NAABB

National Alliance For Advanced Biofuels and Bio-products

FULL FINAL REPORT
SECTION I

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SECTION I
Program Overview

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Work Funded by U.S. DOE-EERE Bioenergy Technologies Office, DE-EE0003046

Executive Summary

In 2010, the Department of Energy Office of Energy Efficiency and Renewable Energy's (DOE-EERE's) Biomass Program awarded $48.6 million for the National Alliance for Advanced Biofuels and Bioproducts (NAABB) consortium. The three-year, one-time investment from the American Recovery and Reinvestment Act (ARRA) was designed to spur the domestic algal biofuels industry and create new jobs. NAABB combined talents from the national laboratories, universities, and industries from across the United States. By the end of the project, NAABB consisted of 39 institutions and had 2 international partners and $19.1M in cost-share.

The main objective of NAABB was to combine science, technology, and engineering expertise from across the nation to break down critical technical barriers to commercialization of algae-based biofuels. The approach was to address technology development across the entire value chain of algal biofuels production, from selection of strains to cultivation, harvesting, extraction, fuel conversion, and agricultural coproduct production. Sustainable practices and financial feasibility assessments underscored the approach and drove the technology development.

NAABB led the algal biofuels field a long way from the Aquatic Species Program, which ended in 1996. In particular, the availability of new molecular biology tools within the past two decades has revolutionized algal biology. In NAABB, we had a successful bioprospecting effort in which thousands of new strains were isolated from the environment and screened for lipid production. Genetic engineering strategies were developed and applied in a pipeline approach to enhance lipid and biomass production. Likewise, innovative adaptive evolution approaches were successfully applied to improve strain performance.

NAABB established a variety of cultivation resources ranging in scale from commercial-scale outdoor testbeds, to large and mid-scale innovative designs, and to small laboratory-scale photobioreactor systems. The testbeds were located in different regions and enabled long-term study of productivity throughout different conditions. The variety of cultivation systems was critical for optimizing sustainable cultivation practices and for efficiently moving strains from the laboratory to outdoor cultivation.

Harvesting is a major contributor to the cost of biofuel production from algae. The challenge of dewatering and concentrating microalgae was addressed in NAABB through a rigorous cross-comparison of innovative technologies that showed the potential to reduce the energy consumption and operating costs of harvesting to a fraction of conventional technologies. Lipid extraction technologies were focused on technologies that utilized wet concentrates of algae to eliminate the energy-intensive step of drying and reduce the use of solvents.

There are a variety of routes that can be used to convert algal lipids into production of hydrocarbons and biodiesel. These routes were explored and compared among NAABB partners to understand the yields and qualities of the fuels produced and what would be needed to meet specifications for biodiesel, green diesel, or jet fuel. In addition, technology for converting whole algae biomass directly to fuel was developed to reduce the time and energy consumed in fuel conversion from algae.

For algal biofuels to be economically viable, it is possible that a coproduct must be produced from the lipid extracted algae (LEA) biomass. In the most extensive study to date, NAABB investigated the use of LEA as an animal feed and protein supplement in a range of animals and the effectiveness of LEA as a fertilizer.

Reduced use of nutrients and water in algal cultivation and reduction in the carbon footprint of new technologies for processing algae into fuel will help to build a sustainable algae biofuels industry. NAABB created the architecture for quantitatively assessing the energy, environmental, and economic viability of NAABB technologies. Data for these assessments were provided by the technology developers in each part of the fuel production process to the sustainability team for their analyses. The bottom line is that if key innovations from NAABB were implemented along the entire value chain to production of algal biocrude, then the cost of algal biofuels could be reduced from a starting baseline of $240 per gallon to a reasonable cost of less than $7.50 per gallon of crude oil. As technologies are further refined, there is the potential to reduce costs even further.

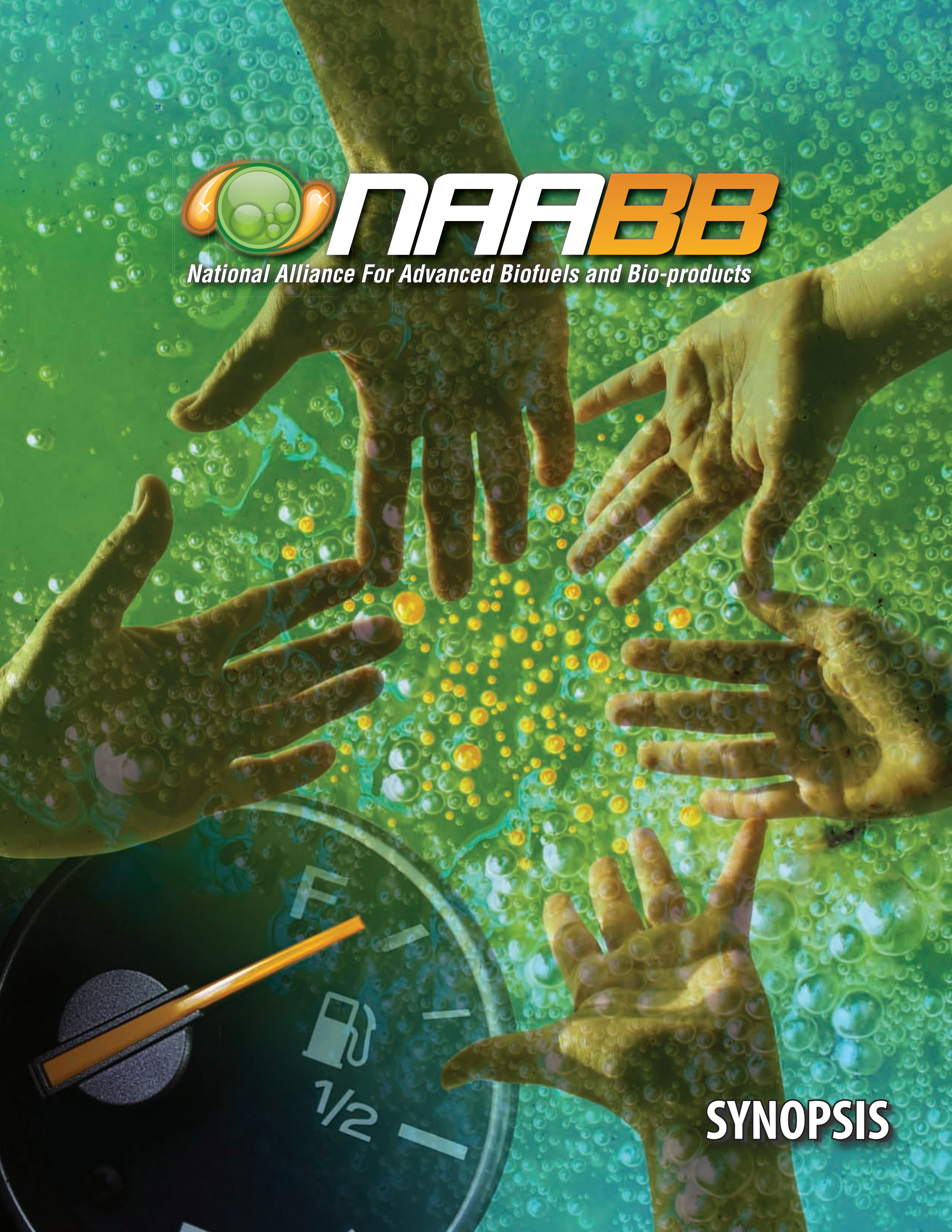
The NAABB consortium will leave a lasting legacy through our technical accomplishments. As of February 2014, NAABB produced 33 intellectual property disclosures, produced more than 100 peer-reviewed publications, and deposited 30 new algae isolates into the University of Texas culture collection. Five students from academic partners completed their graduate thesis research supported by NAABB. Through a partnership with Elsevier publishing, one new journal (*Algal Research*) was created, and one new international conference series (*International Conference on Algal Biomass, Biofuels, and Bioproducts*) was established. One new company (*Phenometrics*) was born from NAABB to produce small environmental photobioreactors for bench-scale algal research. Finally, the integrated upstream-to-downstream approach of NAABB enabled us to take a new strain discovered in the field (*Chlorella* sp. *DOE1412*), sequence it, cultivate it outdoors, harvest it with new technology, and convert it to biocrude oil, all with NAABB partners.

