Sandia Bioscience

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Nutrient Recycling for Sustained Algal Production

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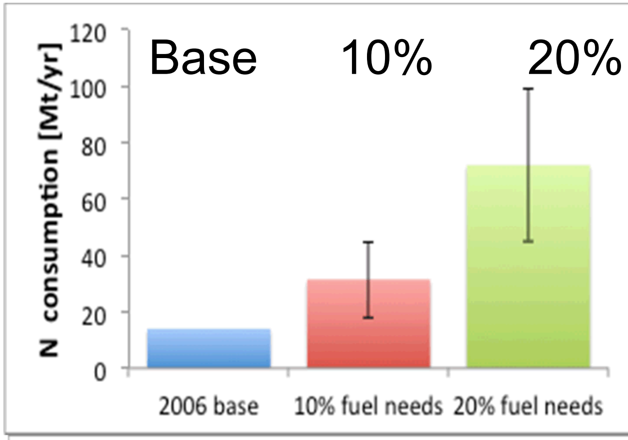
DOE funded project is a partnership between national lab, university and industry

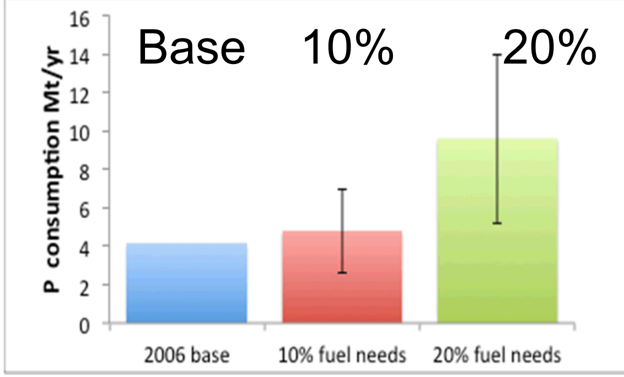
- Sandia National Labs
  - Project Lead
  - Biochemistry
  - Precipitation Science
- Texas Agrilife:
  - biomass production
  - pilot scale field trials
  - Marine species
    - *Nannochloropsis salina*
    - *Phaeodactylum* tricornutum
      - (NAABB strains)
- OpenAlgae
  - TAG extraction
  - DAG extraction
    - Converted phospholipids

Biomass at energy-consumption relevant scales exceeds current nutrient production

Pate, Klise, Wu, "Resource demand implications for US algae biofuels production scale-up," Applied Energy, 88:3377-3388 (2011).

- To meet 10% of U.S. 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'

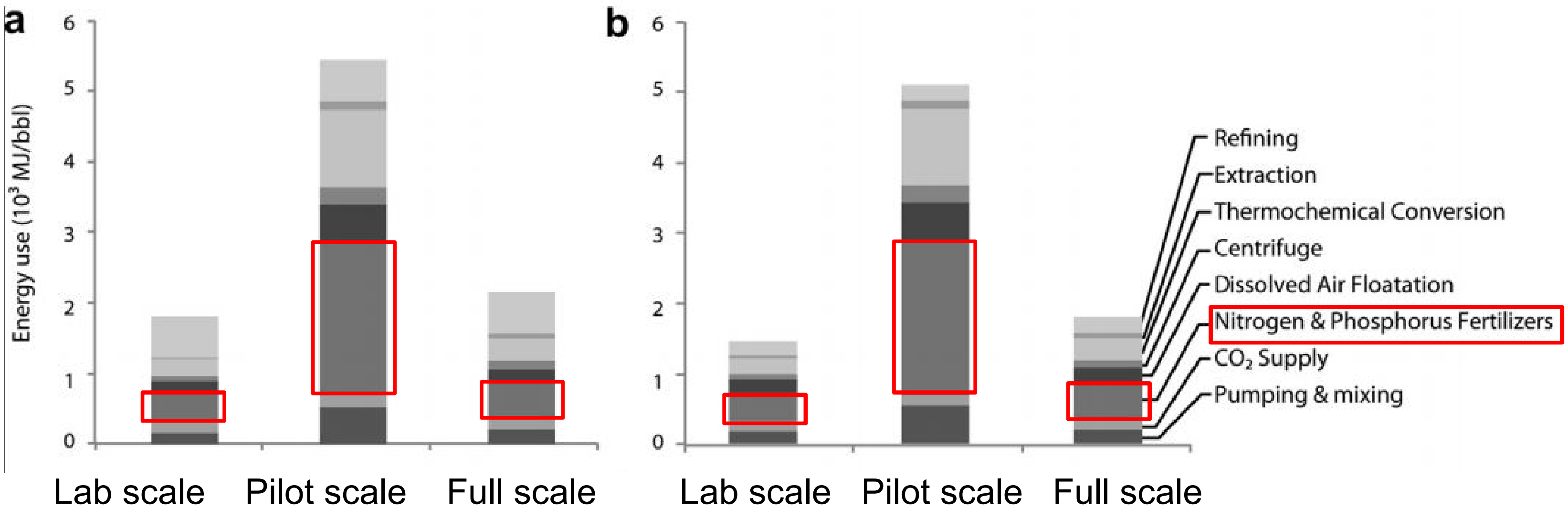




- Food vs fuel: "The Achilles Heel of Algae Biofuels: Peak Phosphate," Forbes, Feb. 2012.

Nutrients constitute a large energy input

- Recent LCA, based on Sapphire data, suggests nutrient costs (even with partial recycle) are substantial.
- Energy use per barrel of (a) diesel and (b) gasoline
- *according to this, N/P is the biggest single energy input into the system, accounting for ~30-40% of the total



Liu, *et al.*, "Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction " *Bioresource Technology,* 148:163-171, 2013.

(LCA based on Sapphire data)

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Need to recycle nutrients
Cannot afford to pass through once only

- Nutrients are needed for biological productivity, not fuel.
- Develop and evaluate processes for nutrient recycling.
  - Two steps:
    - Convert organic N and P to inorganic forms.
    - Separate nutrients from energy products & return to culture.

- **Chemical form of nutrients only important in that it must be bioavailable**

- Target struvite ($MgNH_4PO_4$) as convenient, transportable, fungible nutrient.

- Recovers 1:1 N:P
- Precipitates at accessible concentrations.
  - Experience in waste water treatment industry.
- Involves Mg readily available in seawater
- (and inexpensive otherwise).
- Alternates include Ca and Mg phosphates.

