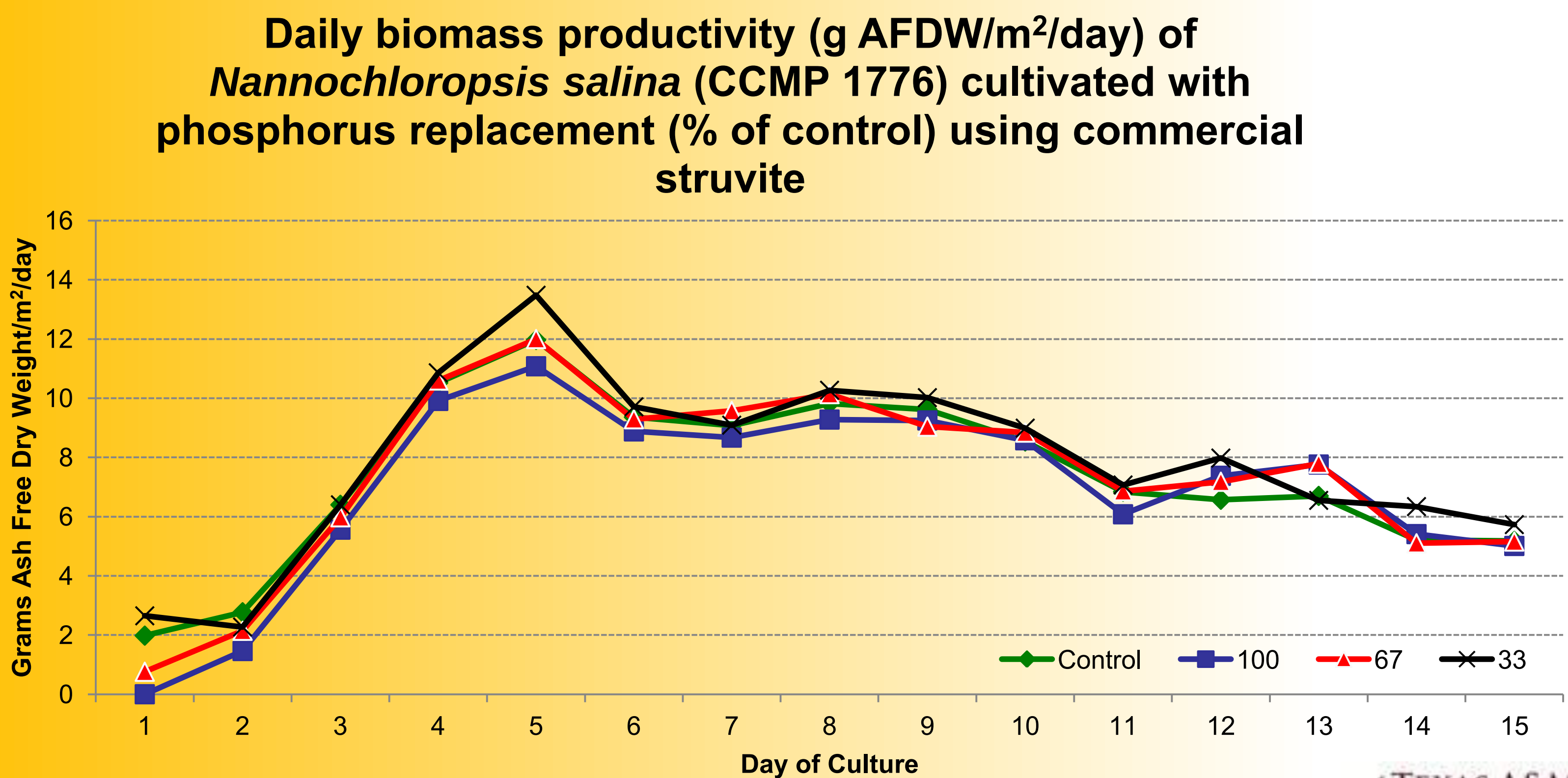
Cultivation with struvite in outdoor raceways

G/N-G Milestone: demonstrate that our expected final product can support algal growth



