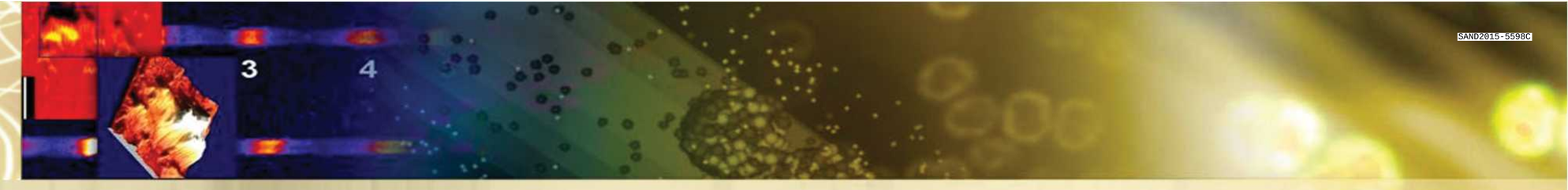
SANDIA BIOSCIENCE



SAND2015-5598C

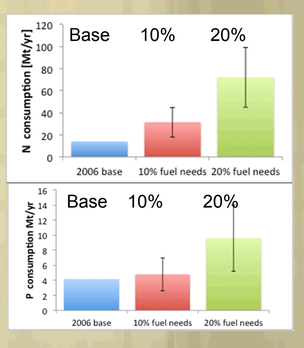
Major Nutrient Recycling for Sustainable Algal Culture.

Todd W. Lane, Ryan W. Davis, Pamela D. Lane, John Hewson, Nick Wyatt, Anthony Siccard, Peter Kipp

Biomass at energy-consumption relevant scales outstrips current nutrient production

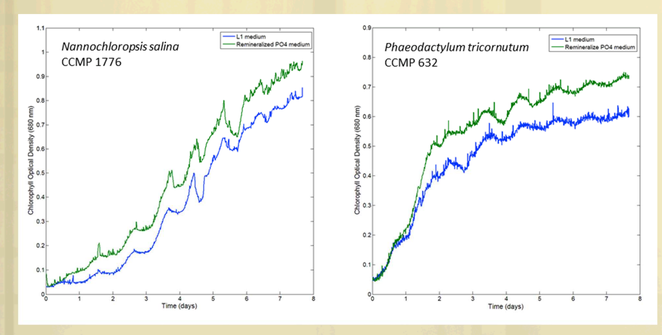
To meet 10% of liquid fuel needs (roughly 30 BGY)
- Algal biomass: 200 – 500 Mt/yr.
- Nitrogen: 18 – 45 Mt/yr
  - Compare 14 Mt/yr in 2006
  - Haber-Bosch process requires energy.
- Phosphorous: 2.4 – 6 Mt/yr
  - Compare 4.1 Mt/yr in 2006
  - P is mined resource.
  - Recent concerns over 'peak phosphate:'

N/P is the biggest single energy input into the system, accounting for ~30-40% of the total

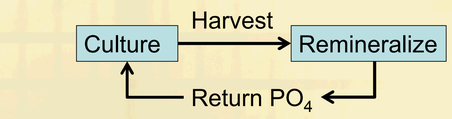


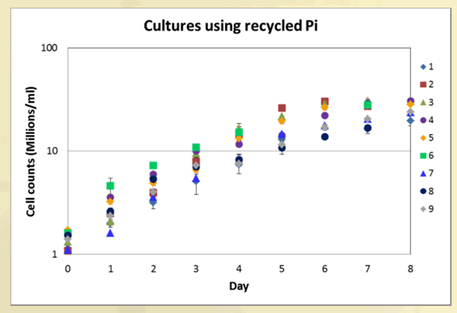
Pate, Klise, Wu, *Applied Energy*, 88:3377-3388 (2011).
Liu, *et al.*, *Bioresource Technology*, 148:163-171 (2013).

