DOE Bioenergy Technologies Office (BETO) 2017 Project Peer Review

Major Nutrient Recycling for Sustained Algal Production

1.3.2.200

3/06/2017

Algae Technology Area Review

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Sandia National Laboratories

Sandia National Laboratories is a multi-program laboratory operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Goal Statement

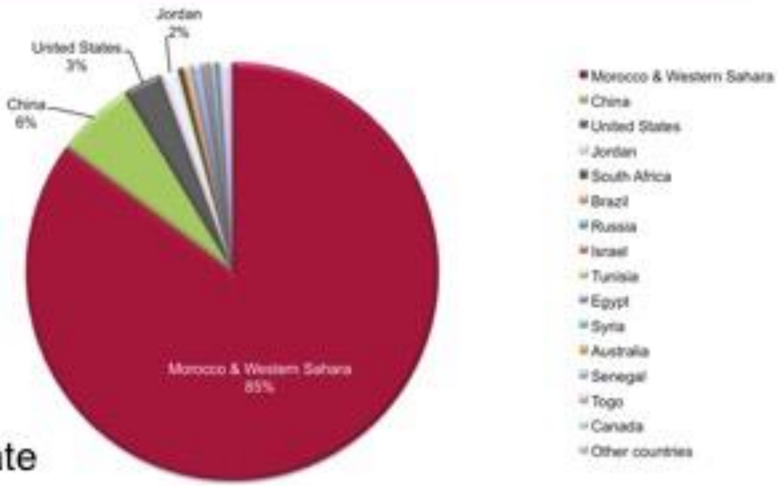
The grand objective of the proposed work is to develop an integrated system for the culture, harvest, and processing of algal biomass that will enable the reuse of major nutrients thus reducing the operational requirement for external nutrients.

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."

ASAP FOA: Develop and demonstrate a process that "Significantly reduces external nutrient input requirements (eg: Nitrogen and Phosphorus) and has the potential to be scaled economically.

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.

Phosphate presents a potential geopolitical risk to energy security

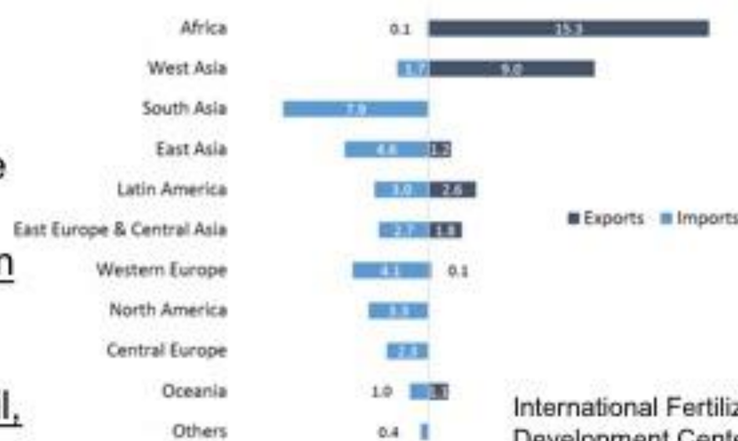


Cordell & White 2011

- 82% of mined phosphate rock is used for fertilizer
- Non-renewable, finite, fossil form of phosphate
- Substantial uncertainty over magnitude of phosphate reserves
    - Large changes in the estimated phosphate reserves
    - Potential for movement of phosphate resources into reserves.
- Quality of the reserves (such as % P and presence of impurities) is generally in decline over time.
- Developed countries are net importers of phosphate rock
- <u>Three countries control more than 85% of the known global phosphate rock reserves</u>

- <u>This concentration of power is far greater than for oil, where the dozen members of the Organization of the Petroleum Exporting Countries control 80% of the world's oil reserves."</u>

Fig. 3 *Phosphate Rock Ex- & Imports, 2011 (Mt.)*



Source: Based on IFAdata (2013a).

International Fertilizer Development Center (IFDC)

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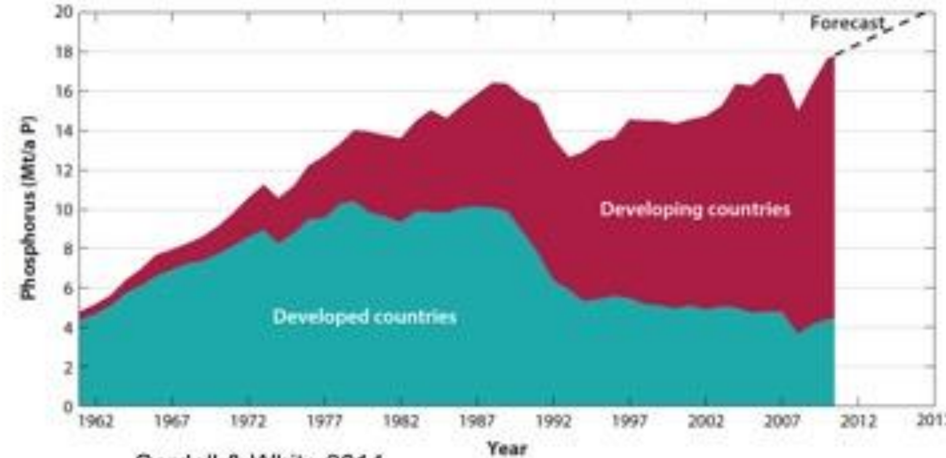
Increased phosphate demand and environmental impact

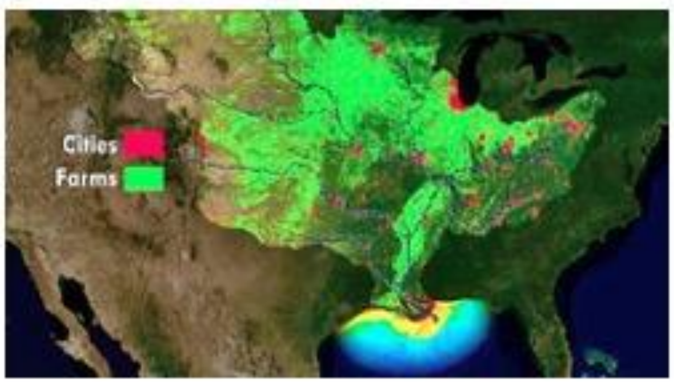
Dietary changes: increased meat consumption drive increased demand
- Transient price spike ~2007
- Instability in prices after 2010

Decrease in phosphate demand due to realization of consequences of over usage and economic downturn in former Soviet states
- -Fertilizer runoff leads to the generation of dead zones

Biofuels production may lead to increased phosphate demand in developing countries



Cordell & White 2014



NOAA 2015

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
  - TAG extraction
  - DAG extraction
    - Converted phospholipids

Quad Chart Overview

Timeline

- Project start date: 3/15/2013
- Project end date:9/31/2016
- Percent complete: 100%

Barriers

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

Budget

	Total Costs FY 12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	$827,743	$636,052	$616,495	64,893
Project Cost Share (Comp.)* AgriLife	$80,059	$45,649	$33,518	0
Project Cost Share (Comp.)* Open-Algae	$195,724	$30,284	$151,049	0

