Project 25 - Major Nutrient Recycling for Sustained Algal Production (9.1.1.3)

5/24/2013

Algae Peer Review

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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.

Goal Statement

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 rapid micriobial mediated process to liberate and capture the nitrogen and phosphorous present in de-oiled algal biomass.
2. Convert phospholipids to DAG & remineralized phosphate, and recover both.
3. Precipitate ammonia and phosphate as struvite and demonstrate a simple struvite recovery process.
4. Demonstrate the utility of struvite as a nutrient to support algal growth.
5. Operate the growth process at a lower overall N:P ratio to favor lipid production and minimize nitrogen cycling.

Quad Chart Overview

Timeline

- Project start date: 03/15/2013
- Project end date: 3/14/2016
- Percent complete: 5%

Barriers

- Barriers addressed
  - FT-A Feedstock availability and cost
  - **FT-B. Sustainable production**
  - Im-F. Cost of production

Budget

Total project funding: $2.682M
- DOE share: $2.145M
- Contractor share: $567K

Funding received in FY12: $0
Funding for FY13: $715K
ARRA Funding: $0

Partners

Interactions/ collaborations

Texas Agrilife (TAMU)
Open Algae LLC

Project management

Project Lead: Todd Lane
Sandia National Labs, Livermore

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).

Project Overview

Grand objective:

Develop an integrated system for the culture and processing of algal biomass that will enable the reuse of major nutrients thus reducing the operational requirement for external nutrients.

Team Members:

Texas Agrilife (TAMU): Cultivation trials and production of biomass

- Characterize growth efficiency on recycled nutrients
- Generate 1-10kg lots of biomass for R&D

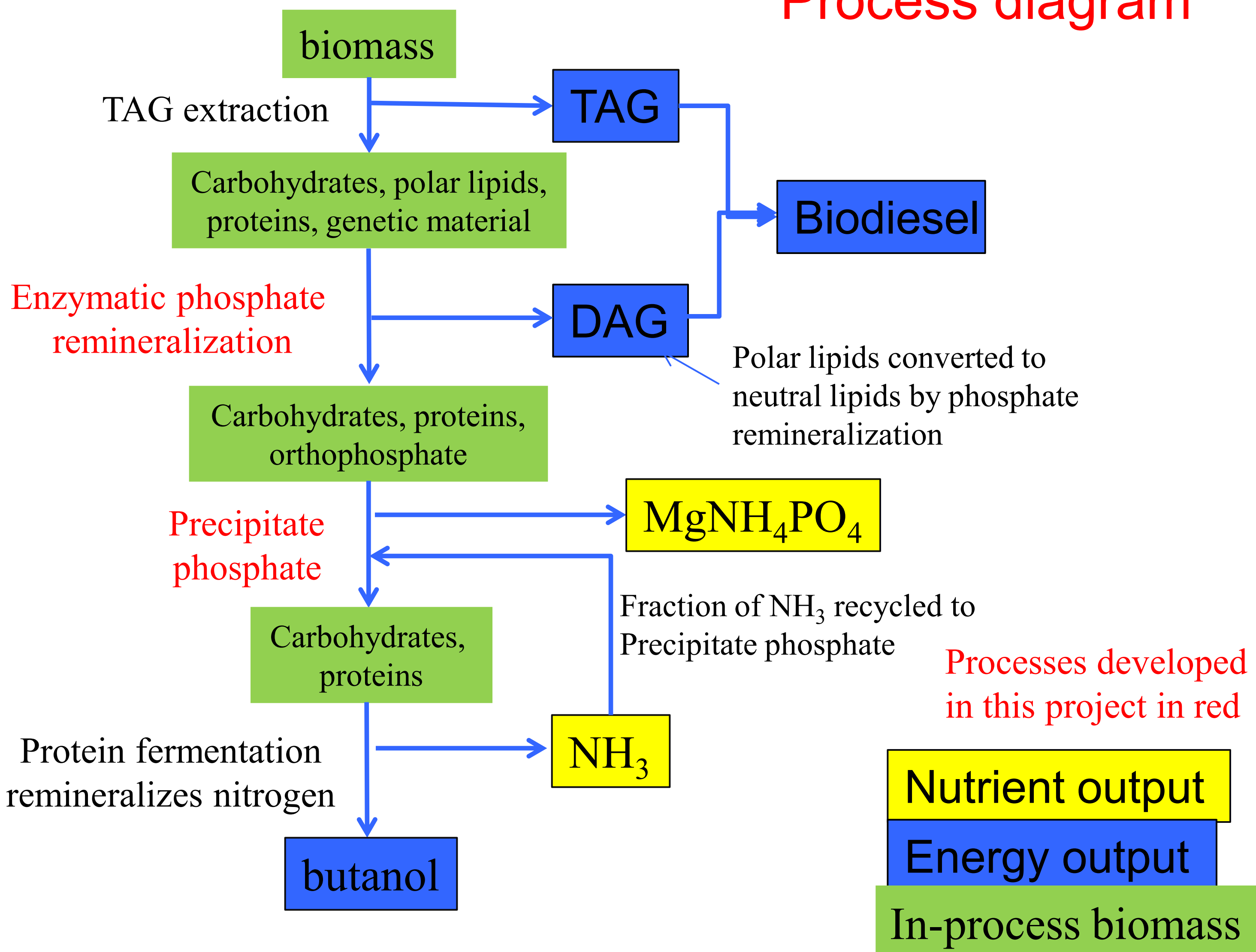
Open Algae LLC: Extraction of neutral lipids (TAGs and DAGs derived from phospholipids)

