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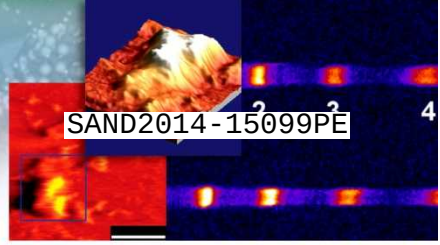

Nutrient Recycling for Sustained Algal Production

Pamela Lane, Ryan Davis, John Hewson,
Anthony Siccardi, Nick Wyatt, **Todd Lane**

4th International Conference on Algal Biomass, Biofuels and Bioproducts.
Santa Fe, New Mexico
18 June 2014

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Nutrient recycling project is a partnership between national lab, university and industry

- LDRD>>DOE
- 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

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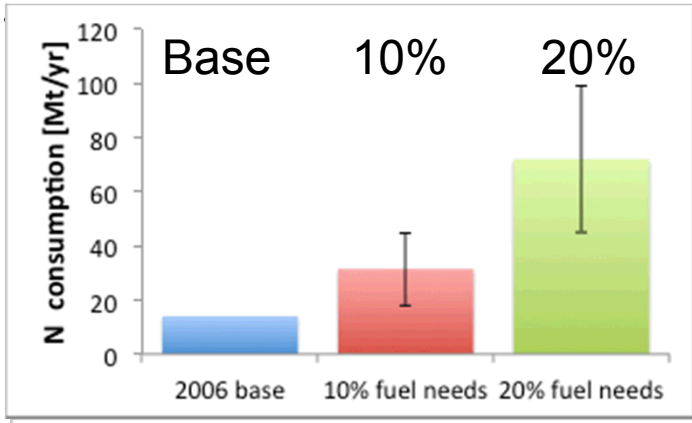
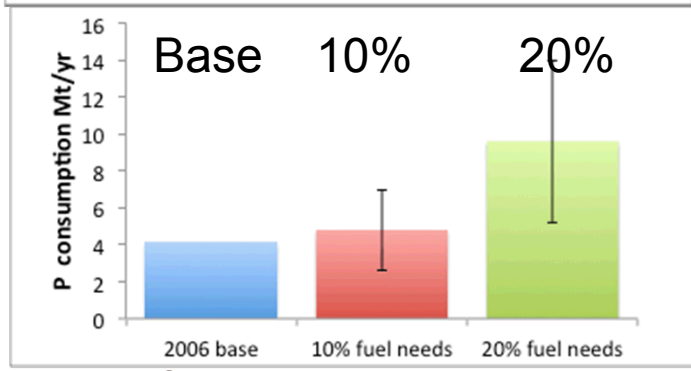
Biomass at energy-consumption relevant scales exceeds current nutrient production

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

- To meet 10% of liquid fuel needs (roughly 30 BG
  - 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:'
      "The Achilles Heel of Algae Biofuels: Peak Phosphate," Forbes, Feb. 2012.

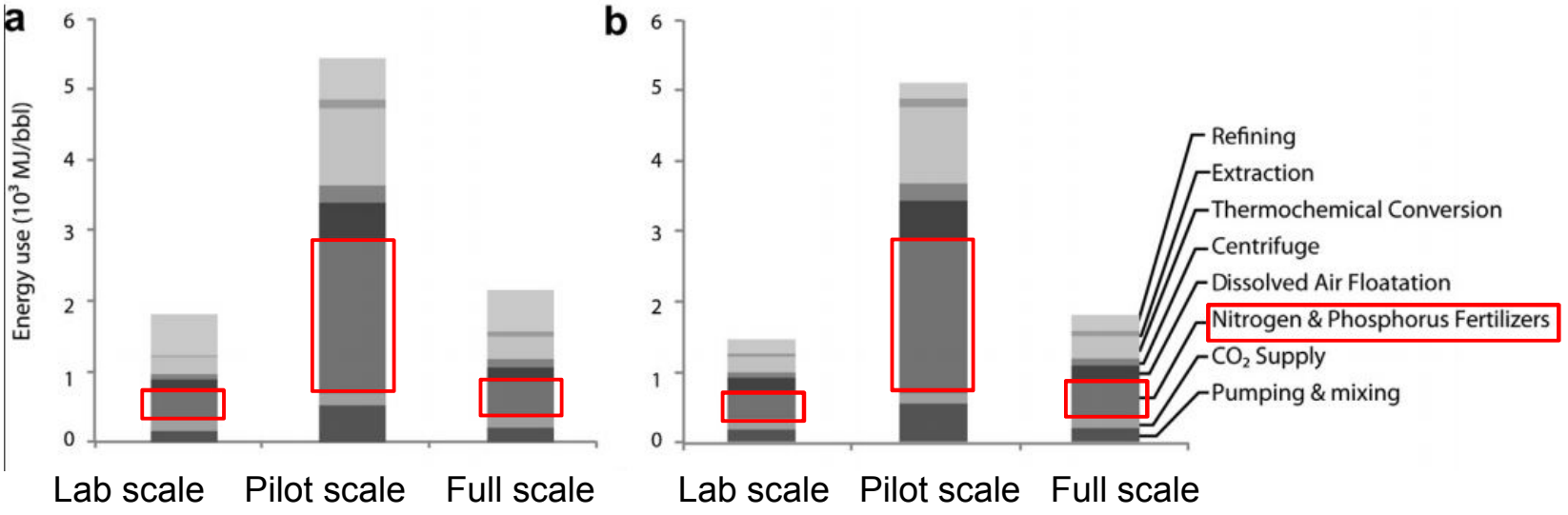




- Food vs fuel:
  - Nutrient use for algae should not compete with food production
- Nutrient run-off has detrimental environmental consequences (algal blooms).

Nutrients constitute a large energy input

- Recent paper 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)