Partners

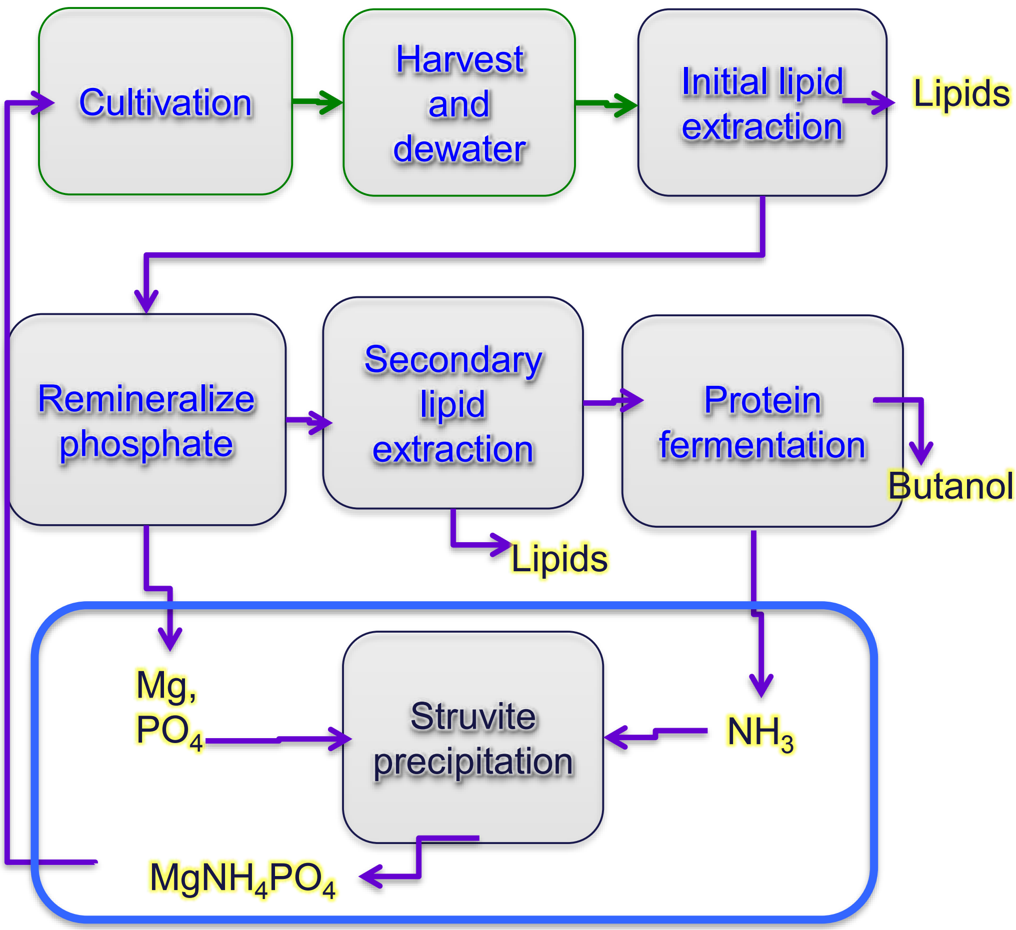
Texas Agrilife (TAMU)
OpenAlgae LLC

1 - Project Overview

The grand objective of the proposed work is to develop an integrated system for the culture, harvest, and 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 de-oiled algal biomass.
2. Convert phospholipids to DAG, remineralized phosphate, and recover both.
3. Remineralize nitrogen and phosphorus as struvite and demonstrate a simple struvite 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)

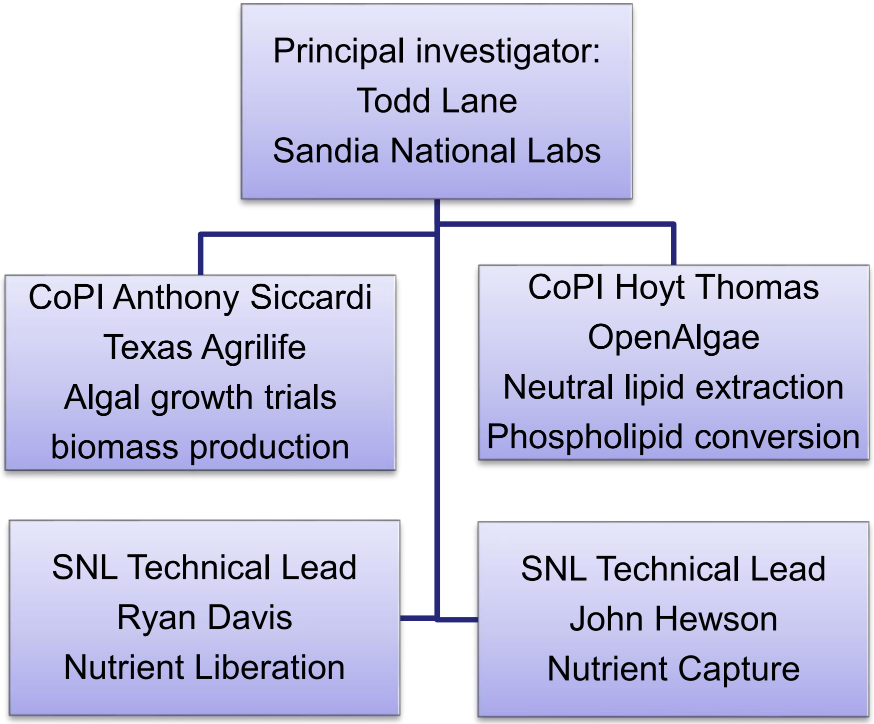


- Non denaturing method of neutral lipid extraction
- Remineralization of cellular phosphate by mild pH and enzymatic treatment
- Conversion of cellular nitrogen by protein fermentation
- Return of recylced nutrients to pond for subsequent biomass production.

Sandia Bioscience

Sandia National Laboratories

Management structure reflects task structure



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
- CoPIs and technical leads are responsible for achieving task milestones
- PI retains ultimate decision-making authority

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 tricornutom*, 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 proccess

