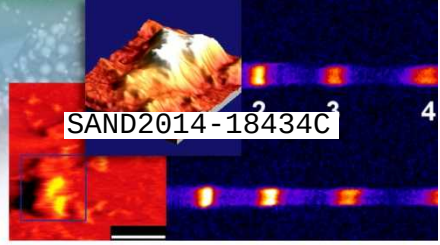
SANDIA BIOSCIENCE



Enhanced growth, crop protection and sustainability

Todd Lane, Sandia National Laboratories

2014 Canada-US Algae Workshop

San Diego, California

3 October 2014

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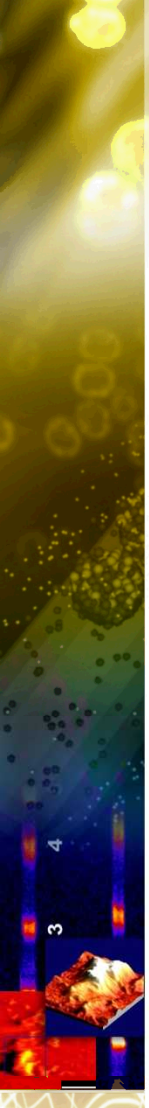
Outline

- Nutrient recycling
- Crop protection: Pathoghen and predator identification
- Probiotics for enhanced growth and crop protection



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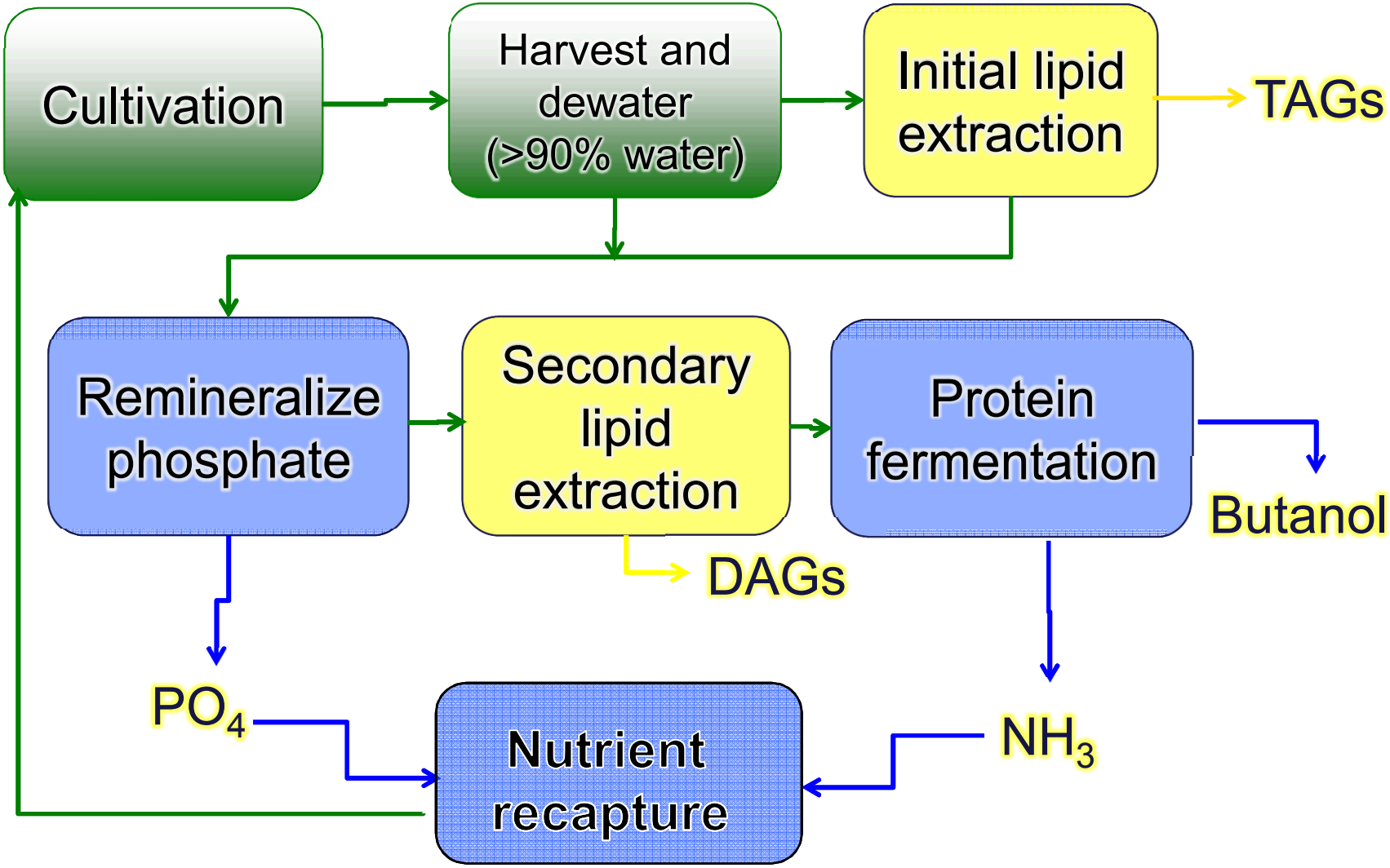
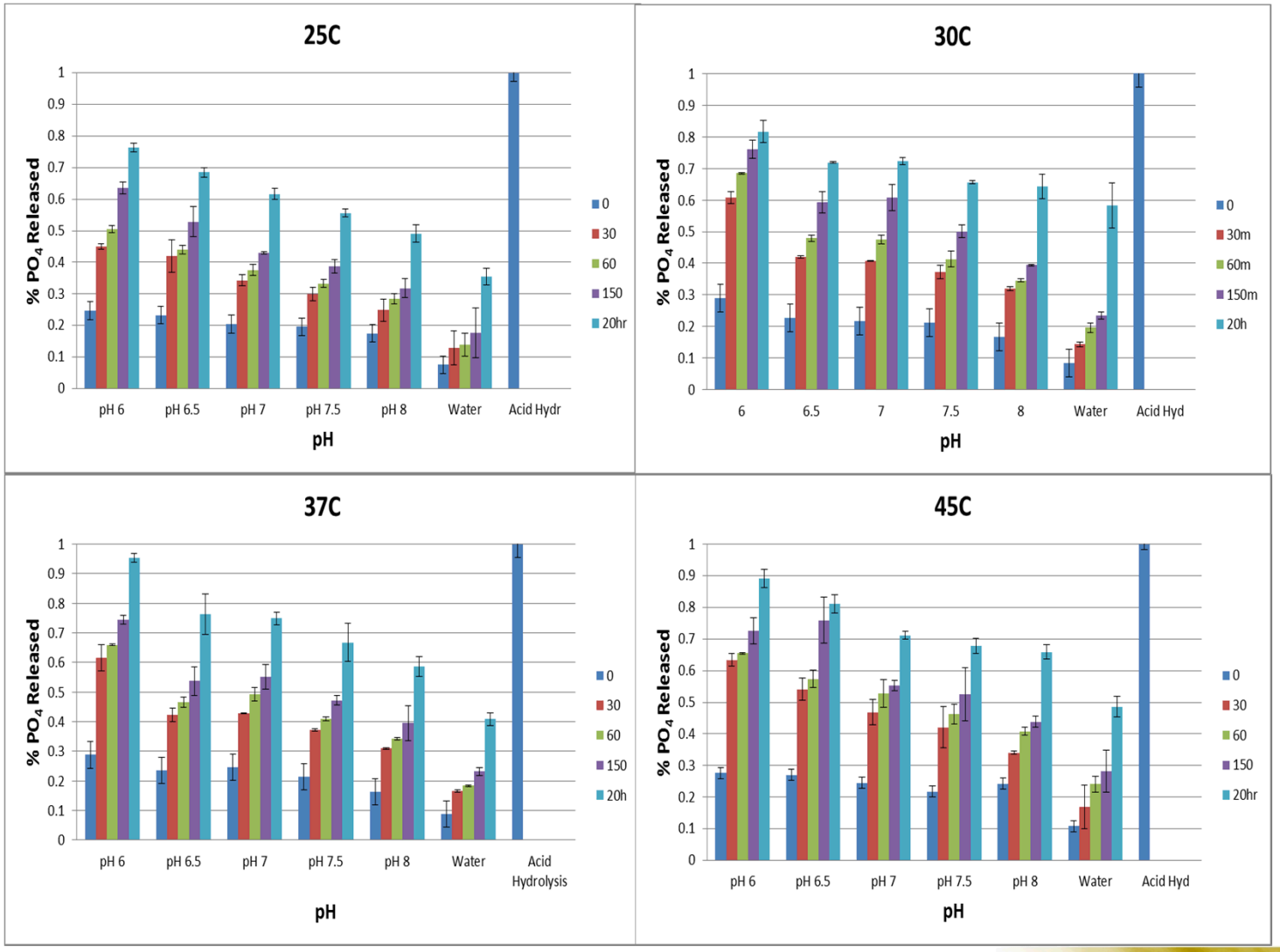
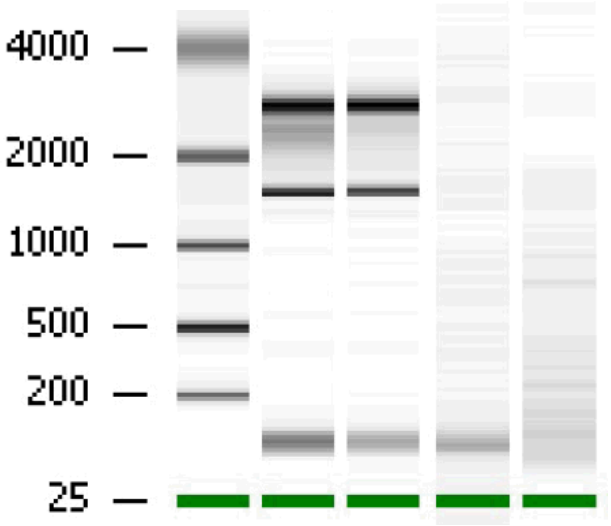
Proposed closed process