Need to recycle nutrients
Cannot afford to pass through once only

- Nutrients are needed for biological productivity, not fuel.
  - N: amino acids (incl. chlorophyll)
  - P: nucleic acids, phospholipids, ATP.
- Our work:
  - 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 not important; 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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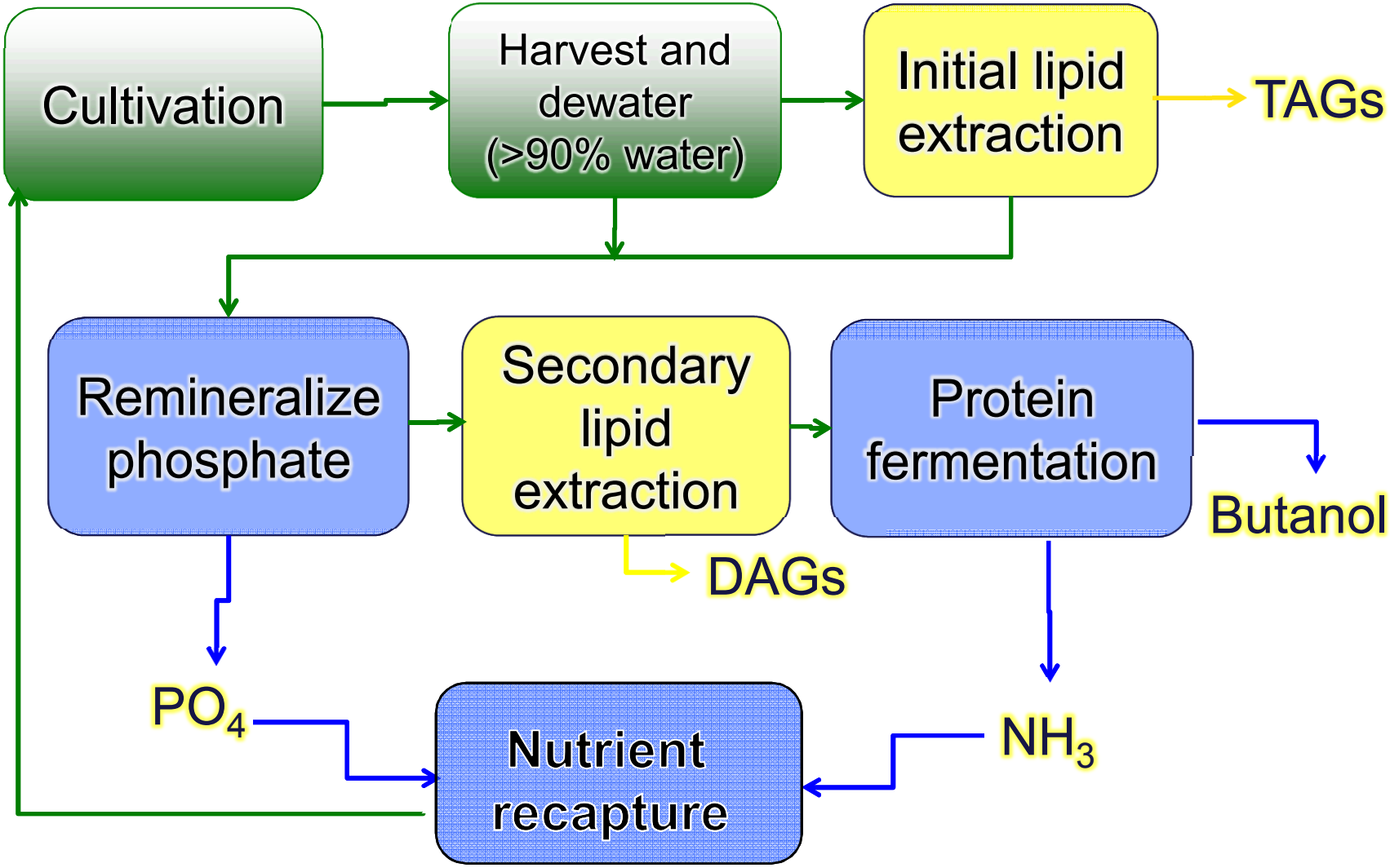
Proposed closed process



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  - TAG extraction
  - DAG extraction
    - Converted phospholipids

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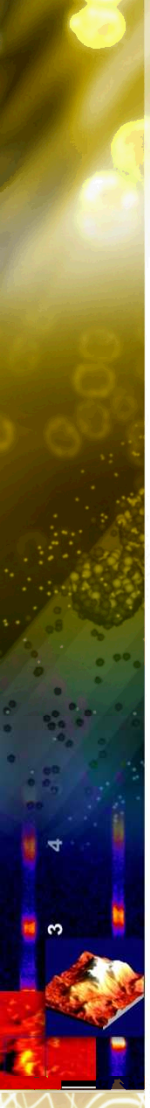
Staged approach to development of a nutrient recycle system

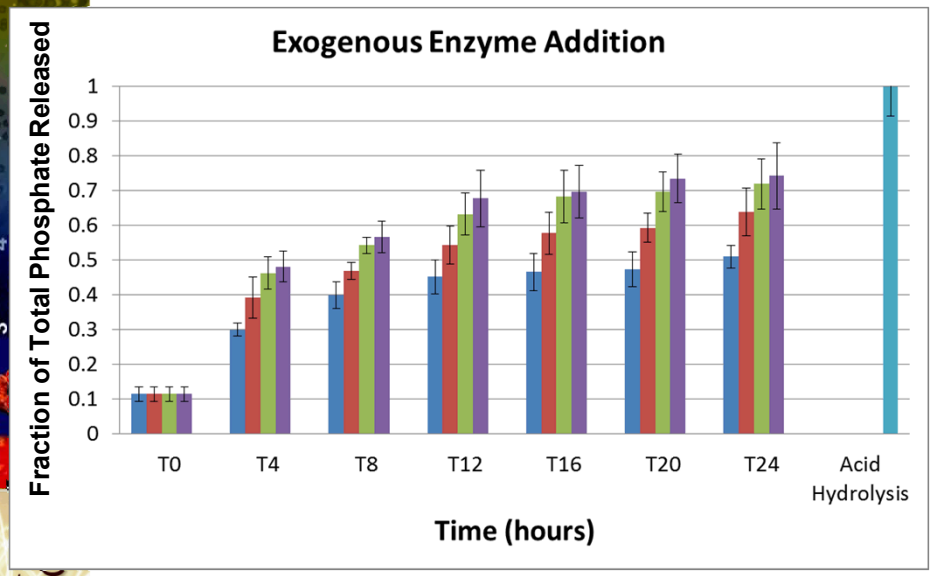
- Test different conditions for remineralization of phosphate and phospholipid conversion.
  - Determine rate of remineralization
  - Optimize reaction conditions (extraction of nutrients from solid phase)
  - Identify recalcitrant pools
  - Minimize reaction time
- Develop microbial consortia with appropriate 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

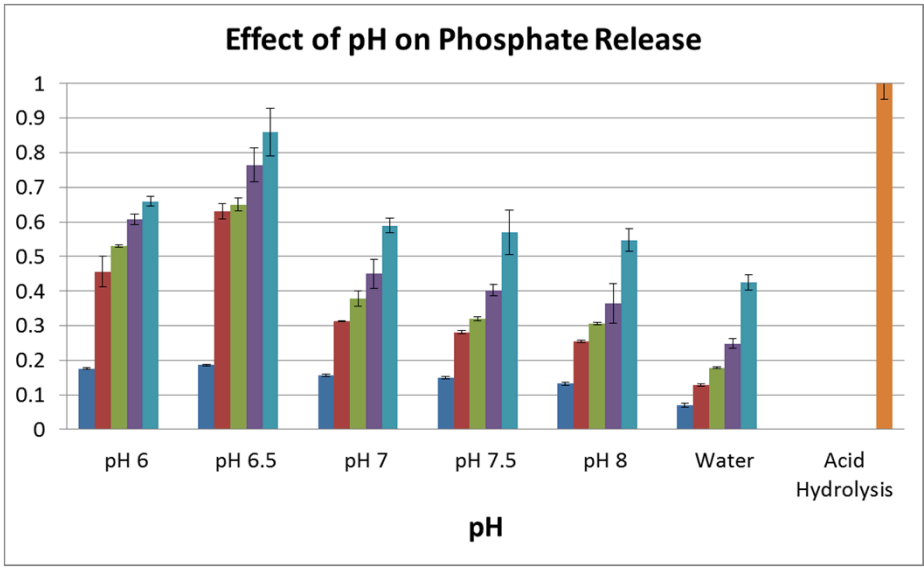


Majority of cellular phosphate rapidly remineralized in non-denatured algal biomass