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
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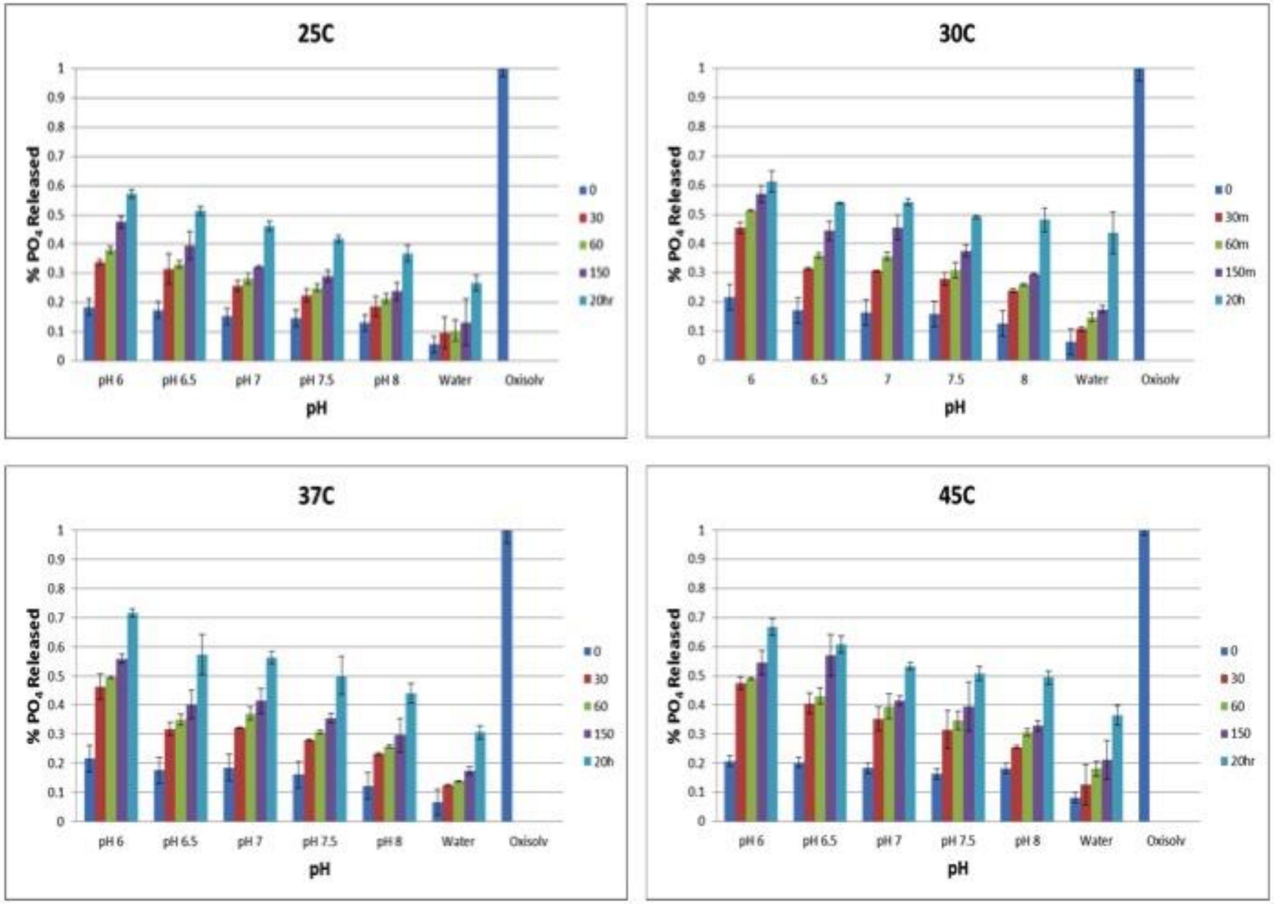
- Soluble, remineralized phosphate can provide 100% of phosphate required for growth of *N. salina* or *P. tricornutum* at laboratory and pilot scale
- Crude struvite can provide 100% of phosphate and large fraction of nitrogen for the growth for the growth of *N. salina* and *P. tricornutum* at laboratory scale and pilot scale.
- Struvite produced from the combination of remineralized phosphate and fermentation liquor can support the growth of *N. salina* at laboratory scale

Optimized reaction releases up to 70% of cellular P

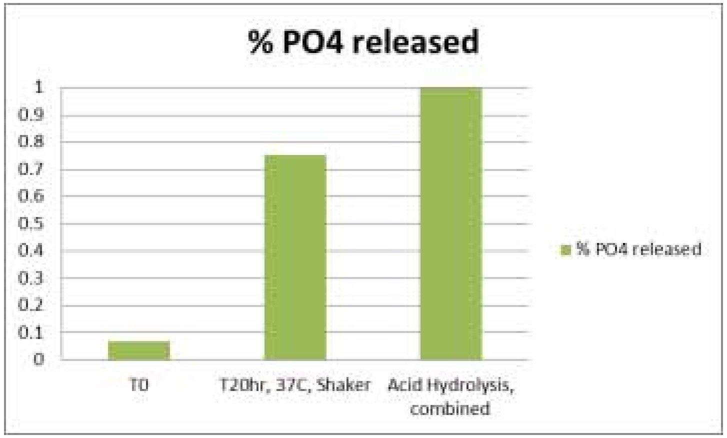


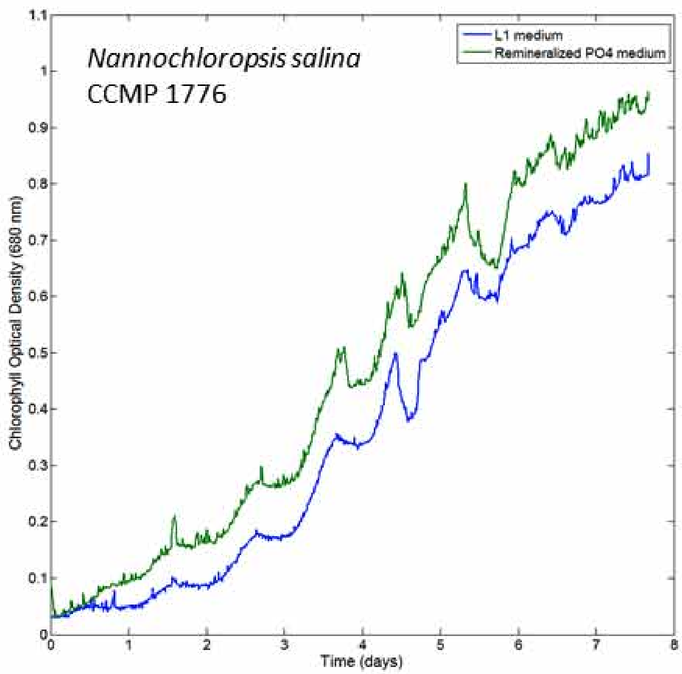
Liberating up
To 70% of total
cellular phosphate
under mild pH
incubation conditions

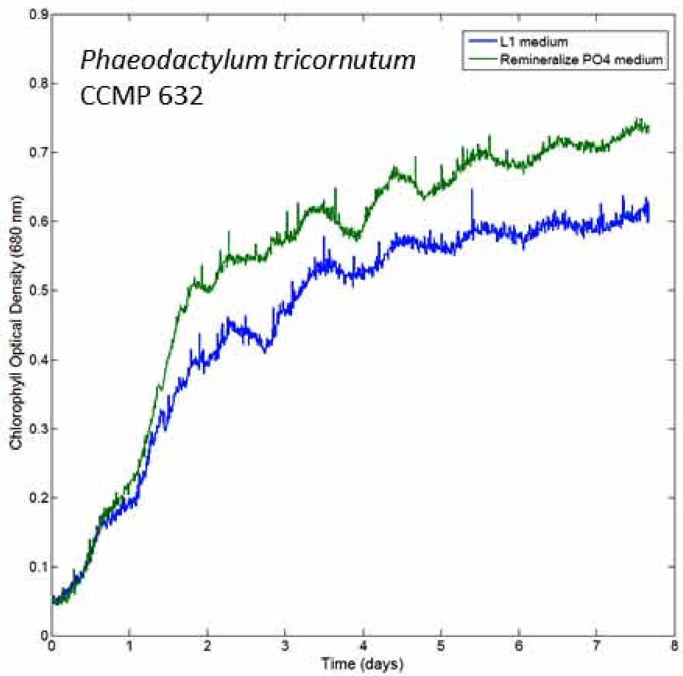
Higher temperature
does speed the
reaction somewhat

