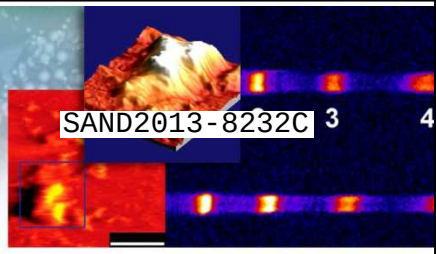
SANDIA BIOSCIENCE



Nutrient Recycling

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

Algae Biomass Summit, Orlando FL
2 October 2013

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.

U.S. DEPARTMENT OF ENERGY | NNSA National Nuclear Security Administration

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

Sandia National Laboratories

Motivation – scale of energy consumption exceeds nutrient production

- To meet 10% of liquid fuel needs (roughly 30 BGY):
  - Algae biomass: 200 - 500 M mt/yr.
  - Phosphorous: 2.4 - 6 M mt/yr
    - Compare 4.1 M mt in 2006: 61 – 146% of recent consumption.
  - Nitrogen (nitrate, ammonium, etc.) 18 - 45 M mt/yr
    - Compare 14 M mt in 2006: 130 – 320% of recent consumption.
- Food-vs-fuel concerns for nutrients.
- Nutrients (fertilizer)
  - Needed for biological productivity, not for fuel.
  - Phosphorous: mined resource, essentially nonrenewable.
  - Nitrogen: Haber-Bosch process has own energy requirements.

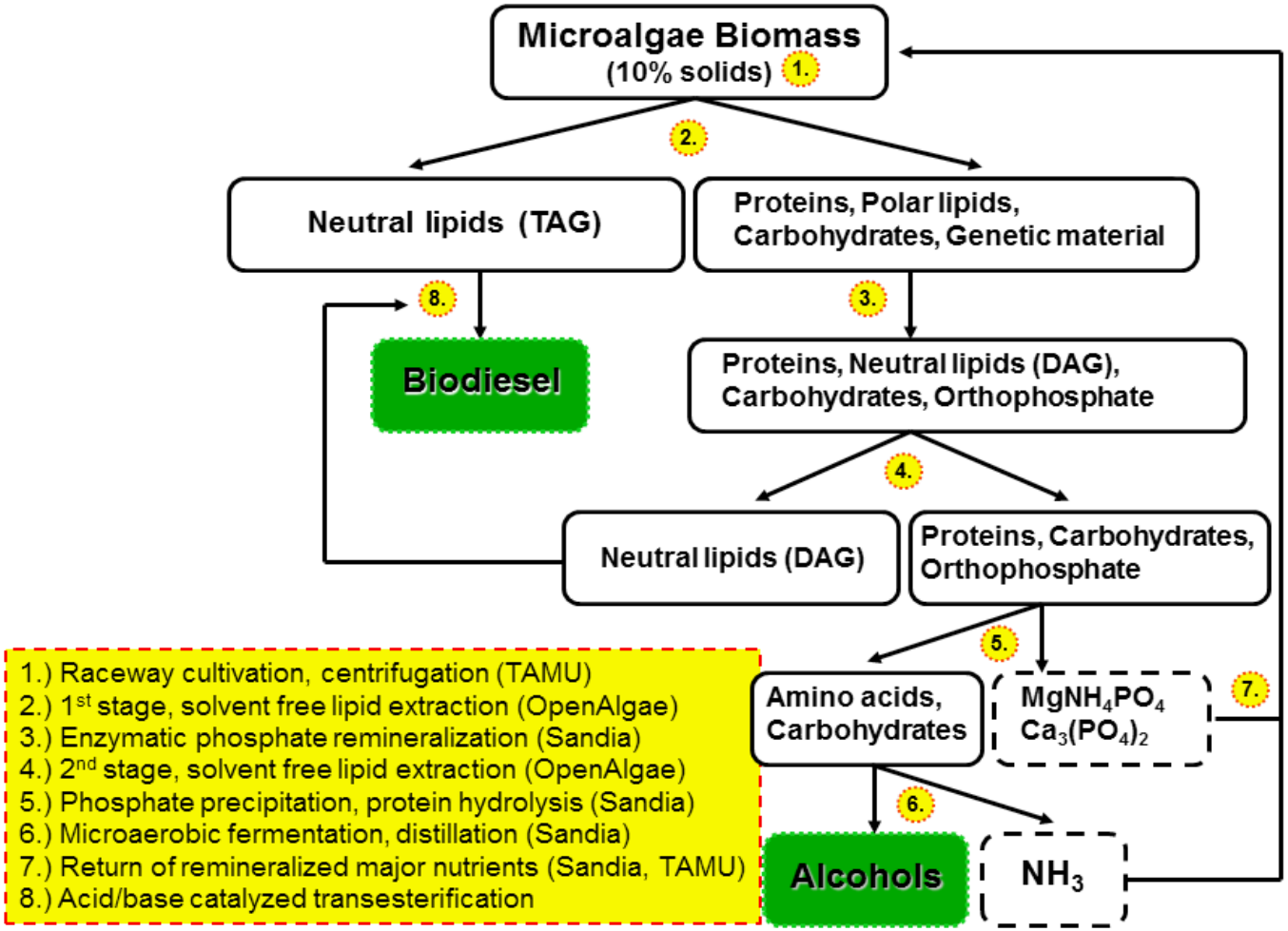
"The Achilles' Heel of Algae Biofuels: Peak Phosphate"
Forbes, Feb 2012

Need to recycle nutrients.

Cannot afford to pass through one time only.