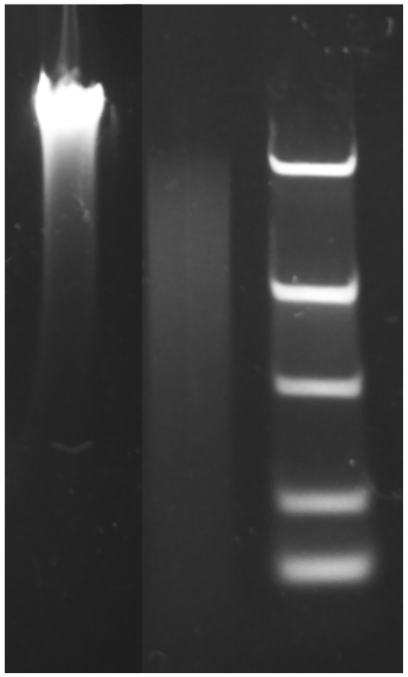
Temperature optimum for Reaction



Accessing phosphate in nucleic acid pool



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

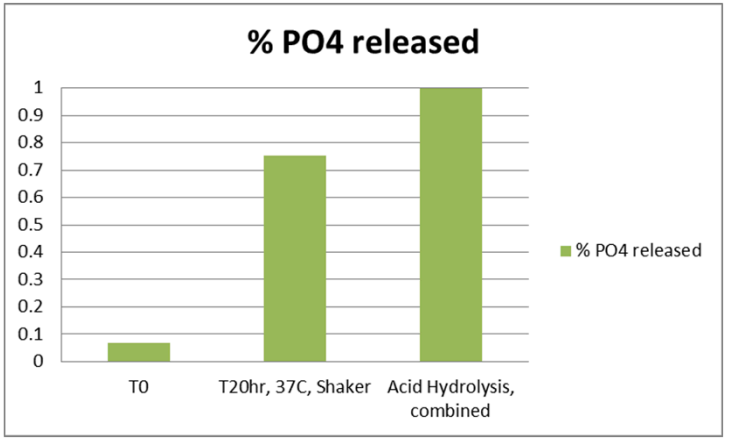


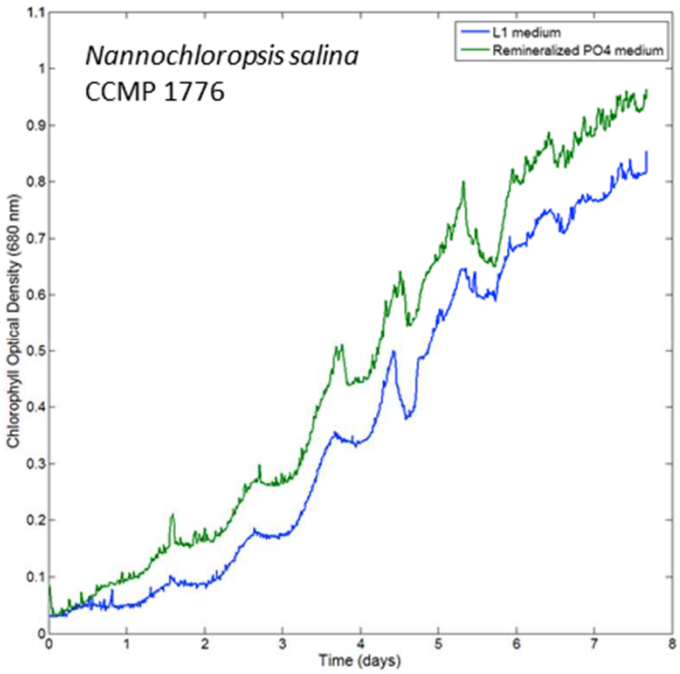
1. Ns DNA before digest
2. Ns DNA after digest (pH6, 37C, 20h)
3. Marker