Equally important, NAABB demonstrated the value of the consortium approach, which joined many hands to reach for new solutions to reduce the U.S. dependence on fossil fuels. The large size of the consortium allowed NAABB great flexibility to pursue the development and scale-up of innovative technologies along the entire value chain to algae biofuel production with breadth as well as depth. We blended basic research with applied research, and by incorporating so many industrial partners our research efforts were balanced at every stage with the needs and realities of industry. Harnessing the diversity of talents that comprised the NAABB consortium was a complex task in itself that required an open and involved management approach.

Consequently, the NAABB consortium provided unprecedented insight into the promise of algal biofuels and the challenges that lie ahead. Since the start of NAABB, several policy documents have been published that address the sustainability of algal biofuels and the importance of technology innovation for achieving a sustainable, commercially viable biofuels industry as a critical part of the emerging national bioeconomy. The NAABB consortium approach to emphasize research and development that promotes environmental and economic sustainability is aligned with the direction provided by the nation's top thought leaders in energy and climate interactions. We are confident that the achievements of the NAABB consortium will have a far-reaching impact on the advanced biofuels and bioproducts industry and will have relevance for many years to come.

NAABB

National Alliance For Advanced Biofuels and Bio-products



SYNOPSIS

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Preface

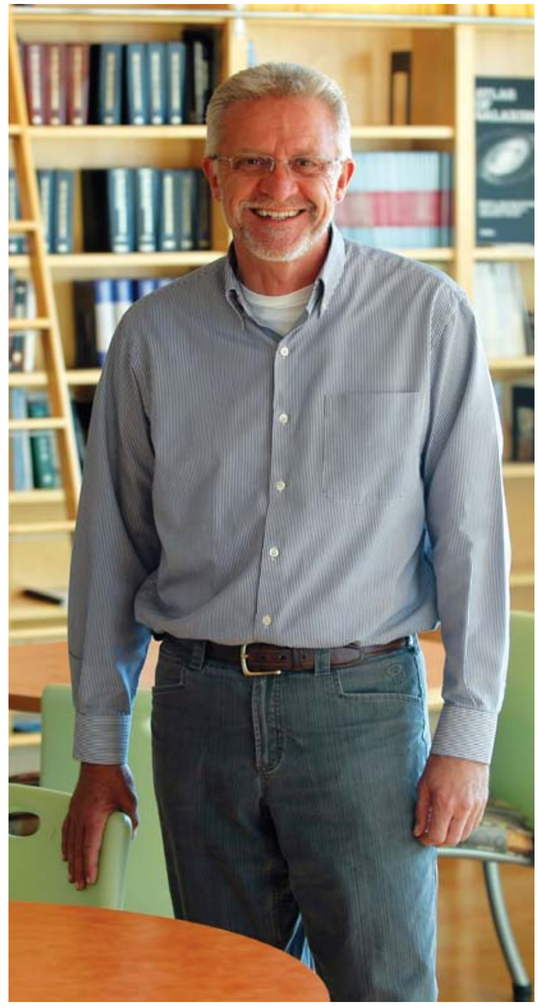
The National Alliance for Advanced Biofuels and Bioproducts (NAABB), an algal biofuels research consortium, was formed to specifically address the objectives set forth by the U.S. Department of Energy, Energy Efficiency and Renewable Energy (DOE-EERE), Office of Biomass Programs (now called the Bioenergy Technologies Office, BETO), under the funding opportunity announcement DE-FOA-0000123, "Development of Algal/Advanced Biofuels Consortia". The American Recovery and Reinvestment Act of 2009 provided the funds for this effort. In this announcement DOE sought consortia that would "synergistically use their unique capabilities to expedite the development of biomass based fuel production pathways." The opportunity allowed and encouraged the participation of industry, academia, and government and/or non-government laboratories, and could include foreign entities, all providing "best-in-class" technical approaches. NAABB specifically addressed Topic Area 1–Algal Biofuels Consortium focused on the following pathways:

- Feedstock Supply–Strain development and cultivation
- Feedstock Logistics–Harvesting and extraction
- Conversion/Production–Accumulation of intermediates and synthesis of fuels and coproducts

In addition to the above, the program asked applicants to address sustainable practices, life cycle and economic analyses, and resource management relative to the proposed pathways. Furthermore, economic and resource issues were to be taken into account throughout the process and should be incorporated into the proposed strategies. At the conclusion of the three-year effort, a report was required from the consortium, detailing the current state of the technologies investigated, including a cost analysis and a life cycle analysis. The cost analysis would include the modeled cost of algal biofuels production based on the experimental and/or operational data gathered through the consortium efforts.

Over its short period of performance from 2010 to 2013, the NAABB consortium achieved its technical objectives, completed a formidable body of research and development, and helped establish a sustainable algal biofuels and bioproducts industry. Now, one year after the formal closure of NAABB, we are proud to share our accomplishments and insights with you in the form of two reports. The document that follows is a Synopsis of the NAABB Consortium, which provides a brief summary of the NAABB accomplishments intended for a broad audience. The Full Final Report is a more detailed technical document consisting of three sections: (1) the NAABB consortium background and organization, (2) the main accomplishments of the NAABB R&D teams, and (3) short summaries of the individual NAABB projects.

We thank the DOE-EERE Bioenergy Technologies Office for their funding, through the American Recovery and Reinvestment Act, and collaborative work in managing this effort with NAABB. Most of all, we thank over two hundred investigators, students, postdoctoral fellows, and supporting staff, representing thirty-nine NAABB institutional partners, who contributed to the success of this program. These reports represent their creativity, hard work, and collaborative spirit over a three-year span.



Jose A. Olivares, Ph.D.
NAABB Principal Investigator and Executive Director

National Alliance For Advanced Biofuels and Bio-products

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

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Executive Summary

Development of liquid transportation fuels from biomass is an essential part of diversifying the U.S. energy portfolio and moving the economy away from fossil fuel sources. The 2007 Energy Independence and Security Act (EISA) set major goals for the reduction of greenhouse gas (GHG) emissions through the development of a biofuel production capacity of 36 billion gallons by 2022. Furthermore, in 2011 the U.S. Department of Energy (DOE) published *The Billion Ton Update*, a study showing that in the future the United States could produce over 1 billion tons of biomass as a resource for transportation fuels, chemicals, and power. To achieve this advanced biofuel production goal, technologies that result in high energy return on investment, are economically feasible, and provide a sustainable approach to resource management will need to be developed and brought into production.