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

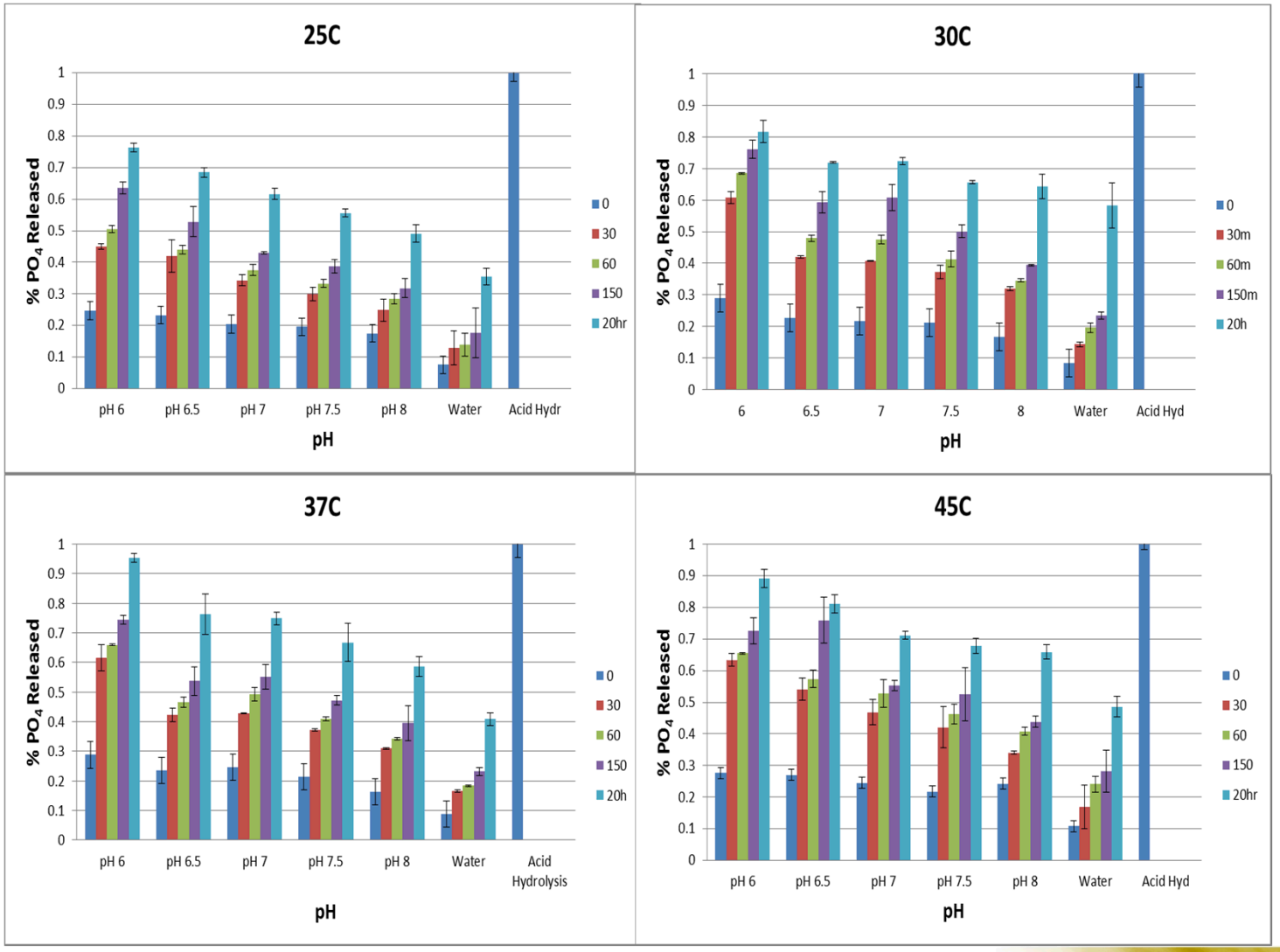




1. No enzyme
2. Alkaline Phosphatase (AP)
3. AP + Benzonase (B)
4. AP + B + Phospholipase D

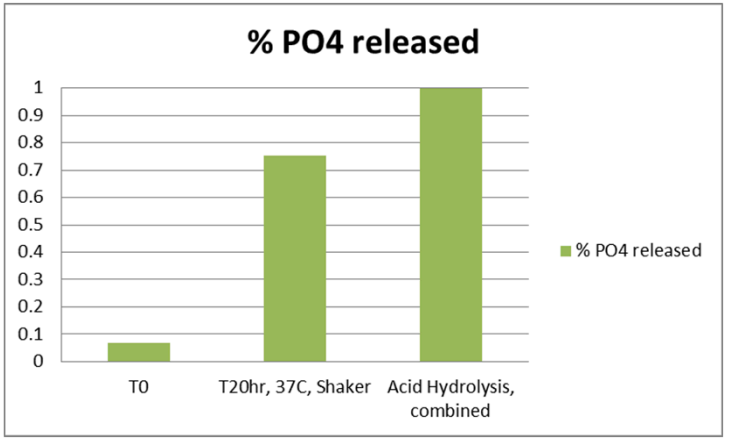
T=0, 30min, 60min, 150min, 20hr, Acid Hydrolysis

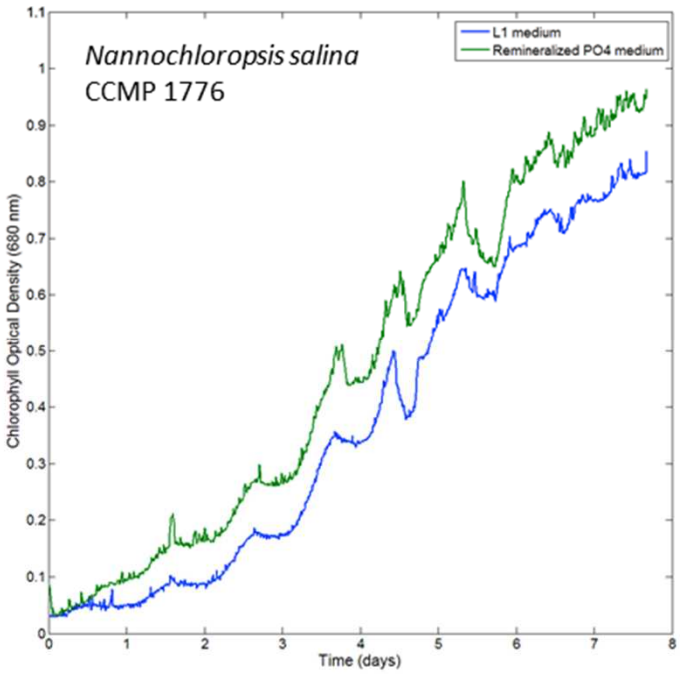
Temperature optimum for Reaction

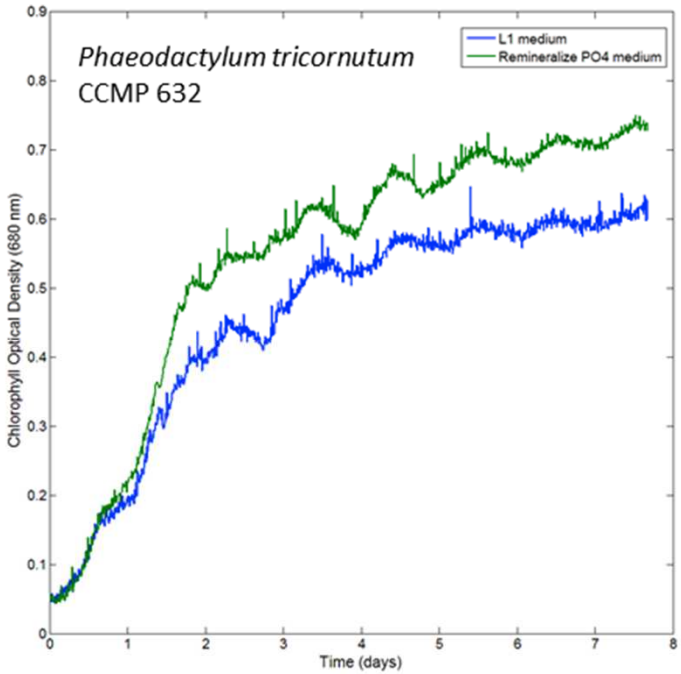


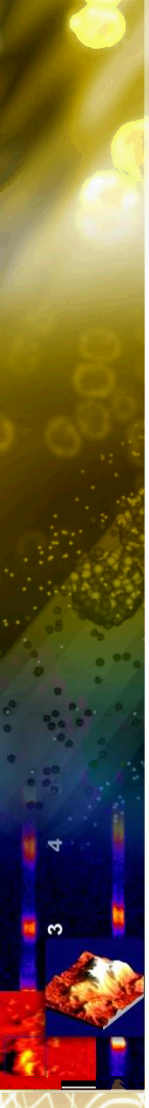
Regrowth of biomass on remineralized phosphate