Sandia National Labs: Project lead, Development of methods for remineralization and capture of nutrients.

1-Approach

Remineralization: The biological conversion of organic forms of nutrients to inorganic forms.

Process diagram



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Laboratories

Development of a microbially-mediated phosphate recycling system

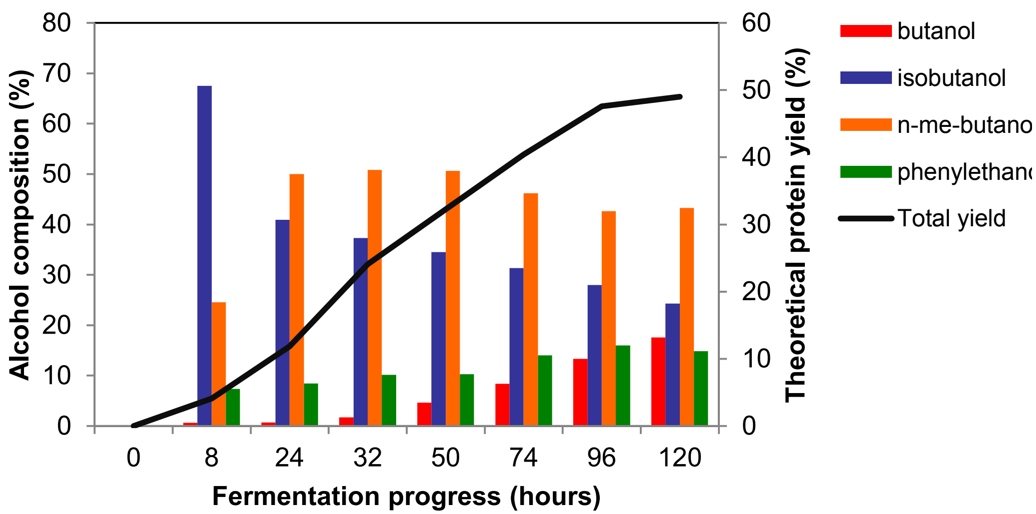
- Test different enzyme cocktails for remineralization of phosphate and phospholipid conversion.
  - Determine rate of remineralization
  - Optimize reaction conditions
  - Identify recalcitrant pools
  - Minimize reaction time
- Develop microbial consortia with appropriate enzymatic activities: test culture supernatants.
  - Identify candidate genes, clone, overexpress
  - Test for protein and activity level
  - Characterize mixed culture supernatants.
- 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

