



# Algae R&D Activities Peer Review

Alexandria, Virginia

March 23, 2015

**José A. Olivares**

*Los Alamos National Laboratory*

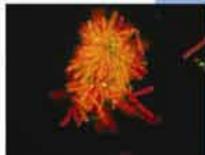
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# Purpose of NAABB

## Algal Biology

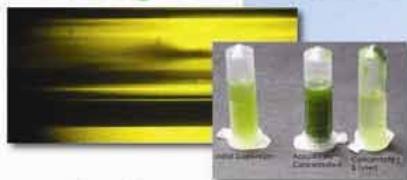


Greater space-time lipid/algal yields

## Cultivation



## Harvesting and Extraction



Novel techniques to reduce cost and environmental impact

## Valuable Coproducts



Livestock feed



Direct energy production



Chemicals for industry use

## Fuel Conversion



High energy-density fungible fuels

# SUSTAINABILITY



CO<sub>2</sub>



Water



Land



Nutrients

NAABB has developed and demonstrated science and technology that will significantly increase production of algal biomass and lipids, efficiently harvest and extract algae and algal products, and establish valuable conversion routes to fuels and co-products.



# NAABB Projected Objectives Outcomes

Objectives	Outcomes
Developing technologies for cost-effective production of algal biomass and lipids	
<b>1.0: Algal Biology</b> - Increase overall productivity of algal biomass accumulation and lipid/hydrocarbon content	Super-performing, safely-deployable, algal biofuel production strains with greater overall productivity and enhanced lipid production
<b>2.0: Cultivation</b> - Increase overall productivity by optimizing sustainable cultivation and production systems	Scalable cultivation practices for various environments. Optimized growth rates and lipid/hydrocarbon concentrations
<b>3.0: Harvesting/Extraction</b> - Develop cost-effective and energy efficient harvesting and lipid extraction technologies	Innovative, low-energy, algal harvesting and lipid/hydrocarbon extraction technologies integrated with cultivation and conversion processes
Developing economically viable fuels and co-products	
<b>4.0: Fuel Conversion</b> - Develop technologies to convert lipids/hydrocarbons and biomass residues into useful fuels	Optimized conversion technologies for algal extracts and whole algae into drop-in transportation fuels
<b>5.0: Valuable Coproducts</b> – Develop a set of valuable coproducts to add profitability and provide flexibility to allow responsiveness to changing demands/opportunities in the market.	New animal and mariculture feed products from algae biomass validated by FDA and cost-effective technologies for the production of large-scale, marketable co-products
Providing a framework for a sustainable algal biofuels industry	
<b>6.0: Sustainability Analysis</b> – Quantitatively assess the energy, environment, economic viability and sustainability of the NAABB approaches to guide our strategy	Sustainable processes and resource management. Life-cycle and economic analyses embedded in advanced system-level models.

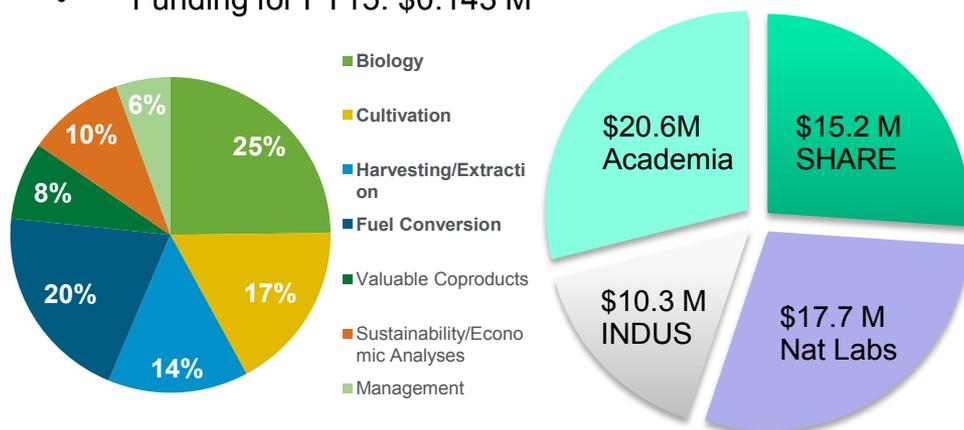


## Timeline

- Project start date: April 5, 2010
- Project end date: September 30, 2013
  - R&D Ended April 4, 2013
- Percent complete: 100%

## Budget (ARRA Funding)

- Total project funding
  - DOE share: \$48.6 M
  - Contractor share: \$15.2 M
- Costs for FY10-12: \$21.3 M
- Costs for FY13: \$9.3 M
- Costs for FY14: \$1.5 M
- Funding for FY15: \$0.143 M



## Barriers Addressed

- Ft-A. Feedstock Availability and Cost
- Ft-B. Sustainable Production
- Ft-C. Feedstock Genetics and Development
- Ft-D. Sustainable Harvesting
- Ft-G. Feedstock Quality and Monitoring
- Ft-N. Algal Feedstock Processing
- Bt-A. Biomass Fractionation
- Bt-B. Biomass Variability
- Bt-K. Biological Process Integration
- Tt-A. Feeding Dry Biomass
- Tt-B. Feeding or Drying Wet Biomass
- Gt-C. Gasification of Biomass
- Tt-E. Pyrolysis of Biomass and Bio-Oil Stabilization
- Tt-G. Fuel Synthesis and Upgrading
- St-A. Scientific Consensus on Bioenergy Sustainability
- St-C. Sustainability Data across the Supply Chain
- St-D. Indicators and Methodology for Evaluating Sustainability
- St-F. Systems Approach to Bioenergy Sustainability

**Partners (Next Slide):** 39 member institutions, Integrated PM team

## Lead Institution: The Donald Danforth Plant Center\*†

\* NAABB Team Management

† NAABB Board of Directors

### National Laboratories

- Los Alamos National Laboratory / New Mexico Consortium\*†
- Pacific Northwest National Laboratory\*†
- Idaho National Laboratory
- National Renewable Energy Laboratory
- USDA - ARS

### Universities

- Brooklyn College
- Clarkson University
- Colorado State University\*†
- Iowa State University
- Michigan State University†
- New Mexico State University\*†
- North Carolina State University
- Texas AgriLife Research / Texas A&M University System\*†
- University of Arizona\*†
- University of California Los Angeles
- University of California Riverside
- University of California San Diego
- University of Pennsylvania
- University of Texas (sub)
- University of Washington
- Washington State University
- Washington University St. Louis

### Industry

- Albermarle Catilin†
- Diversified Energy
- Eldorado Biofuels
- Genifuel
- Cellana†
- Inventure
- Kai BioEnergy
- Palmer Labs
- Phycal
- Reliance Industries Limited
- Pan Pacific, Ltd.
- Solix Biofuels\*†
- Targeted Growth†
- Terrabon
- UOP a Honeywell Company†
- Valicor