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

DOE Nutrient Recycling Project



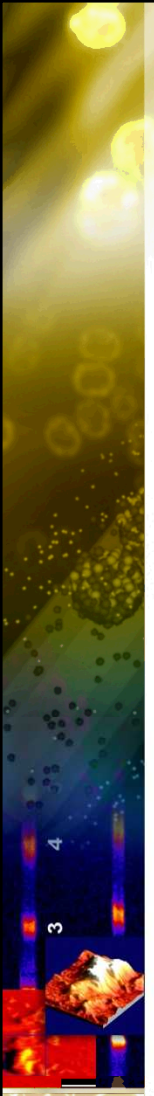
DOE funded project is a partnership between national lab, university and industry

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

SANDIA BIOSCIENCE

Staged approach to development of a one pot reaction

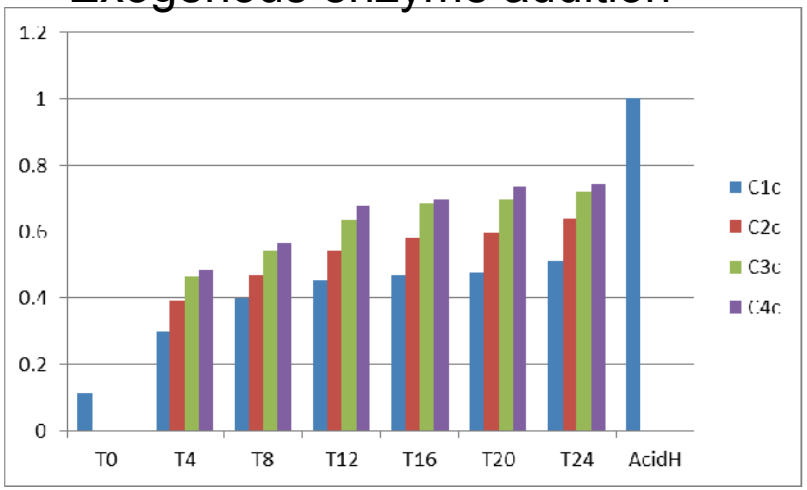
- Test different cocktails for remineralization of phosphate and phospholipid conversion.
  - Determine rate of remineralization
  - Optimize reaction conditions (extraction of nutrients from solid phase)
  - Identify recalcitrant pools
  - Minimize reaction time
- Develop microbial consortia with appropriate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