- ~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









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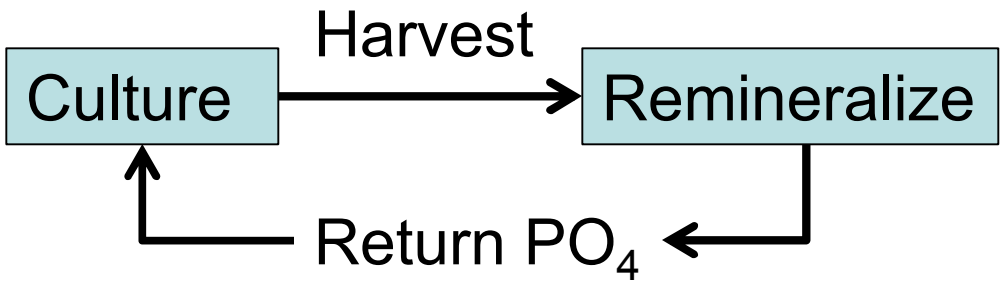
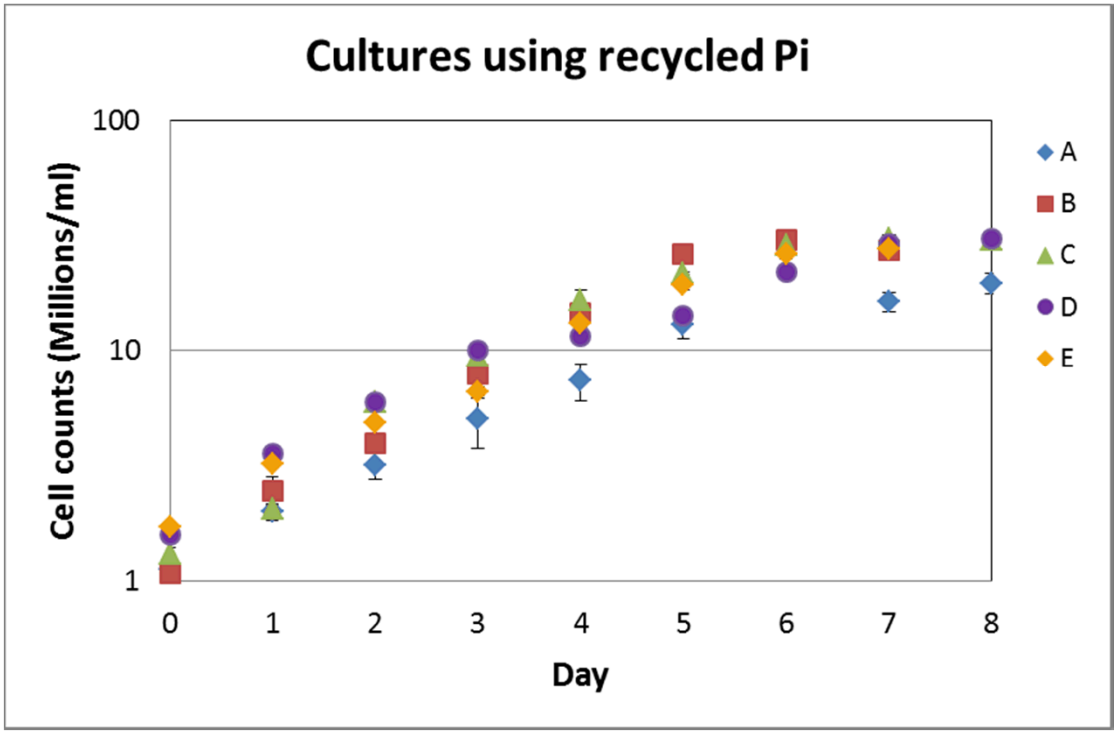
Repeated rounds phosphate remineralization and reuse in *N. salina* culture



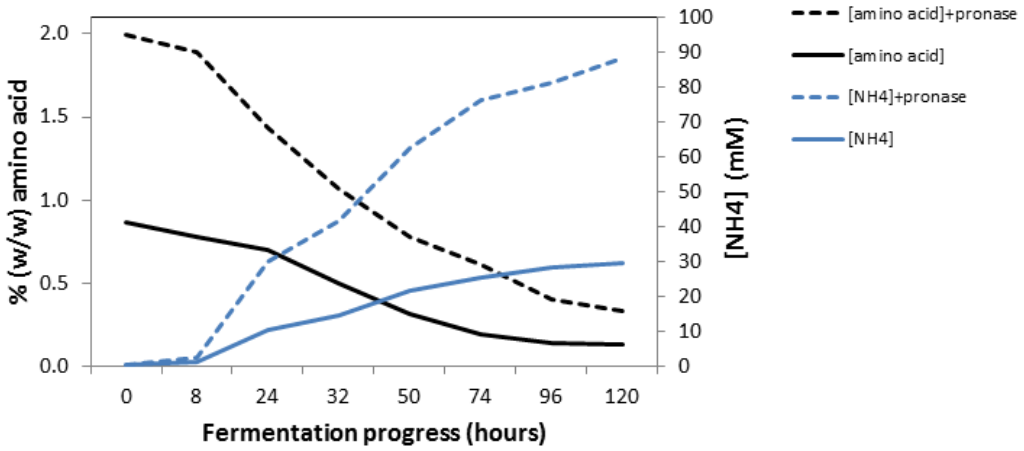


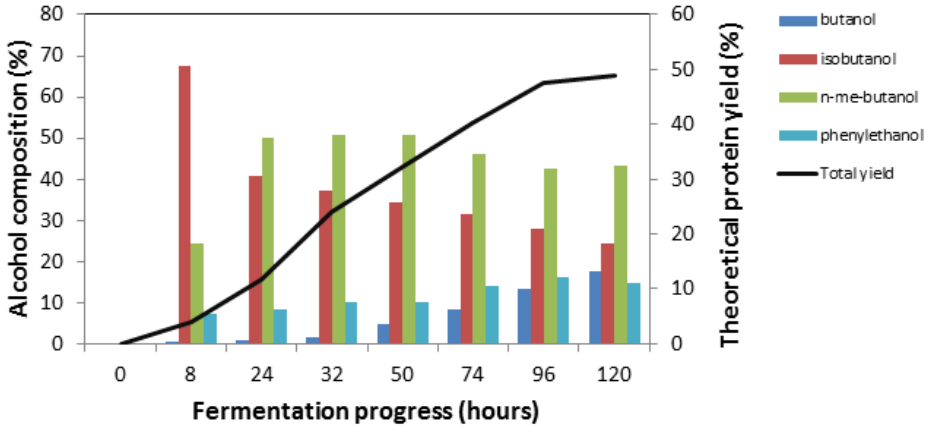
After first round, recycled up to 66% of consumed phosphate

No difference in specific growth rates over the course of 4 rounds of recycle (5 culture rounds)

No evidence of accumulation of growth inhibitors through 4 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.