# NAABB Partners



# Overview – NAABB Integration of Technologies

## Algal Biology

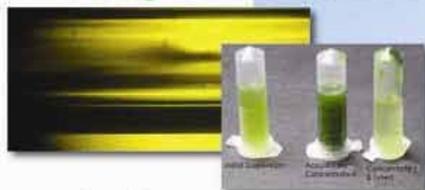


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lipid/algae yields

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# SUSTAINABILITY



CO<sub>2</sub>



Water



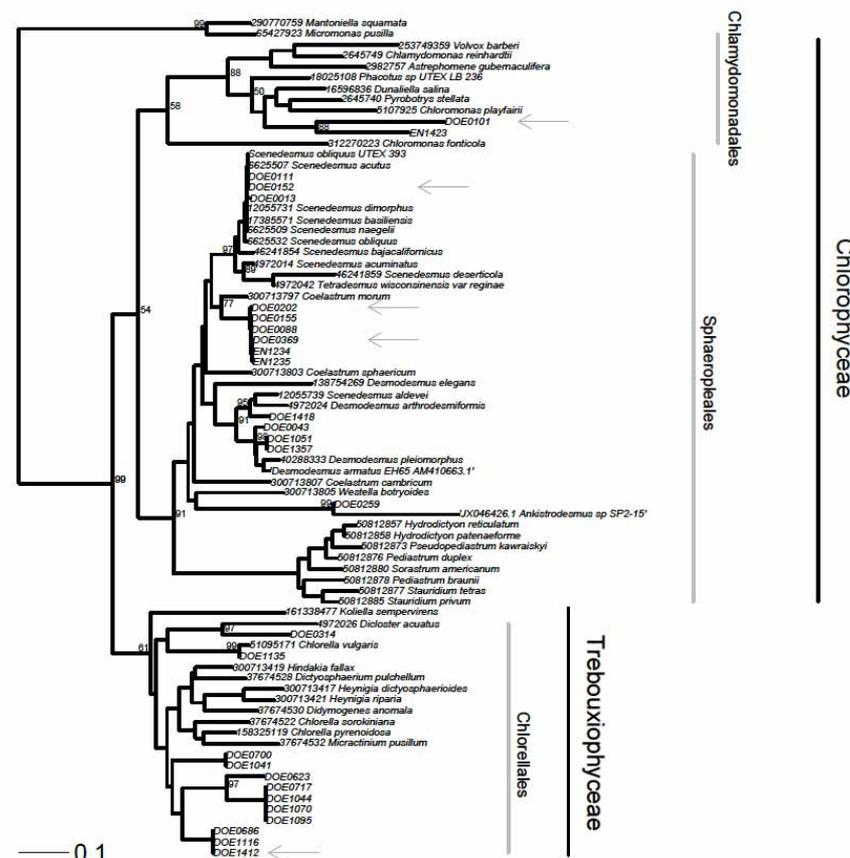
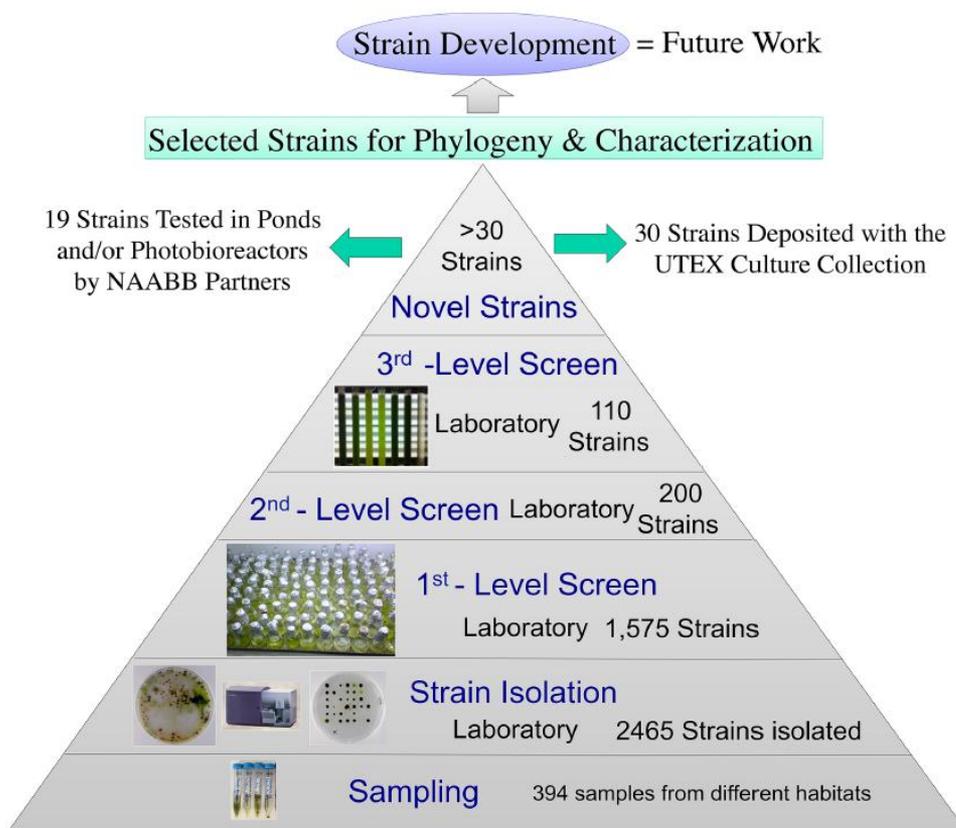
Land



Nutrients



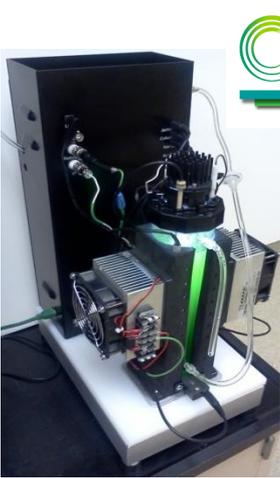
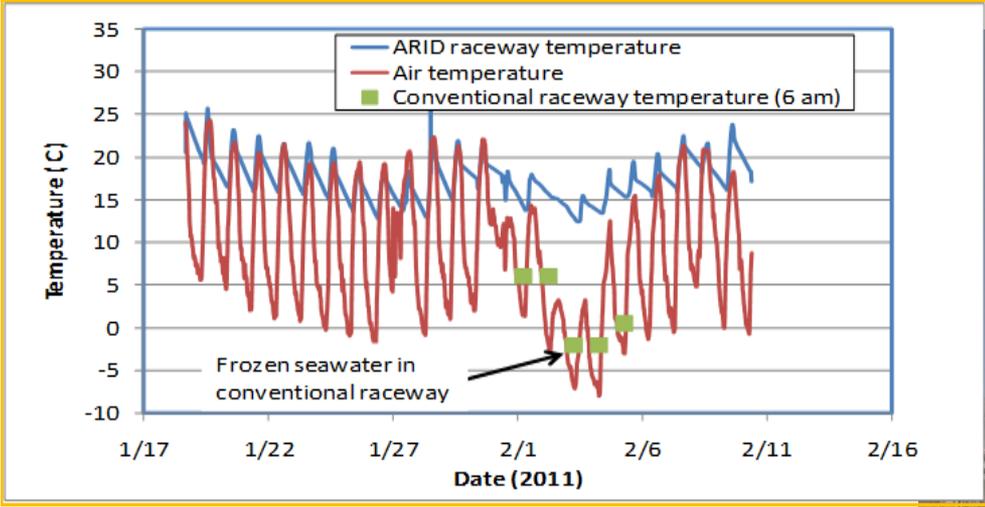
# New Strain Isolation and Development



# Cultivation

- The ARID raceway system has been shown to be cost effective – CAPEX > 8% OPEX < 45%

## Advanced AgroBioFuels, LLC



- A commercial photobioreactor system that mimics a pond environment has been developed and a start-up company, *Phenometrics*, initiated

# • Harvesting

- Current bottlenecks to cost effective production of biofuels from algae – traditional is centrifuge
- Need to concentrate from 1 g/L to 40 or 100 g/L prior to extraction



Electrocoagulation



Filtration

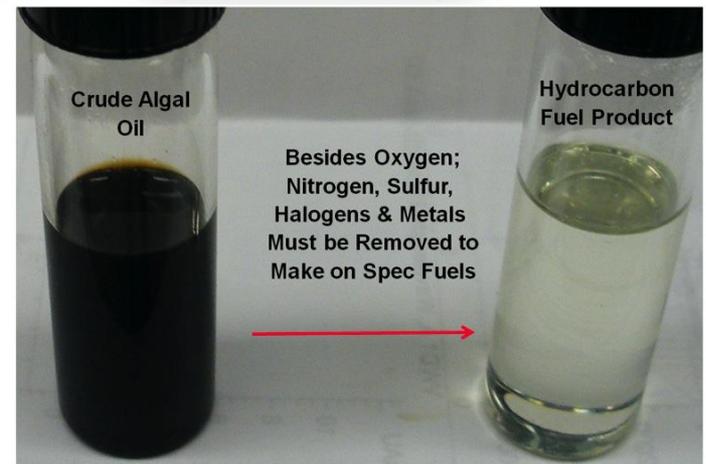
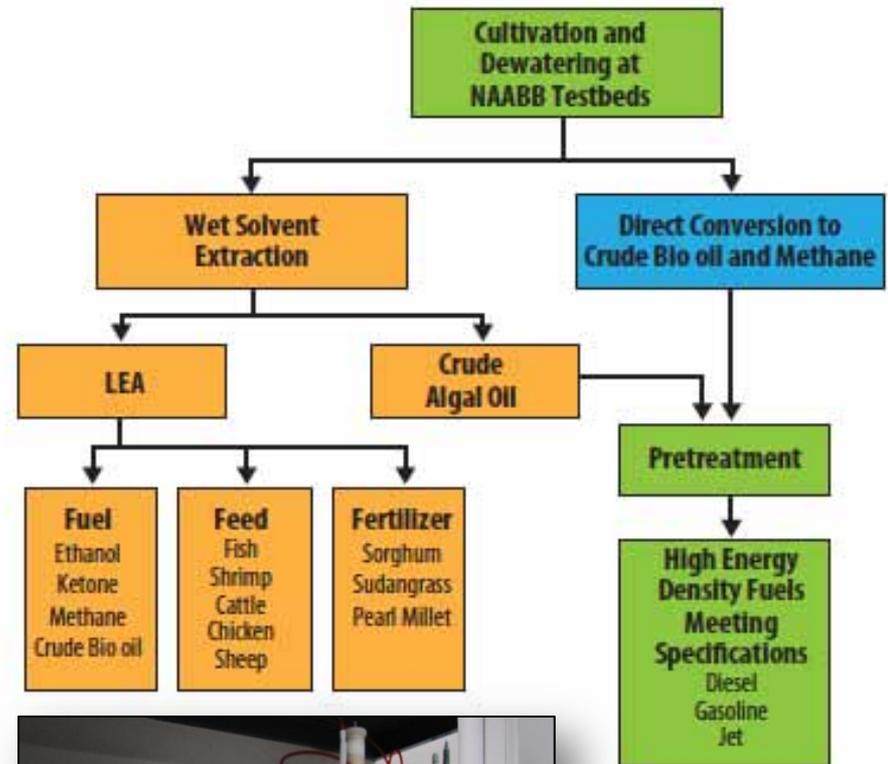