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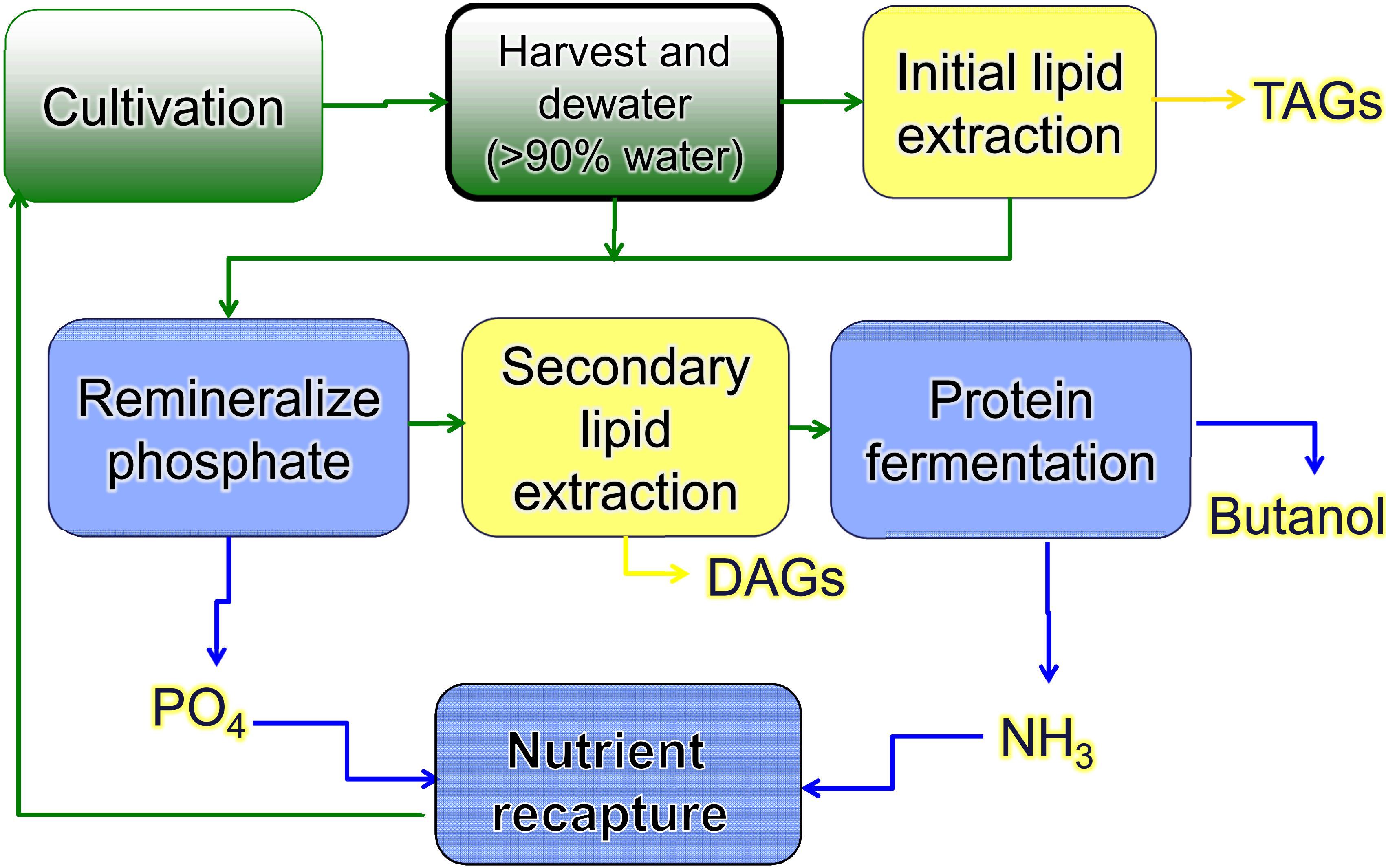
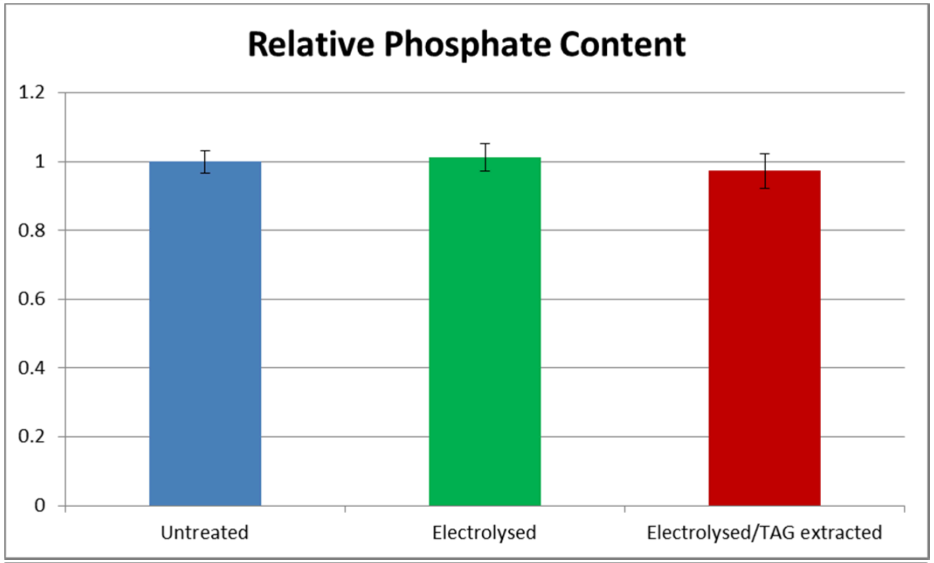
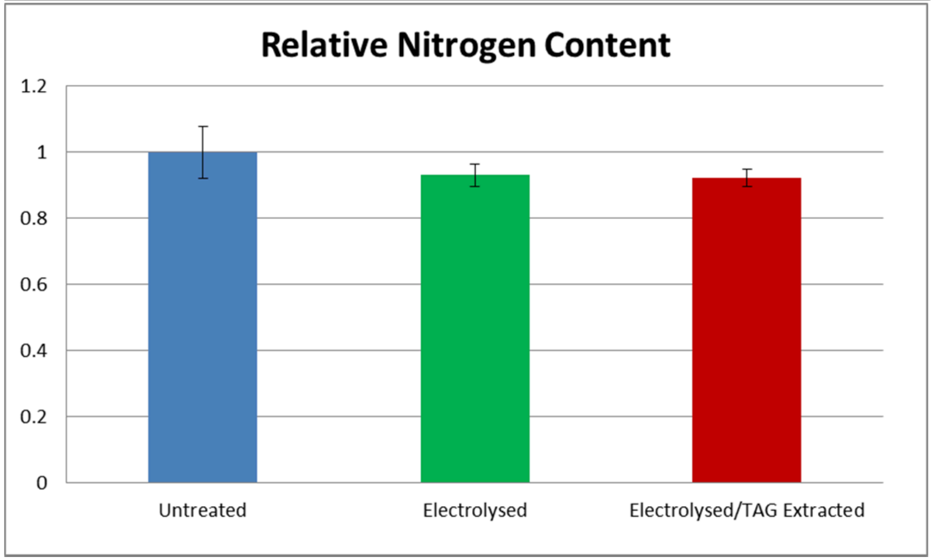
Proposed closed process



Open Algae oil extraction process Preserves N & P in Biomass





Goal: Retain at least 40% of cellular phosphate and nitrogen after TAG extraction by Open Algae

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

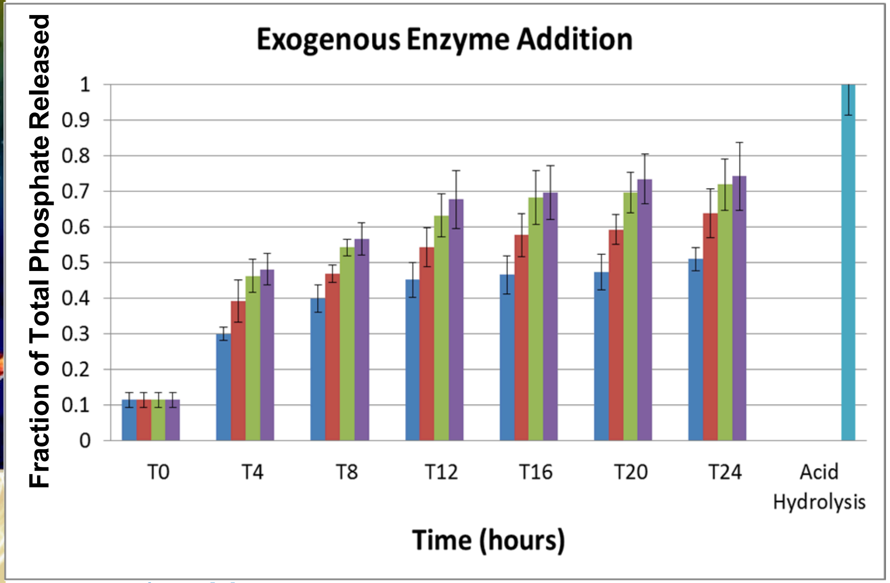
Tested on untreated and TAG extracted, 3 Biological replicates