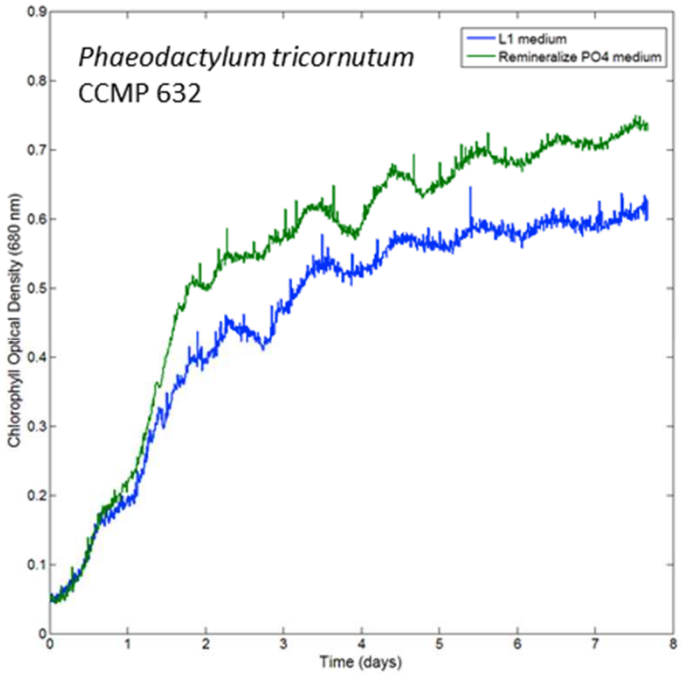
Digest conditions:
Resuspend biomass in water to 1% solids
Add MES pH6 to 50mM
Incubate at 37C, 20 hours

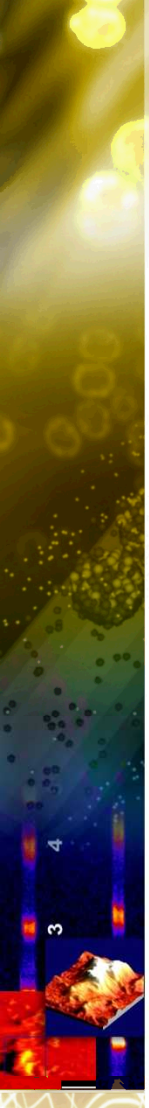
Regrowth of biomass on remineralized phosphate

- ~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
- Growth of *P. tricornutum* and *N. salina* on soluble liberated phosphate









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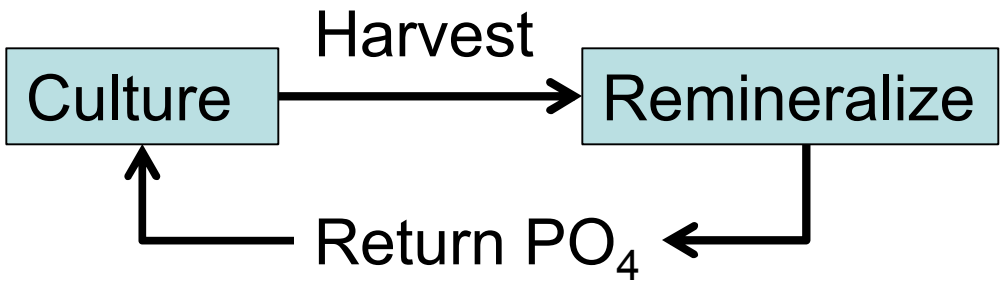
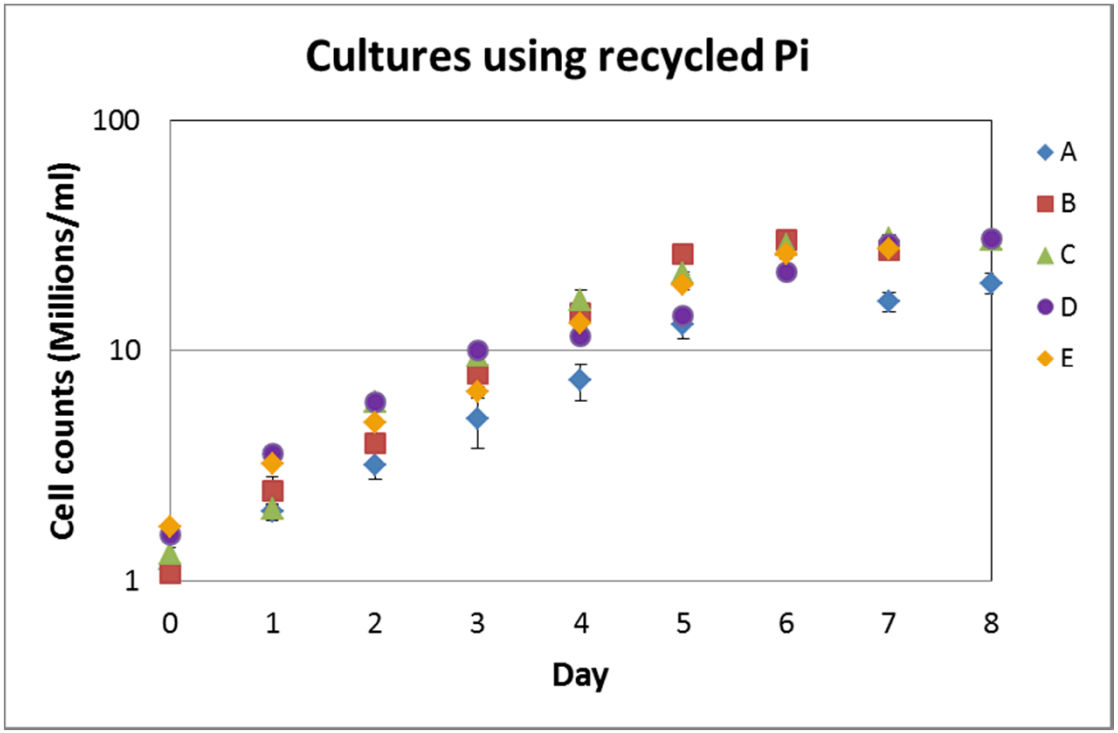
Repeated rounds phosphate remineralization and reuse in *N. salina* culture