There are a number of biomass resources that were not included in *The Billion Ton Update*, including non-terrestrial sources such as algae, because their full impacts were not adequately understood at the time. Although an aquatic organism, algae represent an additional source of biomass with the potential to significantly impact the displacement of fossil fuels if challenges in feedstock production, logistics, and conversion are effectively addressed. For nearly two decades (1978 to 1996), the U.S. DOE had an algal biofuels program called the Aquatic Species Program (ASP). This program made significant advances in the science of algal biology for manipulating lipid content of microalgae and the engineering of microalgal production systems. The ASP concluded that increasing biomass productivity with improved algae strains through biological enhancements should be a central subject for any future U.S. research program in microalgal biofuel production.

To further understand the impacts of algae on overall biomass and liquid transportation fuel production, the DOE funded the National Alliance for Advanced Biofuels and Bioproducts (NAABB) in 2010. Through this effort, NAABB, a consortium of thirty-nine partner institutions, advanced algal biofuel technology development by addressing biological enhancements and biomass productivity. The consortium brought expertise from industry, universities, and national laboratories (Figure 1) that spanned the entire value chain from algal biology to fuel conversion—a strategy that would ensure a thorough evaluation of the production potential of algae-based fuel.

Main Results from the National Alliance for Advanced Biofuels and Bioproducts

In three years, NAABB was able to develop technologies that have the potential to reduce the cost of algae-based biocrude by two orders of magnitude from our starting baseline; that is, from $240 to $7.50 per gallon.

NAABB was managed through the Bioenergy Technologies Office of the U.S. DOE [ref FOA-0000123, "Development of Algal/Advanced Biofuels Consortia] for three years with $48.6 million public funds from the American Recovery and Reinvestment Act of 2009 and $19.1 million in private funds.

Lead Institution ★

The Donald Danforth Plant Science Center, St. Louis, MO

National Laboratories ●

Los Alamos National Laboratory/New Mexico Consortium, Los Alamos, NM
Pacific Northwest National Laboratory, Richland, WA
Idaho National Laboratory, Idaho Falls, ID
National Renewable Energy Laboratory, Golden, CO
United States Department of Agriculture – Agricultural Research Service, Washington, DC

Universities ■

Brooklyn College, Brooklyn, NY
Clarkson University, Potsdam, NY
Colorado State University, Fort Collins, CO
Iowa State University, Ames, IA
Michigan State University, East Lansing, MI
New Mexico State University, Las Cruces, NM
North Carolina State University, Raleigh, NC
Texas AgriLife Research / Texas A&M University System, College Station, TX
University of Arizona, Tuscon, AZ
University of California Los Angeles, Los Angeles, CA
University of California Riverside, Riverside, CA
University of California San Diego, San Diego, CA
University of Pennsylvania, Philadelphia, PA
University of Texas, Austin, TX
University of Washington, Seattle, WA
Washington State University, Pullman, WA
Washington University, St. Louis, MO

Industry ▲

Albemarle Catilin, Ames, IA
Diversified Energy, Gilbert, AZ
Eldorado Biofuels, Santa Fe, NM
Genifuel, Salt Lake City, UT
Cellana, Kailua-Kona, HI
Inventure, Tuscaloosa, AL
Kai BioEnergy, San Diego, CA
Palmer Labs, Durham, NC
Phycal, Highland Heights, OH
Reliance Industries Limited, Mumbai, India
Pan Pacific, Ltd., Adelaide, Australia
Solix Biosystems, Fort Collins, CO
Targeted Growth, Seattle, WA
Terrabon, Bryan, TX
UOP a Honeywell Company, Des Plaines, IL
Valicor, Dexter, MI

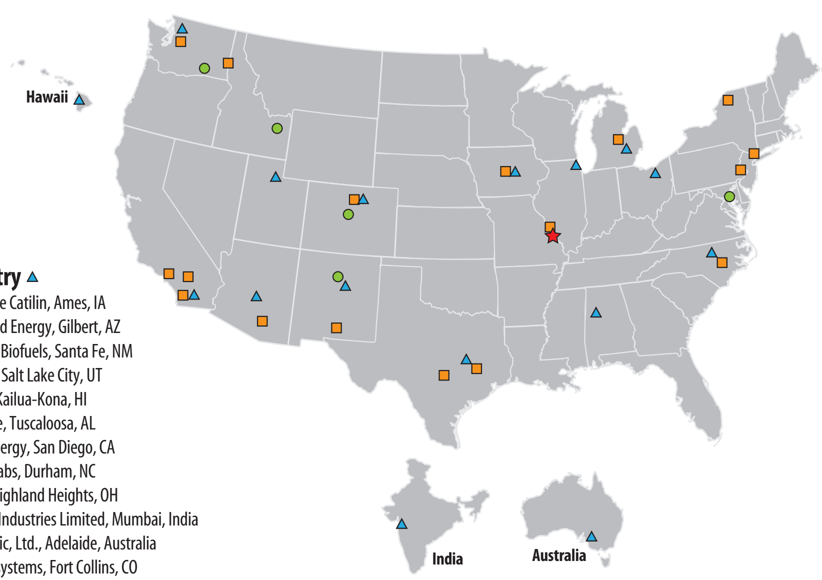


Figure 1. Map showing the locations of the thirty-nine NAABB consortium partners.

This Synopsis of the NAABB Full Final Report highlights the scope, accomplishments, and recommendations of the NAABB research effort, including the following four cost-reducing innovations (Figure 2):

- **New strain development**—Discovery of a new platform production strain, *Chlorella* sp. *DOE1412*, which has the robust ability to produce good oil yield under a variety of conditions. When combined with genetically modified (GMO) versions of the strain the cost of algal biocrude would be reduced by 85%.
- **Improved cultivation**—Development of a new open pond cultivation system, the Aquaculture Raceway Integrated Design (ARID), which uses little energy, extends the growing period, improves productivity, and provides a 16% cost reduction.
- **Low energy harvesting technology**—Demonstrated use of an electrocoagulation (EC) harvesting technology, which is a low-energy, primary harvesting approach using commercially available equipment that provides a 14% cost reduction.
- **High-yield extraction-conversion technology**—Creation of a unique hydrothermal liquefaction (HTL) system that combines extraction and conversion to provide high biocrude yield without the need for extraction solvents, resulting in an 86% cost reduction.

Additional productivity and cultivation gains will be needed to further reduce the cost of biocrude to under $2 per gallon. These improvements will need to come from new developments in algal farms that reduce capital expenditure (CAPEX) requirements by about 50% along with similar cuts in operational expenditure (OPEX) through efficiencies in utilization of major resources, such as water (Table 1).

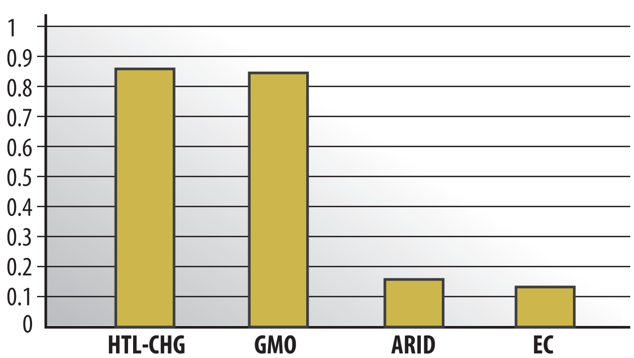


Figure 2. Potential cost reductions (y-axis) that can be achieved with four main NAABB innovations, hydrothermal liquefaction with catalytic hydrothermal gasification (HTL-CHG), a novel genetically modified algae strain (GMO), a unique design open pond cultivation system (ARID), and electrocoagulation harvesting (EC).

Table 1. Average total cost per gallon for biocrude oil ($/Gallon).