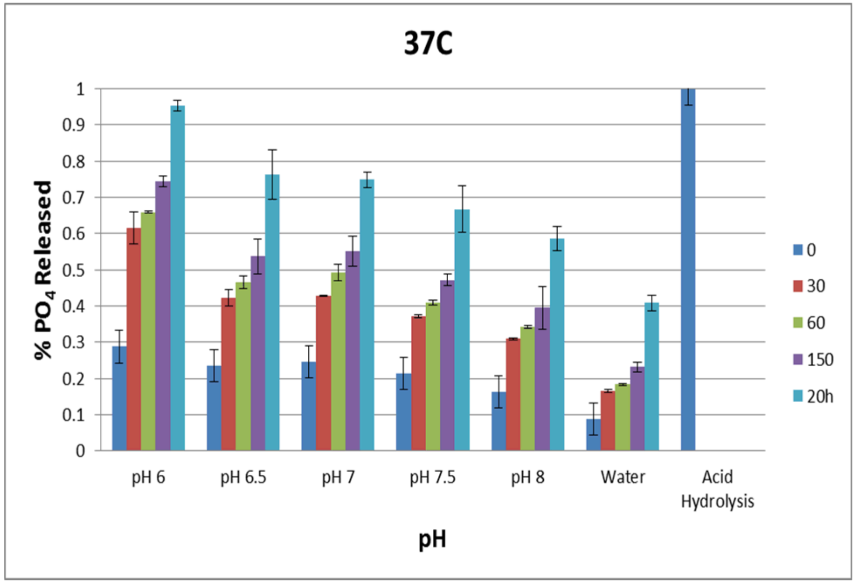
Total cellular phosphate and nitrogen assayed by standard methods (Oxisolv oxidation followed by colorimetric determination)

Cellular phosphate rapidly remineralized in osmotically shocked, non-denatured *N. salina*

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

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

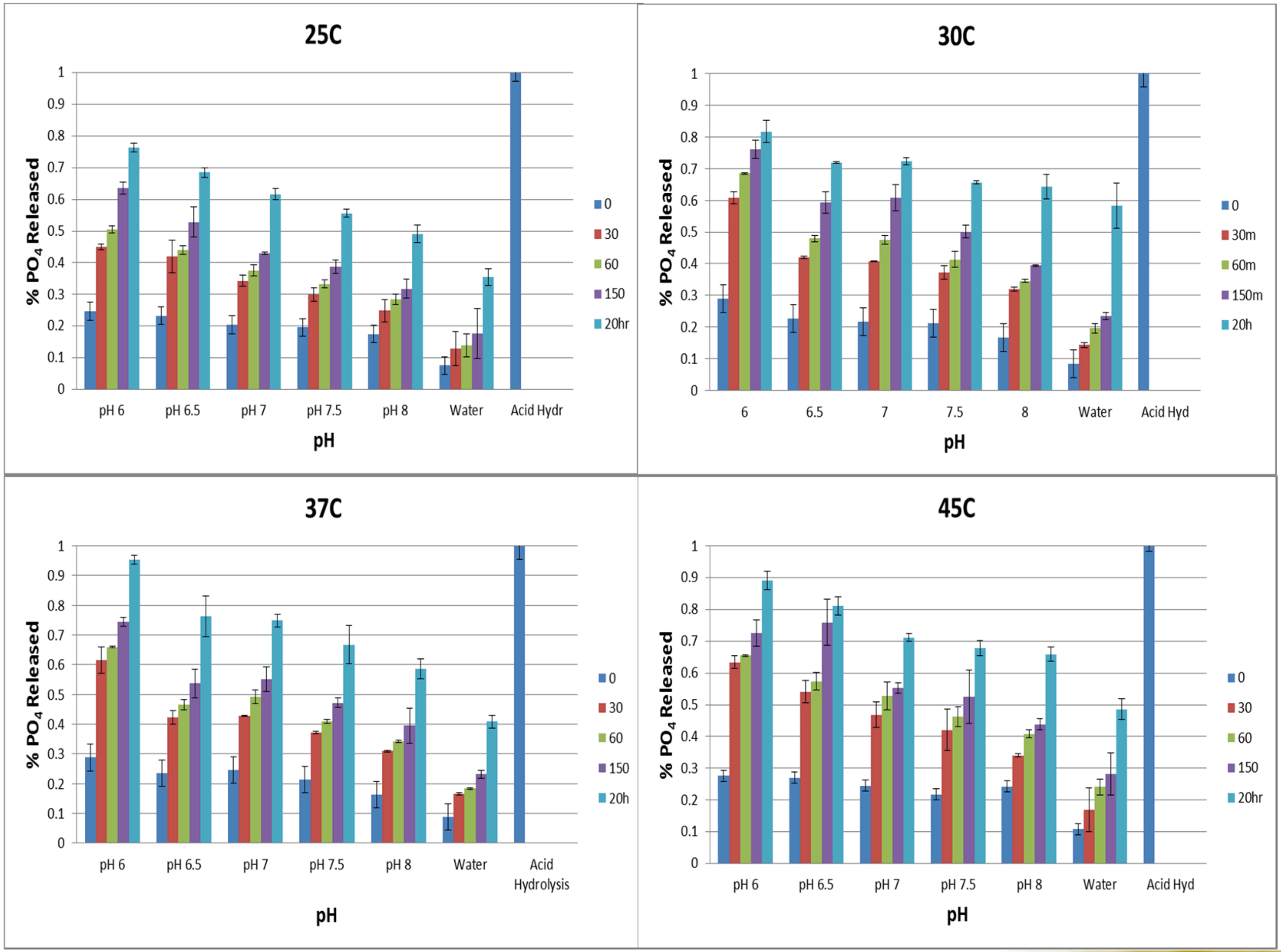




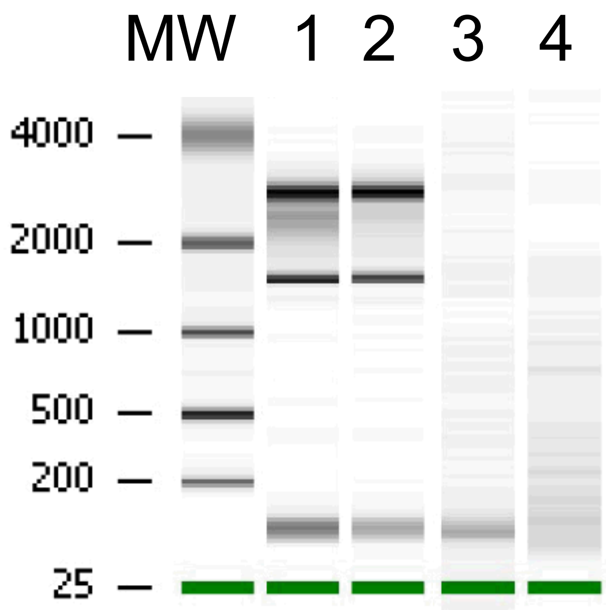
1. No enzyme
2. Alkaline Phosphatase (AP)
3. AP + Benzonase (B)
4. AP + B + Phospholipase D