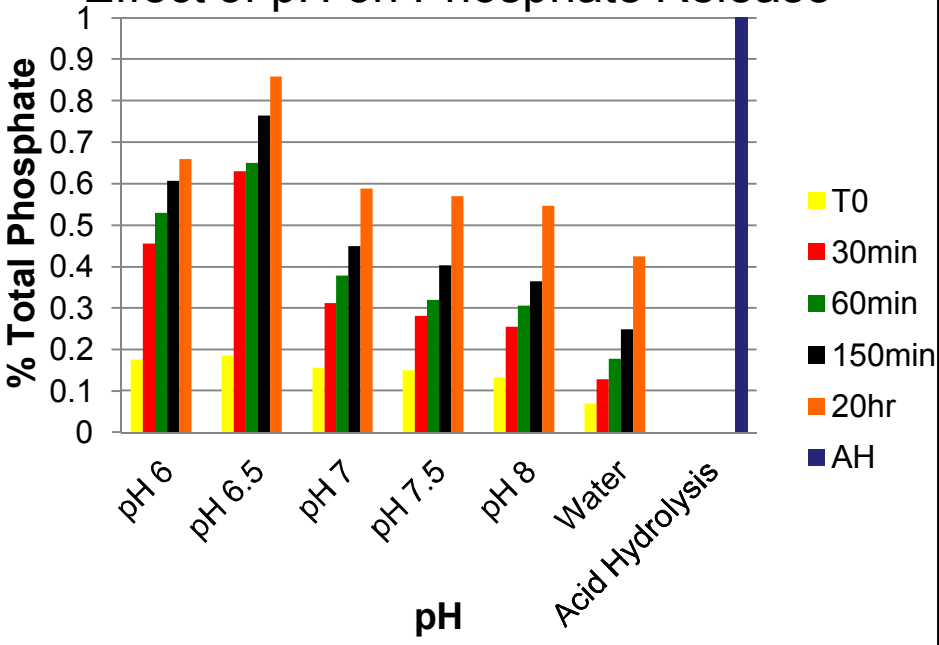
Exogenous enzyme addition



1. No enzyme
2. AP
3. AP + B
4. AP + B + PL-D

AP = alkaline phosphatase
B = benzonase
PL-D = phospholipase D

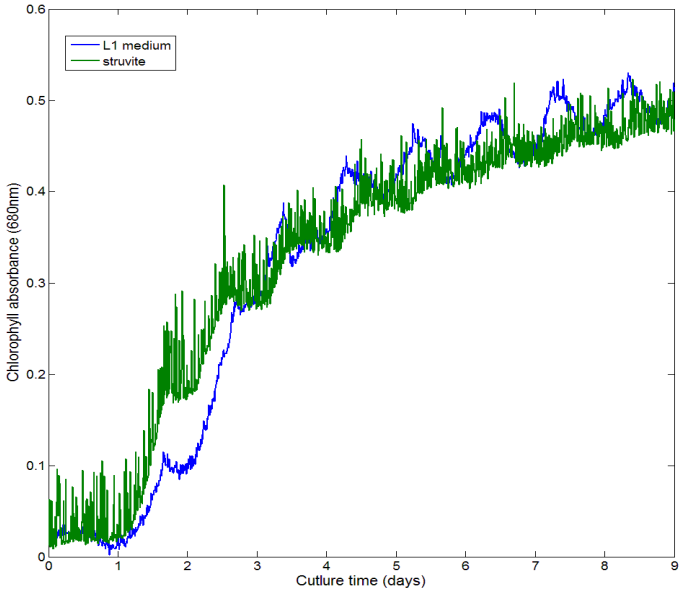
Effect of pH on Phosphate Release

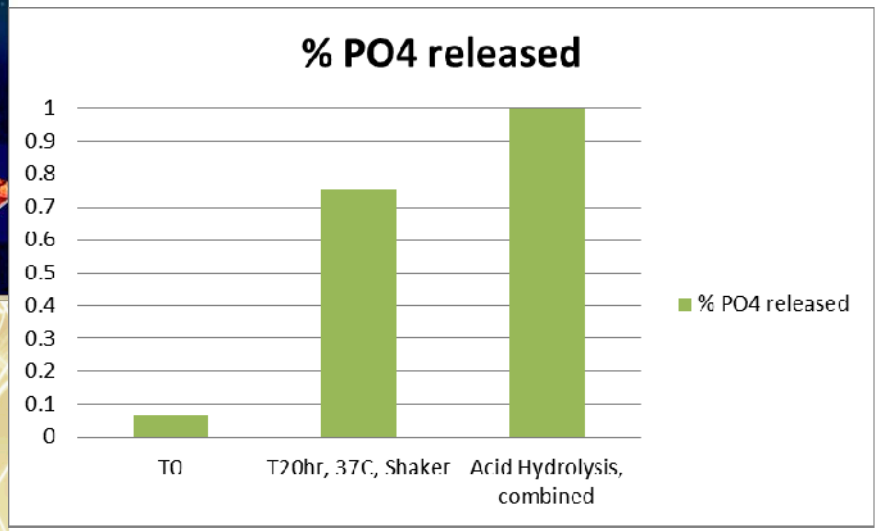


SANDIA BIOSCIENCE

Growth on recycled 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 lab cultures
- Growth of *P. tricornutum* and *N. salina*

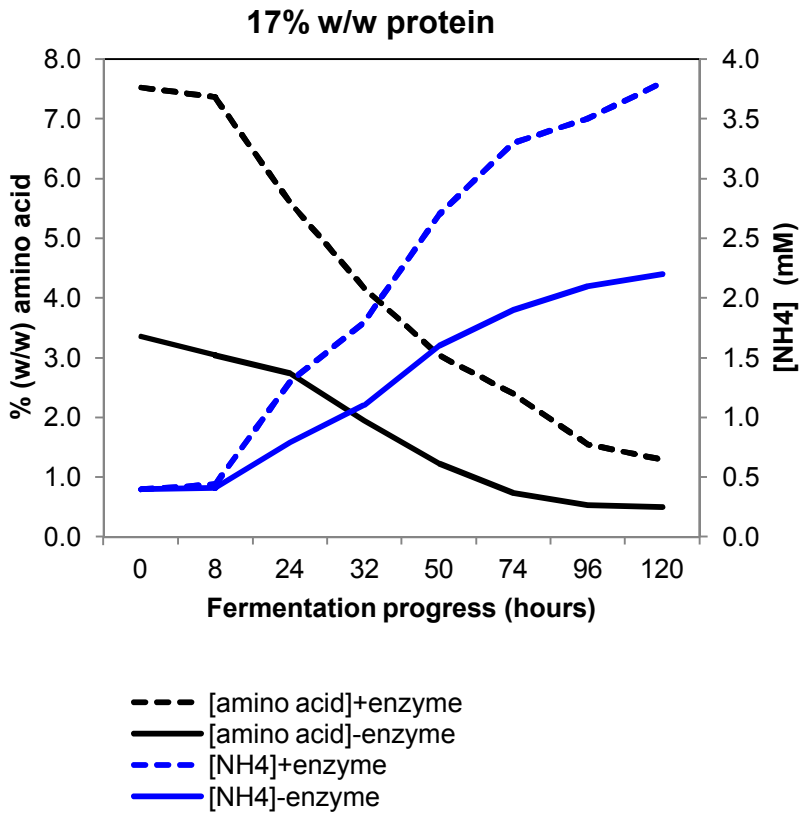




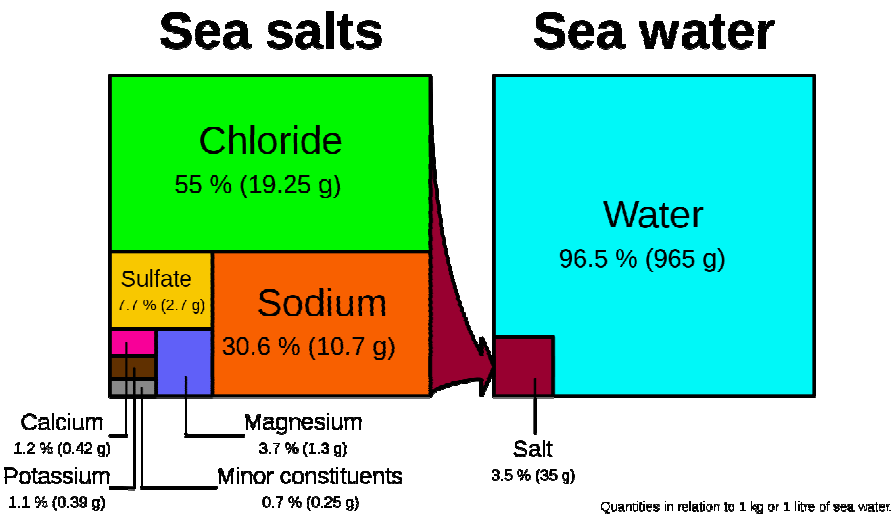
We will take advantage of NH_4 production by Previously developed fermentation process

Subject lipid extracted biomass to microaerobic fermentation using an alcohol tolerant metabolically engineered *E. coli* strain, (Xin Huo, et al *Nature Biotech*, 2011)

1) Mixed alcohols = 50% protein yield using 5-step process:
2) Alcohol components do not significantly vary with biomass type
3) Accumulation of alcohols proceeds in distinct temporal phases: isobutanol -> n-methyl-butanol -> phenylethanol and n-butanol
4) Ammonium is accumulated in fermentation liquor as amino acid breakdown product