Regrowth of biomass on remineralized phosphate

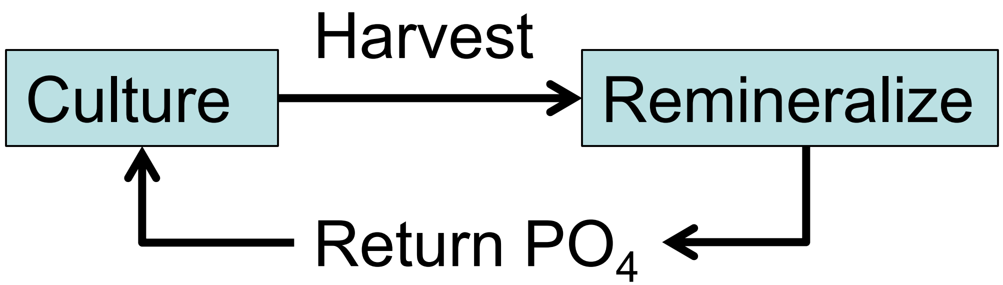
- ~50 gm of 20% solids. *N. salina*
- Diluted to 2% solids pH 6.5, 37° , 20hrs
- Liberated phosphate used to replace total phosphate in algal culture
- Growth of *P. tricornutum* and *N. salina* on soluble liberated phosphate

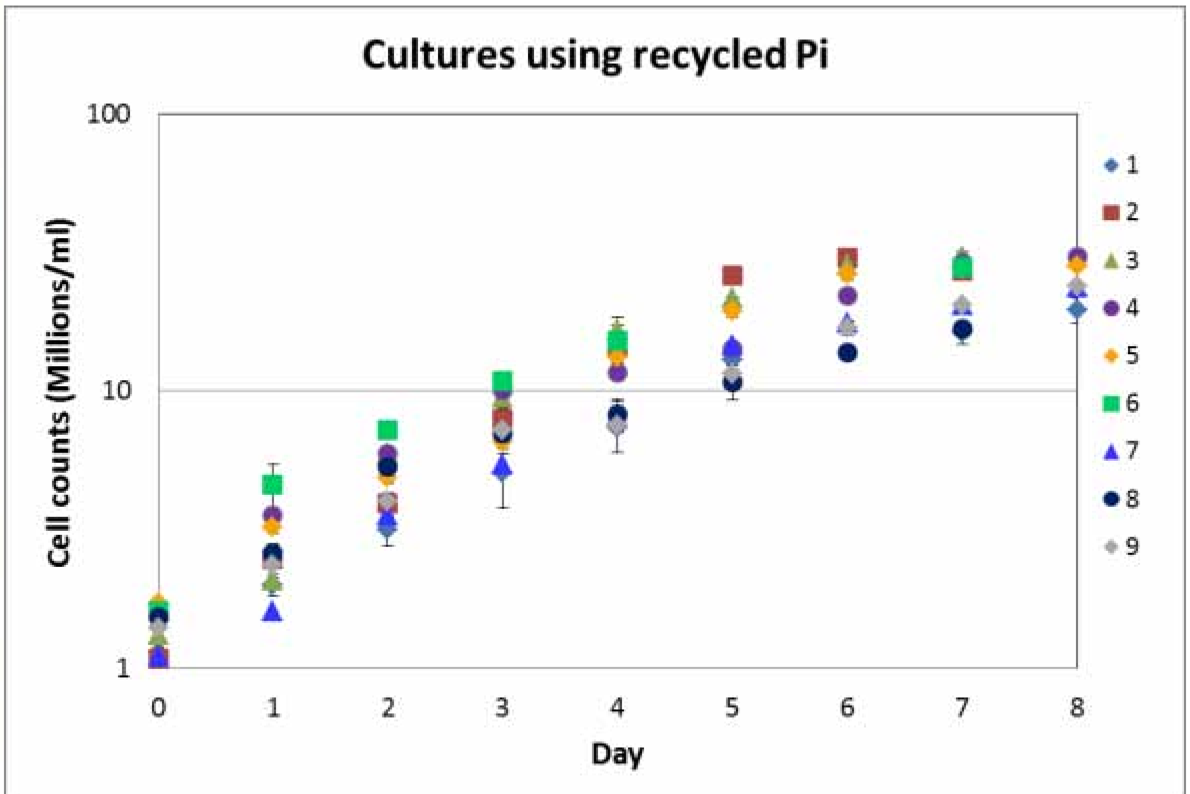






Repeated rounds phosphate remineralization and reuse in *N. salina* culture





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

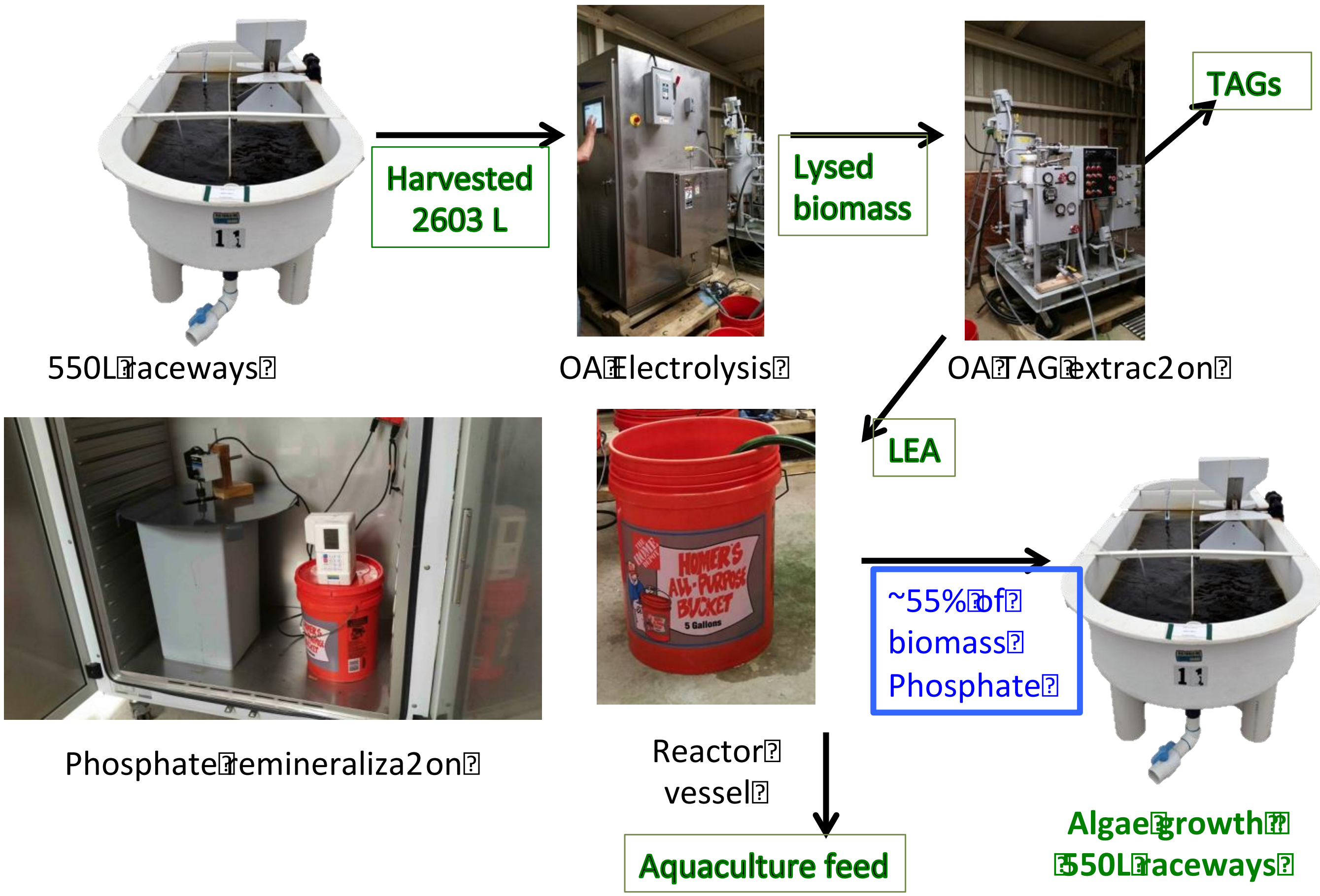
No difference in specific growth rates over the course of 8 rounds of recycle (9 culture rounds)