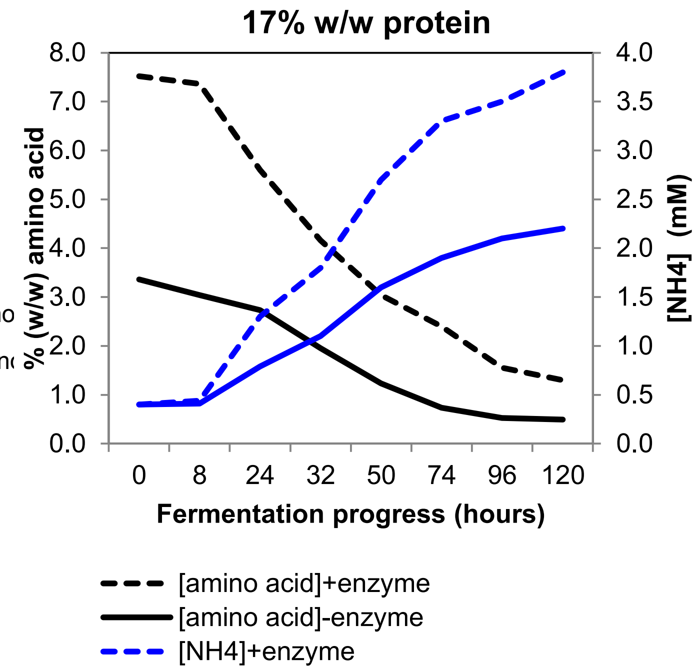
We will take advantage of NH_4 production by Previously developed fermentation process

Goal: Develop process to maximize conversion of microalgal residuals to fuels
Strategy: Subject lipid extracted biomass to microaerobic fermentation using an alcohol tolerant metabolically engineered *E. coli* strain, (Xin Huo, et al *Nature Biotech*, 2011)

Results

1) Mixed alcohols = 50% protein yield using 5-step process:
dilute acid pretreatment -> ethanolic fermentation -> distillation -> enzymatic digestion (proteins) -> microaerobic fermentation (37° C, 96-120 hrs)
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