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

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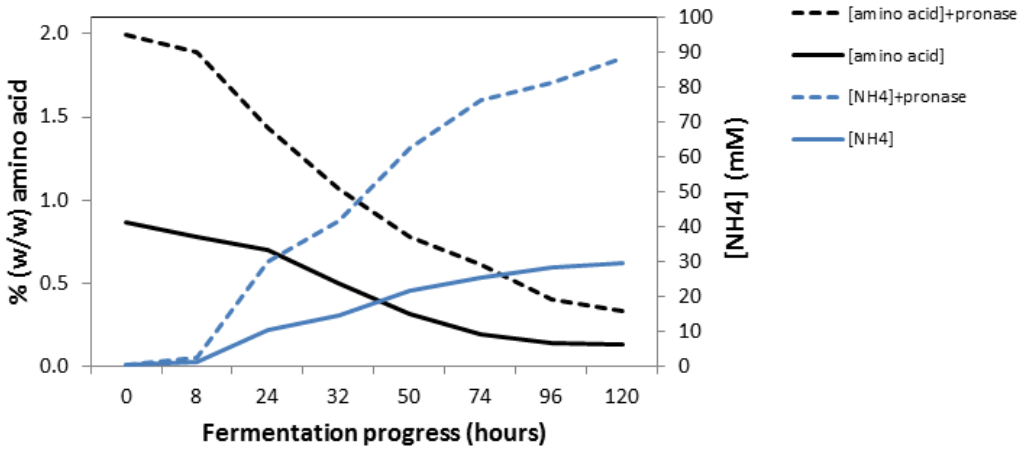
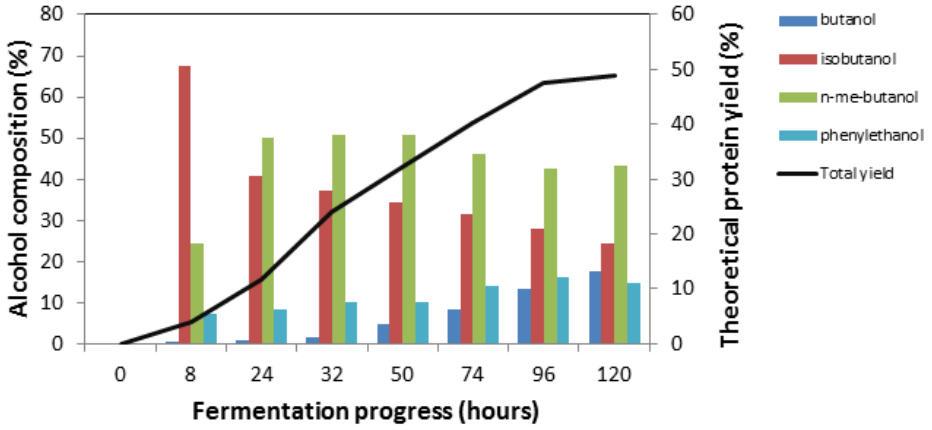
Remineralize N through protein fermentation