Acoustic Focusing

# Technologies for M1: Demonstrate 100L/hr

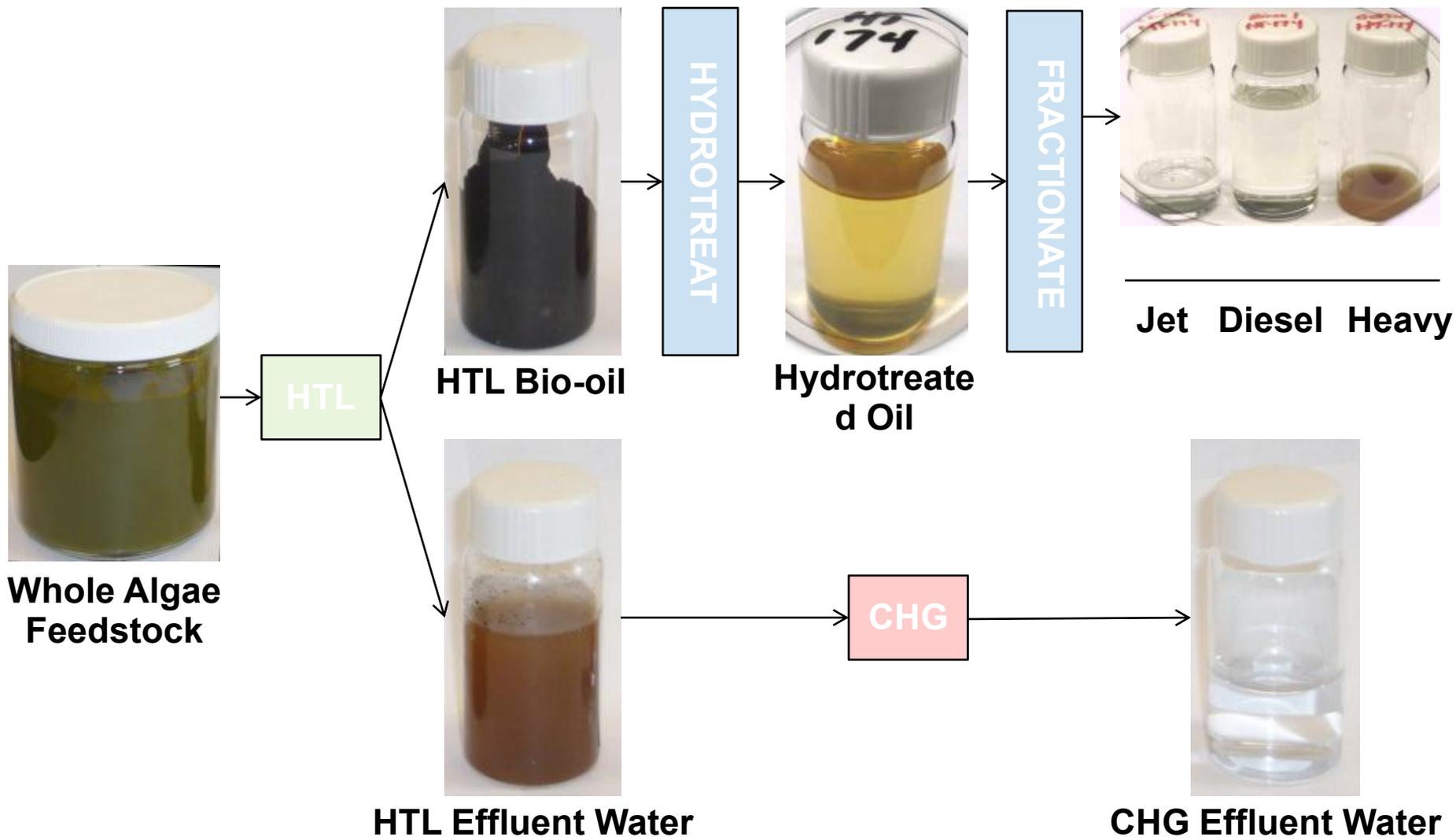
Table 3. Baseline Feasibility Assessment of Harvesting & Extraction Technologies						
Technology	Energy Input (kWh/kg)	Chemical Cost (USD/Kg)	Electricity Cost (USD/kg)	OPEX (USD/kg)	OPEX (USD/Gal)	PEL
<b>Baseline Harvesting Technologies</b>						
Centrifuge Baseline	3.300	0.000	0.264	0.264	1.799	56.98
Dissolved Air Floatation	0.250	0.008	0.020	0.028	0.191	4.317
Spiral Plate Separation	1.418	0.000	0.113	0.113	0.773	24.47
<b>NAABB Harvesting Technologies</b>						
Chitosan Flocculation	0.005	0.055	0.000	0.055	0.377	0.093
AlCl <sub>3</sub> Flocculation	0.120	0.046	0.010	0.056	0.380	2.072
Electrolytic Harvesting	0.039	0.004	0.003	0.007	0.049	0.673
Membrane Filtration	0.046	0.000	0.004	0.004	0.025	0.789
Ultrasonic Harvesting	0.078	0.000	0.006	0.006	0.043	1.347
<b>Baseline Extraction Technologies</b>						
Pulsed Electric Field	11.52	0.000	0.922	0.922	6.280	198.90
Wet Hexane Extraction	0.110	0.001	0.009	0.010	0.068	1.904
<b>NAABB Extraction Technologies</b>						
Solvent Phase Algal Migration	1.648	0.947	0.132	1.079	7.352	28.456
Ultrasonic Extraction	0.384	0.000	0.031	0.031	0.209	6.630
Nanoparticle Mesoporous	0.008	54.35	0.001	54.356	370.463	0.137
Supercritical	1.174	0.000	0.094	0.094	0.640	20.27
The highlighted harvesting technologies were selected for scale-up.						

# • Conversion Lipid Extracts to Fuels



**"Contaminants" for Conversion are "Nutrients" for Cultivation**

# • Combined HTL & CHG Conversion of Whole Algae



Hydrothermal processing technology developed in NAABB is being used in many DOE and privately funded efforts.



FLC award to PNNL and Genifuel

NAABB cost share provides funding for engineering plans and construction of a 1 tonne/day demonstration facility.

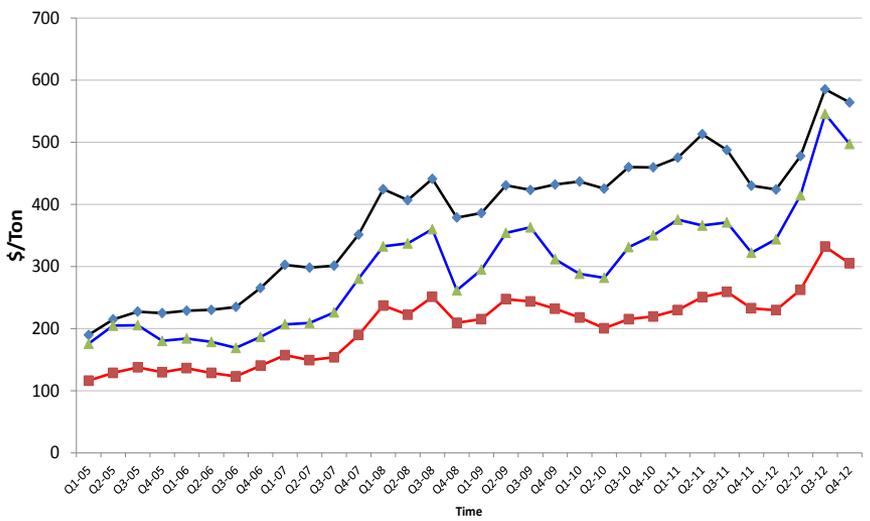
- **Reliance has built pilot, and has also ordered a second system to expand the pilot**
  - In addition, is building a bench-scale lab system
- **Algenol has built a small test system to process residual algae, using it for HTL**
- **Genifuel is working with Southern California Gas to build a bench-scale system to test HTP and produce gas for insertion into their pipeline system**
- **Genifuel is working with the province of Alberta, Canada to process waste wood via HTP**