T=0, 30min, 60min, 150min, 20hr, Acid Hydrolysis

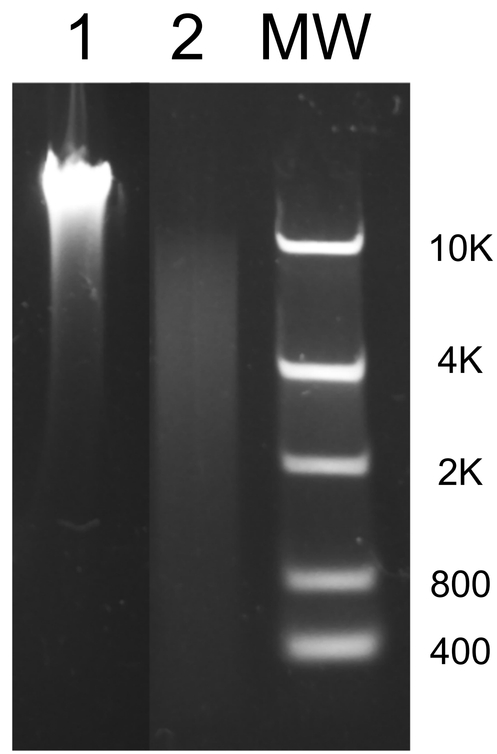
Temperature optimum for remineralization in *N. salina*



Nucleic acid pool is largely degraded by incubation



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

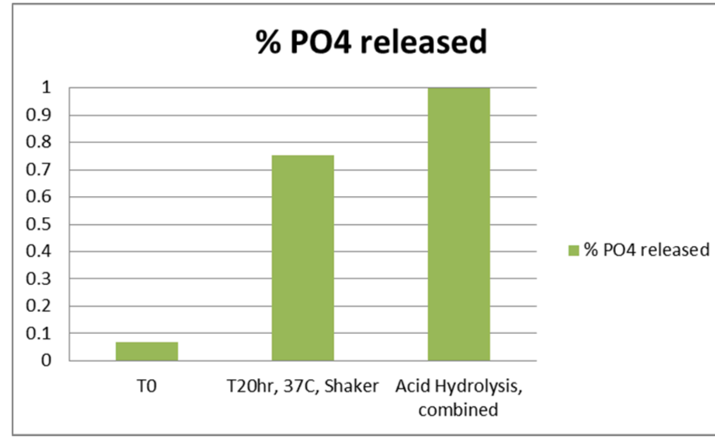


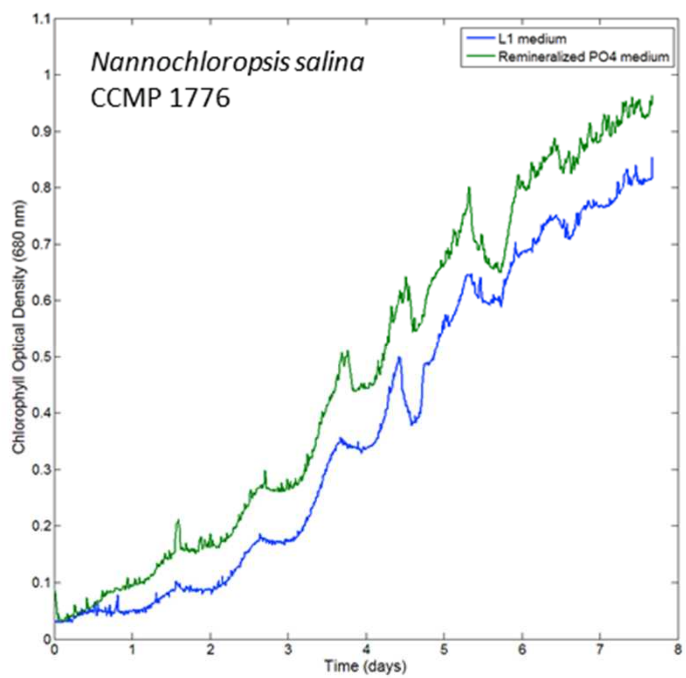
1. *N. salina* DNA before digest
2. *N. salina* DNA after digest--**25X loaded**

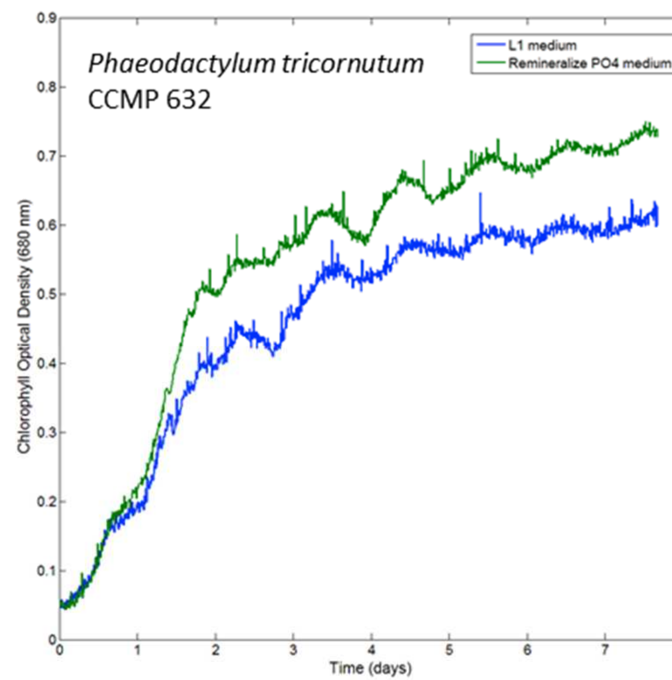
Digest conditions:
Resuspend biomass in water to 1% solids
Add MES pH6 to 50mM
Incubate at 37C, 20 hours

Regrowth of biomass on remineralized phosphate

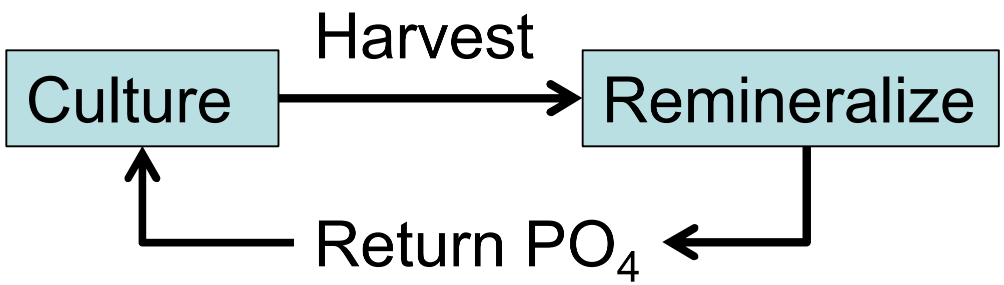
- ~50 gm of 20% solids. *N. salina*
- Diluted to 2% solids pH 6.5, 37° , 20hrs
- Liberated phosphate used to replace total phosphate in algal culture
- <u>Growth of *P. tricornutum* and *N. salina* on soluble liberated phosphate</u>