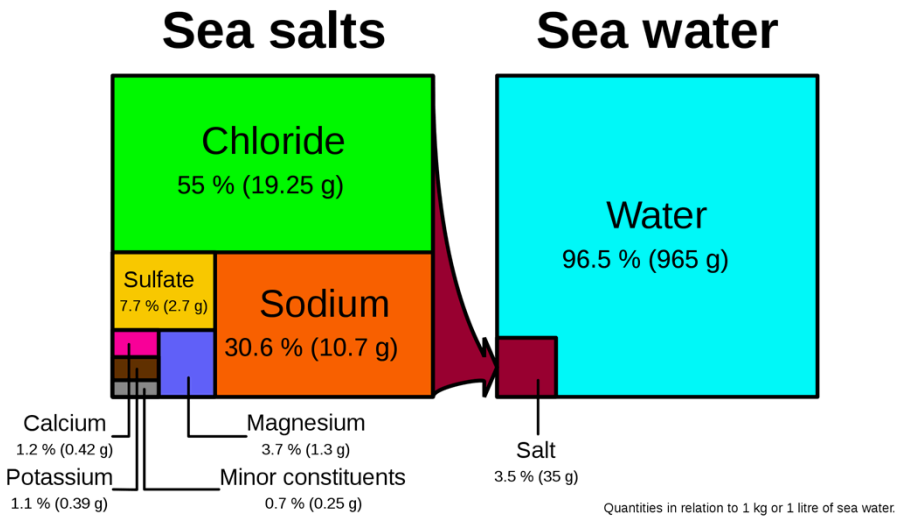




Davis *et al* 2013

- 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>

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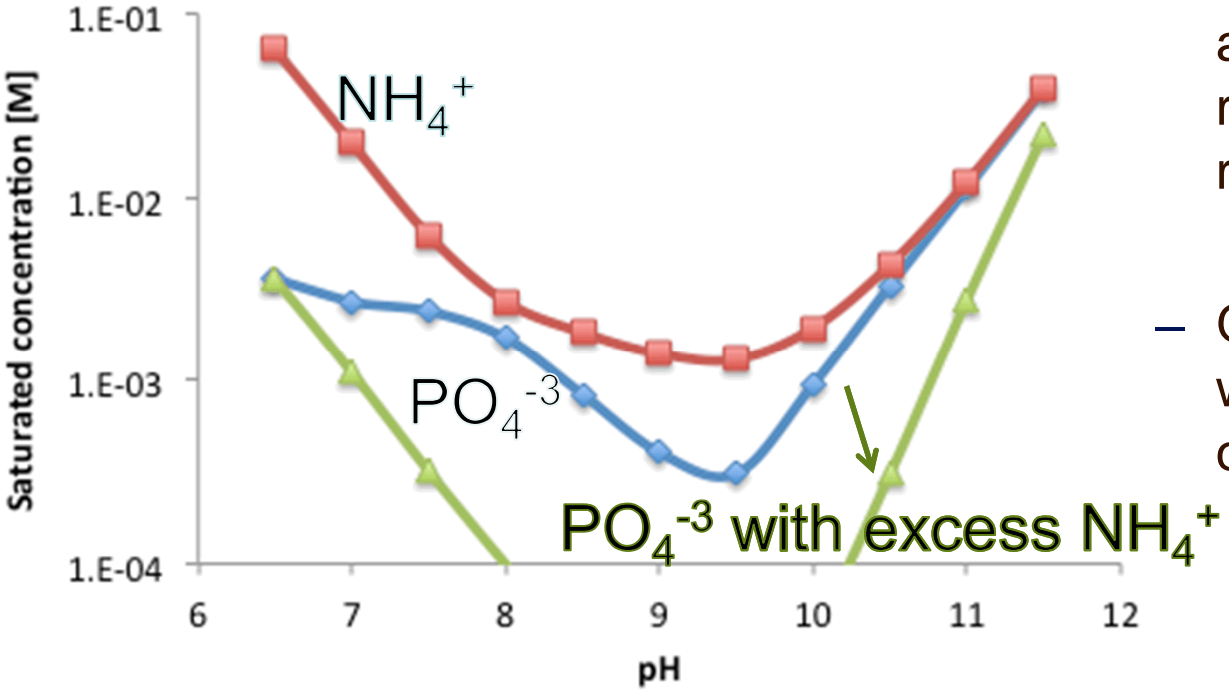
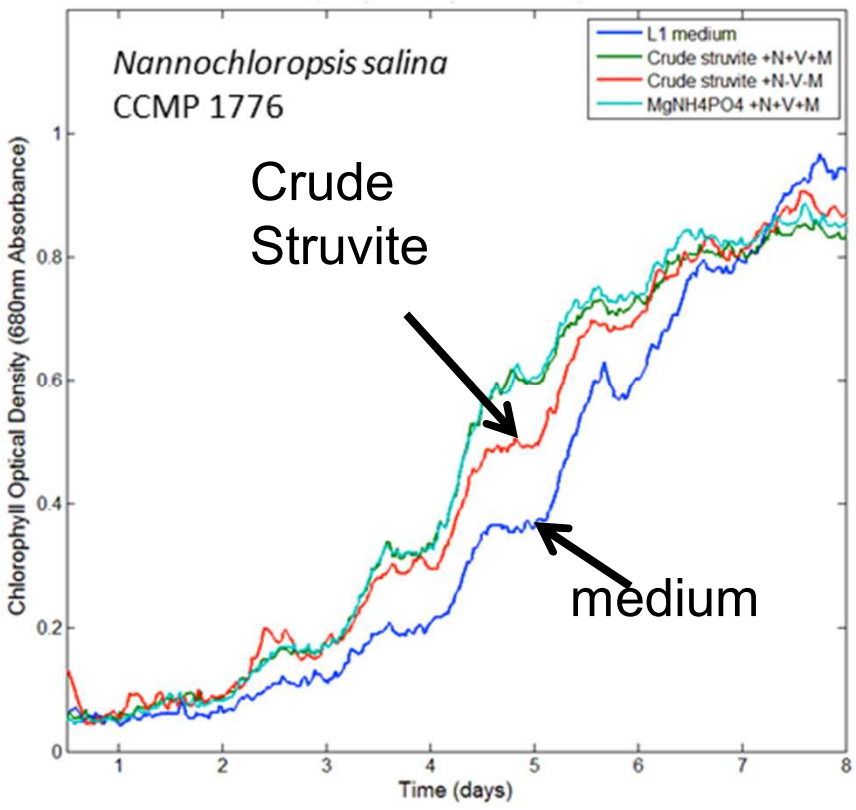
Recover nutrients through precipitation

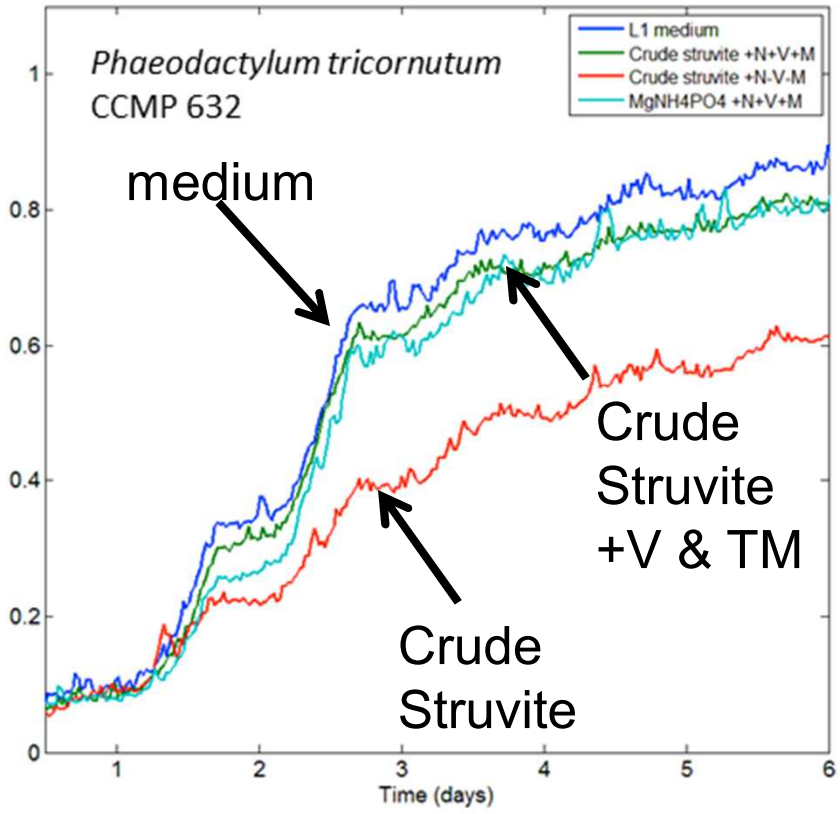
- Struvite ($MgNH_4PO_4$) is useful mineral form of nutrients.
  - Alternates include Ca and Mg phosphates.
- Looking at designing system to maximize recovery – need to measure precipitation kinetics.