# Value of LEA for Shrimp and Fish Feed

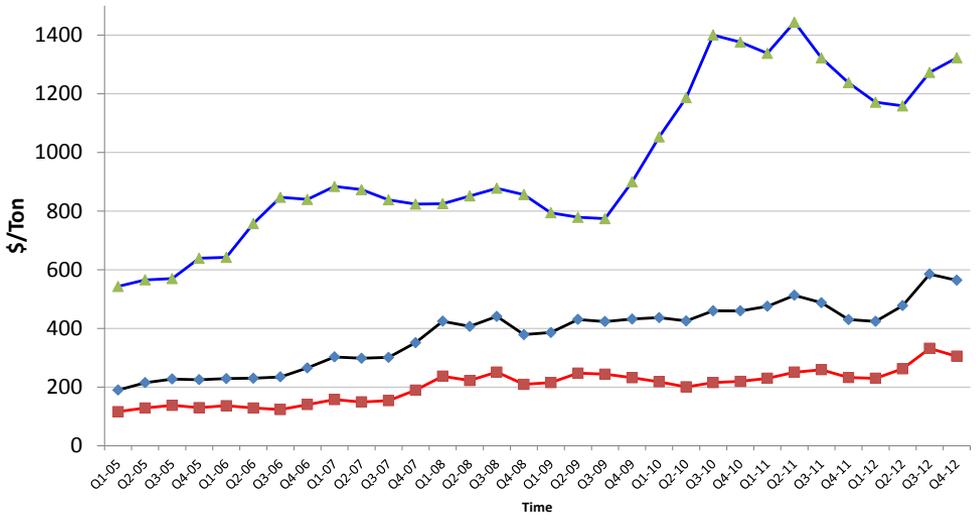


Based on fractions of energy, protein, fat, etc. in LEA and whole algae; the value of these ingredient in mariculture rations are:

- Whole algae averages \$82/ton more than soybean meal – about \$373/ton in 2013
- LEA averages \$94/ton less than soybean meal – about \$200/ton in 2013
- A non-market advantage of feeding LEA to mariculture is it replaces a portion of fishmeal in the ration thus protecting the ocean’s fish population



— Spirulina maxima — NO floc — Soybean meal (high protein)

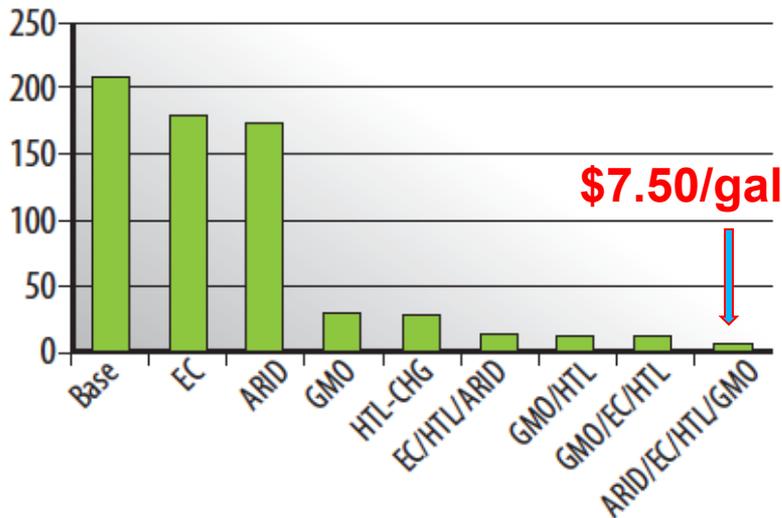


— Spirulina maxima — NO floc — Menhaden Fishmeal

# NAABB Scenarios for Overall Economic Feasibility

**Table 10. Summary of the technologies analyzed for the seven alternative scenarios.**

	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6	Scenario 7
Products	Crude TAG & LEA	Crude TAG & LEA	Crude TAG & LEA	Crude HTL oil & methane			
Cultivation	Open pond w/ liners	Open pond w/ liners	ARID w/liners	Open pond w/ liners	Open pond w/ liners	ARID w/liners	ARID w/liners
Feedstock strain g/m <sup>2</sup> /d	Generic 7.4	Generic 7.4	Generic 9.3	Generic 7.4	Generic 19.4	Generic 9.3	GMO 23.2
Harvesting	Centrifuge	EC	EC	Centrifuge	EC	EC	EC
Extraction	Wet solvent extraction	Wet solvent extraction	Wet solvent extraction	HTL-CHG	HTL-CHG	HTL-CHG	HTL-CHG
Nutrient recycling	No	No	No	Yes	Yes	Yes	Yes
Biomass Production (tons/yr)	119,900	119,900	152,200	119,900	316,800	152,200	378,600
Crude Oil Production (gallons/yr)	4,679,000	5,096,000	6,470,000	13,506,000	42,321,000	20,332,000	51,570,000
Location	Pecos, TX	Pecos, TX	Tucson, AZ	Pecos, TX	Pecos, TX	Tucson, AZ	Tucson, AZ



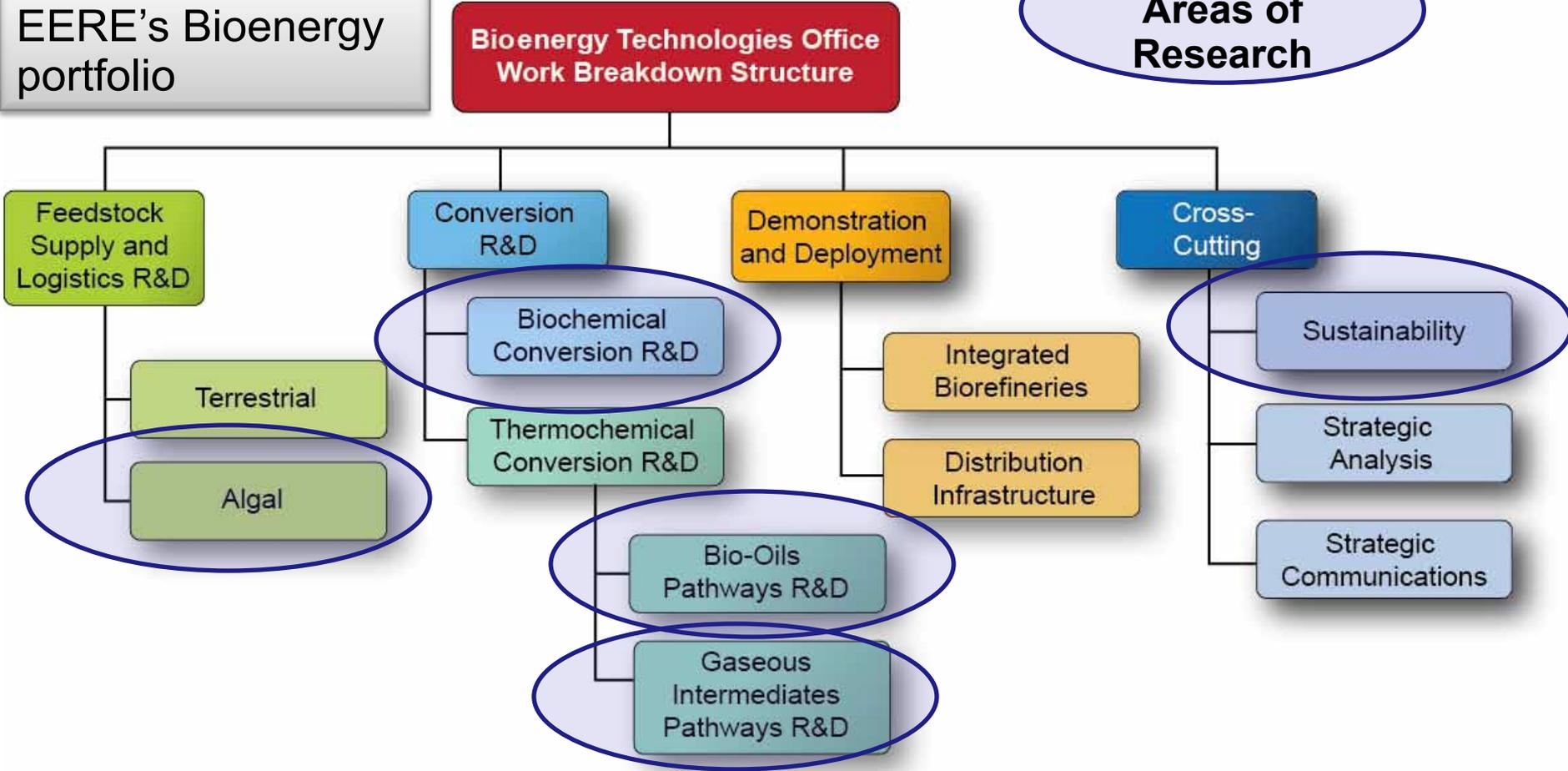
**Table 1. Average Total cost per Gallon for Biocrude Oil (\$/Gallon)**