TEXAS A&M AGRILIFE RESEARCH

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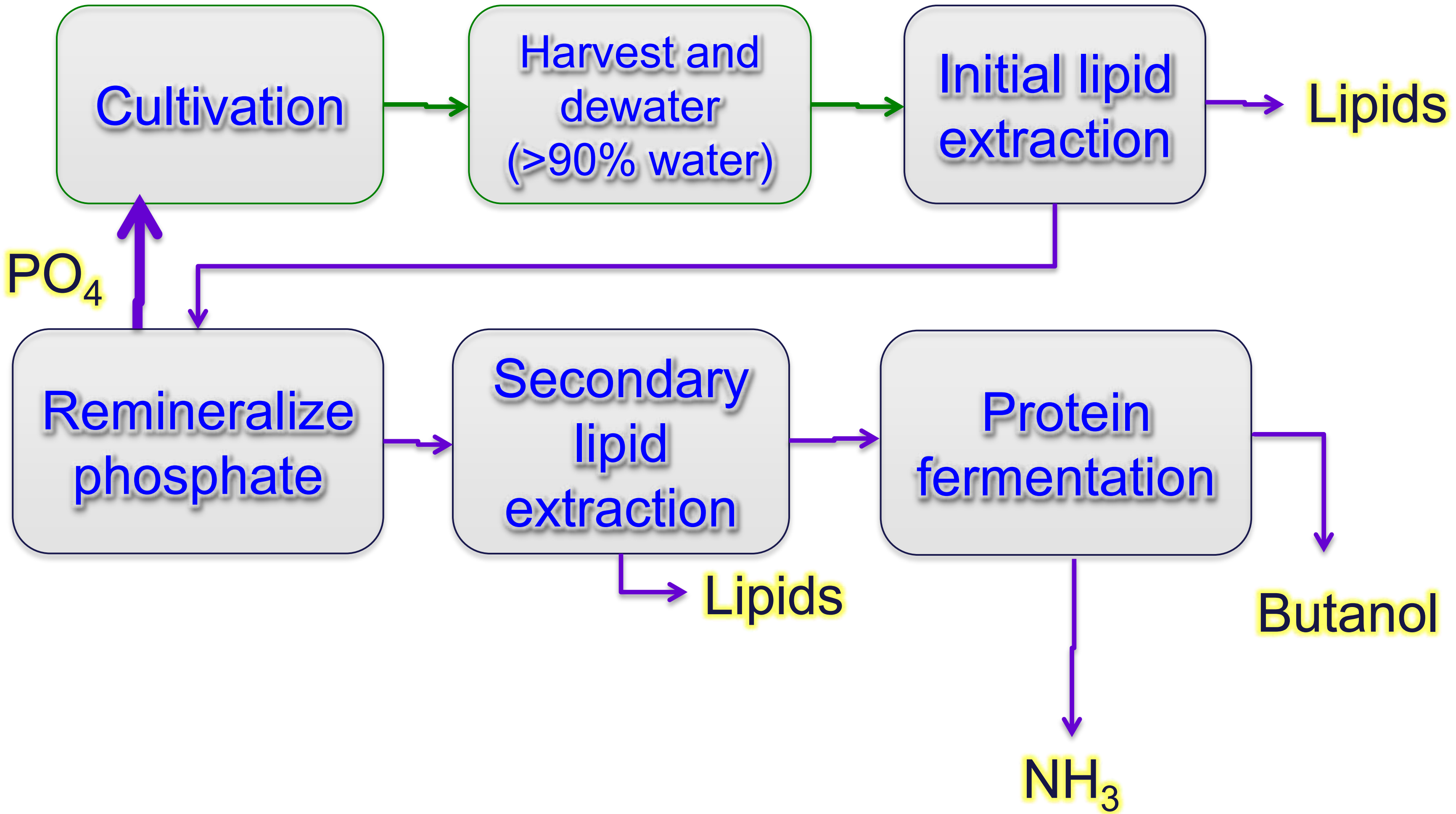
Partial closure of cycle



4 – Relevance

- This project project directly addresses a specific barriers in the BETO MYPP: **Aft-J. Resource Recapture and Recycle**
  - We are developing, and have demonstrated in part, a system for the recapture and recycle of nitrogen and phosphate in algal culture.

- *Our objectives are aligned the Bioenergy Technologies Office, MYPP goals, and are relevant to and algal biomass industry*

- The success of this project will advance the state of technology and positively impact the commercial viability of algal biofuels
  - Reduce demand for nonrenewable nutrients ie phosphate
  - Reduce competition with agriculture for fertilizer.
  - Potentially reduce the cost of nutrients
  - Could extract P & N prior to HTL or other processes

5 – Future Work (12 mos. to end of project)

- Improve yield: From a current best of ~70% P and ~60% N to ~100% recycle
  - Optimize liberation of phosphate from phospholipids
  - Combine our phosphorous and nitrogen remineralization processes
  - Incorporate methods for phospholipase/phosphatase treatment of biomass into the algal oil removal process
- Optimize growth at altered Redfield ratios for the utilization of struvite and reduce the energy required for the recycle of additional ammonium.
- Complete TEA based on current process parameters
- Key Milestones
  - Recycle nitrogen and demonstrate algal growth on recycled N
  - Optimize liberation of phosphate from phospholipids
  - Combine phosphate and nitrogen capture and demonstrate growth
- Decision points: Go/No-Go milestone in October 2015
  - Demonstrate the combined remineralization and capture of both phosphorous and nitrogen from algal biomass in a biologically available form that supports algal growth.
  - Demonstrate the growth of algae utilizing both remineralized N and P from algal biomass

Summary

1. Overview
   - An integrated system for the culture, harvest, and processing of algal biomass that will enable the reuse of major nutrients.
2. Approach
   - Non denaturing lysis and lipid extraction
   - P remineralization by gentle pH treatment
   - N remineralization by protein fermentation
3. Technical Accomplishments/Progress/Results:
   - at 2013 peer review we had been funded for 1 month—very limited results
   - TAG extracted biomass (OA process) retains ~90% of N & P
   - Phosphate can be remineralized, in soluble form by mild pH treatment: 50-70% yield
   - Nitrogen can be remineralized in soluble form by protein fermentation: 57 % yield
   - Soluble, remineralized phosphate can provide 100% of phosphate required for growth
   - Crude struvite can provide 100% of phosphate and large fraction of nitrogen for the
4. Relevance: Displacement of the need for fresh fertilizer inputs in upstream cultivation
5. Future work.
   - Optimize nutrient extraction
   - Develop system for combined capture of both N & P
   - Convert P-Lipids to DAGs and extract with OpenAlgae process

Acknowledgments

DOE EERE BioEnergy Technology Office

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Open Algae

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- Hoyt Thomas
- Stacy Truscott

AgriLIFE RESEARCH
Texas A&M System

OpenAlgae

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Additional Slides