	Fraction Reductions in CAPEX				
Fraction OPEX	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

Outlook for the Future

Through an integrated, multidisciplinary approach, the NAABB consortium has shown that an economically viable, algal biofuel system is feasible. Innovative improvements across the entire value chain were demonstrated, including technologies that maximize biomass productivity and cultivation. Direct pathways to fuels were shown to be the most feasible, minimizing the high-energy consumption involved in dewatering algae, and maximizing the carbon input into the final fuel.

We envision algal biofuels to be a viable competitor in the liquid transportation fuels market after a few more key improvements. A successful algal production farm requires a new approach to construction and cultivation that drastically reduces the cost of construction and its effect on capital layout. Furthermore, the algal farms must implement algae strains and cultivation methods that maximize biomass productivity year-round, such as the NAABB strain and cultivation technologies defined above. Finally, the use of major resources, such as key nutrients and water, need to be minimized and efficiently utilized. Combining technologies and systems in these three areas into a model integrated production and biorefinery system will bring viable algae-based biofuels into the market.

Introduction

The Aquatic Species Program and National Research Council Perspectives

Although the stated goal of the ASP was producing fuel from algae, the research was focused on strain prospecting and cultivation. There were no significant efforts focused on harvesting, lipid extraction, or the conversion of biomass or lipids to fuels. In contrast, NAABB was asked to fully integrate the development of new algal strains; cultivate the strains in large outdoor ponds; and harvest, extract, and convert the resultant oils to fuel products using a variety of different processes within a three-year period.

In October 2012, the National Research Council (NRC), at the request of DOE-EERE, published a report on the sustainable development of algal biofuels. The committee found that sustainable development of algal biofuels would require research, development, and demonstration of the following:

- Algal strains with enhanced growth characteristics and biofuel productivity;
- An energy return on investment (EROI) that is comparable to other transportation fuels or at least improving and approaching the EROIs of other transportation fuels;
- Reactor strategies that use either wastewater for cultivating algae for fuels or recycled water from harvesting systems, particularly if freshwater algae are used;
- Recycling of nutrients in algal biofuel pathways that require harvesting, unless coproducts are produced that meet an equivalent nutrient need; and
- A national assessment of land requirements for algae cultivation to inform the potential amount of algal biofuels that could be produced economically in the United States. That assessment must take into account climatic conditions; freshwater, inland and coastal saline water, and wastewater resources; sources of CO_2; and land prices.

Although the report came out well into the NAABB project, NAABB research was already fully engaged in each of the areas recommended by the NRC.

NAABB Research and Development Framework

NAABB research was structured into a framework (Figure 3) that covered six technical areas with major cross-cutting objectives for algal biofuels production: (1) increasing productivity; (2) reducing energy and cost of producing fuels; and (3) assessing and optimizing sustainable practices throughout the value chain.

NAABB's R&D framework facilitated strong team collaborations within each of the major technical areas and critical interactions between technical areas. This occurred in two ways: (1) objectives for each of the research areas were refined and updated as the needs of upstream and downstream technologies were identified and (2) handoffs of technology improvements, data sets, and intermediate products (e.g., strains, biomass, lipids, and lipid extracted algae (LEA)) allowed for cross-cutting interactions.

This R&D Framework enabled integration of our process development across the entire algal biofuels value chain. The NAABB Process Matrix (Figure 4) included (1) the development of new strains, (2) cultivation processes with these new strains, (3) harvest processing of the algal biomass, (4) extraction processing for crude lipids and LEA, (5) LEA conversion and LEA product trials, (6) direct conversion processes of algal biomass to biocrude, and (7) upgrading lipids and biocrudes to fuels.

A gap analysis of the process matrix allowed us to identify key cost drivers and how they impact technologies upstream and downstream. We also learned where the consortium was missing key R&D elements and were able to address such needs. We took six different algae strains through all or most elements of the process matrix, collecting one-of-a-kind data sets for analysis and model development.

In the following section, we describe some of the technical highlights of the NAABB research teams and conclude each section with our recommendations for future steps.

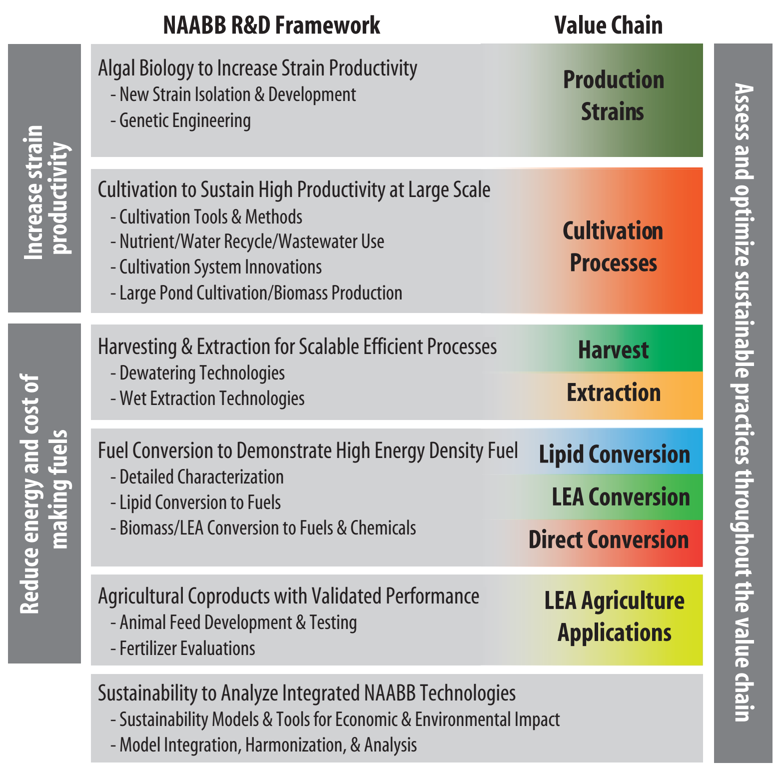


Figure 3. The NAABB R&D Framework.