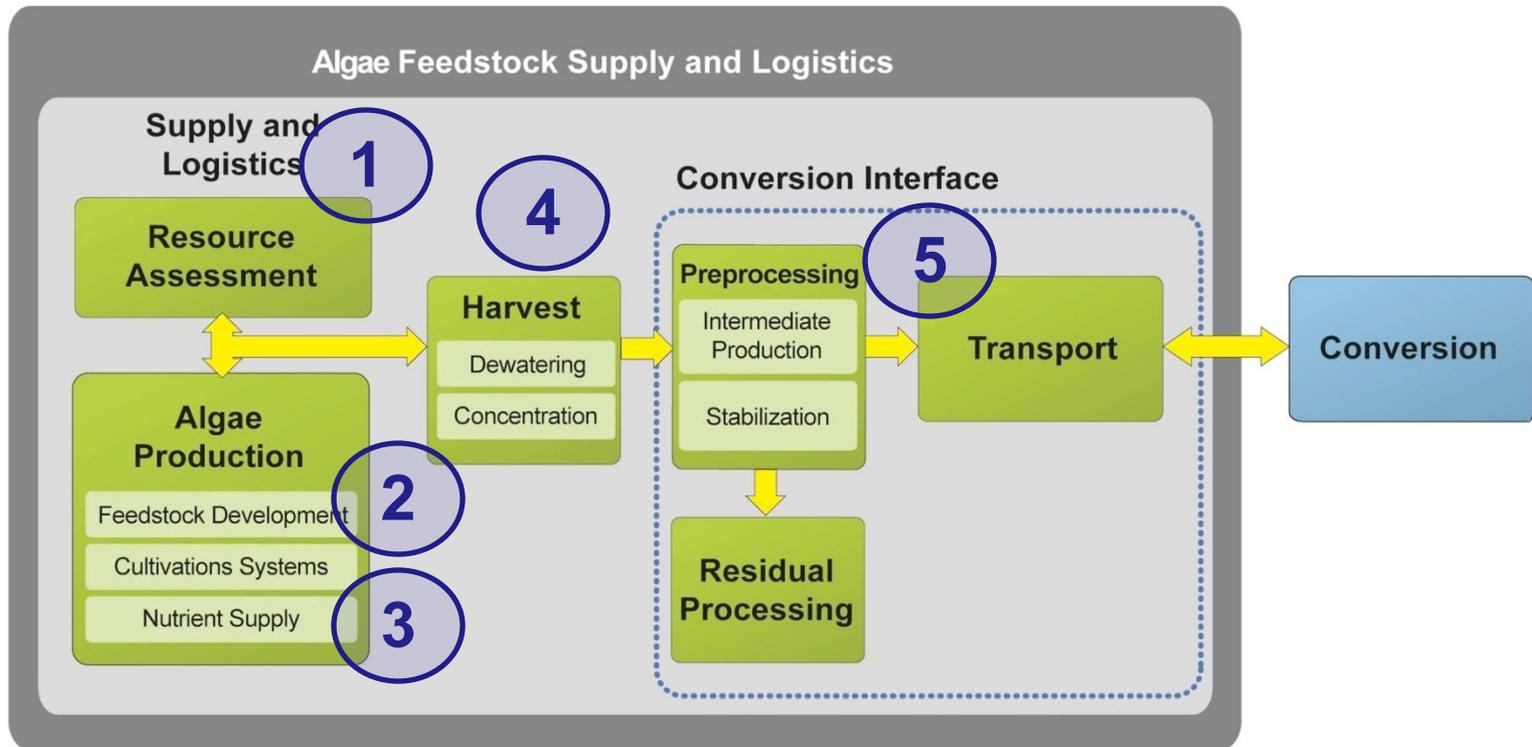
Fraction OPEX	Fraction Reductions in CAPEX				
	0	0.2	0.4	0.6	0.8
0	7.40	6.40	5.40	4.50	3.50
0.2	6.40	5.50	4.50	3.60	2.60
0.4	5.50	4.60	3.70	2.80	1.90
0.6	4.70	3.80	2.90	2.10	1.40
0.8	3.90	3.10	2.30	1.60	0.80

# Relevance

NAABB has a central role in EERE's Bioenergy portfolio

NAABB Areas of Research





NAABB has...

1. Provided a detailed resource assessment through the AISIMS BAT Module
2. Brought five new strains through the value chain (including outdoor cultivation)
3. Demonstrated the ARID cultivation (heat management) and low nutrient cost
4. Evaluated three new harvesting systems at larger scale
5. Combined extraction with conversion technology to reduce cost and demonstrated with NAABB produced algae (with high and low lipid content)

# Critical Factors for Future Success



**Priority 1**  
**Improve Biomass Productivity**  
Target: 2-4 X Increase (g/m<sup>2</sup>)

- Transfer genes to production organisms
- Validate GMO strain outdoors
- Reduce pond crashes

**Priority 2**  
**Improve Extraction-Conversion Yield**  
Target: 2 X Increase in Yield

- Optimize HTL processing/upgrading
- Integrate with CHG Processing
- Enable Nutrient Recycle

**Priority 3**  
**Improve Cultivation-Harvesting Efficiency**  
Target: 1.5X

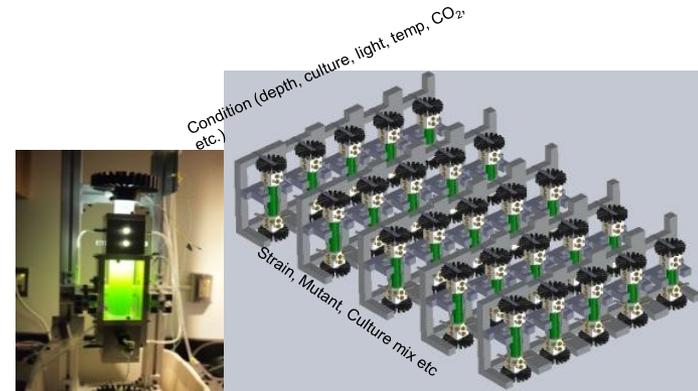
- Reduce energy & extend season
- H<sub>2</sub>O and CO<sub>2</sub> management
- Demonstrate harvesting scale-up

**Integration Tools**

- ✓ Growth models
- ✓ Resource Assessment Models
- ✓ Sensitivity analysis to optimize conversion systems
- ✓ Tools to optimize algae to climate conditions
- ✓ LCA for recycle of water nutrients and energy balances

- >100 scientific publications
- Five theses
- New Journal: **ALGAL RESEARCH** (by Elsevier)
- New Conference Series: *International Conference on Algal Biomass, Biofuels and Bioproducts*
- Deposited 30 most productive algae strains into UTEX culture collection
- 33 Intellectual Property Disclosures
  - Molecular biology tools – 10
  - Cultivation – 5
  - Harvesting and Extraction – 7
  - Fuel conversion – 8
  - Co-products and other – 3
- 2 New Companies

## Advanced AgroBioFuels,

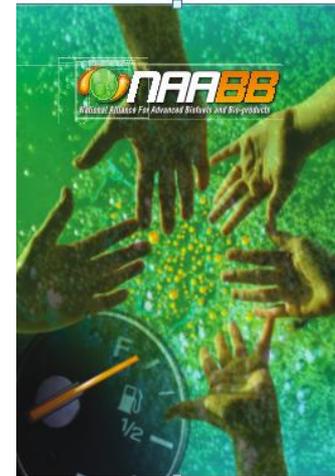


- **NAABB Final Report to DOE-BETO**

<http://www.energy.gov/eere/bioenergy/downloads/national-alliance-advanced-biofuels-and-bioproducts-synopsis-naabb-final>

- **NAABB Final Report Webinar**

<http://www.energy.gov/eere/bioenergy/downloads/webinar-algal-biofuels-consortium-releases-groundbreaking-research-results>



- **Virtual Special Issue of NAABB publications:**

<http://www.journals.elsevier.com/algal-research/virtual-special-issue/virtual-special-issue-the-national-alliance-for-advanced-bio/>



# ADDITIONAL BACKGROUND SLIDES

# RESPONSES TO PREVIOUS REVIEWERS' COMMENTS



We want to thank the DOE for the opportunity to respond to the Peer Review Committee comments. We found that the comments reflected some very positive views that the NAABB program is making an impact, even this early on in the performance period. There were also a number of areas where the committee raised concerns or issues with our program. Since the Peer Review comments to our presentation were extensive and not organized in any logical manner, we have distilled and arranged these according to performance area, for ease in response and cohesiveness.

**1. Project Performance and Management: The approach of forming a large public-private consortium to develop algal biology, cultivation, harvest, extraction, fuel conversion, TEA, and sustainability analysis in a very short time was a requirement of the FOA. This was daunting...**

**Response:** We thank the reviewers for the thoughtful comments and critique. The management team spent considerable effort integrating the project and insuring each individual PI understood his/her role in the team they were supporting and to the success of the consortium success overall. As one reviewer pointed out, consistency can always be improved. We will devote a chapter to our public Final Report on the consortium approach, what worked well and where improvements could be made.