No evidence of accumulation of growth inhibitors through 8 recycles

Response to peer review

Pilot scale phosphate remineralization

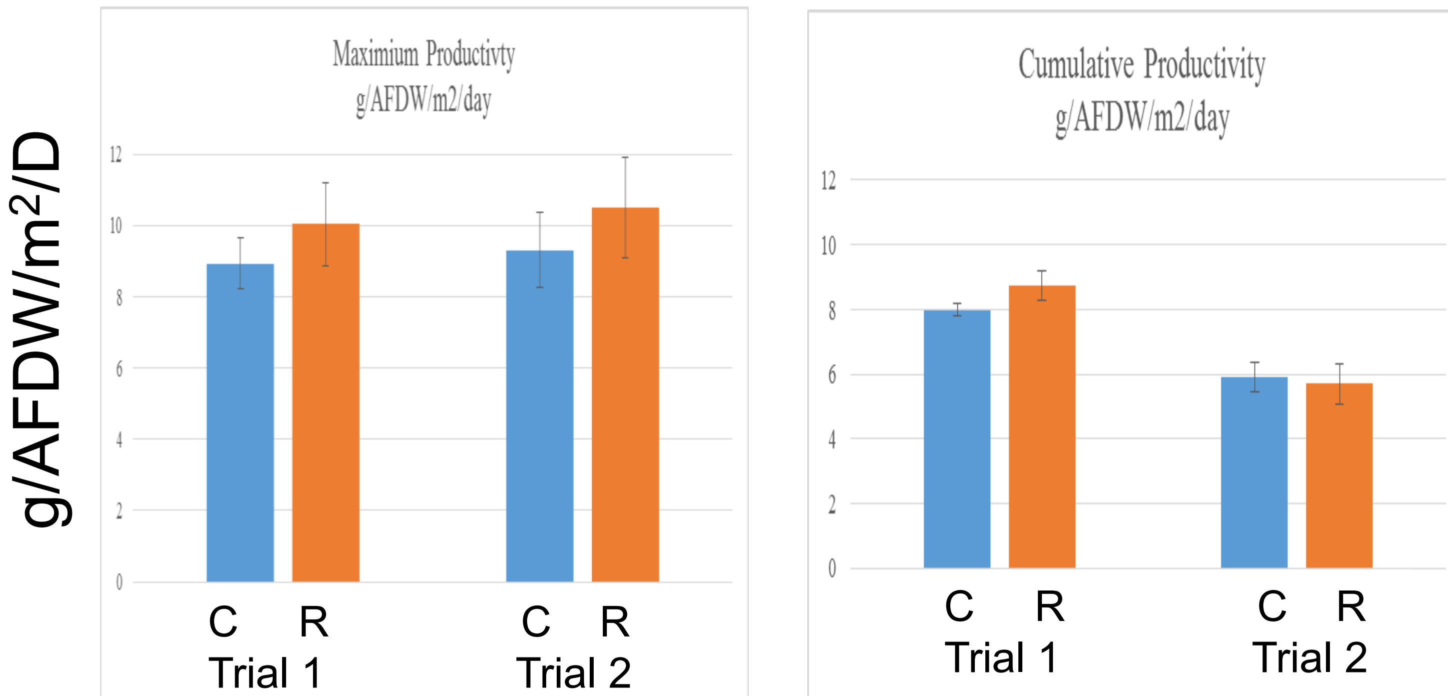
Pilot scale phosphate remineraliza1on pathway



Phosphate reuse at pilot scale

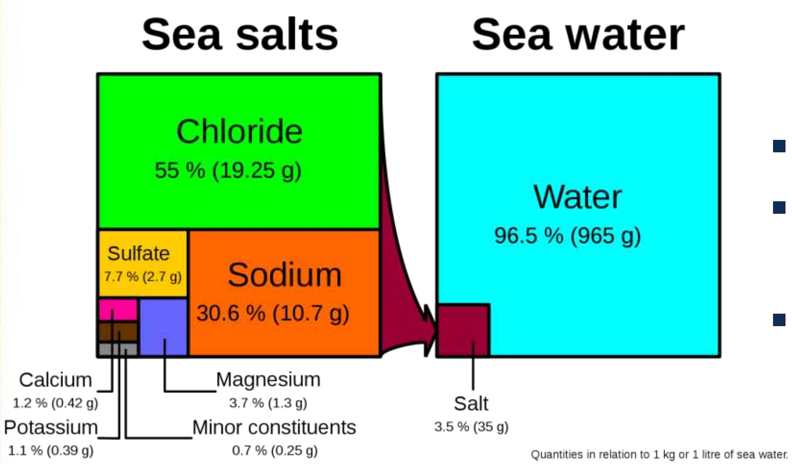
P Recycle Trial 1 April 2016
10:1 N:P Ratio (0.49 m Mol/L/N : 0.05 m Mol/L/P)

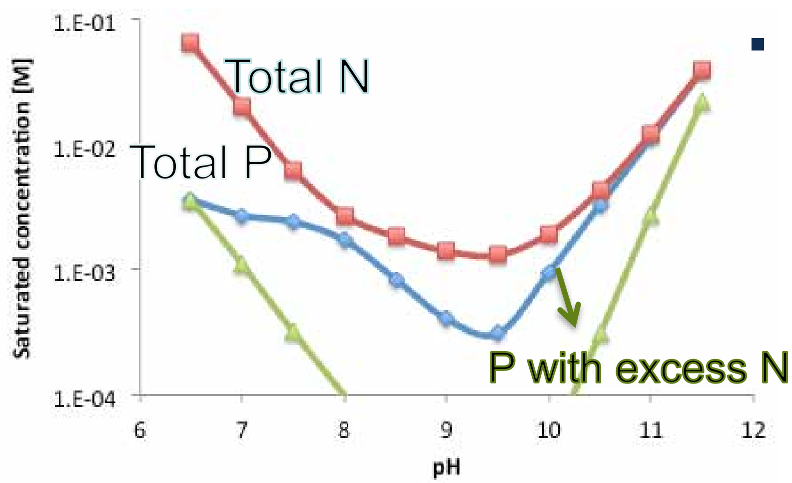
P Recycle Trial 2 Sept 2016
10:1 N:P Ratio (0.49 m Mol/L/N : 0.05 m Mol/L/P)



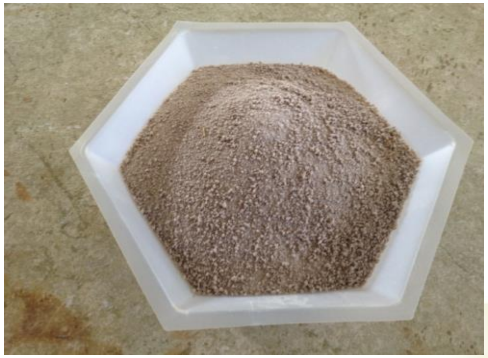
C: New phosphate control
R: Recylcled phosphate