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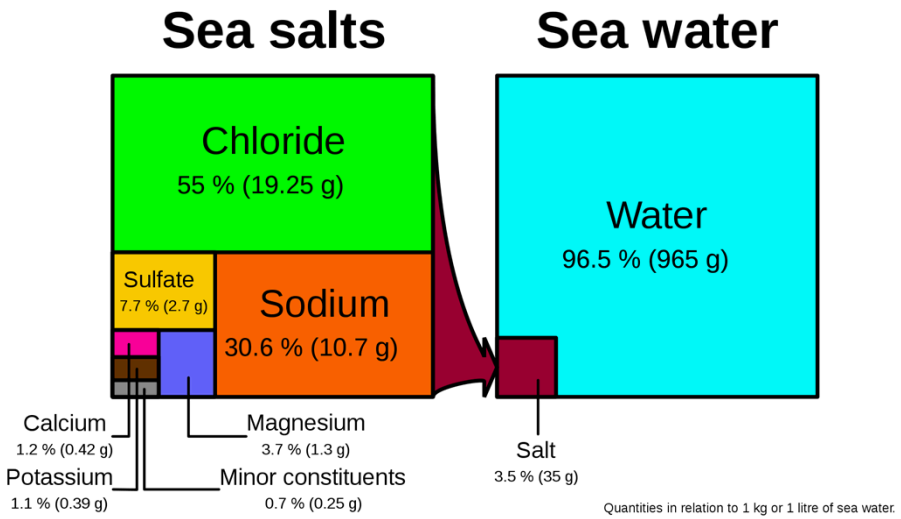
In current system, significant Mg is carried over with biomass



$NaNO_3$ (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>

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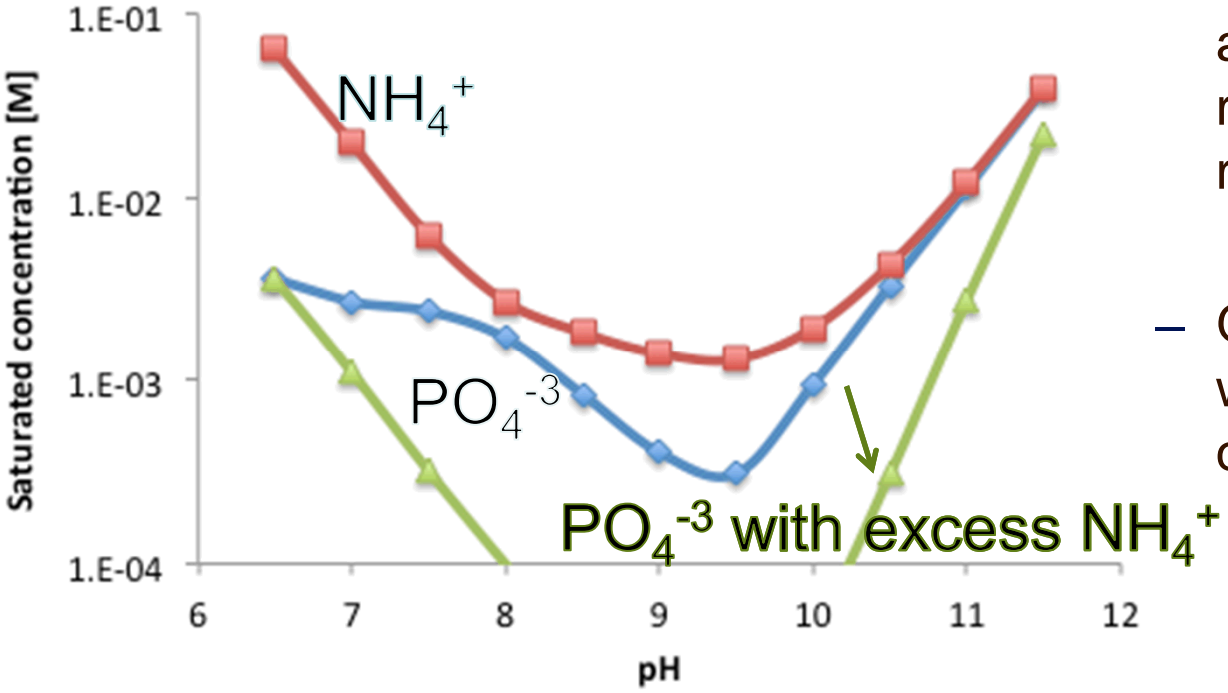
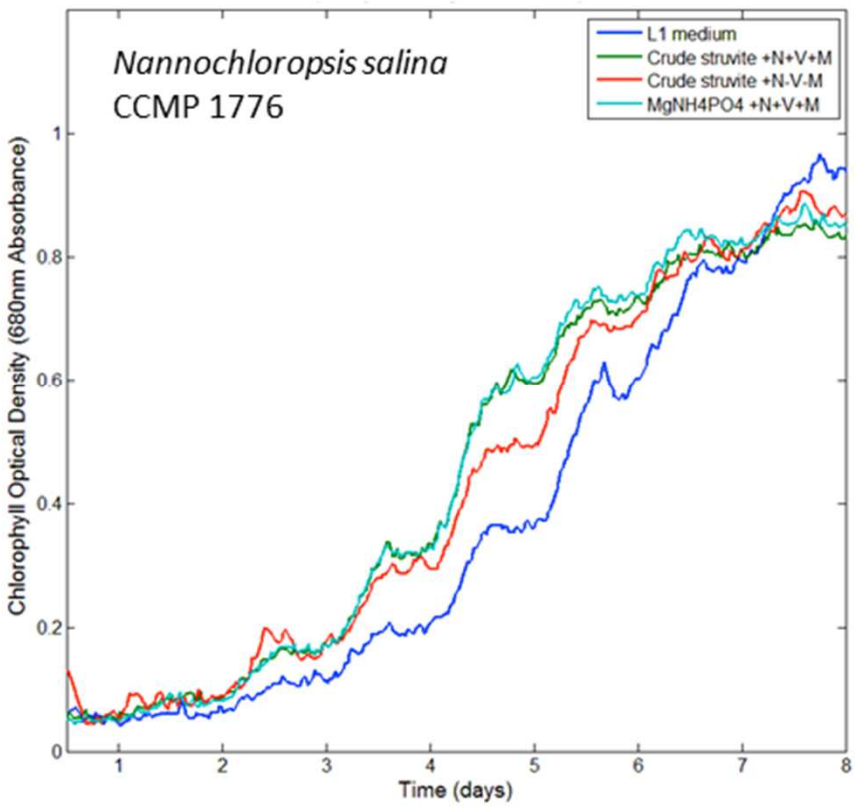
Recover nutrients through precipitation