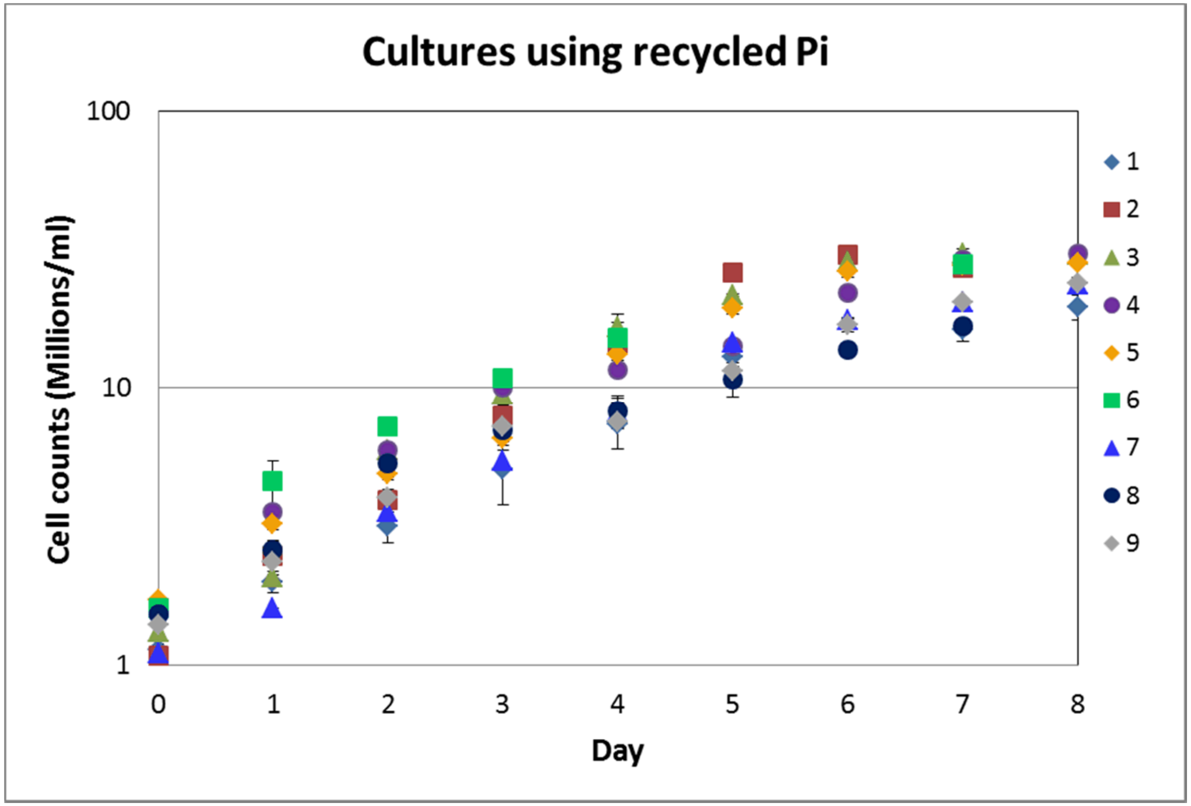






Repeated rounds phosphate remineralization and reuse in *N. salina* culture



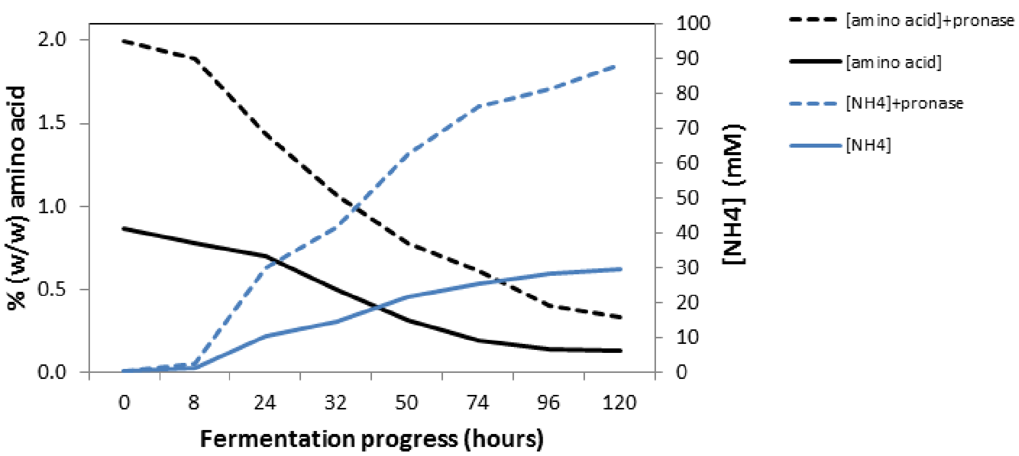


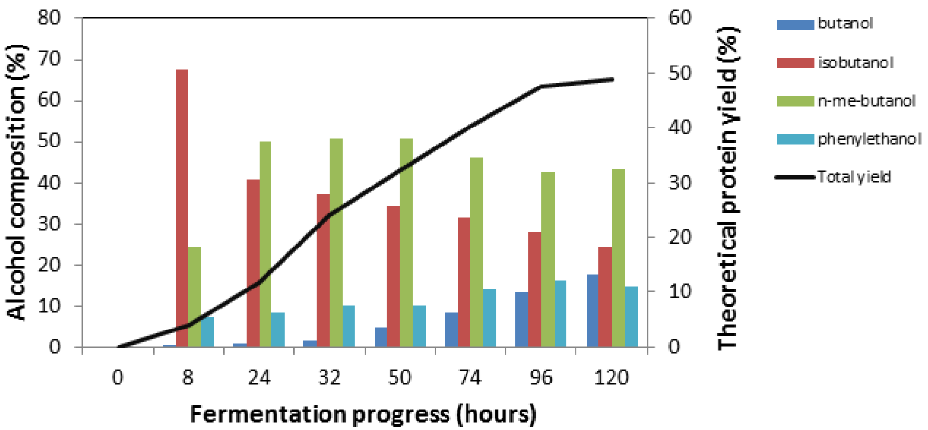
After first round, recycled up to 66% of consumed phosphate

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

Remineralize N through protein fermentation

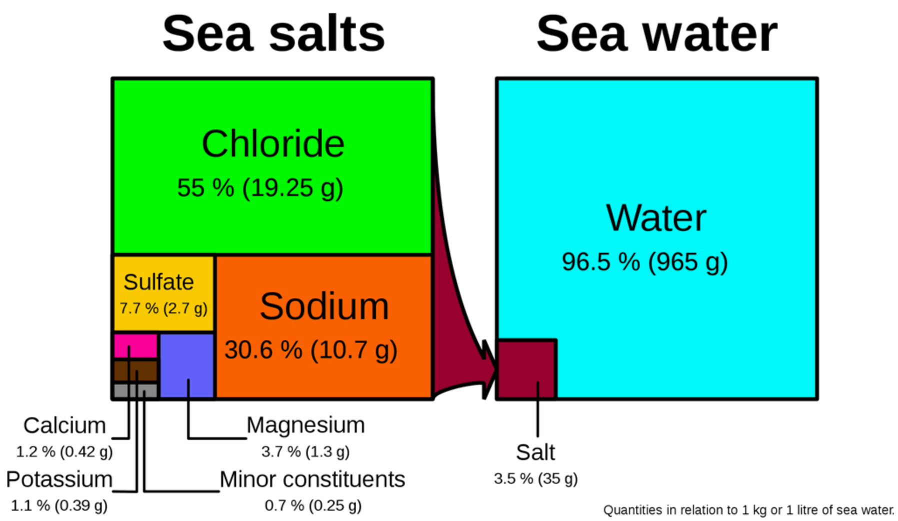




Davis *et al* 2013

- Amino acid fermentation yields ammonium and higher alcohols.
  - Huo et al., Nature Biotechnology, 29(4):346-351, 2011.
- Proteins recalcitrant to dilute acid hydrolysis. Adding enzyme mix more than doubles amino acid availability.
- Resulting ammonium available at moderate concentrations.