Soluble recycled phosphate will support repeated rounds of algal growth



- ~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





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

Project Overview: A BETO funded partnership between national lab, university and industry

Laboratory to pilot/field scale

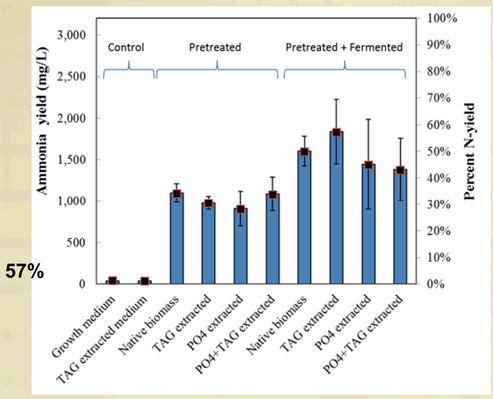
Sandia National Labs: Project Lead
- Biochemistry, Precipitation Science

Texas AgriLife (TAMU):
- Biomass production, Pilot scale field trials
- Marine species:
  - *Nannochloropsis salina* & *Phaeodactylum* tricornutum

OpenAlgae
- TAG extraction
- DAG extraction from Converted phospholipids

Cellular nitrogen is converted to ammonium by fermentation

- 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

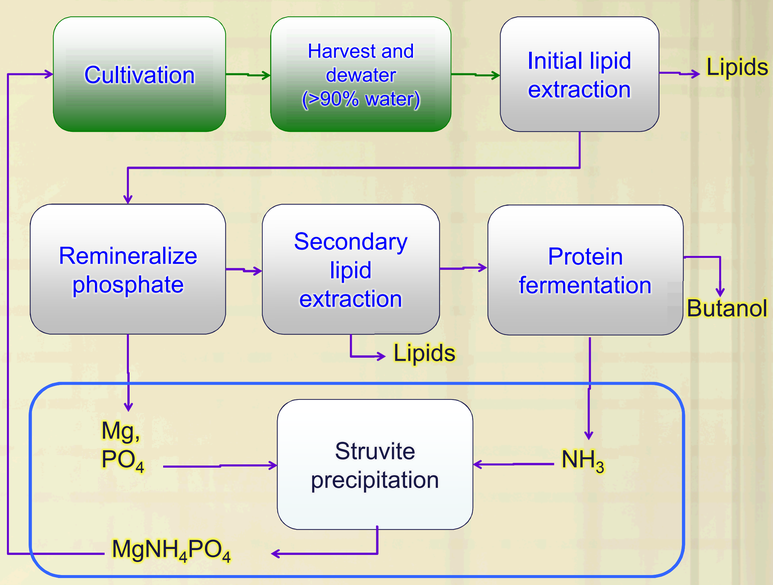


Fermentation of acid and pronase treated biomass results in the liberation of cellular nitrogen in the form of ammonium (left). Liberated ammonia combines with phosphate and residual Mg to form sruvite (right).

Overall goal: Integrated major nutrient recycling.

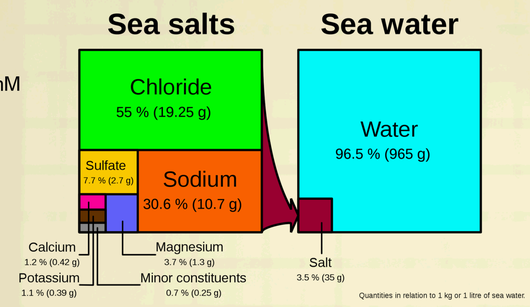
The grand objective of the proposed work is to develop an integrated system for the culture, harvest, and processing of algal biomass that will enable the reuse of major nutrients thus reducing the operational requirement for external nutrients. To meet this overall objective, the team will:

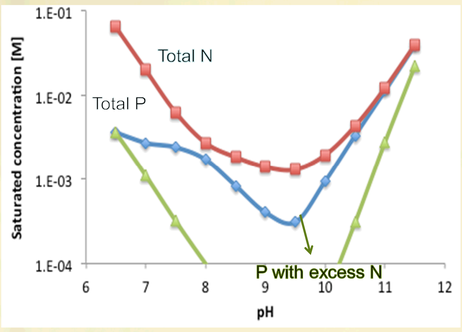
1. Develop a process to liberate nitrogen and phosphorous present in de-oiled algal biomass.
2. Convert phospholipids to DAG, remineralized phosphate, and recover both.
3. Remineralize nitrogen and phosphorus as struvite and demonstrate a simple struvite recovery process.
4. Demonstrate the ability of 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.