- 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





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

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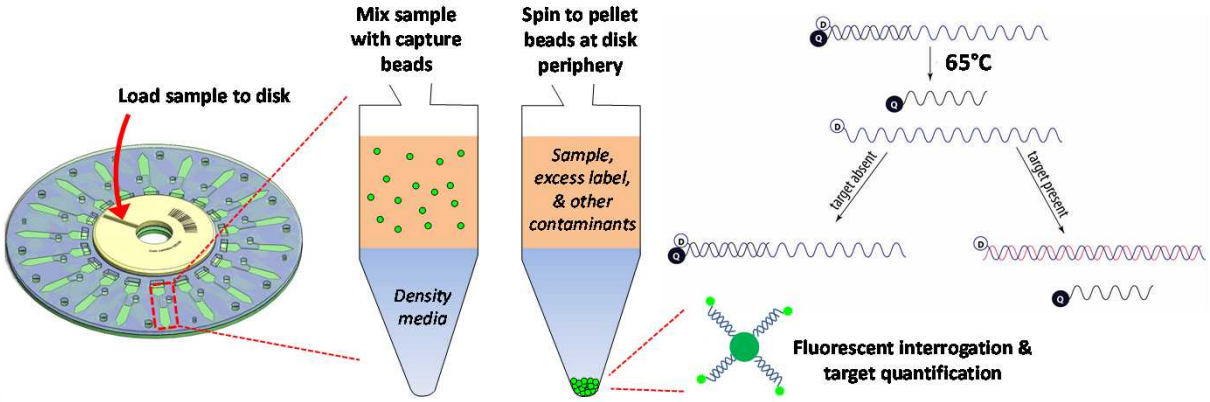
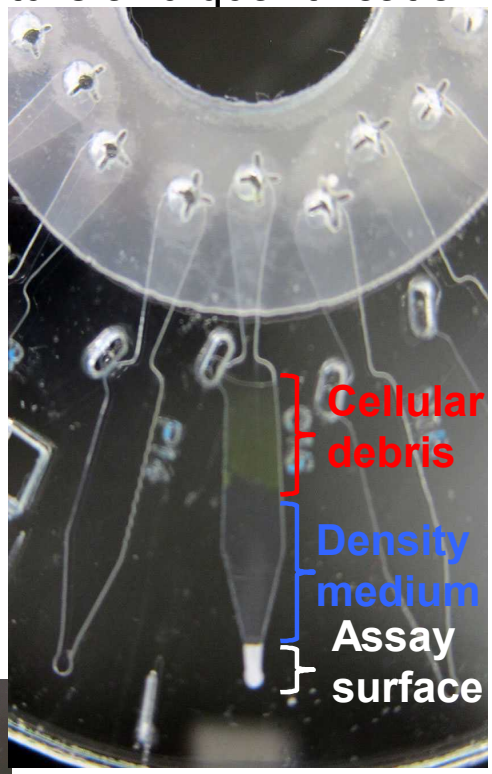
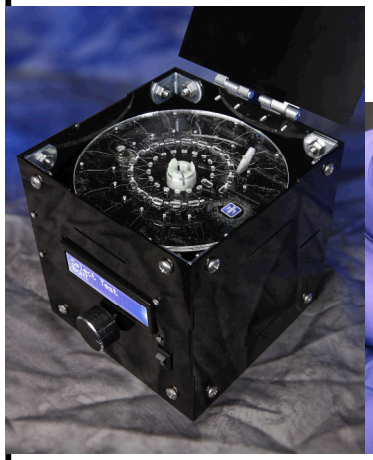
SpinDx™ has the necessary characteristics for a field assay for pond management

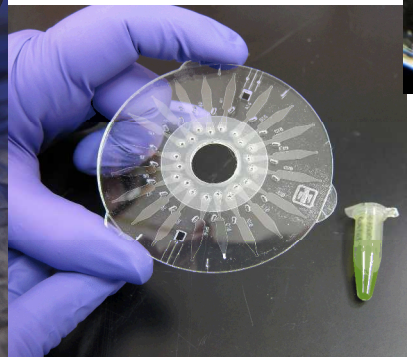
- FRET-based bead hybridization assay enabling capture and quantification of pathogen-specific RNA/DNA signatures





- Assay time: approx 30 min
- 36 channels per disc
- Potential for multiplexed assays in each channel
- Low reagent costs
- Low material costs
- Low instrument cost ($1000)
- Fieldable





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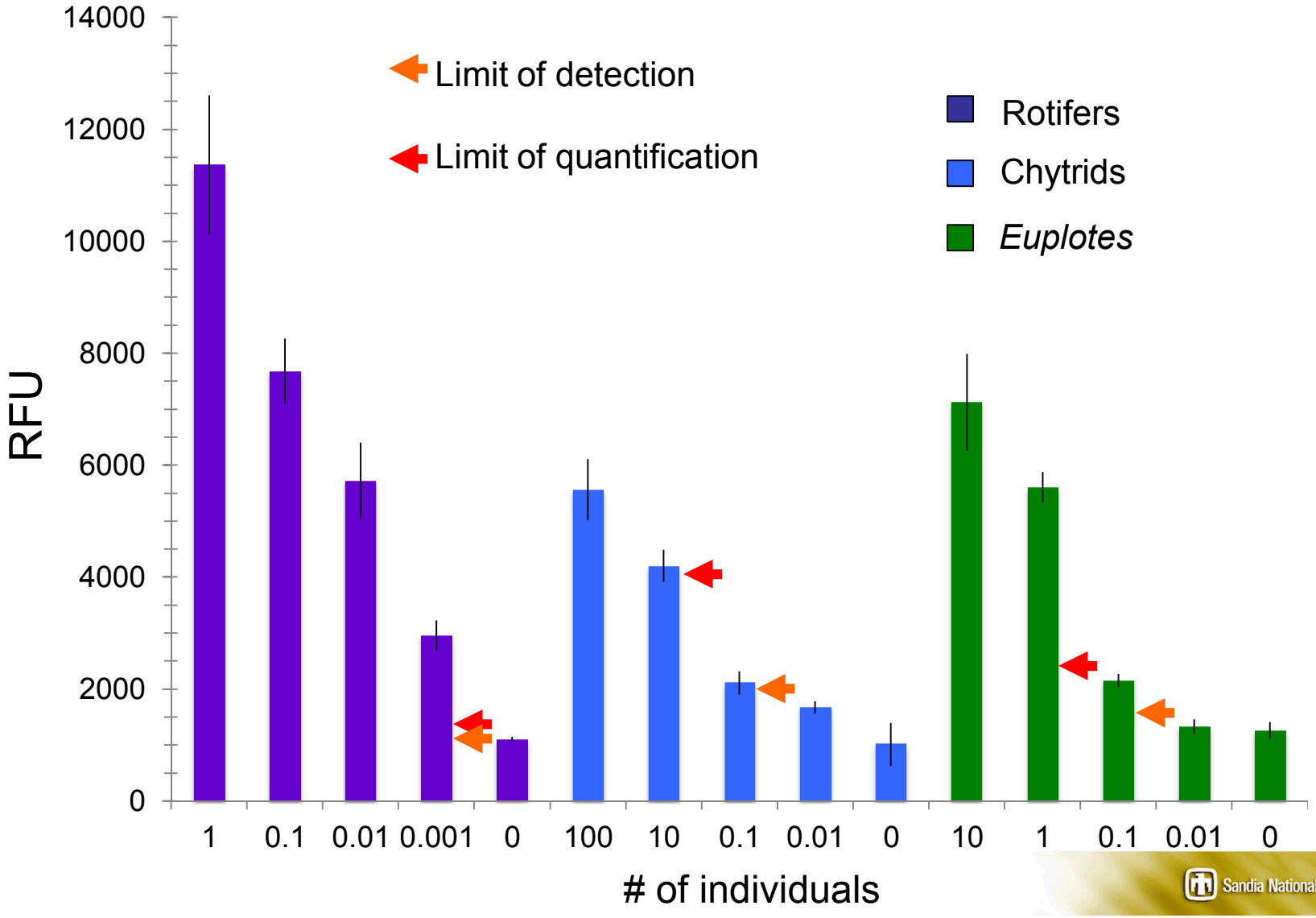
SpinDX detection/quantification of pest species