In current system, significant Mg is carried over with biomass



$NaNO_3$ (M)	KH_2PO_4 (M)	Fe (mg/g)	Mg (mg/g)
0.006	0.0003	3.37	77.3
0.006	0.0003	4.75	67.1
0.006	0.001	3.86	78.1
0.006	0.001	3.81	98.3
0.003	0.0003	2.83	82.0
0.003	0.0003	2.91	93.5
0.003	0.001	3.46	74.8
0.003	0.001	2.41	74.4

Encarnação *et al* J. of Agri. Sci. and Technol. A 2 (2012) 824-832

Depending on chemical makeup of growth medium, significant extracellular Mg may be carried over with biomass

Internal Mg stores may also be significant: 3-4 mMol Mg/gm AFDW

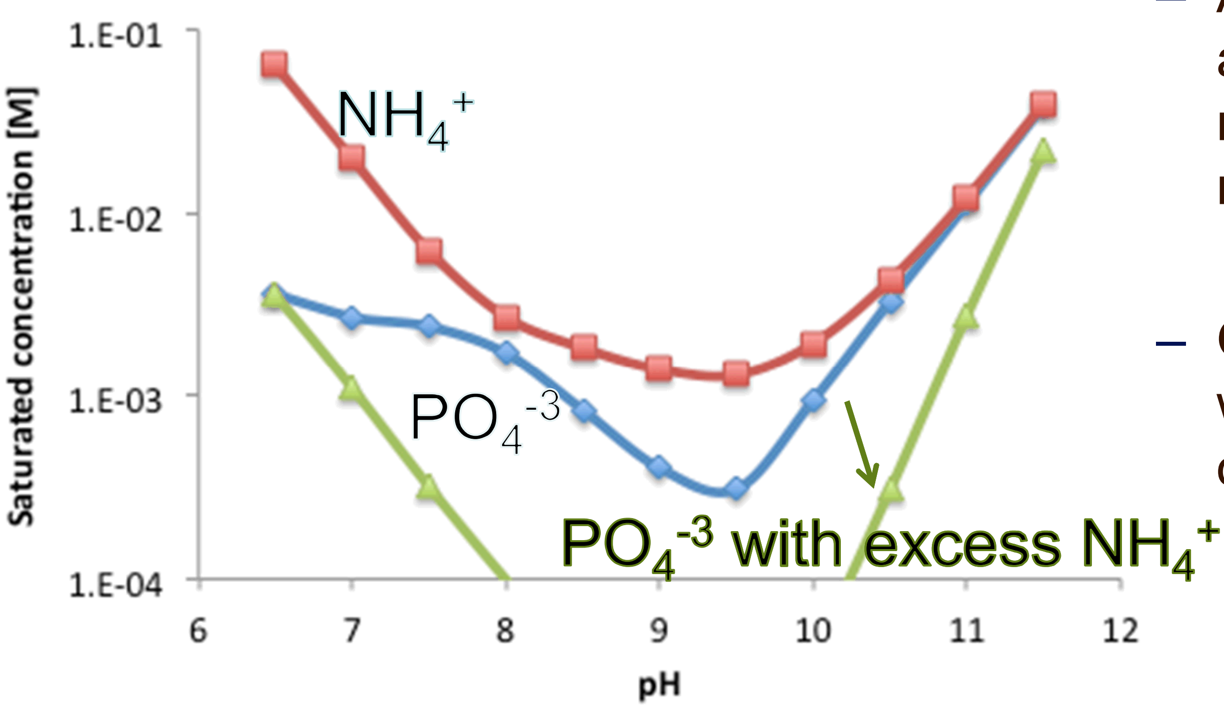
The resulting P/Mg ratio may promote the formation of struvite: $MgNH_4PO_4$

<u>The formation or utilization of struvite does not alter the chemistry of the pond or biomass</u>