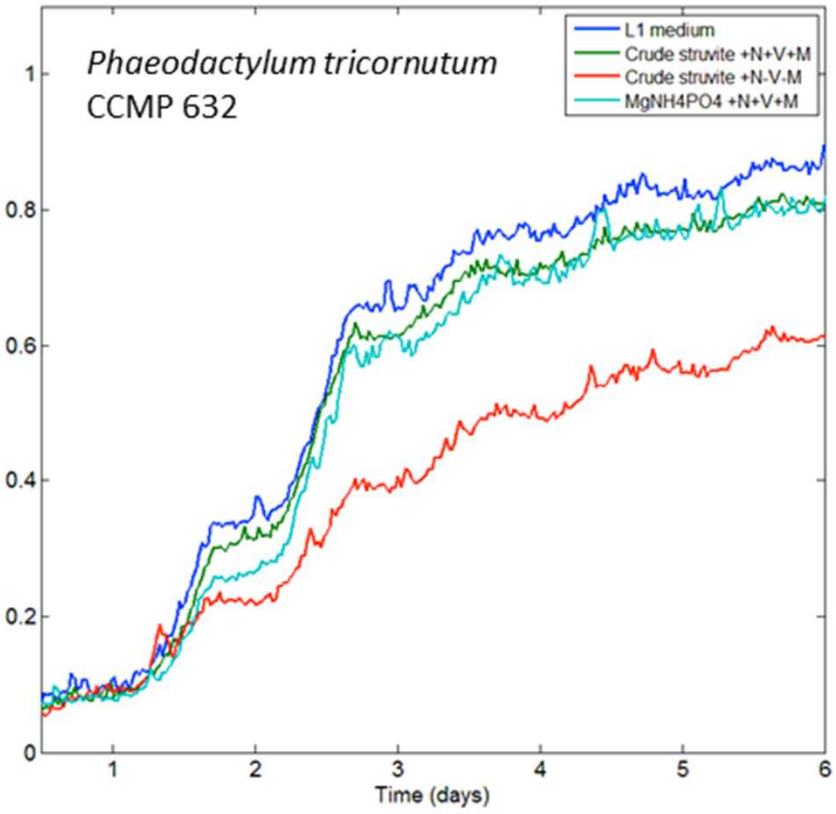
- Struvite ($MgNH_4PO_4$) is useful mineral form of nutrients.
  - Alternates include Ca and Mg phosphates.
- Looking at designing system to maximize recovery – need to measure precipitation kinetics.

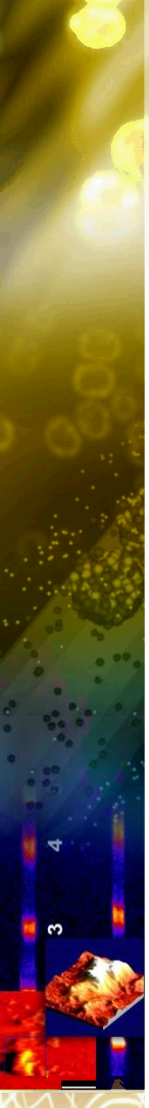


- At concentrations available in nutrient recovery, potential to recover >90% PO_4^{-3}.
- Outstanding issues with effect of organics on kinetics.

Struvite can replace "new" nutrients in microalgal culture







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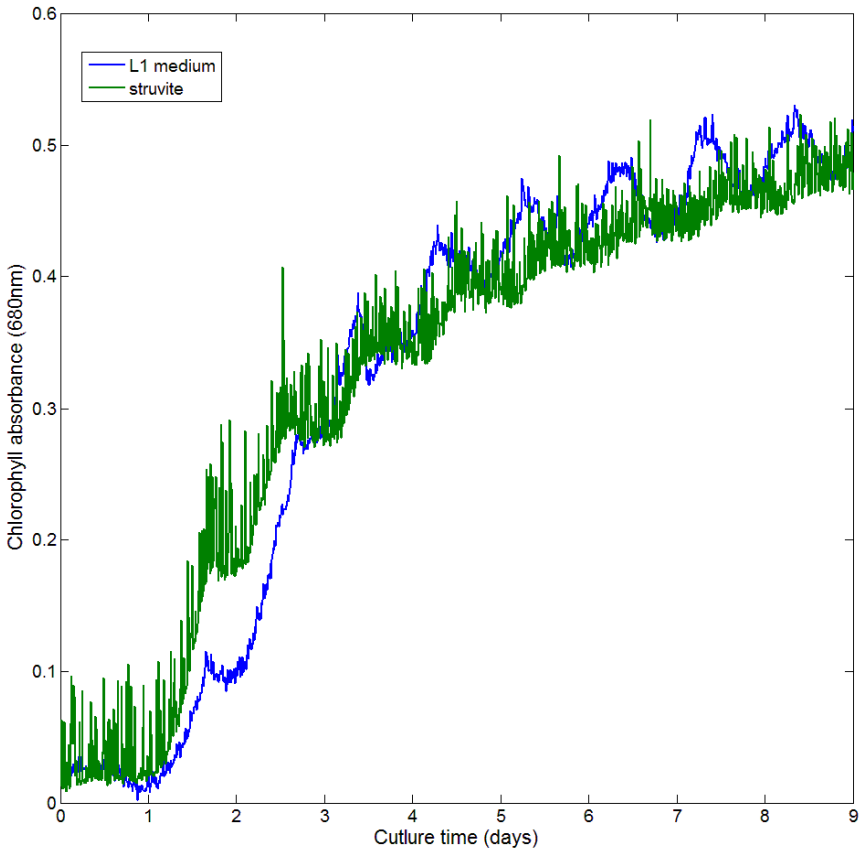
Cultivation with excess struvite

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



- Multicultivator, sinusoidal diurnal cycle, peak 1000 μE, 21 to 24 C

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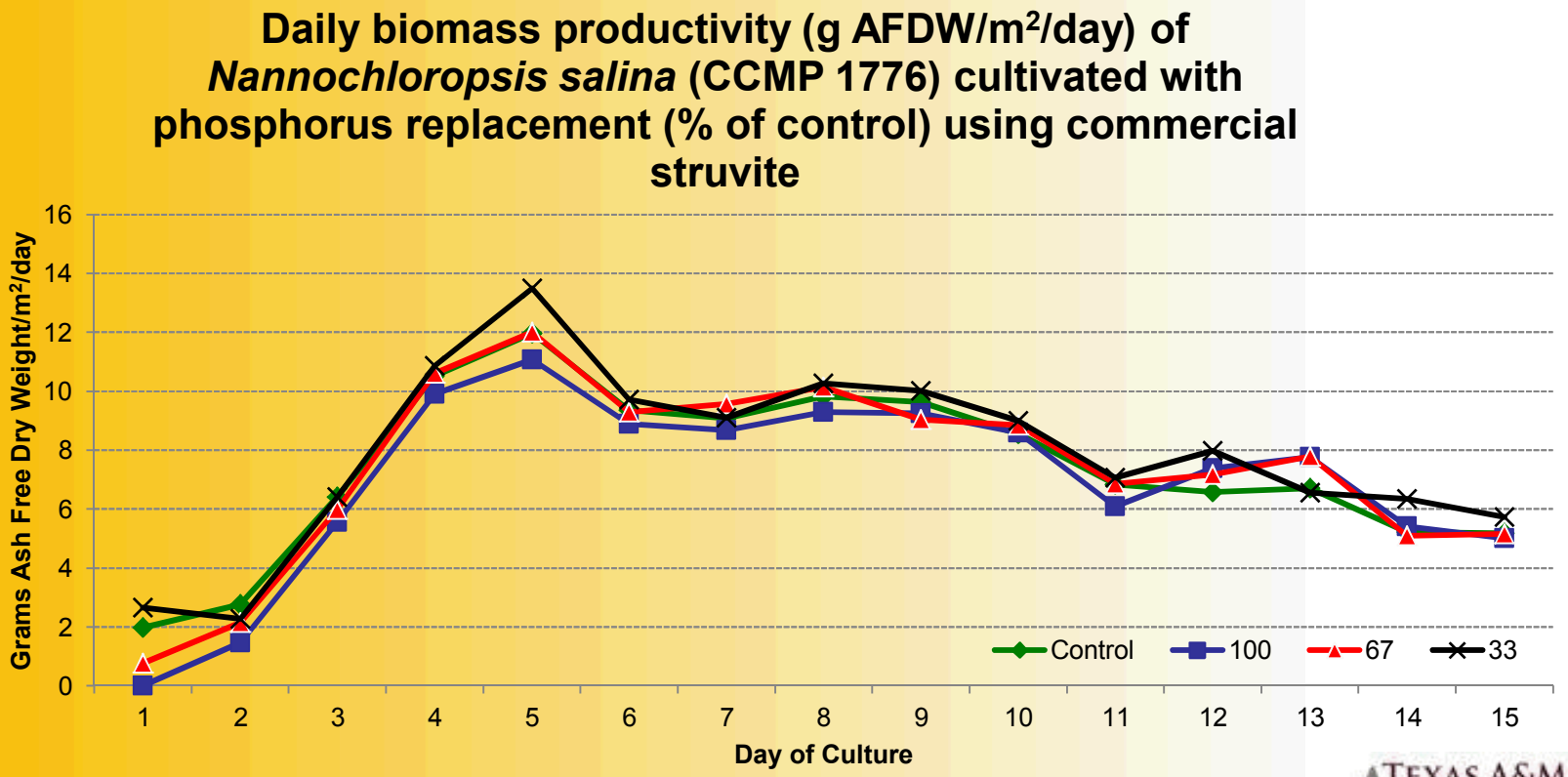
Struvite Supplementation in raceway cultures: (*Nannochloropsis salina*)