In current system, Mg carried over with biomass may result in struvite fomation



$NaNO_3$ (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

3-4 mMol Mg/gm AFDW

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

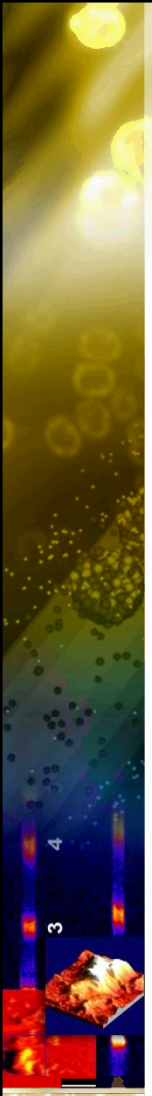
Internal Mg stores may also be sigificant: Macronutrient

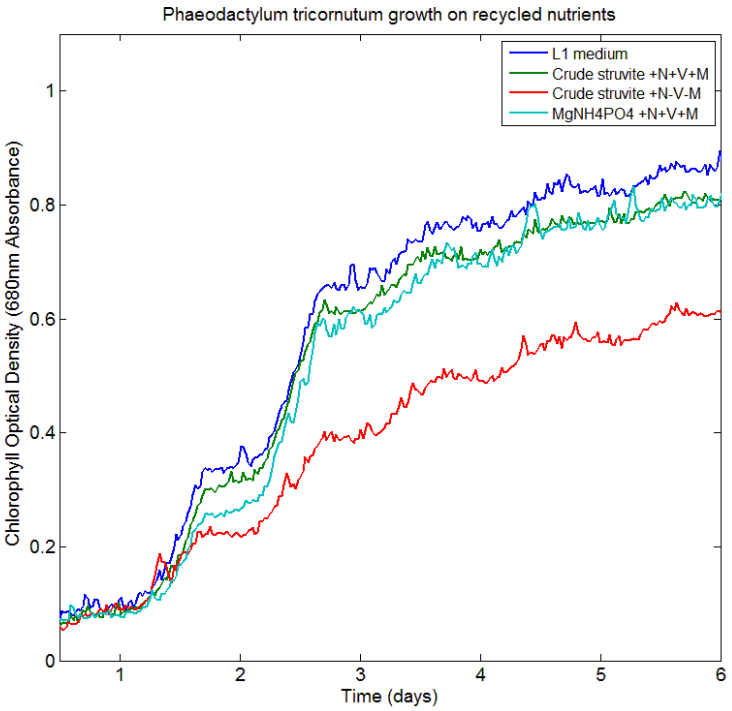
The resulting P/Mg ratio may promote the formation of struvite: $MgNH_4PO_4$

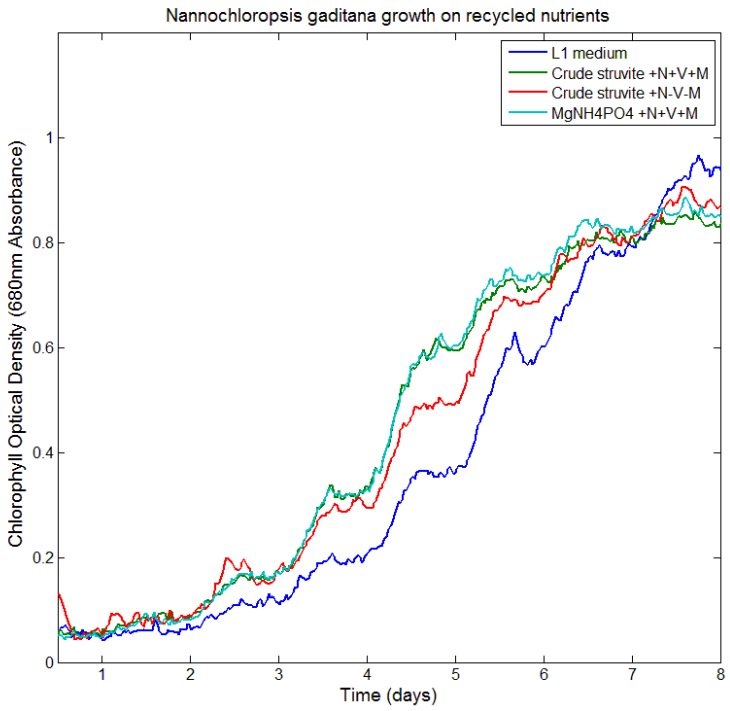
We will not alter the chemistry of the pond or biomass to form or use struvite