<u>No requirement for new Mg</u>

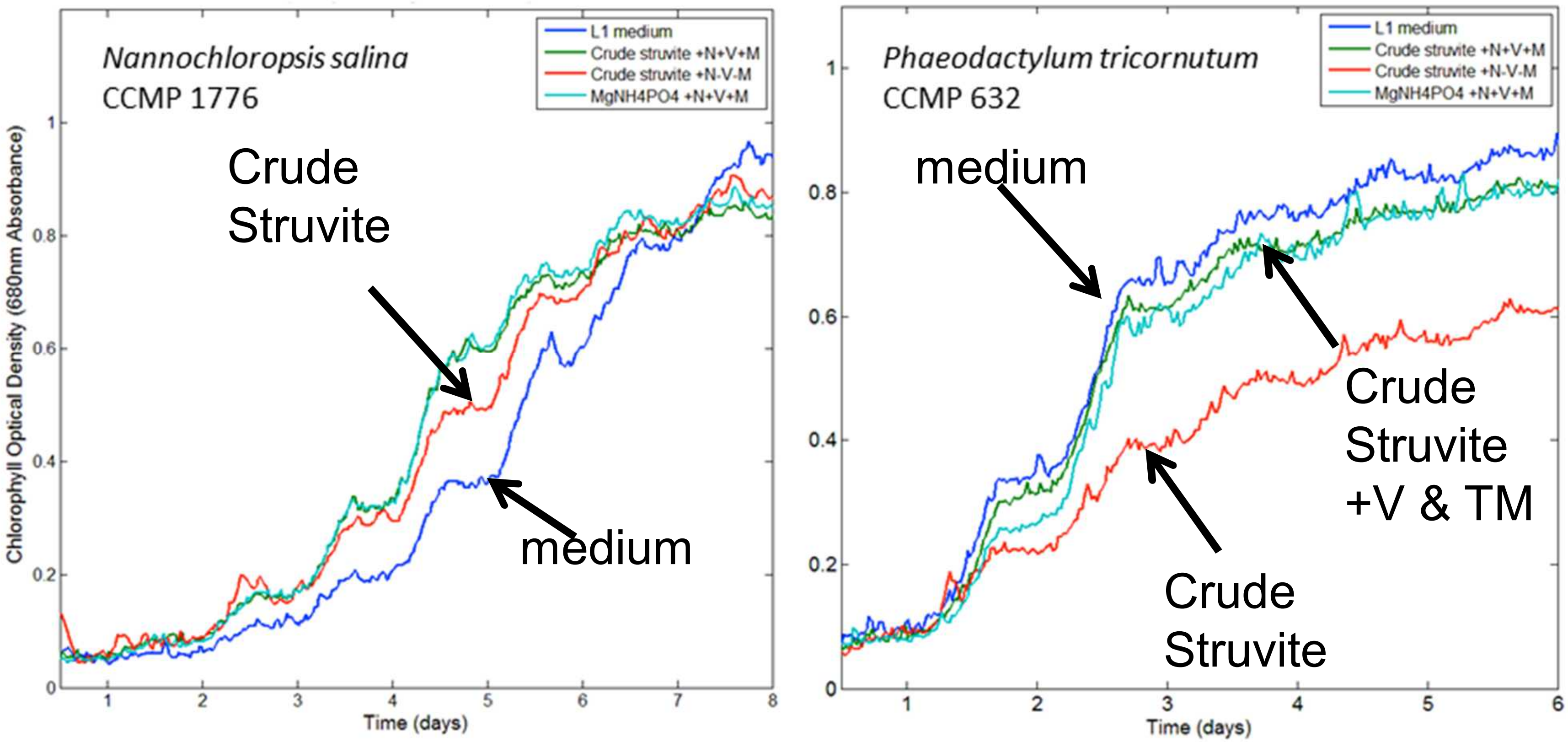
Recover nutrients through precipitation

- Struvite ($MgNH_4PO_4$) is useful mineral form of nutrients.
  - Alternates include Ca and Mg phosphates.
- Looking at designing system to maximize recovery – need to measure precipitation kinetics.



- At concentrations available in nutrient recovery, potential to recover >90% PO_4^{-3}.
- Outstanding issues with effect of organics on kinetics.

Struvite can replace "new" nutrients in microalgal culture



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

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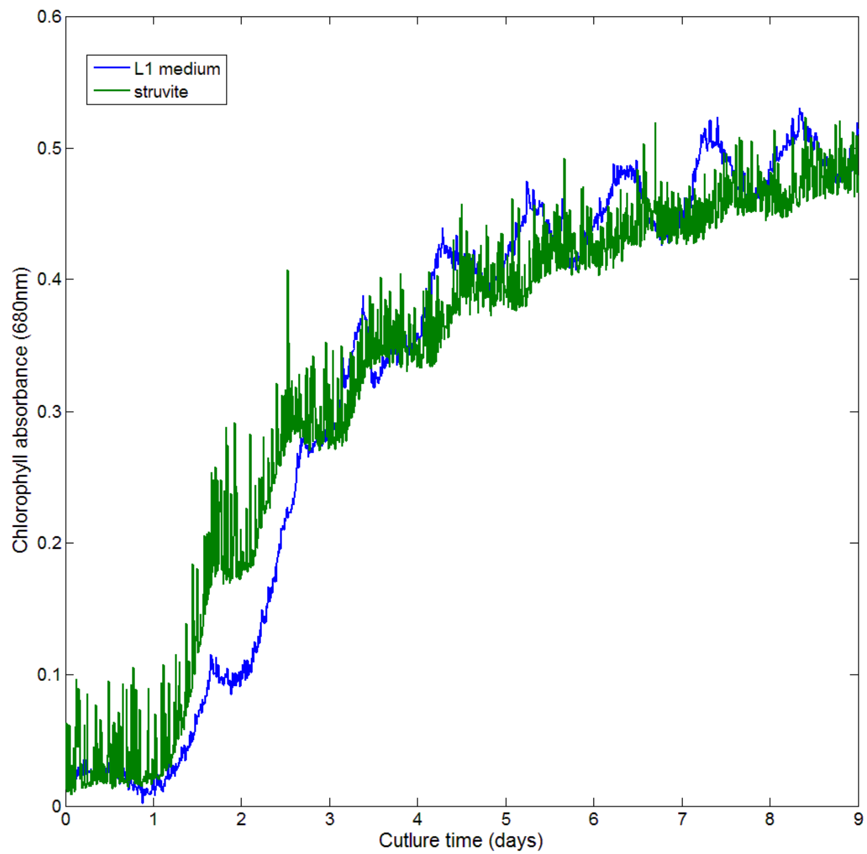
Cultivation with excess struvite

- Use enough struvite to provide recommended N, excess P (16x).
- 2/3 struvite does not initially dissolve (noisy absorption signal).