Struvite is the likely form of recycled phosphate & nitrogen



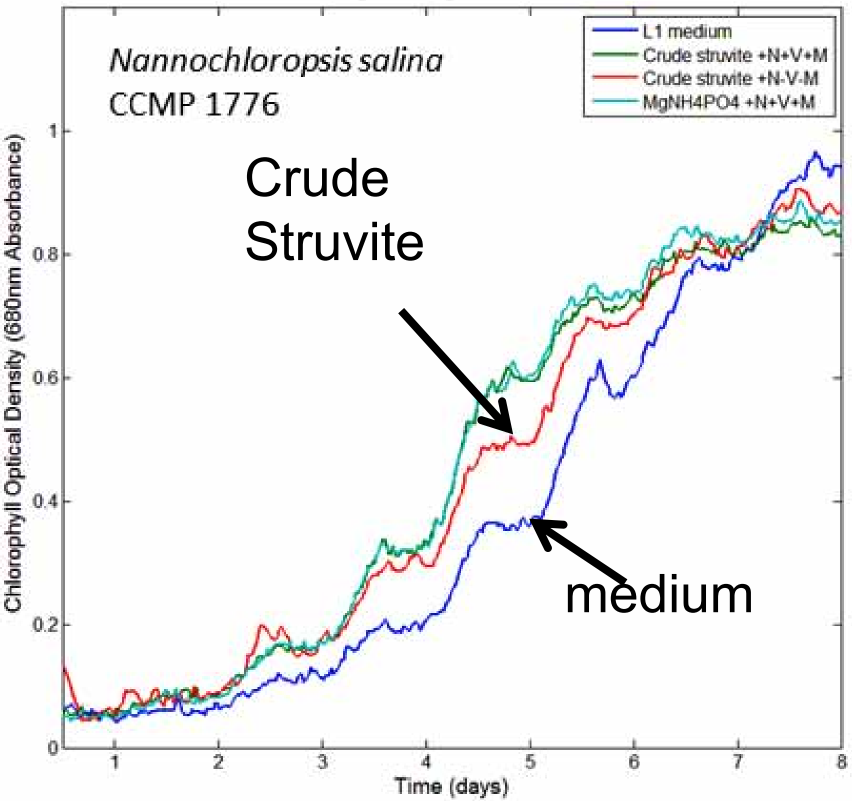


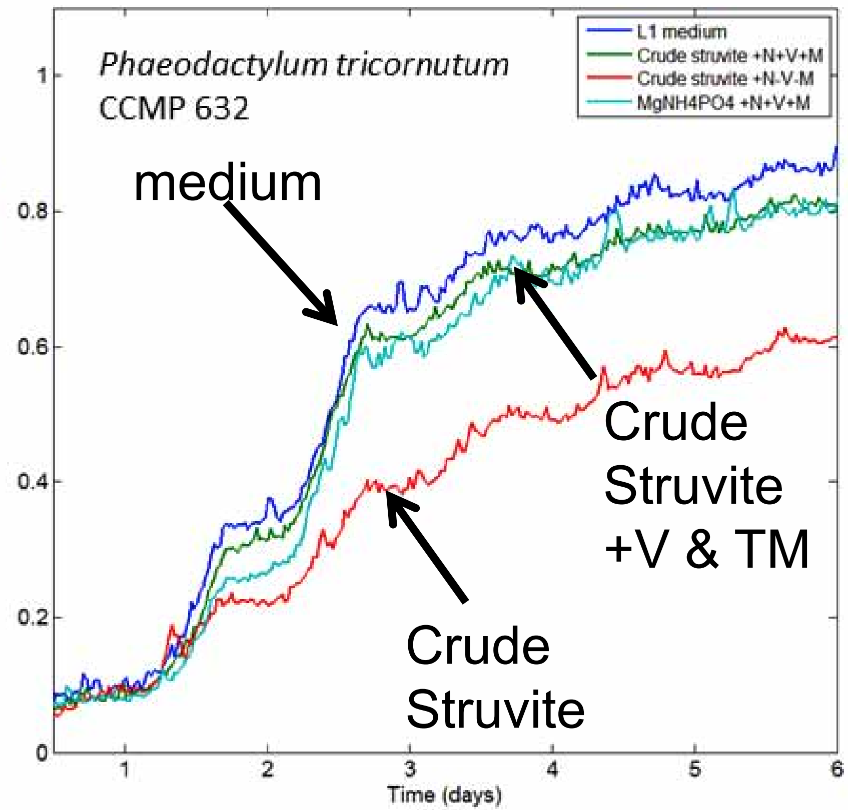
- It has been shown that struvite can be formed with purity levels up to 95% when using seawater and brine as the Mg source (Liu et al., 2013)
- Mg is 50mM in seawater
- If algae dewatered to 10% one can expect 5mM Mg to remain.
- Depending on excess NH4/NH3 used in precipitation, estimate 80-99% potential PO4 precipitation.
- Recovers 1:1 N:P
- Precipitates at accessible concentrations
- Trials conducted at anticipated raceway phosphorus levels
  - pH 7.8, 8.2