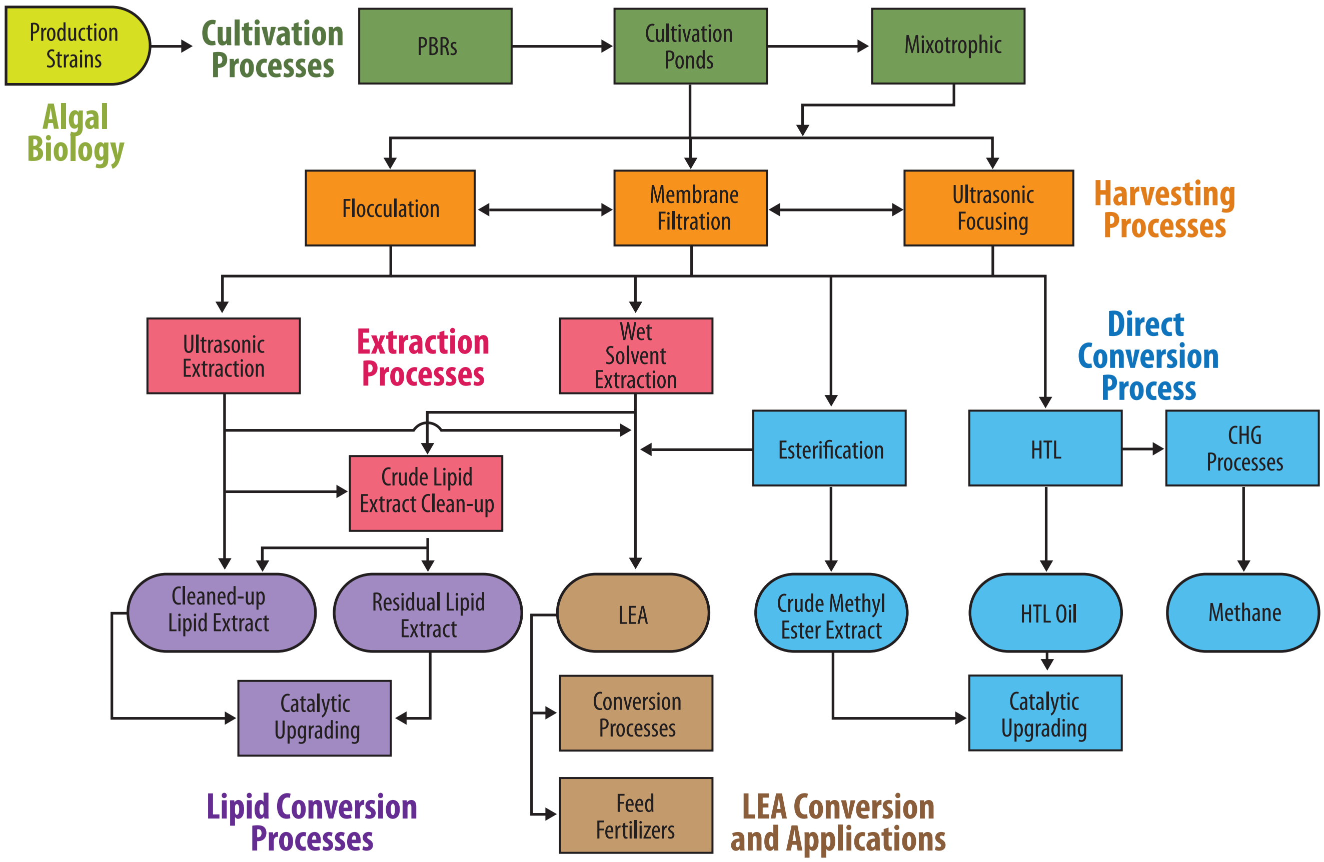


Figure 4. The NAABB Process Matrix.

Technical Highlights of NAABB R&D

Algal Biology

At the start of the NAABB consortium in 2010, little was known about the molecular basis of algal biomass growth or oil production. Very few algal genome sequences were available and efforts to identify the best producing wild species through bio-prospecting approaches had largely stalled since the efforts of the ASP. Furthermore, algal genetic transformation and metabolic engineering approaches to improve biomass and oil yields were in their infancy. However, genome sequencing and transcriptional profiling were becoming less expensive, and the tools to annotate gene expression profiles under various growth and engineered conditions were just starting to be developed for algae. It was in this context that an integrated Algal Biology Team effort was formed for NAABB to develop super-performing algal biofuel production strains with greater productivity of algal biomass accumulation and lipid/hydrocarbon content. To achieve this goal, NAABB took two parallel approaches: (1) identify and improve naturally high-producing strains and (2) develop new strains with high productivity through genetic modification. Both approaches were underpinned by a systems biology effort to characterize the gene regulation and metabolic flux in lipid and hydrocarbon biosynthesis pathways and by development of new technology, resources, and approaches for strain improvement.

Screened 2200 isolates to find the best candidates for biofuel production

Over the last few decades many strains of algae have shown potential for lipid-based biofuel production. While NAABB initially focused on further development of a few model strains, we recognized that there was also a need to survey nature to find superior cultivation candidates. An important thrust of NAABB was the isolation and characterization of algal strains that have potential for rapid biomass accumulation and lipid production under large-scale cultivation conditions. We developed protocols for isolating and characterizing lipid-producing algae from various geographic areas, environments, and seasons; then winnowing down the isolates through a multi-tiered screening procedure to select those with the greatest potential for high-productivity cultivation (Figure 5).

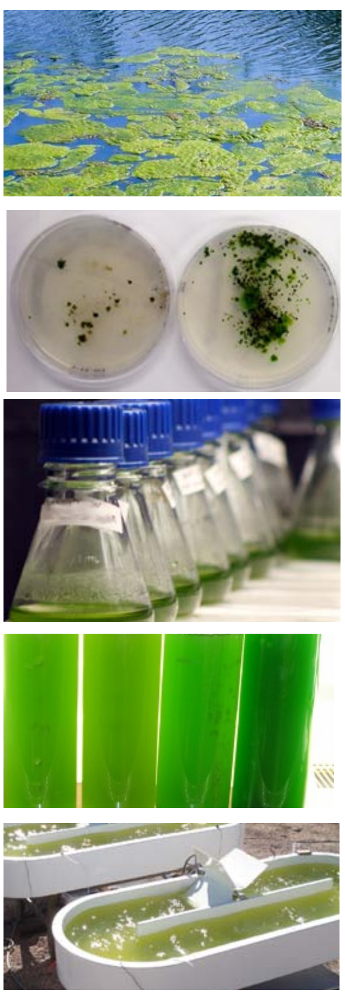
Approximately 400 samples were collected across the continental United States from various habitats, including soil, freshwater, brackish water, marine, and hyper-saline environments. From these samples, over 2200 independent strains were isolated, and over 1500 of those were subjected to a preliminary screen for oil accumulation. Strains were isolated by traditional culture methods using a variety of growth media for initial plating and by high throughput fluorescence-activated cell sorting to screen for high lipid content. Combining these approaches provided wide diversity and large numbers of isolates. Once isolated, we carried out high throughput screening using 96-well plates to identify strains that grew well autotrophically and accumulated lipids. These data were compared to the biomass productivity of the benchmark strain, *Nannochloropsis salina CCMP1776*. Hundreds of algal strains were assembled into a catalogued culture collection. Thirty of the best strains that approximated or exceeded the biomass productivity of *N. salina* were deposited in the University of Texas culture collection (UTEX). Several strains were examined extensively by other consortium members and cultured in one or more of the NAABB testbed facilities. NAABB took one of these new strains *(Chlorella* sp. *DOE1412)* through the entire NAABB process.

Completed genome sequencing on eight new algae strains

NAABB took advantage of new genome sequencing technologies including the Illumina, 454, and Pacific Biosciences platforms, which were complemented by the development of novel computational tools to sequence, assemble, and annotate high-quality algal genomes and transcriptomes quickly. A major accomplishment of the NAABB consortium was the sequencing and assembly of eight high-quality algal genomes from three independent phyla, the greatest biodiversity of algae sequences at that time (Table 2). NAABB also created two complementary web-based platforms for more accurate gene annotation and display, analysis, and distribution of "omics" bioinformatics (Figure 6).

Identified fifty gene targets for improving biomass and oil yield

To develop gene models and understand the connection between genes and certain characteristics, over 250 transcriptomes were sequenced and analyzed using new bioinformatic tools. Many of the gene expression studies were completed under nitrogen deprivation or other stress or growth conditions to monitor changes in gene expression during lipid induction. We provided extensive transcriptome sequences to analyze genes involved in lipid production in the model strain, *Chlamydomonas reinhardtii*. The RNA sequence data were also used to generate gene models and functional annotations for production strains *N. salina*, *Picochlorum* sp., and *Auxenochlorella protothecoides*.