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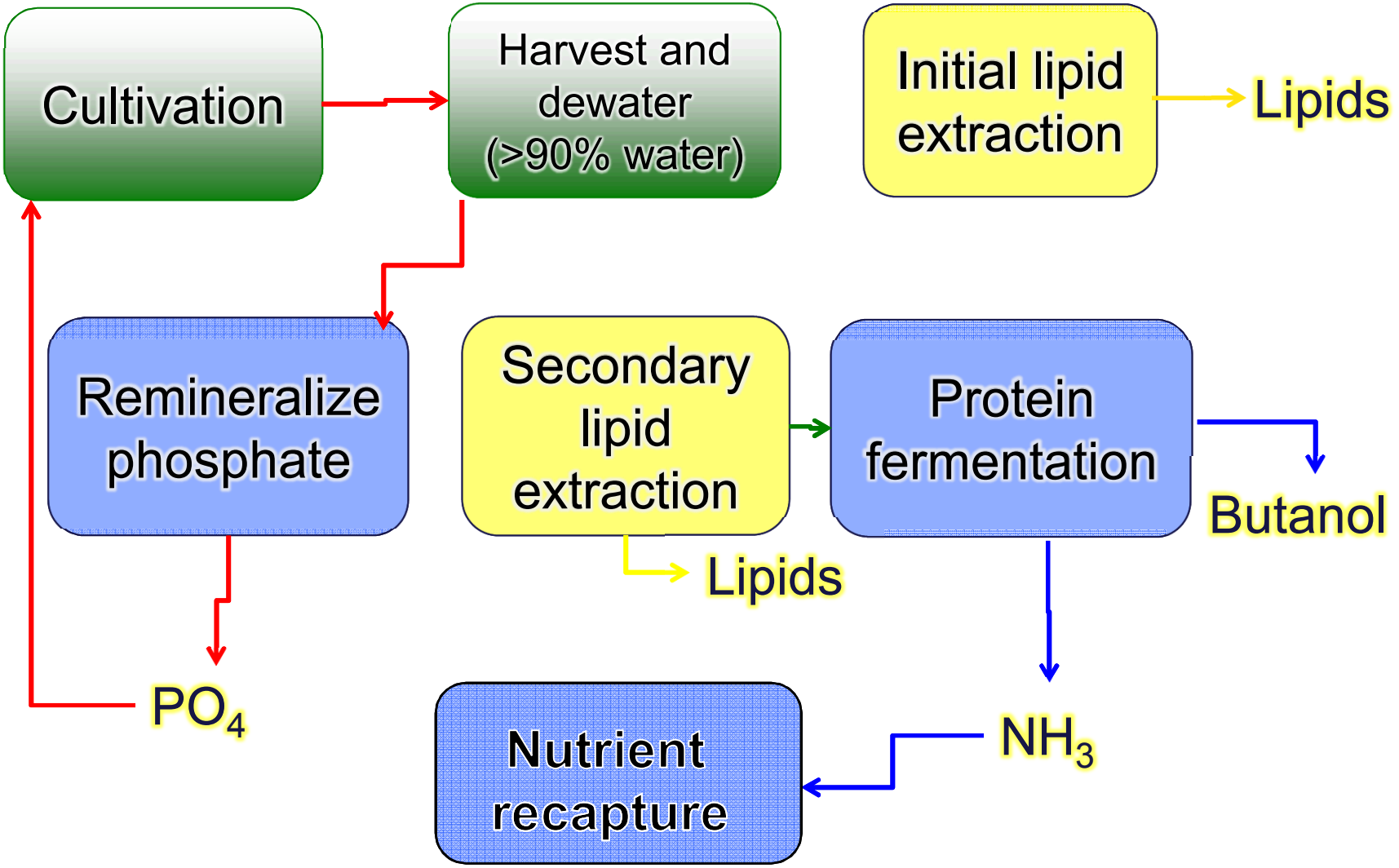
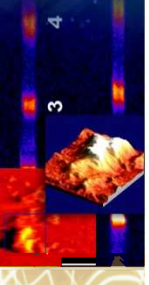
TEXAS A&M AGRILIFE RESEARCH

Partial demonstration of closed process



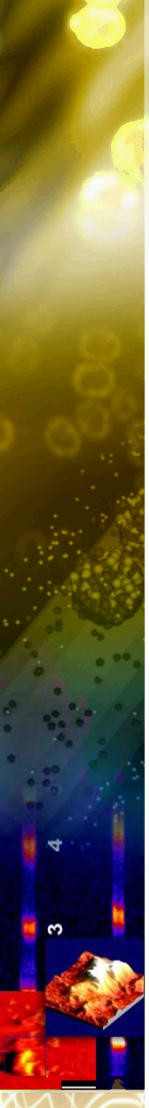
Future work

- Complete and report the results of experiments to determine and compare the relative growth rates of *Phaeodactylum tricornutum* and *Nannochloropsis salina* on struvite versus standard nutrients.
- Complete the characterization of reservoirs of recalcitrant phosphate remaining in algal biomass after initial remineralization.
- Demonstrated the repeated recycle (10X) of phosphate in laboratory culture: Characterize growth inhibition; if any.
- Demonstrate solvent free extraction of TAGs from biomass provided from Texas Agrilife.
- Characterize the phosphate pools in extracted versus unextracted biomass.



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*
- 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.



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Acknowledgments

DOE EERE BioEnergy Technology Office

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- Ryan Davis
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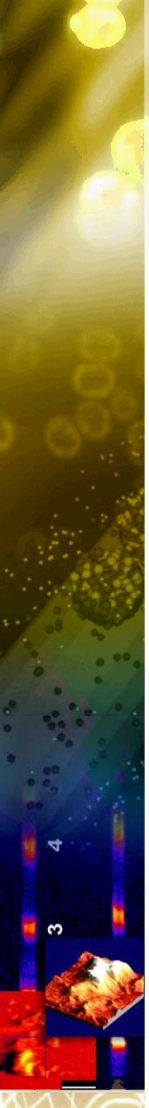
Texas Agrilife

- Anthony Siccardi

Open Algae

- Peter Kipp
- Hoyt Thomas
- Stacy Truscott



AgriLIFE RESEARCH
Texas A&M System

OpenAlgae

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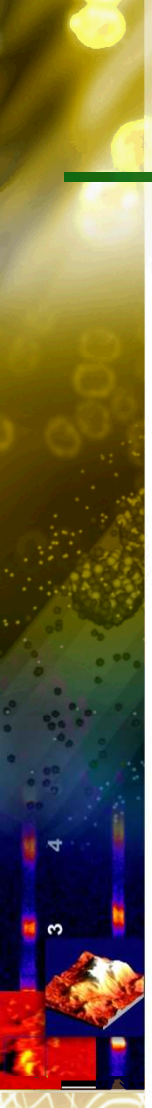
Experimental Design (Mono-Culture)