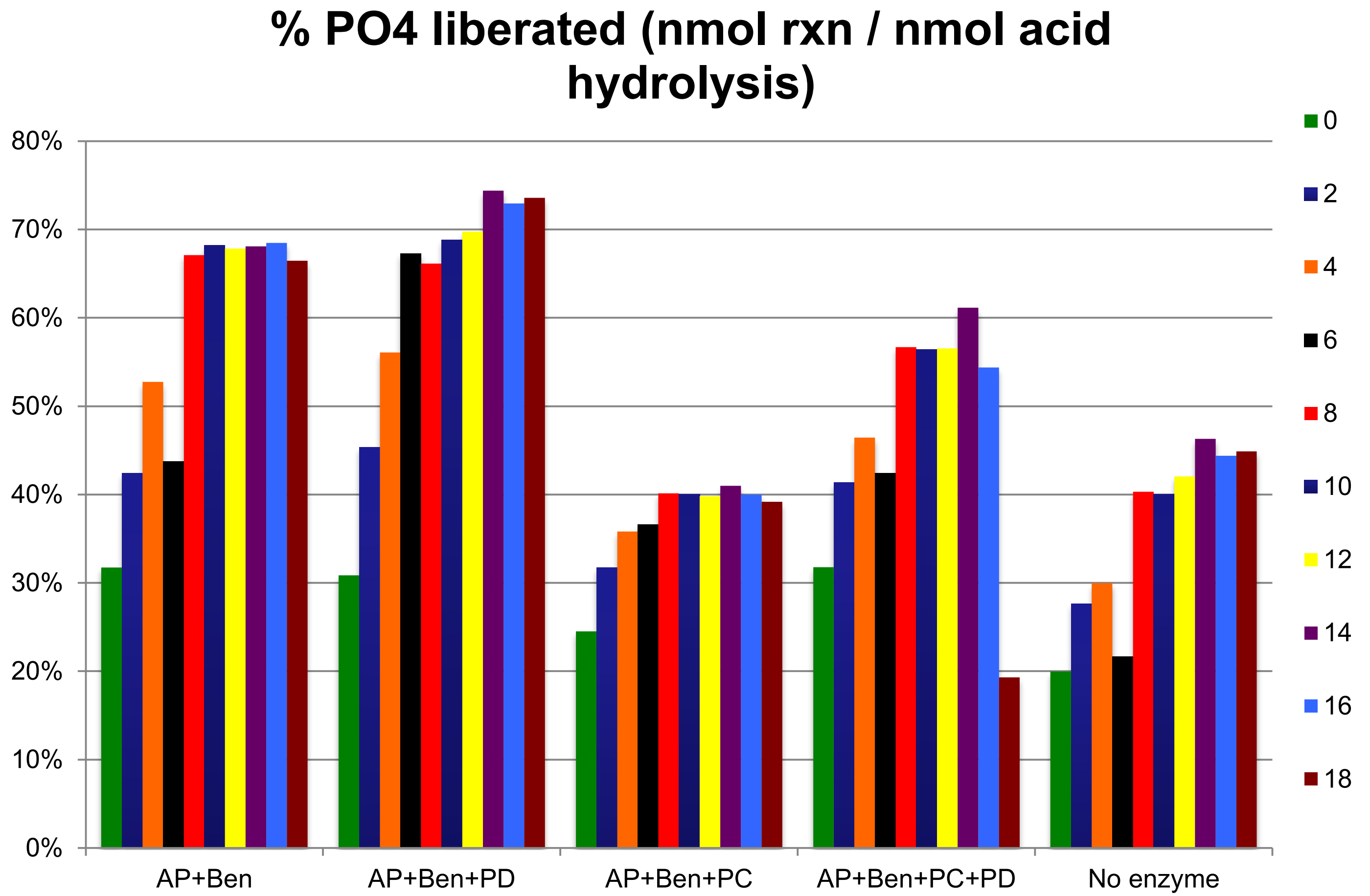


Key objectives and Milestones

Milestones		Completion Date
Go /No Go	Residual biomass obtained after TAG extraction must contain at least 40% of the original P & N content of the untreated biomass. The recaptured nutrients must contain at least 20% of the original P & N content of the untreated biomass The final product of the mineralization (expected to be struvite) must be suitable as a nutrient for algal growth.	Q6
Final	Demonstrate remineralization of >66% of major nutrients in residual biomass and growth of algae on recycled nutrient. Determine optimal growth conditions that limit outside P & N based on recycled on P & N Demonstrate linked initial TAG extraction/P extraction/diacylglyceride extraction.	Q12

2-Technical accomplishments.

Remineralization of "total" cellular Phosphate



3 - Relevance

- ***BETO Multi-Year Program Plan (2012) milestone:***
  - By 2018, demonstrate at non-integrated process development unit scale, an open cultivation system design without use of traditional plastic liners and with integrated nutrient and water recycling that demonstrates algal productivity of 25 gallons per square meter per day AFDW (corresponding to 2,500 gallons processed oil or equivalent biofuel intermediate per acre per year)
- ***sustainability target***
  - By 2022, evaluate, quantify, and document sustainable integrated pilot-scale production of biofuels from… algae

- The objectives of the project will contribute to meeting these goals by developing and demonstrating methods for integrated nutrient recycle

- Project results will benefit algal industry by reducing costs and potential food versus fuel conflict.