No requirement for new Mg

Struvite can replace “new” nutrients in microalgal culture





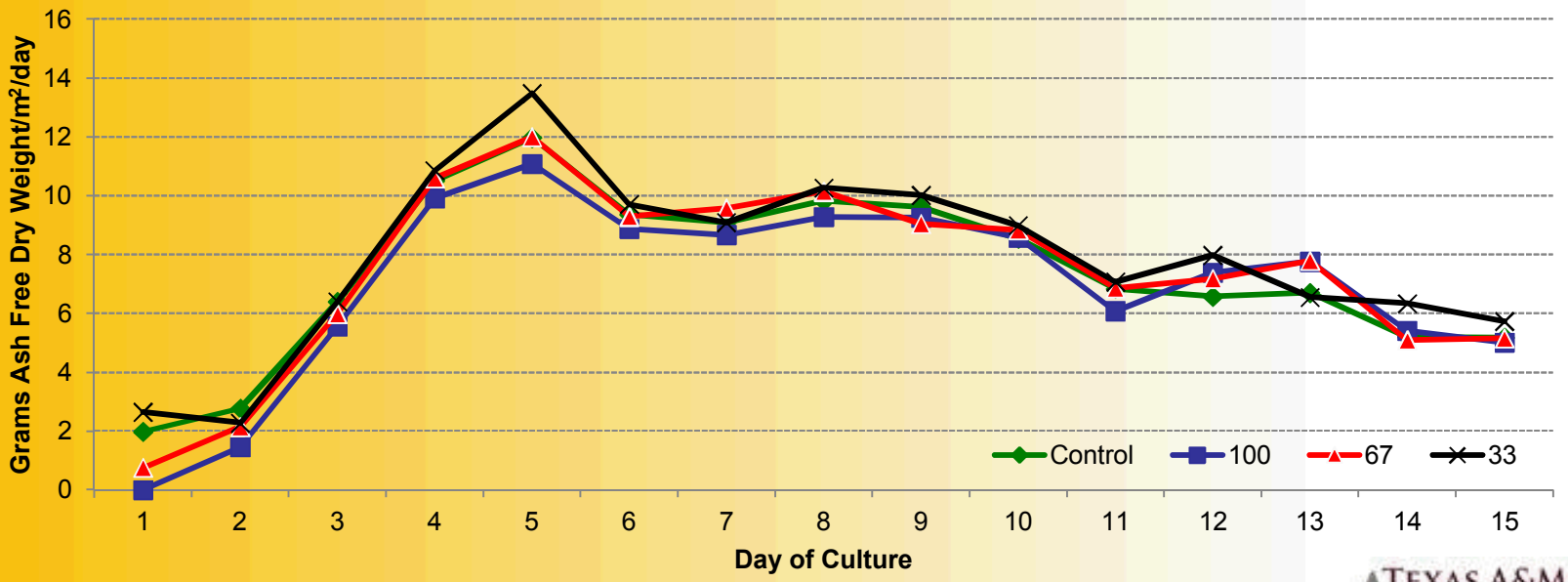


SANDIA BIOSCIENCE

Sandia National Laboratories

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

Daily biomass productivity (g AFDW/m^2/day) of *Nannochloropsis salina* (CCMP 1776) cultivated with phosphorus replacement (% of control) using commercial struvite



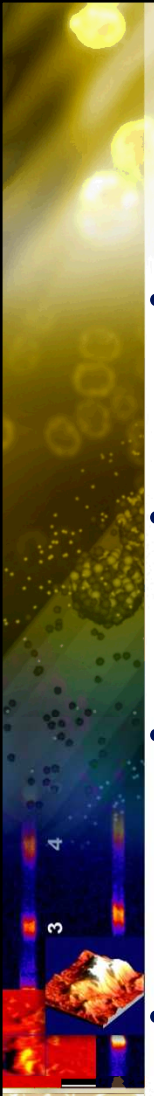
SANDIA BIOSCIENCE

TEXAS A&M AGRILIFE RESEARCH

Sandia National Laboratories

Summary

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



SANDIA BIOSCIENCE

Sandia National Laboratories

Acknowledgments

DOE EERE BioEnergy Technology Office

Sandia National Labs

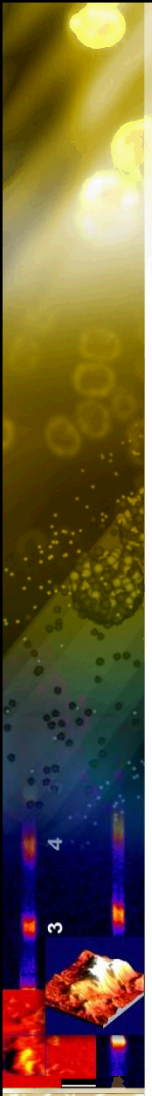
- Ryan Davis
- John Hewson
- Pamela Lane
- Nicholas Wyatt
- Deanna Curtis

Texas Agrilife

- Anthony Siccardi

Open Algae

- Peter Kipp
- Hoyt Thomas



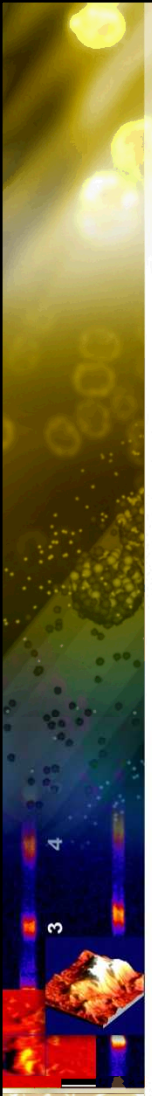
SANDIA BIOSCIENCE

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

Sandia National Laboratories

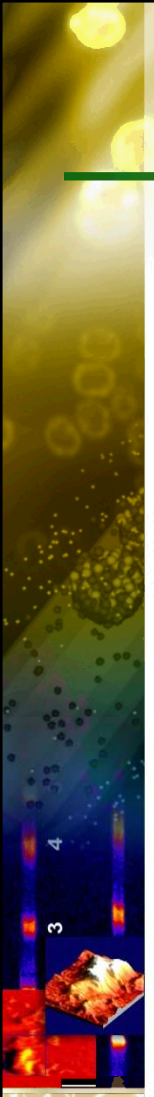
Spare slides



SANDIA BIOSCIENCE

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)

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

Final Biomass Productivity (g AFDW/m^2/day)

Final 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)	Final biomass productivity (g AFDW/m^2/day)
Control	5.18±0.32^a
100	5.01±0.38^a
67	5.16±0.61^a
33	5.73±0.53^a

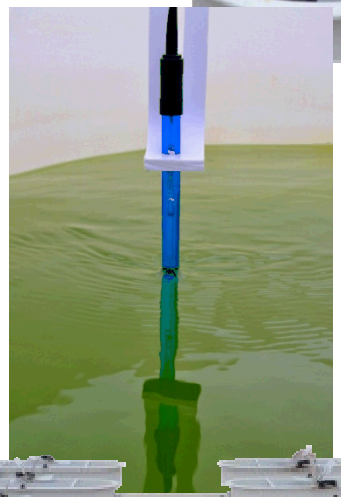
[1] Means with similar superscript in the same column are not statistically different (p>0.05)