- 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

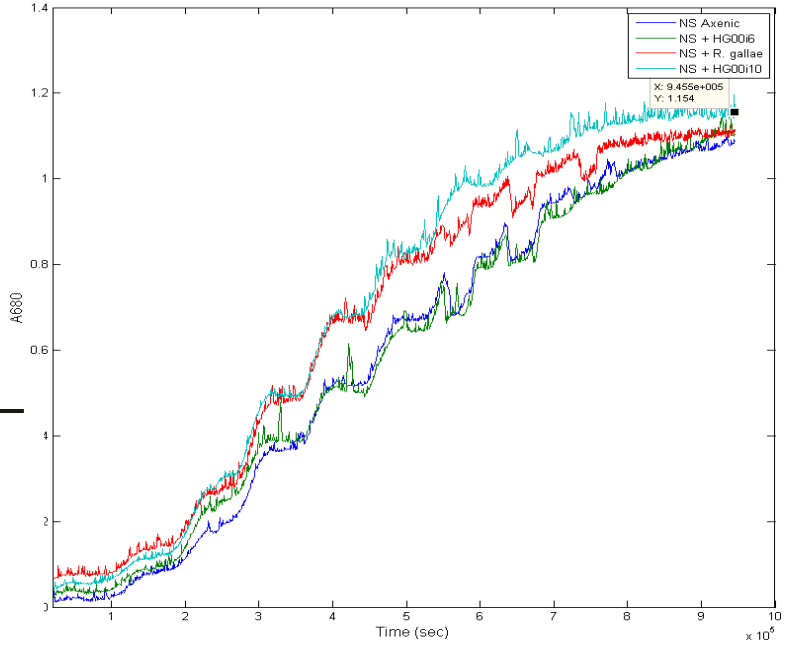
[1] Means with similar superscript in the same column are not statistically different ($p>0.05$)

[2] N = 3 raceways

[3] Standard deviation

Probiotic bacteria increase *N. salina* growth rates and apparent Nutrient Use Efficiency

- Bacterial algal co-culture in *N. salina* specific growth rate (μ) and final titer



	N. salina if cocultured				
	Growth rate (r)	Total cell numbers (1000× cells)	Closest species in NCBI	Physiology Traits	References
HG00i3	0.55±0.02	25,819±2,889	*Alteromonas* sp. AKA07-3	N. A.	N. A.
HG00i6	0.58±0.07	31,091±1,199	*Marinobacter adhaerens* HP15	a diatom-interacting marine microorganism dinoflagellate cultures	Stand Genomic Sci 3 (2), 97-107 (2010)
HG00i10	0.57±0.01	26,749±3,210	*Alteromonas* sp. DG1302		Green,D.H., Hart,M. and Moss,C. submitted
None Addition Control	0.46±0.03	13,290±435			

DOE BER Special Focus Area (LLNL): A systems Biology Approach for Microbial Symbiosis: How Algal-Bacterial Interactions Control Resource Allocation in Biofuel Producing Communities collaboration with

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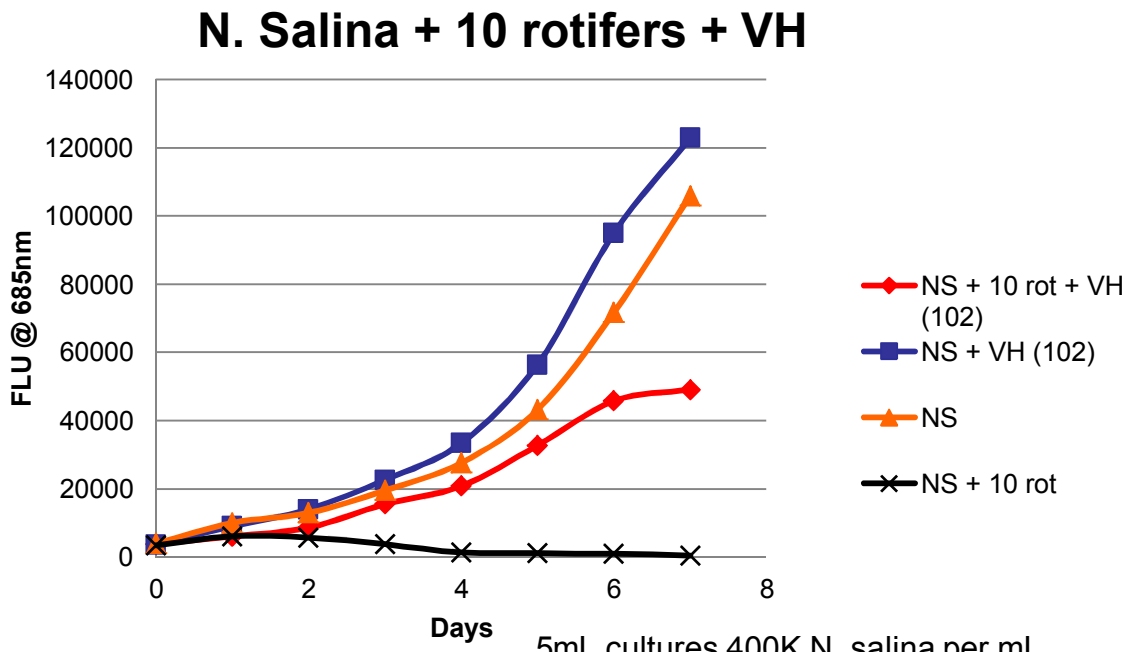
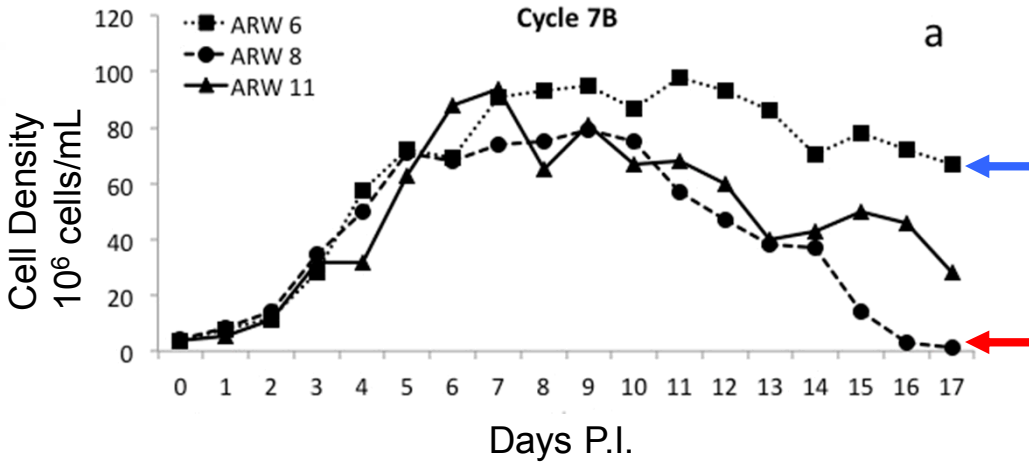
Specific bacterial strains can defend against predation





5mL cultures 400K N. salina per mL, ~20 bacteria VH per mL (~100 total) and ~2 rotifers per mL (10 total).

NS: N. salina grown alone

NS + 10 rot : N. salina + 10 rotifers per 5 mL culture

NS + VH: N. salina plus 100 cells of bacterial strain VH per 5 mL culture

NS + 10 rot + VH: all of the above.

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