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

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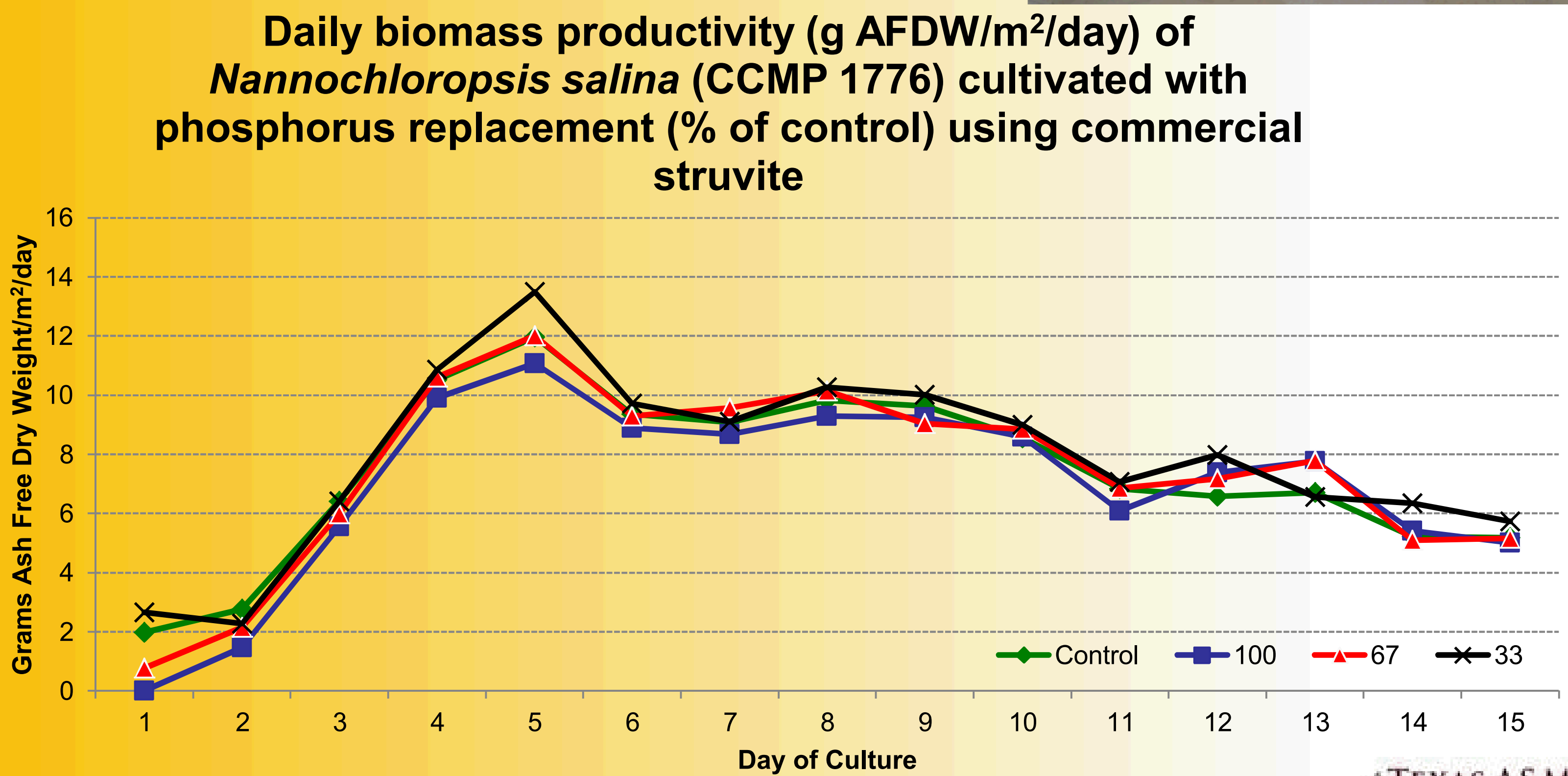
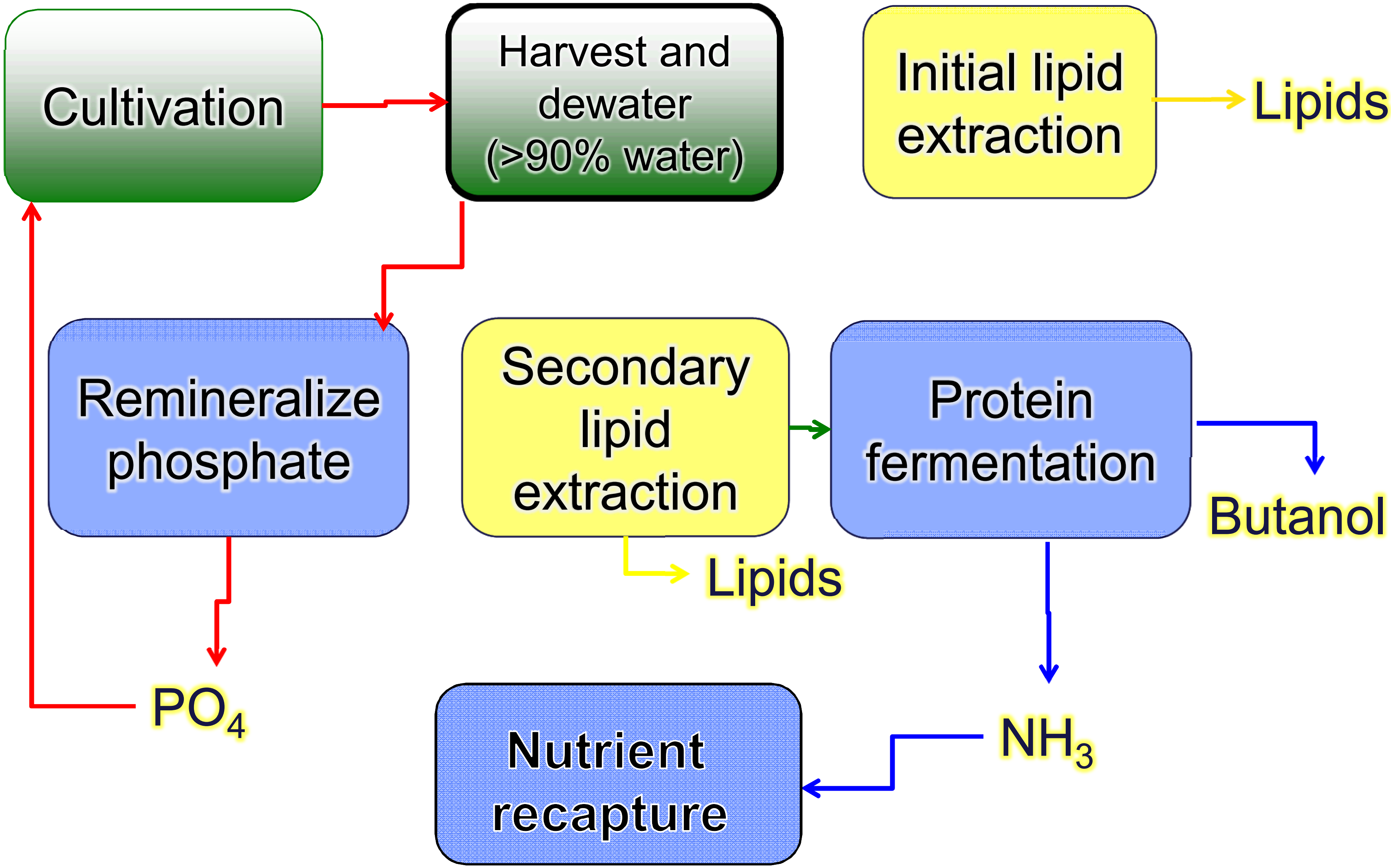
Struvite Supplementation in raceway cultures: (*Nannochloropsis salina*)



Partial demonstration of closed process



Summary

- Phosphate can be remineralized, in soluble form, from non denatured *N. salina* biomass by enyzmatic digest or mild pH treatment

- Soluble, remineralized phosphate can provide 100% of phosphate required for growth of *N. salina* or *P. tricornutum.*

- Appear to be accessing phosphate from the NA pool,   P-lipids TBD

- No evidence of any accumulation of growth inhibitory compounds with 66% recycle

- 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.

Acknowledgments

DOE EERE BioEnergy Technology Office

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- Peter Kipp
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- Stacy Truscott

AgriLIFE RESEARCH
Texas A&M System

OpenAlgae

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Staged approach to development of a nutrient recycle system

- Test different conditions for remineralization of phosphate and phospholipid conversion.
  - Determine rate of remineralization
  - Optimize reaction conditions (extraction of nutrients from solid phase)
  - Identify recalcitrant pools
  - Minimize reaction time
- Develop microbial consortia with appropriate enzymatic activities: test culture supernatants.
  - Identify candidate genes, clone, overexpress
  - Test for protein and activity level
- Grow microbial consortia on residual algal biomass—expressing enzymes *in situ* and converting amino acids to ammonium.
  - Optimize growth conditions (limit conversion to microbial biomass)
  - Optimize enzyme production on residual biomass
  - Limit uptake of inorganic phosphate by microbial consortium

Experimental Design (Mono-Culture)

- Stocking: Cultures of *Nannochloropsis salina* (CCMP 1776) were stocked into 12 outdoor 3 m^2 fiberglass raceways to achieve an initial stocking density of ~0.15 g/L afdw at 5 cm depth
- Nutrient Mix: "ODI" mix composed of ammonium sulfate, phosphoric acid and ferrous sulfate
- Experimental Design:
  - Control: supplemented with ODI nutrients at a 16:1 N:P ratio
  - Struvite: supplemented with commercial struvite to replace 33, 67 and 100% of the phosphorus in the control treatment
  - Water depth in each raceway was gradually increased to a final depth of 20 cm providing a total working volume of 550 L
- Parameter Monitoring: Raceways were monitored daily for solar radiation, rainfall, wind-speed, pH, temperature, salinity, afdw, ammonia, nitrite, nitrate, and phosphate

Day 5 biomass productivity (g AFDW/m^2/day) of *Nannochloropsis salina* (CCMP 1776) cultures with phosphorus replacement using commercial struvite[1,2,3]

Phosphorus Replacement Level (% Control)	Day 5 biomass productivity (g AFDW/m^2/day)
Control	11.98±2.22[a]
100	11.08±0.50[a]
67	12.00±1.25[a]
33	13.48±0.70[a]

[1] Means with similar superscript in the same column are not statistically different (p>0.05)

[2]N = 3 raceways

[3]Standard deviation