[2] N = 3 raceways

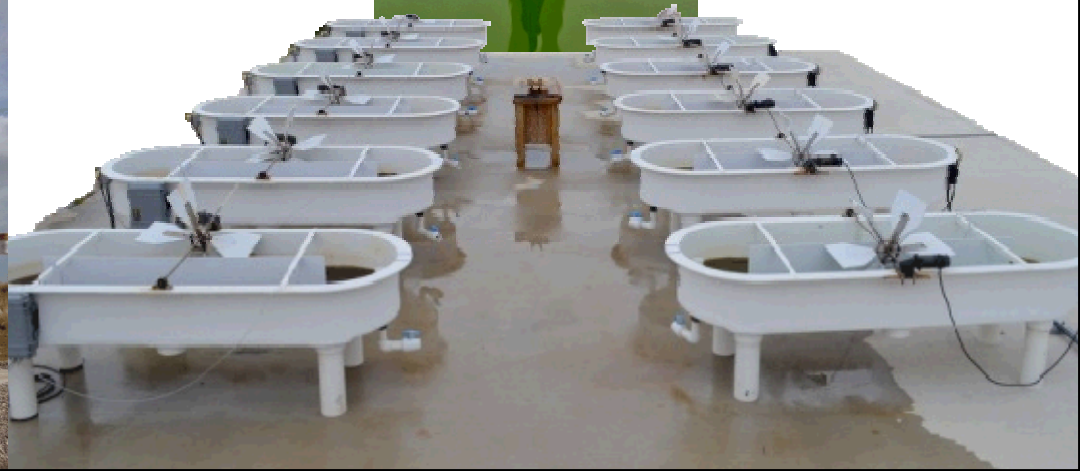
[3] Standard deviation

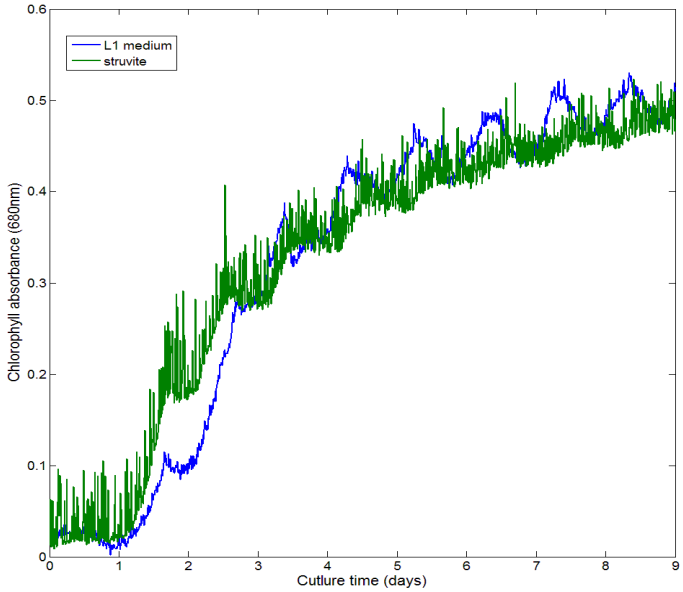

American Marine Inc.
7.86
PINPOINT pH Controller


11




AGRILIFE

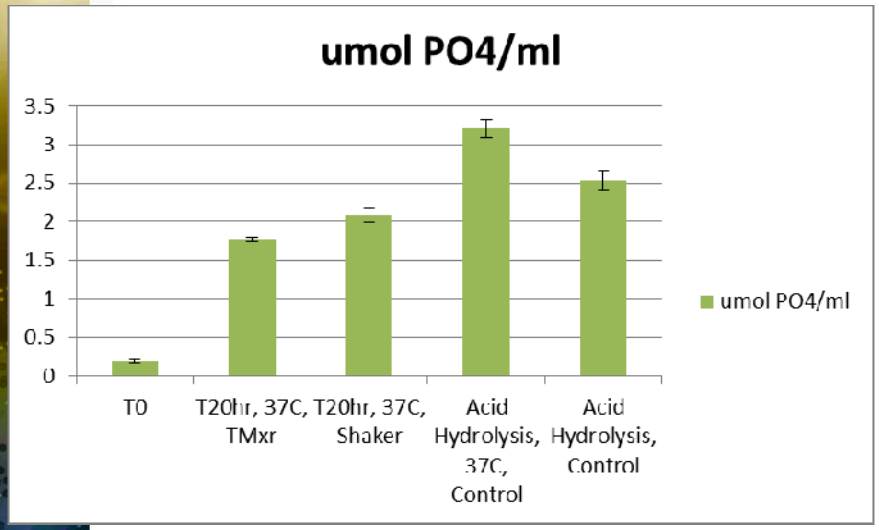




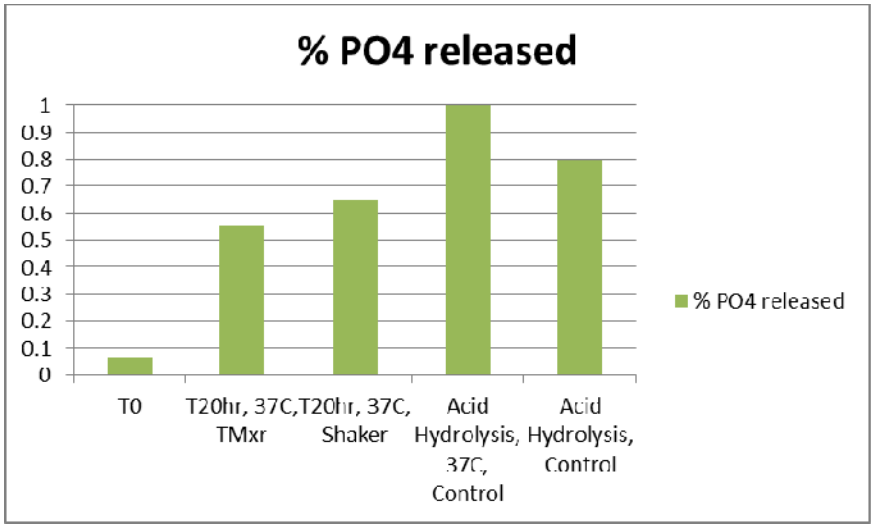
SANDIA BIOSCIENCE

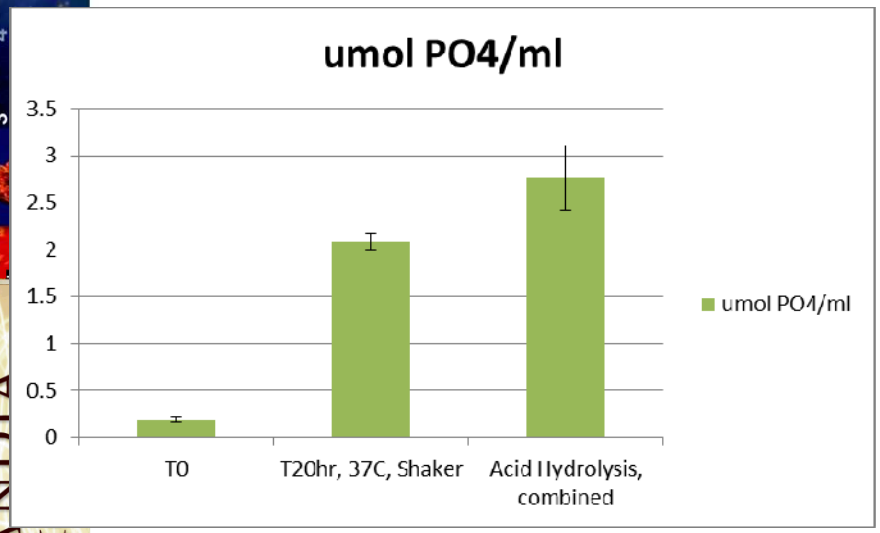
Sandia National Laboratories

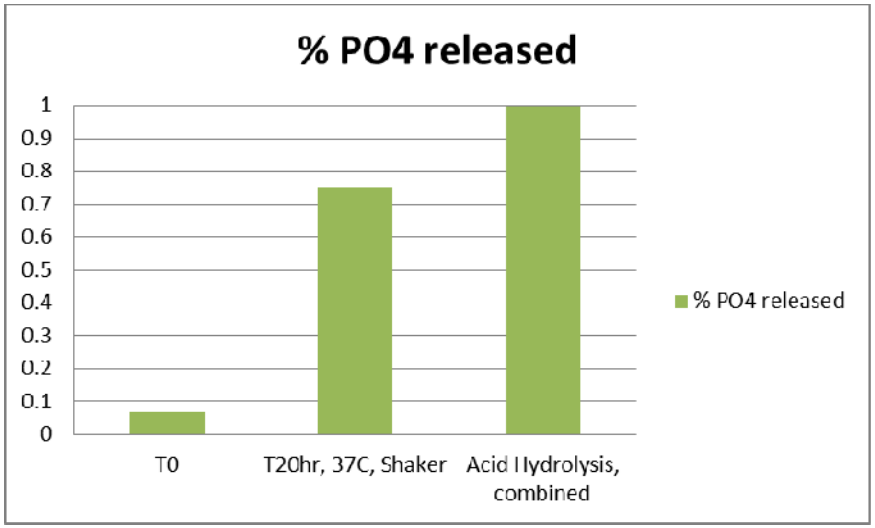
umol PO4 released from 1ml of TX biomass (2% solids); umol/ml = mM