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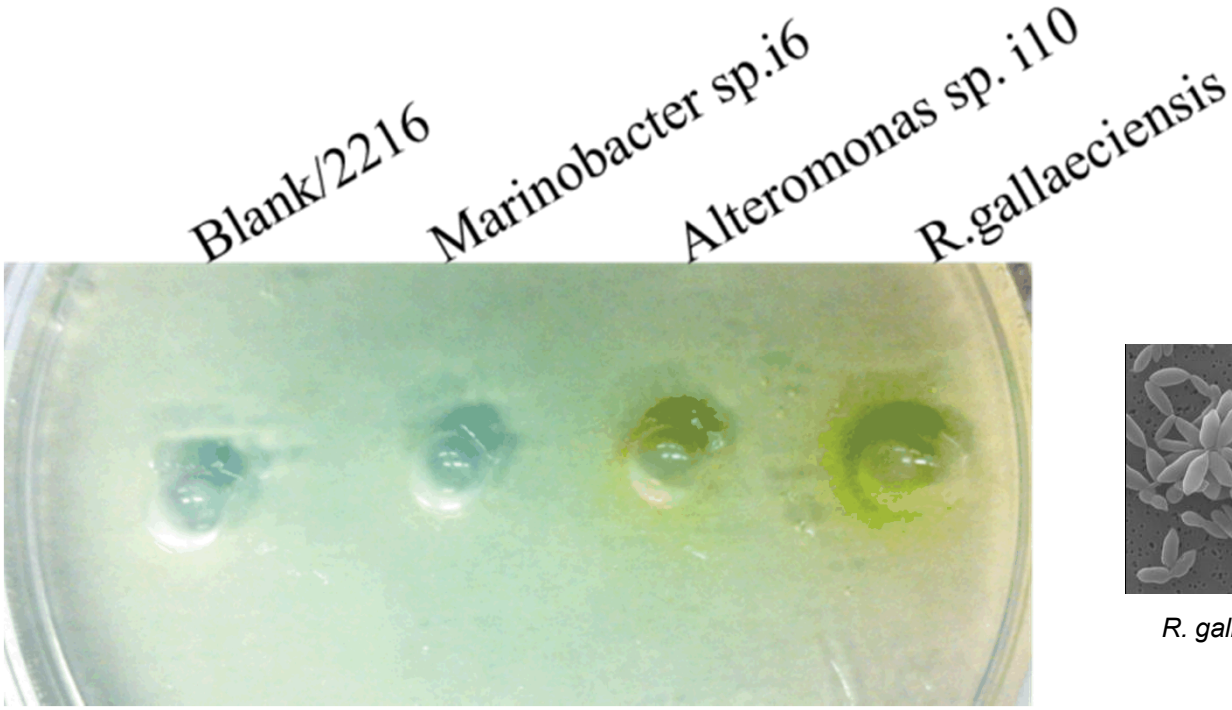
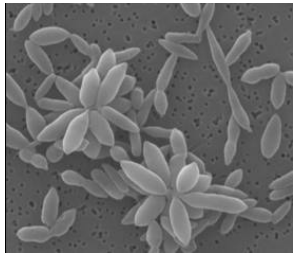
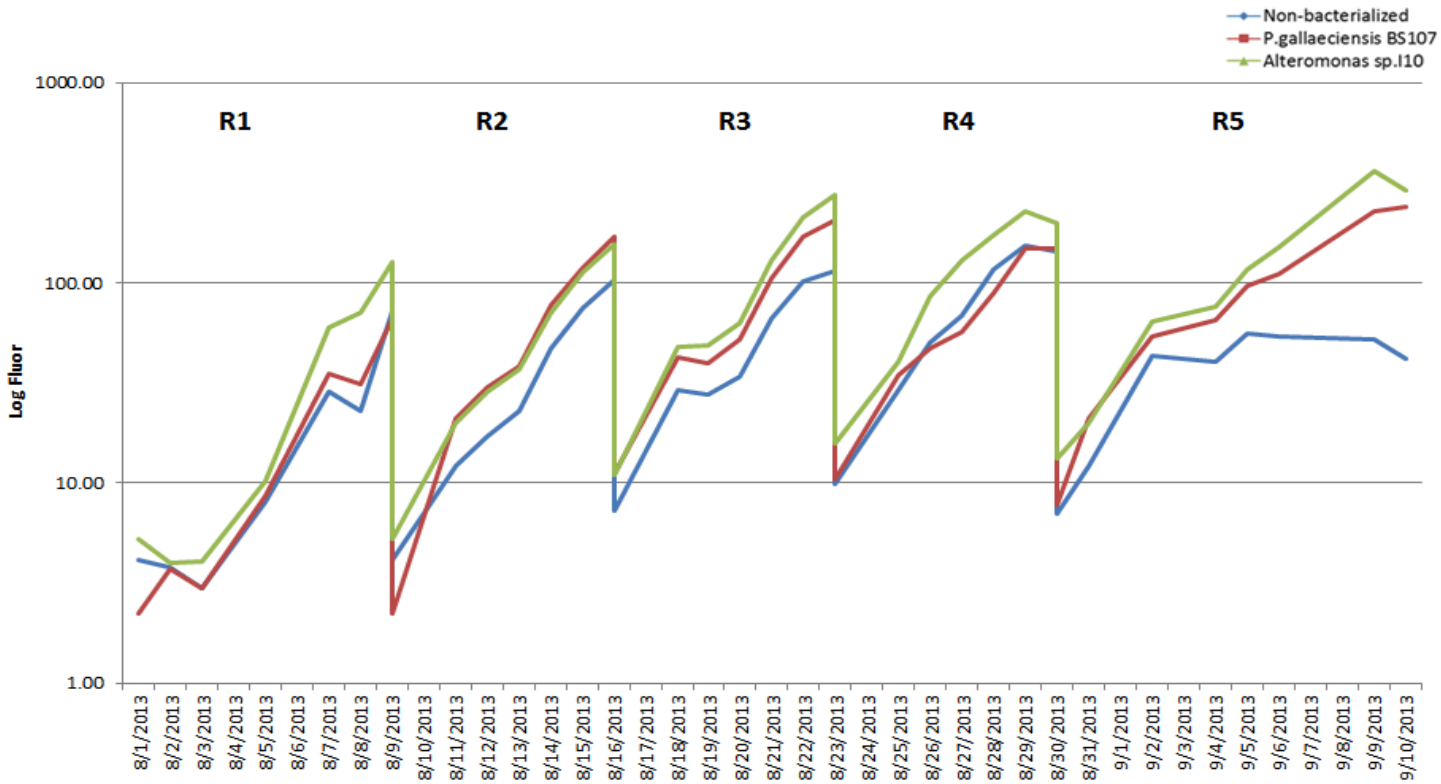
Co-culture of N. salina with isolated strains improves growth on plates