**2. Project Met Objectives: There was (1) an apparent lack of improvement in cultivation productivity in spite of the extensive algal screening and (2) the number of strains deposited in culture collections and (3) linking best options together through each processing step. We will address these in reverse order.**

**Response:** NAABB leadership met with NABC leadership multiple times to compare best practices and in fact we had a common individual who sat on the executive leadership team of both consortia. The main difference between the two consortia are (1) the technology readiness level (TRL) at the onset of the work (i.e. the relatively mature TRL level for NABC technologies vs. algal enterprise) and (2) an understanding that NAABB was structured to have an impact on the entire value chain of the enterprise from developing new organisms, new agronomic practices, new harvesting as well as understand how all the efforts fit together to produce a final product slate—with an economic understanding. To tie all the best options together required that each be scaled up to a level that we could produce the quantity of material to move through the entire process from cultivation to fuel production (in continuous flow reactors). Indeed it was our goal to do so, and this is exceptionally challenging to do in ... Continued in next slide

## 2. Continued...

**Response:**...three years starting at the TRL level that we were starting. We are very pleased, even proud of the fact, that we took three NAABB-based algal strains through the entire process of outdoor cultivation through fuel production at a scale required to get high quality data. These data and information will be presented in the Final Report. We will be able to provide detailed information but also commentary on the lessons learned through integrating the process. This is the first time such a breadth of information will be available (from start to finish) in the open literature to our knowledge. This accomplishment should not be overshadowed by the fact that at the same time we have additional technologies moving through the value chain that have the potential to be yet further improvements.

Algae productivity is key to making an algal biofuels industry profitable (and possible), hence we understand the value of preserving strains for future researchers. We made a commitment to DOE at the onset to deposit the 30 best strains to be preserved and we met that promise. This is a question on quality vs quantity. More can be deposited, but this will require additional resources for preparation of strains and preservation. Further, NAABB established a contract with UTEX for three years for preservation of the strains. Further resources will need to be allocated to help these strain collection banks in maintenance and we recommend that the DOE establish a mechanism for this type of preservation. Otherwise, we fear a similar fate will be experienced with the loss of many of the strains from the Aquatic Species Program in the early 90's.

The critique that is scientifically most interesting is the “apparent lack in cultivation productivity in spite of extensive algal screening.” Some of this is an understanding of “optimal—carefully controlled” vs “realistic—outdoor real-world conditions.” We will comment on our approach and what we learned.

NAABB had several levels of screening in selecting strains. The first was in small lab cultures comparing growth rate and lipid content to our baseline strains (*N. salina* (saltwater) and *A. protothocoides* (freshwater)). If the new isolates performed better than these baseline strains they were selected for further cultivation studies. Next, strains were screened to determine maximal productivity and temperature profile. Highly productive strains were assessed further by biomass growth models and experimentally in small ponds. The best performers were moved into large scale production ponds. There are many reasons why productivity in large ponds did not meet lab expectations in certain experiments. 1) media differences from using well water with high salinity, 2) the season the species was tested may have been too cold or even too hot, 3) cultures became contaminated or overrun with predators, 4) rain and or wind events caused culture disruptions. The reviewer suggestion about evolutionary pressure may be another factor as well.

## 2. Continued...

We now have a strain that is significantly better in outdoor cultivation than the baseline and other strains optimized. This is *Chlorella sorokiniana* DOE-1412, which came from the prospecting work from Jurgen Polle. We believe we will have others as well and hope to test the other strains in follow-on projects. Bottom line: we have demonstrated improved strains; we hope to have more demonstrated in follow-on work; and we certainly know more about how to design realistic criteria for strain selection than we did at the onset.

We agree that there is much value to research that shows negative outcomes; and NAABB will be analyzing all of the cultivation data to try and leverage the understanding from both positive and negative outcomes for the community in our Final Report.

Some additional miscellaneous comments:

The program was set up to develop technologies that would lead towards economic and energetically feasible liquid transportation fuels. Therefore the work performed in animal feed as a byproduct of this effort attempted to understand the value of the byproducts without further development or enhancement. Nevertheless, we agree that for a focused effort in the animal feed industry, increasing protein content would be an excellent goal. At the same time, economic models did not bear out that the ag-coproducts was the best approach to bring down cost, and this needs to be balanced in future planning.

In the overall Peer Review presentation we reserved about 1/4<sup>th</sup> to our modeling efforts. Due to the complexity and number of these efforts included in the NAABB program, it was impossible to include many of the details. These are currently being written up into the NAABB Final Report and appendices of individual task area reports for the DOE. A number of manuscripts have already appeared in the peer-reviewed literature with some of these details, and several others are in preparation for publication.

We agree that even more analytical tools than what was presented in our presentation are needed, including further standardization of methods acceptable to this industry.

## 2. Continued...

NAABB had multiple conversion technologies as part of the conversion task. Fuels were made using multiple algal feedstock and multiple conversion technologies. These data were presented in the review. For example:

Albemarle-Catlin – transesterification to biodiesel via their T-300 solid catalyst

UOP and Diversified Energy – processes to take lipids to green diesel and jet fuel (kerosene) via decarboxylation, hydrotreatment, and isomerization

We apologize if we left the reviewers with the impression that we developed transformation tools for *B. braunii*. Our efforts were focused on understanding metabolic processes in *B. braunii* that could be transferred to other organisms. Within this effort, we point to the development of isoprenoid pathways into *C. reinhardtii* by NAABB's Dr. Joshua Yuan, Texas A&M.

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NAABB did not use DAF, but we selected centrifugation as the baseline to compare other harvesting technologies being developed. The NREL model does include DAF but this was not used to do a direct comparison with electroflocculation.

The Phenometrics ePBR was developed as a research tool for cultivation studies that could more closely mimic an open pond. These unique features include programmable vertical illumination, temperature control, pH control, gas mixture and pond depth to mimic any environmental situation. As such, they have been deployed for a variety of experimental approaches where they are serving as an invaluable resource. These include: 1) laboratory preparation of strains; 2) development of preliminary nutrient conditions prior to outdoor cultivation; 3) development of full outdoor models using small scale indoor systems. The validation of the ePBRs against outdoor cultivation is an ongoing effort. In discussions with Dr. David Kramer (Michigan State U.), developer of the ePBR system, there is total agreement that full statistical analysis of this system against outdoor cultivation ponds is needed, especially if these systems are to be used for 3 above. Unfortunately, there was not enough time and resources available to do this within the NAABB program.

We agree that optimizing cultivation agronomics is important in sustaining high productivities. We did have efforts focused on media development, monitoring, predator control, and operational strategies for cultivation but we were not able to focus on these in our review presentation.

## 2. Continued...

Our efforts in conversion technologies were mainly focused on understanding the value that each brought to the table and limitations and challenges that algae feedstock presented to these technologies. We feel that these were presented along with areas that were improved by the NAABB effort. In doing so, we were able to identify some serious challenges for the lipid extraction route and do some preliminary testing and evaluations of the HTL route, which appears to look very promising.

The FARM model provides another perspective to the other DOE models as it is focused around financial matters and probabilities for achieving profitability. Since the model has been harmonized with the other DOE models it is complementary. Numerous improvements in the DOE-NREL model appeared following our two day meeting to harmonize the models. The expanded NREL model benefitted from our harmonization efforts.

The NAABB program did not attempt to address regulatory issues for GM cultivation, as this was beyond the scope and scale of this program. As a point of clarification, 8 genomes have been sequenced with different levels of annotation and analysis associated with these. Our hope is to release these to public databases within 6 months of ending the program. A number of manuscripts are currently being prepared describing this body of work.

The Valicor wet hexane extraction process is efficient in extracting algal lipids. Some details were provided in the review, more will be provided in the Final Report. .

### **3. Project Contributions to Meeting BETOs and Industry's Goals: NAABB is easily the most relevant completed project in the DOE BETO portfolio...**

**Response:** We thank the reviewers for recognizing the contributions of the NAABB consortium to the BETO program. Through the peer reviewed publications and Final Report we hope that NAABB will provide an important contribution to algal biofuels industry.

### **4. Critical Factors for Success, Key Challenges, and Advanced the State of Technology: NAABB was able to demonstrate a complete run of a potential pathway for algal biofuel production...**

**Response:** We thank the reviewers for recognizing the contributions and leadership of the NAABB consortium in these areas. Dr. Olivares is appreciative and humbled by the comments from the reviewers, but asks that the recognition be given to all of the NAABB management team and partners for their individual contributions. This is a great example where the results are much greater than would be indicated by the sum of the whole.

We fully agree with the reviewers that further work needs to be done in bringing multiple traits into production strains and showing effective productivity in outdoor environments. NAABB has developed the tools needed to do this moving forward.