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

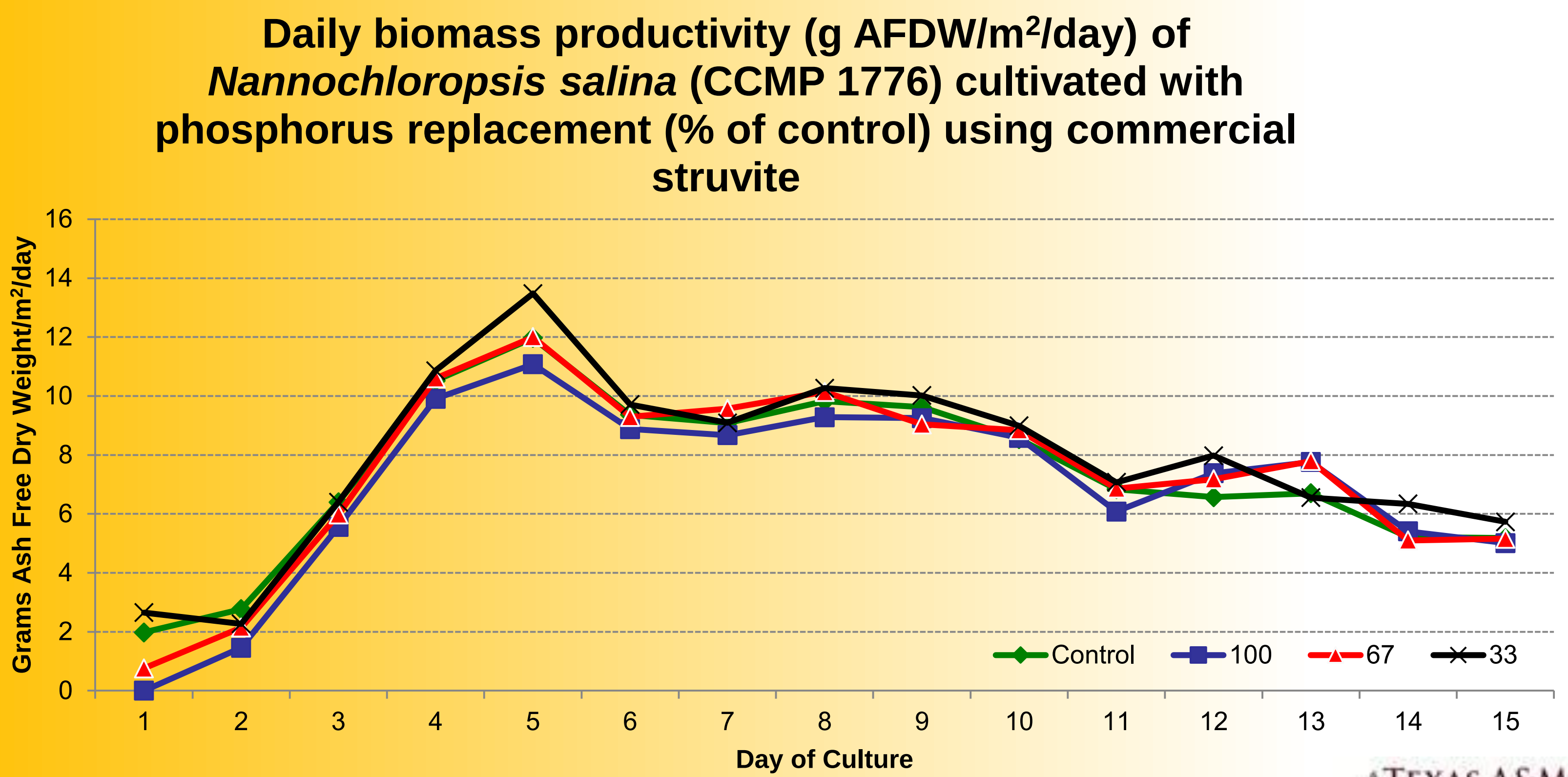




Multicultivator, sinusoidal 16/8 LD cycle,
peak 1000⌠E, 21 to 24 C

Cultivation with struvite in outdoor raceways

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



Regrowth of N. salina on recycled N & P

The N-remineralization yield was 57% (±14%) of theoretical

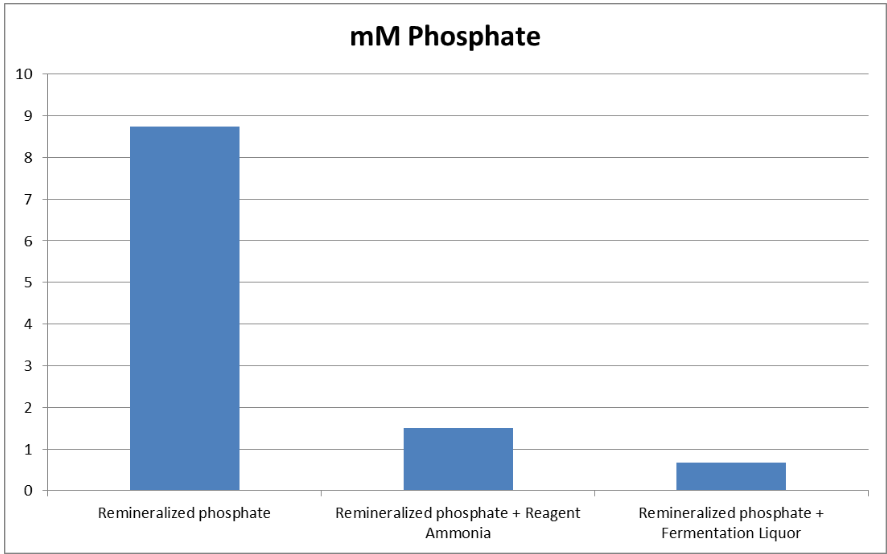
Remineralized Phosphate combined with

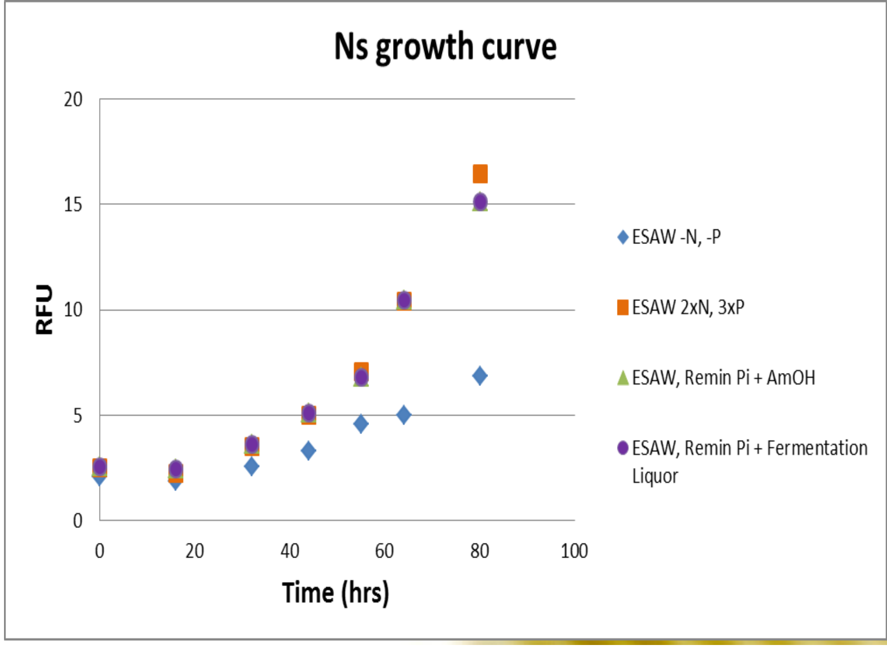
Reagent NH_4OH
Fermentation liquor

Loss of phosphate from soluble phase & formation of ppt presumed to be struvite

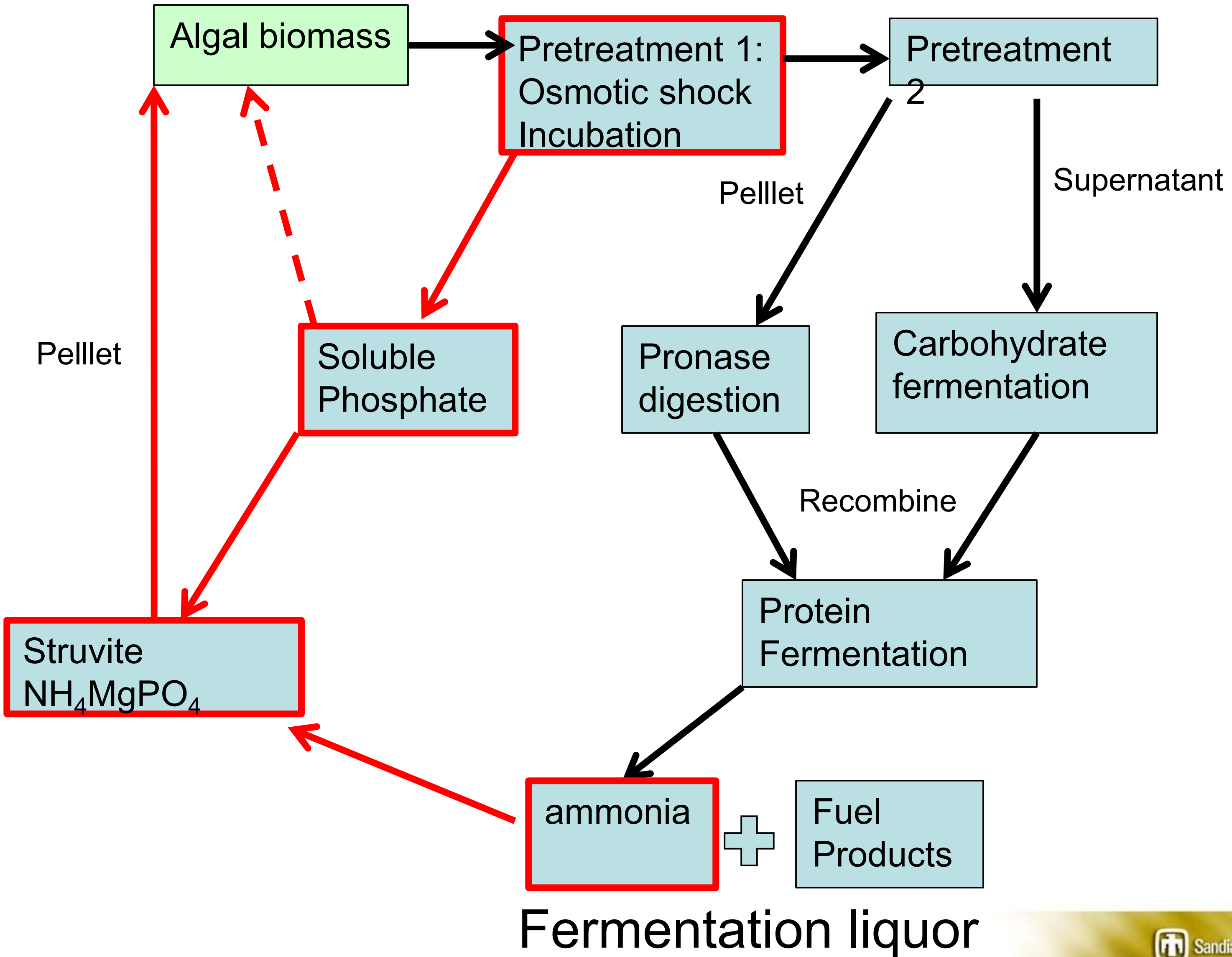
Replace total phosphate and an equimolar amount of nitrogen