Struvite is a likely form for recycled N and P

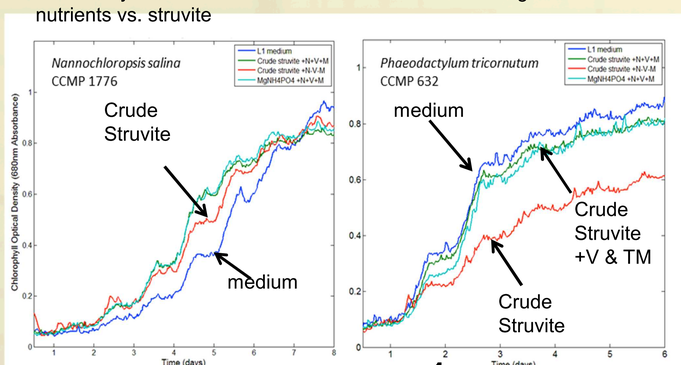
Mg is 50mM in seawater
- If algae dewatered to 10% one can expect 5mM Mg to remain.
- Depending on excess NH4/NH3 used in precipitation, estimate 80-99% potential PO4 precipitation.
- Recovers 1:1 N:P
- Precipitates at accessible concentrations.
  - Experience in waste water treatment industry.
- Alternates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





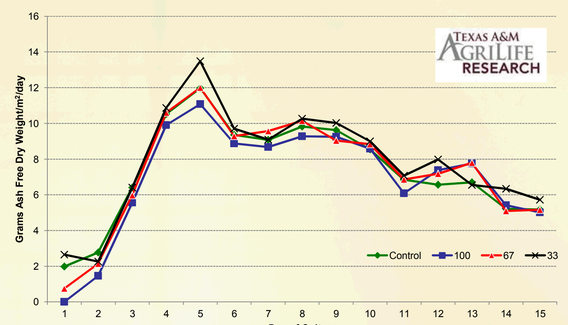
Struvite is a source of nutrients for algal production

Laboratory cultures of N. salina and P. tricornutum using standard nutrients vs. struvite



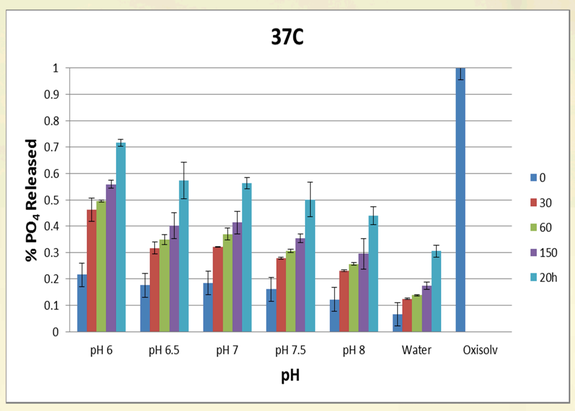
Multicultivator, sinusoidal 16/8 LD cycle, peak 1000⌈E, 21 to 24 C

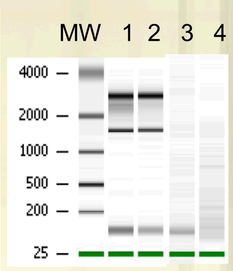
Daily biomass productivity of *N. salina* cultivated with phosphorus replacement (% of control) using commercial struvite



Cellular phosphate can be remineralized by incubation of disrupted biomass

Up to 70% of total cellular phosphate is remineralized by incubation at slightly acidic pH. The majority of this phosphate is derived from the nucleic acid pools



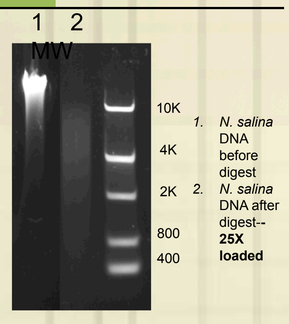


1. *N. salina* RNA before digest
2. *N. salina* RNA before digest
3. *N. salina* RNA after digest
4. *N. salina* RNA after digest

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

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

Future directions

- Improve yield: From a current best of ~70% P and ~60% N to ~100% recycle
  - Optimize liberation of phosphate from phospholipids
  - Combine our phosphate and ammonium 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
  - Optimize liberation of phosphate from phospholipids
  - Combine phosphate and nitrogen capture

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