### **5. Future Work and Remaining Issues: NAABB elucidated several critical pieces of work that still need to be accomplished: extracting crude lipids and cleaning up the crude lipid extract are foremost among them...**

**Response:** In our last slide for the presentation, we did provide a high level overview of the areas for future work. The NAABB Final Report will provide more details on areas that we think will require further work.

GM testing in outdoor ponds will be a challenge. How to address this challenge is a section we are considering in our Final Report.

DAF was not one of the technologies evaluated for harvesting in NAABB.

Please see section 2 for further details on the Phenometrics ePBR system.

The Final Report will include the details of the animal feed studies that were done under the NAABB program, all of which we were not able to present in the Peer Review.

**6. Project Coordination with Other Institutions: NAABB has produced extensive publications covering key aspects of research. Project closeout plans were not presented, but the consortium has extensive, valuable data that should be made available to the broadest extent possible...**

**Response:** We thank the reviewers for recognizing the contributions and achievements of the NAABB consortium in these areas.

**7. Please provide an overall assessment of the project based on the above criteria. Please note that these comments will be featured in the Final Peer Review Report: The obvious criticism -- the lack of linking together all the best technological processes discovered by NAABB -- may only be obvious in retrospect, as there were many moving pieces. This historical effort was well coordinated and well-led...**

**Response:** We thank DOE and the reviewers for their time and effort in this Peer Review. The quality of the comments and the critical assessment was excellent and provides the NAABB executive leadership team with a detailed analysis of areas that can be more clearly articulated in our Final Report, which will be made public. More importantly it also provides DOE with an assessment of gaps and areas for future program focus.

During the past 3 years of NAABB we've made significant technical progress, have had exciting success and learned a lot along the way about technology advances that are still needed to make algae biofuel production commercially viable. We agree with the reviewers on the importance of capturing both the negative and positive results from NAABB and integrating these into a final assessment of the progress and recommendations for future research.

One of the take-home messages for the NAABB executive team is the challenge in effectively communicating our results. The challenge the consortium took on was to carry out impactful research along the entire value chain of the enterprise which included developing new organisms, new agronomic practices, new harvesting techniques, an evaluation of conversion processes and analysis as well as understand how all the pieces fit together to produce a final product slate—along with an understanding sustainability questions and economics.

To tie all the best options together required that each individual process be scaled up to a level that we could produce the quantity of material to move through the entire value chain from cultivation to fuel production--in continuous flow reactors. Indeed it was our goal to do so, and this is exceptionally challenging to do in three years starting at the TRL level that we were starting. We are very pleased, even proud of the fact, that we took a number of strains into outdoor processing, four into large scale reactors and with extensive data on two salt water strains and two fresh water strains through fuel conversion at a scale required to get high quality data. ...

## 7. Continued...

These data will be presented in the final report. We will be able to provide detailed information but also commentary on the lessons learned through integrating the process. This is the first time such a breadth of information will be available (from start to finish) in the open literature to our knowledge. This accomplishment should not be overshadowed by the fact at the same time we have additional technologies moving through the value chain that have the potential to be yet further improvements.

In this effort we carried out our mission to evaluate as many technologies as possible along the entire value chain. We did not go into this project with a pre-conceived idea of which technologies, or strains, would be the most promising. Fully integrating all of the best technologies or strains as they emerged *and* demonstrating this complete process was beyond what was possible in this 3-year project. To help one understand the undertaking, the best strains from the screening effort were being fully vetted around the midpoint of the second year. The same with the down select of harvesting technologies—that then needed to be scaled-up if they were to provide sufficient quantities of material to the conversion team. Demonstrating the best strains from start-to-finish was indeed an accomplishment when one considers the challenges at each step that were overcome.

The NAABB leadership team and members worked extensively to pull down barriers between teams focused on biology, cultivation, and so forth and to understand the impacts from one technology along the entire chain. In this sense we did translate key best results through-out the enterprise, but there is much more to do. We linked the best technologies together in both experimentation, as far as practical and in our analysis—using the highest quality and consistent data available. And we are going to continue to analyze the data as we complete the Final Report.

NAABB provided both a depth and breadth of technologies and expertise not before assembled. The internal “peer review” that was embedded in our approach enabled cross-fertilization of ideas and forced the highest quality out of each project. By having several projects focused on each step in the value chain of algae biofuels production, we enabled the agility to pursue new discoveries and quickly correct technical problems. Some examples are: 1) The strain bioprospecting effort yielded 30 strains that performed better than our bench mark strain (*N. salina*). From this effort, 4 strains were taken to large outdoor cultivation with excellent results.

## 7. Continued...

2) The development of tools to evaluate and model productivity under simulated conditions in full light and temperature controlled ponds that were validated against outdoor ponds productivity and the Phenometrics ePBR system, which became available mid-way through the program to provide algal biologists with an invaluable research tool for optimizing cultivation conditions and for evaluating GMO strains. 3) The harvesting and extraction projects went through a rigorous TEA and down-selection process after 18 months so that resources could be focused on the technologies that were the most promising in terms of lowering the costs of harvesting and extraction. 4) Evaluation of conversion technologies against the cost constraints of harvesting and extraction. 5) Detailed analysis and modeling.

This will be recorded in our Final Report which will detail where we started, the hypothesis driven science that led us where we ended up, and what still needs to be done.

Again we thank the Peer Review Panel for their comments and suggestions and pointing out where we could do more to communicate effectively what NAABB set out to do and what it accomplished.

# PUBLICATIONS, DISCLOSURES AND PRESENTATIONS



## Algal Biology

<p>Boyle, N.R., M.D. Page, B. Liu, I.K. Blaby, D. Casero, J. Kropat, S. Cokus, A. Hong-Hermesdorf, J. Shaw, S. J. Karpowicz, S. D. Gallaher, S. Johnson, C. Benning, M. Pellegrini, A.R. Grossman, and S.S. Merchant. Three acyltransferases and nitrogen-responsive regulator are implicated in nitrogen starvation-induced triacylglycerol accumulation in <i>Chlamydomonas</i>. <i>The Journal of Biological Chemistry</i> 287 (2012): 15811–15825.</p>
<p>Goodson, C., R. Roth, Z.T. Wang, and U.W. Goodenough. Structural correlates of cytoplasmic and chloroplast lipid body synthesis in <i>Chlamydomonas reinhardtii</i> and stimulation of lipid body production with acetate boost. <i>Eukaryotic Cell</i> 10 (2011): 1592–1606.</p>
<p>Merchant, S.S., J. Kropat, B. Liu, J. Shaw, and J. Warakanont. <i>Chlamydomonas</i> as a reference organism for understanding algal triacylglycerol accumulation. <i>Current Opinion in Biotechnology</i> 23 (2012): 352–363.</p>
<p>Kropat, J., A. Hong-Hermesdorf, D. Casero, P. Ent, M. Pellegrini, S.S. Merchant, and D. Malasarn. A revised mineral nutrient supplement increases biomass and growth rate in <i>Chlamydomonas reinhardtii</i>. <i>The Plant Journal</i> 66 (2011): 770–780.</p>
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<p>Henley, W.J., R.W. Litaker, L. Novoveská, C.S. Duke, H. D. Quemada, and R. T. Sayre. Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. <i>Algal Research</i> 2 (2013): 66–77.</p>
<p>Ramos, A. A. J. Polle, D. Tran, J.C. Cushman, E.S. Jin, and J.C. Varela. The unicellular green alga <i>Dunaliella salina</i> Teod. as a model for abiotic stress tolerance: genetic advances and future perspectives. <i>Algae</i> 26 (2011): 3–20.</p>

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Hickman, J.W., K. M. Kotovic, C. Miller, P. Warrener, B. Kaiser, T. Jurista, M. Budde, F. Cross, J. M. Roberts, and M. Carleton. Glycogen synthesis is a required component of the nitrogen stress response in <i>Synechococcus elongatus</i> PCC 7942. <i>Algal Research</i> 2 (2013): 98–106.
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- Xie, S, S. Sun, S.Y. Dai, J. S. Yuan. Efficient coagulation of microalgae in cultures with filamentous fungi. *Algal Research* 2 (2013): 28–33.
- Arudchelvam, Y. and N. Nirmalakhandan. Energetic optimization of algal lipid production in bubble columns: Part 1: Evaluation of gas sparging. *Biomass and Bioenergy* 46 (2012): 757–764.
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## Conversion-Fuel

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# NAABB Invention Disclosures and Patent Applications (in Bold) as of 5/06/2013