1. Sample

2. Isolate

3. Screen

4. Characterize

5. Validate

Figure 5. Flow diagram of the broad temporal, climatic, and geographic survey approach to isolate and characterize algal biofuel candidate strains. Panel 1, Sample-An example sampling site; Panel 2, Isolate-Isolates from fluorescence-activated cell sorting on an agar plate; Panel 3, Screen-First-tier screening in traditional flask cultures; Panel 4, Characterize-100 mL bubble columns for characterizing the most promising candidates; Panel 5, Validate-Cultivation of the most promising strains in 200 L NAABB testbeds.

Table 2. NAABB algal genome projects.			
Genome	**Code**	**Assembly Quality**	**Size, Mbp**
Picochlorum sp.	NSC	Improved high quality draft	15.2
Auxenochlorella protothecoides UTEX25	CPI	Improved high quality draft	21.4
Chrysochromulina tobin	CAF	High quality draft	75.9
Nannochloropsis salina CCMP1776	NSK	Improved high quality draft	27.7
Tetraselmis sp. *LANL 1001*	TSG	Standard draft	220
Chlorococcum sp. *DOE 0101*	CPT	Standard draft	120
Chlorella sp. *DOE1412*	CSJ	Standard draft	55
Chlorella sorokiniana Phycal 1228	CSJ	Standard draft	55

Figure 6. Scientist viewing the Algal Functional Annotation Tool, a bioinformatics resource developed by NAABB.

This information enabled construction of metabolic pathways and comparative genomics analyses. Although the experimental protocols were distinct for each organism, several trends emerged from comparing these transcriptomes. Among these were the identification of specific genes that code for proteins and enzymes that are involved in lipid production and accumulation. Through analyzing the roles of these molecules, we identified over fifty gene targets for improved biomass yield and oil production. Genetic engineering approaches to increase carbon flux through some of the identified biosynthetic pathways are now being developed and applied.

Developed an algal transformation pipeline to increase biomass yield and lipid production

A major deliverable of the NAABB program was to demonstrate proof-of-concept for increasing biomass productivity and oil accumulation in genetically engineered algae. The primary approach used was to engineer the model freshwater algae, *C. reinhardtii*. Because these engineering efforts first required results from the genome sequencing and transcriptomics experiments described above, the engineering efforts were initiated in the last fourteen months of the NAABB program by developing an algal transformation pipeline. Essential to this pipeline was the development by NAABB of a robust bioreactor array to rapidly test the phenotype of the engineered strains.

Photobioreactor Array for Phenotype Characterization

A major accomplishment of NAABB was the design and commercialization of a new type of "environmental photobioreactor" (ePBR) that simulated the key abiotic features of a pond that have the greatest influence on algal productivity including light intensity and quality, temperature, and gas exchange (Figure 7). These ePBRs are used in the laboratory to predict the productivity of algal strains under production pond conditions. In addition, the ePBR was designed to be small and relatively inexpensive so that it could be arrayed in a laboratory to rapidly compare algal strains or growth conditions in parallel.

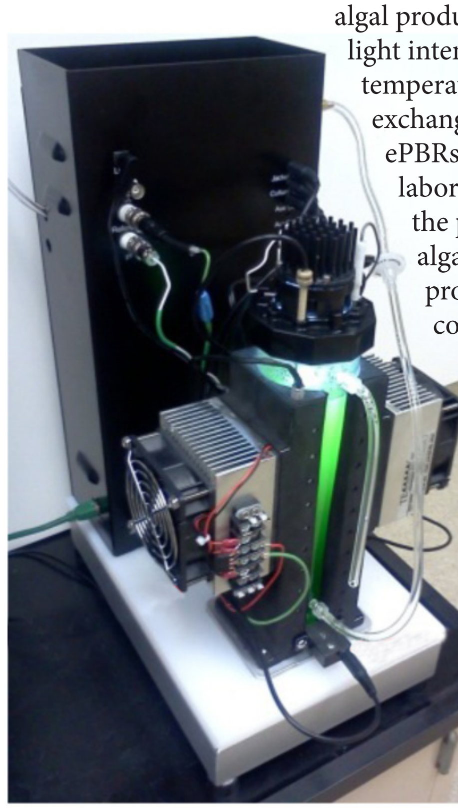


Figure 7. The Environmental Photobioreactor (ePBR).

Optimizing Light-Harvesting Antenna Size

Previous studies had demonstrated that intermediate-sized, light-harvesting antenna were optimal for growth in *C. reinhardtii*. We compared the growth rates of algae in which the accumulation of chlorophyll b was light-regulated so that it decreased at high light levels. This regulation was achieved by controlling the expression and binding of specific binding proteins. As shown in Figure 8, as much as a two-fold increase in biomass was achieved with the best performing transgenics when grown in ePBRs mimicking a typical summer day. *This gene (trait) conferred the greatest increase in biomass productivity of any tested by the NAABB consortium.*

We demonstrated improvement in oil accumulation without a deficit in biomass accumulation using a variety of metabolic engineering strategies. Oil accumulation levels increased as much as five-fold without affecting growth rates. We also found that transformants that overexpressed the enzyme fructose bisphosphatase had significantly increased growth.

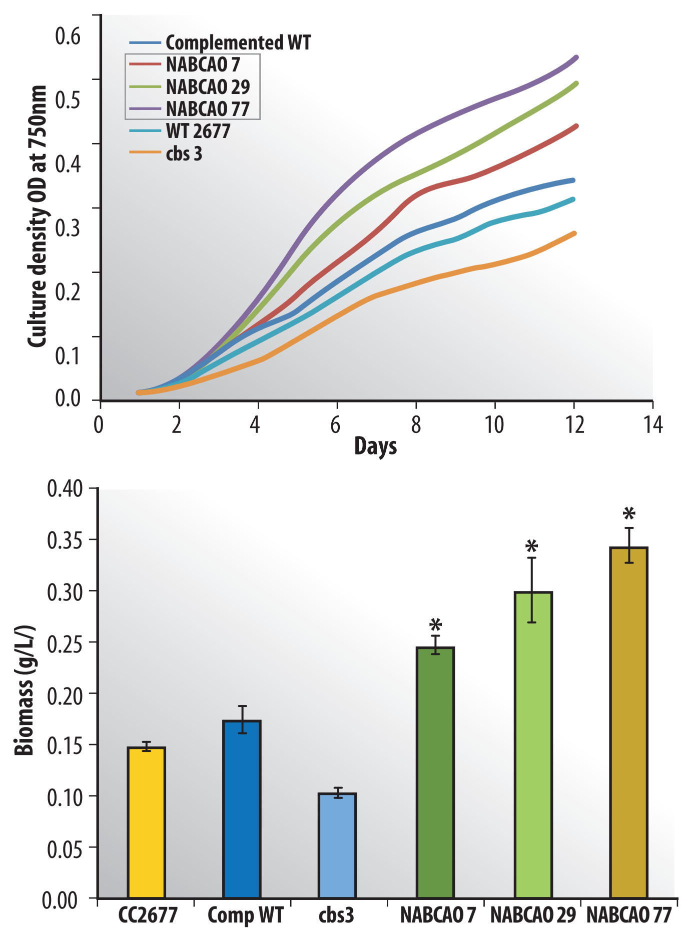


Figure 8. Cellular (top) and dry weight (bottom) productivity of wild type (WT) and transgenic algae with self-adjusting light-harvesting antenna. The NABCAO lines have been engineered to self-adjust the ratios of their chlorophyll binding proteins, and hence peripheral light-harvesting antenna size, in response to changing light levels or culture densities.