4 - Critical Success Factors

"Those few things that must go well to ensure success"

Technical

- Quantitative Recovery and recycling of nutrients
  - Must return the maximal amount of biologically accessible nutrients to the pond

Market/Business

- Reduction of energy and material costs
- Develop the most cost effective means to the desired result
  - Must develop and evolve methods to require lowest level of inputs and highest level of return
  - Linking fuel generation with nutrient recycle
  - Creating integrated methods
- Creation of savings over potential nutrient costs.

Potential challenges

- Various algal strains may require substantially different enzyme cocktails for fast and efficient nutrient remineralization.
  - We will target our approach for two distinct algal species, *Nannochloropsis salina* and *Phaeodactylum tricornutom*, that have been identified as preferred by the National Alliance for Advanced Biofuels and Bioproducts (NAABB), and are actively co-cultured in open raceway environments.
- The microbial strains that will be employed for the remineralization process may sequester a substantial quantity of the recycled nutrients.
  - maximize enzymes expression per unit of microbial biomass
  - Genetically disrupt efficient phosphate transport, reduce “luxury uptake”
- The solubility and stoichiometric ratio of N to P present in struvite may prove to be inefficient as a nutrient source for culturing algae to high concentrations.
  - We anticipate this to some extent based on the N:P proportions dictated by the Redfield ratio for phytoplankton, but reduced N:P ratios favor high lipid yields, and our plan incorporates a secondary mechanism for nitrogen supplementation using ammonia.

Future Work

- **FY13**
  - Complete baseline growth dataset
  - Characterize algal growth on struvite
  - Characterize the enzymatic removal of phosphate from residual biomass
  - Characterize the nutrient retention during TAG extraction
- **FY14**
  - Initial development of microbial mediated phosphate remineralization process
  - Combine with amino acid fermentation system
  - Demonstration of Go/No Go milestones
- **FY15**
  - Refinement of integrated biomass fermentation process
  - Fully characterize growth of algae on recycled nutrients
  - Demonstrate linked extaction of TAG, Phosphate and DAG

Summary

- Relevance of objectives
  - Without recycling of nutrients fertilizer prices will increase, there will be a food vs fuel conflict, and algal biofuels may not be sustainable
- Approach
  - We will created and integrated system for the conversion of residual algal biomass to fuel and at the same time recapture major nutrients
- Technical accomplishments
  - We have demonstrated the remineralization of phosphate from algal biomass
- Future work
  - Creation of a one pot system for recapturing nutrients while producing fuel from residual biomass
  - Demonstration of the growth of algae on recycled nutrients
- Success factors and challenge
  - Creation of an effective and low cost process

Acknowledgments

DOE EERE Bioenergy Technologies Office

Sandia National Labs

- John Hewson
- Ryan Davis
- Deanna Curtis
- Pamela Lane
- Anne Ruffing
- Todd Lane

TAMU

- Anthony Siccardi

Open Algae

- Peter Kipp

Extra Slides

Two major processes: remineralization and separation

Remineralization

- Convert organic N and P to inorganic forms: HPO4 and NH4 in solution.
- Ferment organic components to butanol: maximize energy yield from biomass.
- Use microbial consortia to carry out remineralization processes.

Separation

- Separate nutrients from solution to avoid costs of transporting water.
- Struvite precipitation for P recovery.
- Ammonia distillation, assisted by pH adjustment to recovery NH4.
- Subsequent butanol distillation.

Need to identify process barriers, alternate (undesired) paths.

Struvite to recycle nutrients

- Struvite, $MgNH_4PO_4$, precipitates with precursors at mM levels and pH > 8.
  - Figure shows precipitate formation at mM precursor concentrations measured by Nick Wyatt.
  - With excess N, P removal expected to exceed 90% (PHREEQC predictions).
- Struvite precipitation used in nutrient recovery from wastewater: low concentrations are problem.
- Struvite used as fertilizer.