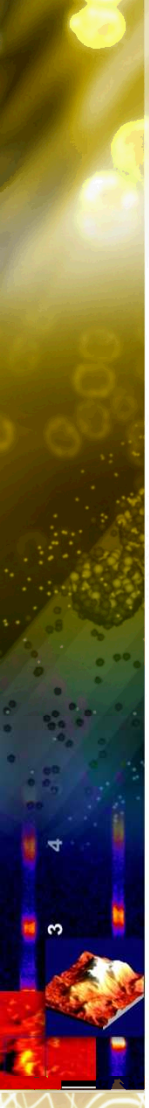


R. gallaenciensis

Probiotics + *N. salina* co-culture in outdoor mesocosms



8-week outdoor cultivation period (20L mesocosms)



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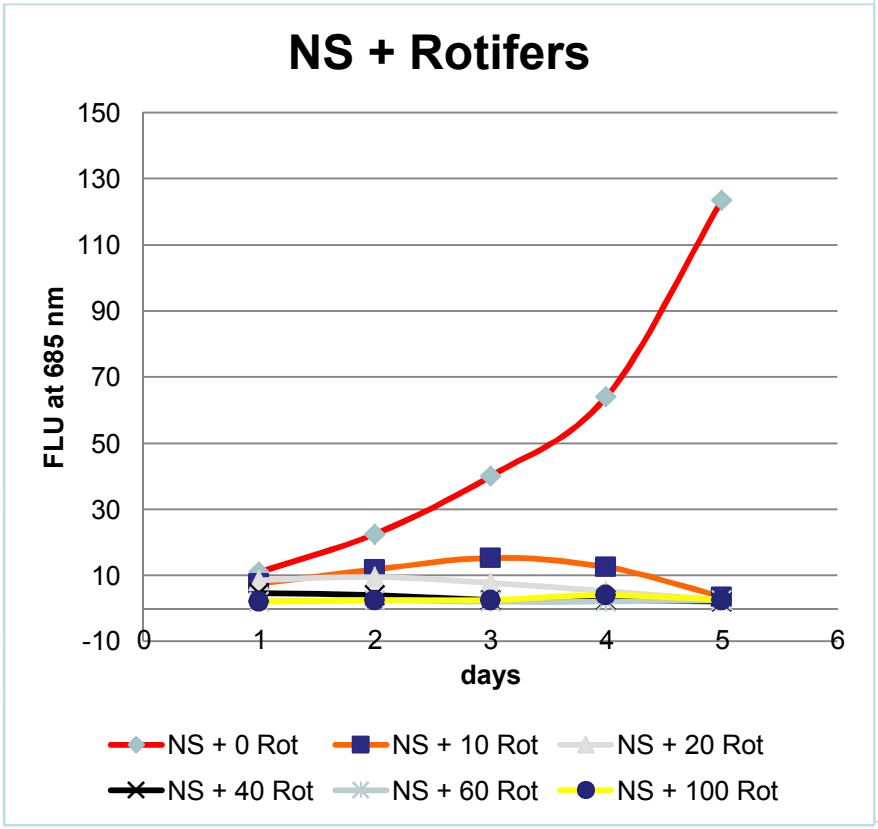
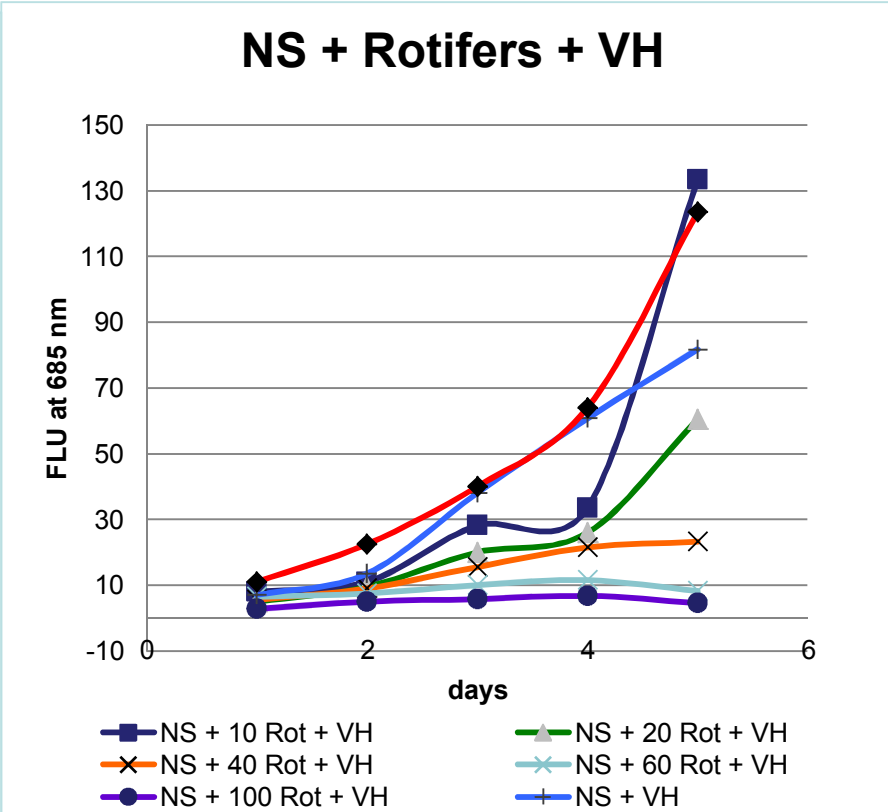
Probiotic strain Vh mitigates Rotifer grazing





Acknowledgments

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Open Algae

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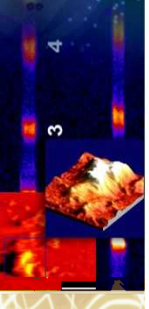
- Hoyt Thomas
- Stacy Truscott



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