Additionally, engineering self-adjusting photosynthetic antennae into *C. reinhardtii* resulted in a significant two-fold increase in biomass accumulation. Overall, we demonstrated that systems biology studies can be used to direct metabolic engineering in complex algal systems and that *C. reinhardtii* can be a robust platform for testing novel gene constructs. While our *C. reinhardtii* model is likely to have the greatest implications for engineering closely related production strains such as *Chlorella* sp., our transcriptomics studies encourage us to test these engineering strategies in other production strains. Moreover, combining traits, such as overexpression of fructose bisphosphatase with expression of self-adjusting antenna could result in a significant leap towards a sustainable algal biofuels industry.

Developed new molecular tools for improving production strains

The literature on algal transformation is filled with numerous reports of requirements for strain-specific sequences in gene promoters and terminators, which are the parts of the gene sequence that code for the beginning and end. No universal promoters for algal gene expression have been reported. The genome sequence information obtained by NAABB allowed us to design species-specific vectors through which gene expression could be targeted. NAABB developed chloroplast-targeted transformation systems for *A. protothecoides* and *Chlorella* sp. *DOE1412* and stable nuclear transformation systems for the marine algae, *N. salina* and *Picochlorum* sp.

Additionally, NAABB identified antimicrobial peptides that kill bacteria and rotifers without harming algae. We expect that this new class of agents will help to protect algae cultivation ponds against invasion by predators and reduce the loss of crops due to pond crashes.

Directed evolution resulted in new strains with 50% improved oil yields

Our adaptive evolution efforts considered both the need to increase algae production per unit area and the need to reduce inputs in order to reduce the cost per barrel of algal lipids. Greater lipid production on a per cell basis was achieved by using fluorescence-activated cell sorting to isolate stable algal lines with greater lipid production. Algal cultures with varying levels of neutral lipids showed distinct separation when stained with the fluorescent dye BODIPY. This rapid flow cytometry assay was used to isolate a hyper-performing subpopulation of the algal strain *Picochlorum* sp. A *Picochlorum* culture was starved of nitrogen, the culture was stained with BODIPY, and a population of high BODIPY-stained cells were sorted and cultured. After multiple rounds of culturing and sorting we isolated a stable population that produced lipids at approximately twice the rate of the parent strain (Figure 9).

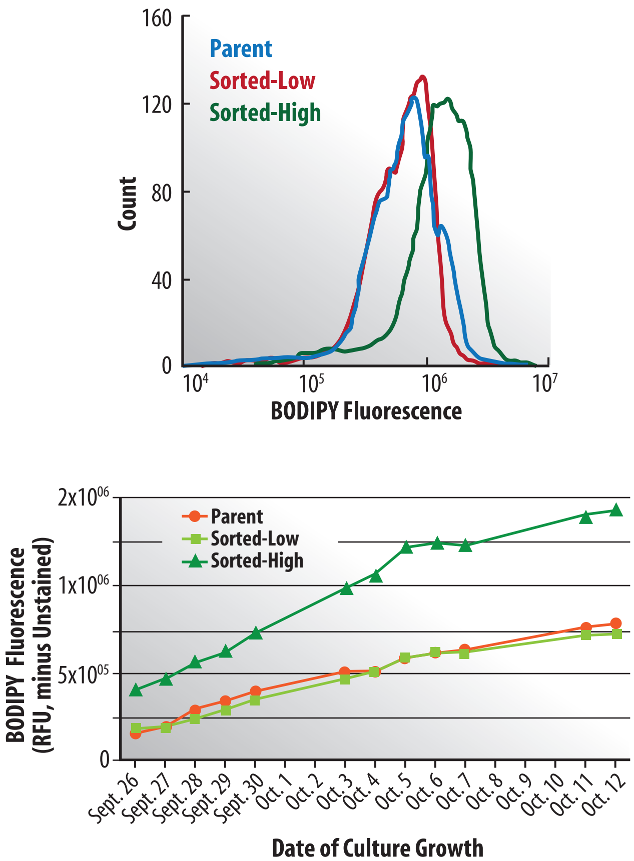


Figure 9. (Top) Histograms of BODIPY fluorescence of parent and sorted populations. (Bottom) During nitrogen starvation, all cultures accumulated lipids, with the sorted-high population outperforming the parent on all days (average 2X improvement).

Demonstrated the impact of lipid remodeling on cellular structure

Quick-freeze deep-etch electron microscopy (QFDEEM) was used to follow lipid body formation in two strains of special interest to NAABB, *C. reinhardtii* and *Nannochloropsis* sp., with the goal of uncovering unique and common characteristics in lipid body (LB) formation between these diverse species. Using this technology, NAABB scientists observed that multiple parts of the algae cell are involved in lipid body formation (Figure 10). By understanding more about this process, new strategies can be developed to enhance lipid production through genetic manipulation or cultivation techniques.

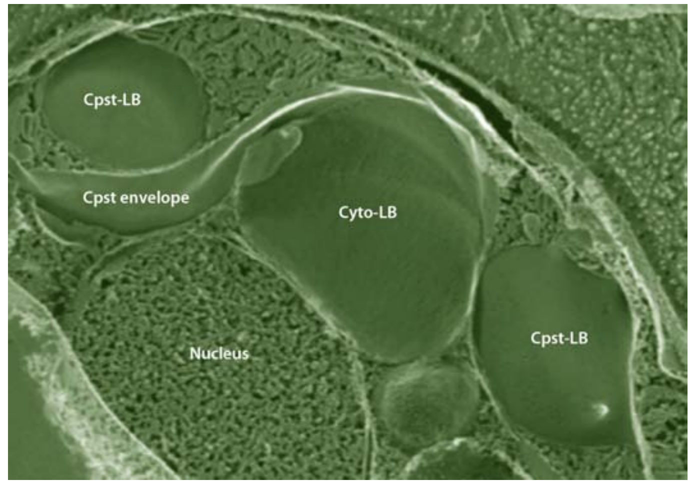


Figure 10. Cytoplasmic and chloroplast lipid bodies in the *sta6* strain of *C. reinhardtii*.

Algal Biology—Next Steps

NAABB's systems biology approaches led to the development of new genetic tools and potential targets to improve algal growth and lipid productivity. Our approach has been tapped but by no means exhausted. Further research is warranted to continue genomic sequencing of algae in general, and of algal biofuel production strains in particular, in order to expand the knowledge base needed to support future strain improvement efforts.

Additional strain options will continue to be needed to ensure the availability of robust strains for different environments. While NAABB's *Chlorella* sp. *DOE1412* represents a new production strain for fresh and impaired waters, new saltwater tolerant strain options would be valuable for coastal regions.

Future engineering of production strains with self-adjusting light-harvesting antennae is a promising approach to increase productivity to commercially viable levels. Because such modifications will not be found in nature there is a critical need to develop standards and regulatory protocols for the safe use of genetically modified algae in testbed facilities.

Cultivation

The cultivation of microalgae in large open raceways and photobioreactors in various forms has been practiced over the past fifty years to produce high-value nutritional products. Consequently, the engineering and performance challenges associated with large-scale cultivation of microalgae are fairly well understood. The NAABB Cultivation Team focused on several of these major challenges including (1) identifying robust production strains that will perform reliably outdoors in specific geographic locations and associated growth seasons; (2) developing methods for cultivation in low-cost media using agricultural-grade nutrients, wastewater sources, and media recycling; and (3) developing and demonstrating enhanced cultivation system designs and operational methods that improve productivity and reduce cost. Our approach was to assemble a variety of working testbed facilities with conventional design for process engineering and technology testing. In addition to standard ponds, we also had development testbeds and indoor growth systems available for evaluating the effects of new concepts on cultivation productivity and cost.