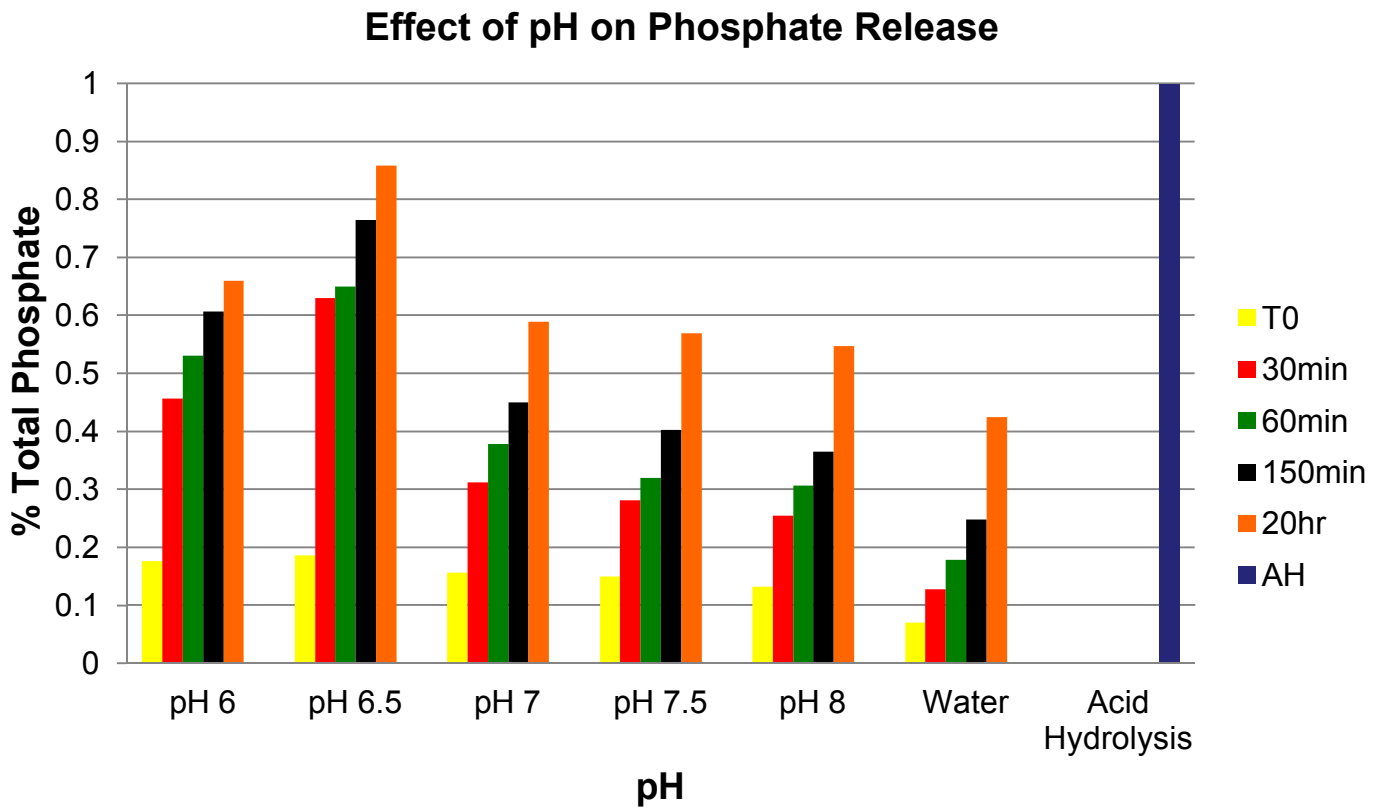


% PO4 release TX biomass (2% solids)

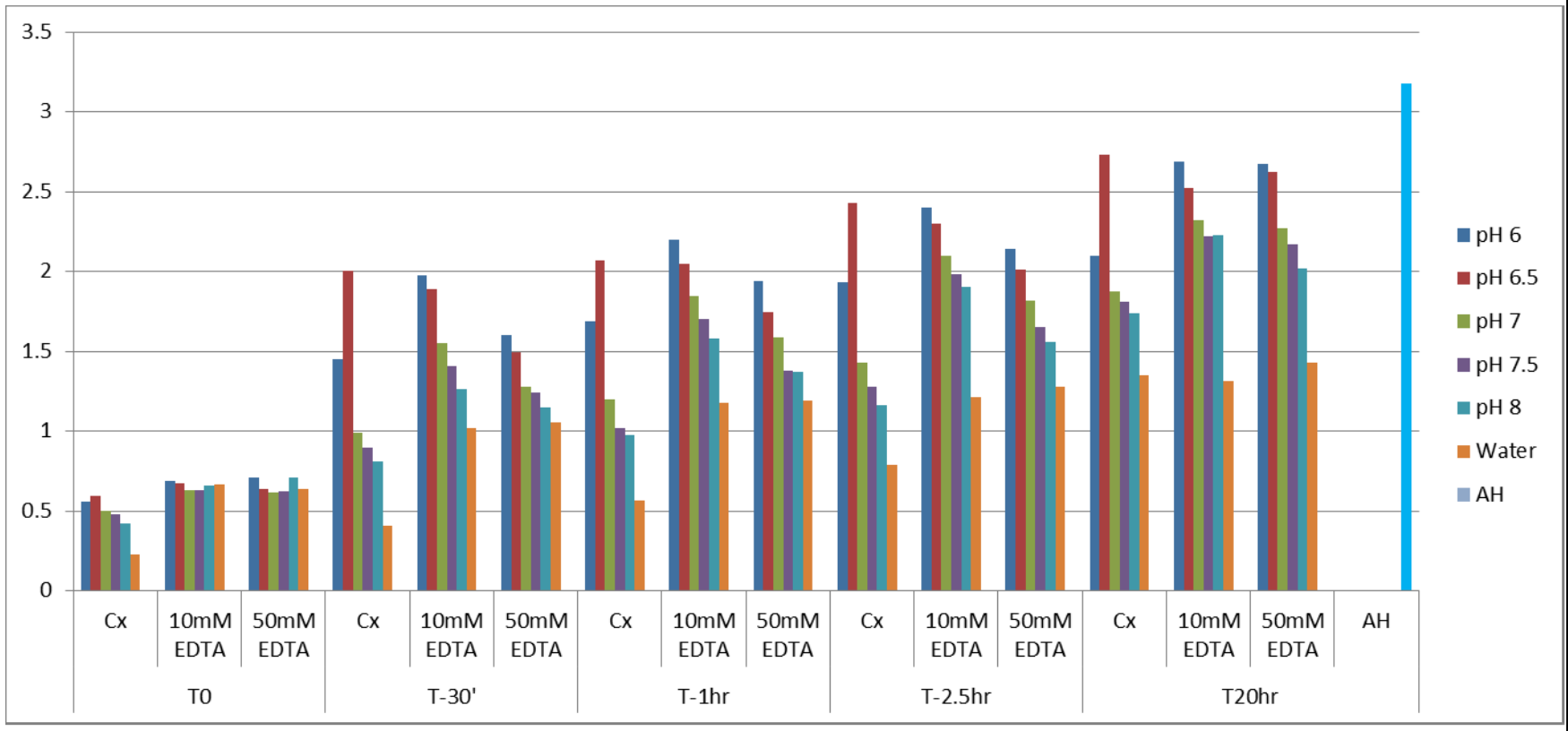







Effect of pH on Phosphate Release
% Total Phosphate
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
pH 6
pH 6.5
pH 7
pH 7.5
pH 8
Water
Acid Hydrolysis
pH
T0
30min
60min
150min
20hr
AH

pH/EDTA effect on phosphate released



SANDIA BIOSCIENCE

Sandia National Laboratories