	Disclosing Member	ID Number	Title
1.	CSU	09-051	Bioconversion Of Extracted Algal Biomass Into Fuels And Other Chemicals
2.	Danforth	DDPSC0017	Developing A Transgenic Photosynthetic Organism That Can Auto-Regulate Its Light Harvesting Antenna Size
3.	Danforth	DDPSC0019	Exposure To Decane Leads To Oil Induction In Algae At Single Cell And Autospore Stages
4.	El Dorado		Methods And Apparatus For Forced Genetic Adaptation And Commercial Scale Growth Of Algae In Challenged Water As Well As A System For Algae-Based Treatment Of Challenged Water
5.	<b>El Dorado</b>	<b>US Provisional</b>	<b>Method And Apparatus For Greenhouse Gas Regulation Using Algae To Create A Strategic Algae Reserve Energy Supply</b>
6.	<b>Genifuel</b>	<b>Patent # US7,905,930 B2</b>	<b>Two-Stage Process For Producing Oil From Microalgae</b>
7.	Genifuel	USPTO12/683369	Closed-Loop System For Growth Of Aquatic Biomass And Gasification Thereof
8.	ISU		Selective Absorption Of Tocopherol By Pentafluorophenyl-Functionalized Mesoporous Silica Nanoparticles
9.	LANL	S121847	Identification And Creation Of Algal Strains For The Purpose Of Drop-In Transportation Fuels: <i>Tetraselmis Sp.</i> Lanl1001 And Others
10	LANL	S129149	Isolation Of A High Lipid-Content Subpopulation Of <i>Nannochloris Sp</i>
11.	LANL	129180	Facile Isotopic Synthesis Of Isoprenoid Precursors

# NAABB Invention Disclosures and Patent Applications (in Bold) as of 5/06/2013



	Disclosing Member	ID Number	Title
12.	LANL	<b>S121345</b> <b>US No. 13/652,296</b> <b>10/15/12</b>	<b>Method And Apparatus For Acoustically Manipulating Biological Particles</b>
13.	LANL	S129415	Hydrogel-Based Integrated Environments For Microalgae Cultivation
14.	LANL	S129181	Functionalization Of Sialic Acid: Odorant Derivatives And Adsorbtion/Attachment To Natural And /Or Man Made Surfaces
15.	LANL	S121085	Reverse-Flow Submerged Forward Osmosis For Clean Water Recharge To Algae Raceway Systems
16.	LANL	S129406	Optical-Driven Emulsion Destabilization
17.	LANL	S129598	Acoustic-Driven Emulsion Destabilization
18	MSU	TEC2011-0021	Photobioreactor/Sensor Array
19.	NMSU		Extractive Conversion Of Wet Algae To Biodiesel Under Supercritical Methanol Conditions
20.	NMSU		Extractive Conversion Of Dry Algae To Biodiesel Under Microwave Irradiation
21.	PNNL/Genifuel	17184	Sulfate Removal From Hydrothermal Environment
22..	TAMU	3273	Solvent-Phase Extraction Of Algal Oils Via Surface Functionalized Migration Of Algal Cells From Aqueous-Phase To Solvent-Phase

# NAABB Invention Disclosures and Patent Applications (in Bold) as of 5/06/2013

	Disclosing Member	ID Number	Title
23.	TAMU	3379	Production Of High-Quality Bio-Oil, Bio-Char And Synthesis Gas From Microalgae Using Pressure Reactor And The TAU Fluidized-Bed Pyrolyzer
24.	TAMU	3521	Metabolic Engineering Of Algae For Aviation Fuel Production
25.	TAMU	3333	Agrobacterium And Glass Beads-Based Transformation Of Different Green Algae Strains
26	Targeted Growth	TARG-022_00US	Modified Photosynthetic Micro-Organisms For Continuous Production Of Carbon-Based Products
27.	Targeted Growth	TARG -022_01US	Modified Photosynthetic Micro-Organisms For Continuous Production Of Carbon-Containing Products
28	Targeted Growth	TARG -023_00US	Modified Diacylglycerol Acyltransferase Proteins And Methods Of Use Thereof
<b>29.</b>	<b>UOP</b>	<b>H0031677 US 12/14/11; PCT 9/5/12</b>	<b>Comparison Of Metals And Phosphorous Removal Of Crude Algae Oil Extracts Using Acid Washing Alone And In Combination With Base And Water Washing</b>
30.	UOP	H0036528	Removal Of Metals From Algal Oil By Acid Washing And Combined Base/Acid Washing
31.	UOP	H0037456	Production Of Large Molecular Weight Paraffinic Waxes From Hydroprocessing Of Algal Oils
32.	UOP	H0037780	Removal Of Chloride From Triglyceride Oils Using A Combination Of Hot Base And Acid Washing
33.	WUSTL		Method For Obtaining Buoyant Triacylglycerol-Filled <i>Chlamydomonas reinhardtii</i>

- **NAABB Researchers Integrate Algae Genome Data in New Web-based Tool** ,  
[http://www.naabb.org/images/Technical/Vol%201\\_No%201\\_Nov1-2010.pdf](http://www.naabb.org/images/Technical/Vol%201_No%201_Nov1-2010.pdf)
- **NAABB Industry Member Catilin Generates Biodiesel from Algal Oil** ,  
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- **NAABB researcher, Dr. Rose Ann Cattolico of University of Washington, sets new standard for measuring algal lipids**  
[http://www.naabb.org/images/Technical/Vol%201\\_No%203\\_Nov15-2010%20final.pdf](http://www.naabb.org/images/Technical/Vol%201_No%203_Nov15-2010%20final.pdf)
- **NAABB researcher, Dr. Shuguang Deng of New Mexico State University, directly converts algal biomass to biodiesel**  
[http://www.naabb.org/images/Technical/Vol%201\\_No%205\\_Nov29-2010%20final.pdf](http://www.naabb.org/images/Technical/Vol%201_No%205_Nov29-2010%20final.pdf)
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- **NAABB spearheads broad collaboration on economics models for algal fuels**  
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- **NAABB Researchers Complete Sequence of Two Algal Genomes**  
[http://www.naabb.org/images/stories/Vol\\_1\\_No\\_9\\_Mar21-2011.pdf](http://www.naabb.org/images/stories/Vol_1_No_9_Mar21-2011.pdf)
- **NAABB Researcher, Pete Silks, of Los Alamos National Laboratory, Develops Process for Converting Algal Oil to Alternative Aviation Fuel**  
[http://www.naabb.org/images/stories/Vol\\_1\\_No\\_10\\_Mar21-2011.pdf](http://www.naabb.org/images/stories/Vol_1_No_10_Mar21-2011.pdf)
- **Researching the genetics of B. braunii microalga, a potential renewable biofuel source**
- **A first use of oil and gas produced water as a medium for algae grown for biofuel production-pilot scale testing**
- **Michigan State University's Algal Photo Bioreactor Licensed by Phenometrics, Inc.**

- **1<sup>st</sup> International Conference on Algal Biomass, Biofuels, and Bioproducts.**  
St. Louis, MO; July 17-21, 2011.
  - *Conference Co-chairs: Richard S. Sayre, The Donald Danforth Plant Science Center; and José A. Olivares, Los Alamos National Laboratory*
- **2<sup>nd</sup> International Conference on Algal Biomass, Biofuels, and Bioproducts.**  
San Diego, CA; June 10-13, 2012.
  - *Conference Co-chairs: Richard S. Sayre, The Donald Danforth Plant Science Center; and José A. Olivares, Los Alamos National Laboratory*
- **3<sup>rd</sup> International Conference on Algal Biomass, Biofuels, and Bioproducts.**  
Toronto, Canada; June 16-19, 2013.
  - *Conference Co-chairs: Richard S. Sayre, The Donald Danforth Plant Science Center; and José A. Olivares, Los Alamos National Laboratory*
- **4<sup>th</sup> International Conference on Algal Biomass, Biofuels, and Bioproducts.**  
Santa Fe, NM; June 17-20, 2014.
  - *Conference Co-chairs: Richard S. Sayre, The Donald Danforth Plant Science Center; and José A. Olivares, Los Alamos National Laboratory*
- **5<sup>th</sup> International Conference on Algal Biomass, Biofuels, and Bioproducts.**  
San Diego, CA; June 7-10, 2015.
  - *Conference Co-chairs: Richard S. Sayre, The Donald Danforth Plant Science Center; and José A. Olivares, Los Alamos National Laboratory*

- **2010 Algae Biomass Summit.** Phoenix, AZ; Sept. 2010.
  - Session: Analysis, Renewability, and Life Cycle Assessment  
*Co-Chairs: Meghan Starbuck, New Mexico State University and Ron Pate, Sandia National Laboratories*
  - Session: Conversion of Algal Biomass and Lipids into Practical Fuels  
*Chair – Anthony Marchese, Colorado State University*
  
- **242<sup>nd</sup> American Chemical Society.** Denver, CO Aug. 28<sup>th</sup> – Sept. 1st, 2011.
  - Symposium: Recycling Carbon: Catalyzed Conversion of Non-Food Biomass to Fuels and Chemicals
    - Sponsored by the Division of Industrial and Engineering Chemistry
    - *Co-Chairs: John C. Gordon, Los Alamos National Laboratory; George Kraus, Iowa State University; L.A. "Pete" Silks, Los Alamos National Laboratory; Ryan West, Procter and Gamble*