No apparent deleterious compounds carried over by phosphate precipitation with fermentation liquor





Fermentation and nutrient recycle



4 – Relevance

- This project project directly addresses a specific barriers in the BETO MYPP: **Aft-J. Resource Recapture and Recycle**
  - We are have developed and demonstrated 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
- We met two of the goals of the ASAP FOA:
  - Our process significantly reduces external nutrient input requirements (eg: Nitrogen and Phosphorus) and has the potential to be scaled economically.
- 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

Summary

- 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
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Summary

- OpenAlgae TAG extraction process retains ~90% of N & P
- Phosphate can be remineralized, in soluble form, from non denatured *N. salina* biomass by mild pH treatment at pilot scale
- This phosphate can can provide 100% of phosphate required support algal growth
- This method is potentially scalable and economic.
- Nitrogen can be remineralized in soluble form from *N. salina* biomass by protein fermentation.
- The fermentation liquor combined with solubilize phosphate will form struvite that can support the growth of *N. salina* at laboratory scale
- 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 and pilot scale.

Acknowledgments

DOE EERE BioEnergy Technology Office

Sandia National Labs

- Ryan Davis
- John Hewson
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- Hoyt Thomas
- Stacy Truscott

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Texas A&M System

OpenAlgae

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy
BIOENERGY TECHNOLOGIES OFFICE

Publications, Patents, Presentations, Awards, and Commercialization

Presentations

1. "Nutrient recycling for pilot scale algal production." Algal Biomass Summit Glendale AZ October 2016
2. " Major nutrient recycling at laboratory and pilot scale for sustainable algal production.", 6th International Conference on Algal Biomass, Biofuels and Bioproducts. San Diego, CA 15 June 2016
3. "Integrated, Recycle of Nitrogen and Phosphorus in for Sustainable Algal Production." Algal Biomass Summit Washnington DC. 1 October 2015
4. "Nutrient Recycling for Sustained Algal Production", 5th International Conference on Algal Biomass, Biofuels and Bioproducts. San Diego, CA 7-10 June 2015
5. "Major Nutrient Recycling for Sustainable Algal Production." Algae Biomass Summit, San Diego, CA, 2 October 2014
6. "Utilization of Struvite to Replace Traditionally used Nutrients for the Production of Microalgae in Mixed and Mono Cultures." Algae Biomass Summit, San Diego, CA, 2 October 2014
7. "Nutrient Recycling for Sustained Algal Production" 5th Congress of the International Society for Applied Phycology Sydney, Australia 22-27 June 2014
8. "Nutrient Recycling for Sustained Algal Production" 4th International Conference on Algal Biomass, Biofuels and Bioproducts. Santa Fe, New Mexico 15-18 June 2014
9. "Struvite Precipitation as a Path for Nutrient Recycling" 4th International Conference on Algal Biomass, Biofuels and Bioproducts. Santa Fe, New Mexico 15-18 June 2014
10. "Nutrient Recycling" Lab Special Topic Presentation. Biomass Program Staff Meeting 12/3/2013
11. "Recovery of nutrients from biomass for nutrient recycling" Algae Biomass Summit, Orlando FL, Sept 30-Oct 3 2013
12. "Major nutrient recycling for sustained algal production" Algae Biomass Summit, Orlando FL, Sept 30-Oct 3 2013

Publications

1. Davis, R.W., Siccardi A., Wyatt N., Hewson J., and **Lane T.W**. 2015. Growth of Mono- and mixed cultures of *Nannochloropsis salina* and *Phaeodactylum tricornutum* on struvite as a nutrient source. *Bioresource Technology* 198:577-585
2. Lane PD, Davis RW, Lane TW "Recycling of phosphate for sustainable *Nannochloropsis salina* culture". Target journal: *Bioresource Technology*

Patents

"High rate algae culture using recycled struvite as a nutrient source" – Hewson, J., Davis, R., Wyatt, N., and Lane, T. SD13112 – PROVISIONAL APPLICATION FILED ON 06.25.14

Responses to Previous Reviewers' Comments

Reviewers have pointed out the need to carry out technoeconomic Analysis (TEA) and lifecycle Analysis (LCA) on our processes. TEA and LCA are outside the scope of the funded SOPO, so our ability to carry out such analyses is very limited. If additional resources were made available from BETO we would be able carryout robust and detailed analyses in collaboration with experts at the NREL and ORNL.

A reviewer suggested that we extend our work to include cyanobacteria. We can carry out limited nutrient extraction trials with cyanobacterial biomass as suggested by the reviewer. However, we believe that our ability to carry out additional experiments with cyanobacteria are limited under the scope of the currently funded SOPO. If further funding was made available we would be able to carryout more extensive cultivation and nutrient recycling experiments with cyanobacteria.

A reviewer suggests that long-term issues include optimization for additional algal feedstocks. We agree that this is indeed important. Our nutrient recycling processes are already being developed for a diversity of algal lineages and preliminary results indicate that our methods are likely to be generalizable to a variety of algal feedstocks.

Are reviewer stated that " These technologies are also not, at this point, compatible with a pathway like HTL". We respectfully disagree on the potential compatibility of our nutrient recycling processes with HTL. There are few limits on biomass treatment prior to HTL—that being one of the strengths of the process. The phosphate remineralization process, that we have developed and demonstrated, would have no impact on the suitability of the residual biomass for HTL. Likewise, nitrogen recycling by protein fermentation can be employed upstream of HTL and, unlike the nitrogen containing raffinate from HTL, is likely to result in a nutrient product that is not contaminated with growth inhibiting compounds.