Developed a microalgae growth model to evaluate the best strain and climate pairings

To accelerate the transition of promising microalgae from the laboratory into large outdoor ponds, NAABB developed and tested an integrated stepwise strategy for screening strains to select strains with high biomass productivity potential as shown in Figure 11.

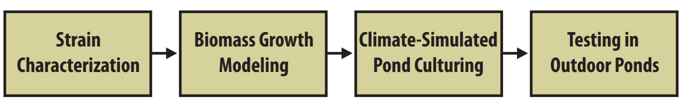


Figure 11. NAABB stepwise strain screening strategy.

A key component of this strategy was development of a microalgae biomass growth model. This model utilizes experimentally determined species-specific parameters from detailed laboratory studies to predict biomass productivity outdoors in open ponds. The model was validated using outdoor pond cultivation data. The biomass growth model, in conjunction with the biomass assessment tool (BAT), enables the prediction of monthly and annual biomass productivities of a given strain in hypothetical outdoor pond cultures located at any geographic location. Furthermore, an indoor raceway pond with temperature control and LED lighting to simulate sunlight spectrum and intensity was designed and successfully operated under climate-simulated conditions (Figure 12). This system allows one to simulate the climate conditions any place in the United States and determine how a specific algal strain will perform at a specific location of interest and season. This innovative modeling capability combined with the LED system can be used as a low-risk and cost-effective way of screening strains and geological locations for high biomass productivities in outdoor ponds to find the best match between a given strain, climate, and season. In addition the process can also be used for identifying the optimum pond operating conditions, thereby accelerating the scale-up of promising high-productivity strains while quickly eliminating sub-optimal candidates.

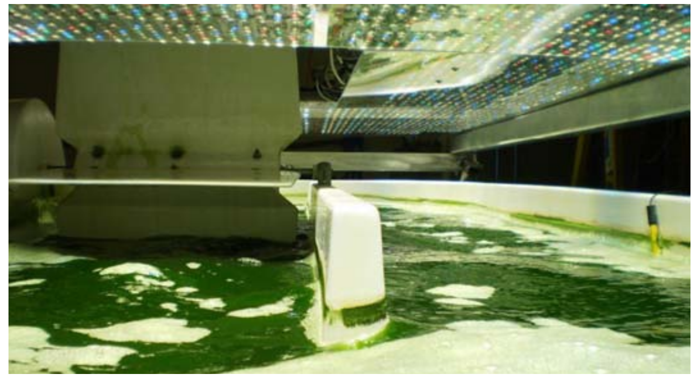


Figure 12. Environmental Simulated Culture System: Indoor raceway pond with temperature control and LED lighting to simulate sunlight spectrum and intensity and climate for any potential production site.

Developed ARID, a unique low-energy pond system that maintains optimum conditions

A key NAABB advancement in developing and operating innovative cultivation systems was the modeling, testing, and design improvements of the ARID pond culturing system (Figure 13). This system provides improved temperature management, maintaining water temperatures within the optimum range for a given microalgae strain throughout the year. Modeling results and measurements demonstrated that water temperatures during the winter in Tucson, Arizona, remained 7–10°C warmer than in conventional raceways. As a result of better temperature management, the ARID system had significantly higher annual biomass productivities compared to conventional raceways. In addition, the ARID design encompassed engineered reductions in the energy use for pumping and mixing through use of a solar powered pumping system and baffled flow system. Cultivation in the ARID system had significantly higher energy productivity (biomass produced per unit energy input) than conventional raceways. By extending the growing season through modulating temperatures combined with lower energy requirements, the impact of the ARID system could be profound. The ARID system could significantly increase annual biomass productivities with lower operating cost for any microalgae strain of choice.

Figure 13. The ARID pond cultivating system.

Conducted and analyzed large-scale cultivation trials on eight algae strains

An important aspect of NAABB was the scale-up of new strains in large-scale culture to assess performance and to provide biomass for downstream processing and analysis. Two large-scale testbeds were utilized: the Texas AgriLife Research facility at Pecos, Texas (Figure 14) and the Cellana facility in Kona, Hawaii (Figure 15).

At Pecos, five algae strains, starting with *N. salina* as the baseline strain and four other strains isolated by the Algal Biology Team, were scaled-up and cultivated at large scale. For each algae strain, the media was optimized, productivity was determined (lipid and ash content), and batches were grown in 23,000 L open ponds with paddlewheels. New production media formulas were developed with 90% lower cost than laboratory media.

At Kona, Cellana's ALDUO™ large-scale cultivation "hybrid" system of PBRs and open ponds was used to cultivate three promising marine strains in their production facility for NAABB. On average, a productivity of 10 $g/m^2/day$ was obtained at both sites for the various strains cultivated at large scale and over 1500 kg AFDW (ash free dry weight) of algal biomass was produced to support downstream processing studies.

Finally, at Eldorado Biofuels, NAABB demonstrated use of impaired water from oil and gas production in outdoor growth.

Closed System Photobioreactor (PBRs)

Open System Open Raceway Ponds

Figure 15. Cellana's large-scale hybrid cultivation system in Kona, Hawaii.

Figure 14.The large-scale NAABB testbed developed at the Texas AgriLife Research facility, in Pecos, Texas.

Cultivation—Next Steps

Moving forward, the characterization of new strains using the LED climate simulation system to optimize conditions for outdoor cultivation is an effective approach to reduce the risk of implementing new cultivation conditions and growing new strains at different large-scale locations.

The large-scale outdoor NAABB testbeds were key resources for evaluating new pond designs (e.g. ARID) and outdoor performance of promising new production strains. Research will be needed on an ongoing basis to optimize sustainable cultivation practices and implement them at large scale.

Future challenges will include developing crop management strategies, such as seasonal crop rotation, and demonstrating them on a large scale to extend growing seasons and overall yearly productivity.

Harvesting and Extraction

Once a selected algal strain has been cultivated, the algal biomass is harvested from the cultivation pond and the harvested algae is typically dried before lipid extraction. Because these steps involve removal and concentration of very dilute concentrations of algae from very large volumes of water, harvesting algae and extracting the lipids are estimated to be significant cost drivers in the biofuel production process. Therefore, the goal of the Harvesting and Extraction Team was to develop low-energy, low-cost harvesting and extraction technologies that could feed biomass and lipids into highly efficient fuel conversion processes. NAABB focused on new harvesting and extraction technologies that would:

- Be easy to integrate with cultivation facilities to limit pumping and power requirements;
- Have low environmental impact, i.e., low or no hazardous chemical or solvent use to enable recycling of water and nutrients with minimum treatment;
- Be capable of high volume processing (at least 100–1000 L/h); and
- Be demonstrated with real-world cultivation samples from the NAABB cultivation testbeds.

In the first half of the NAABB program, NAABB researchers investigated five harvesting and four extraction technologies. Data were collected at lab scale on their performance as well as energy balance and cost factors. Mid-way through the program, these data were provided to the Sustainability Team to conduct a techno-economic analysis of the nine innovative harvesting and extraction technologies compared to baseline technologies (Table 3). Comparisons were on the basis of energy input, chemical costs, electricity cost, operating costs, and parasitic energy loss (PEL).

Evaluated nine innovative harvesting and extraction technologies at lab scale

Based on the results of the TEA study, three of the NAABB harvesting projects and one wet extraction technology were selected to progress to field studies at larger scale:

- Ultrasonic harvesting, a process applying a standing acoustic wave in a flow-through system to gently aggregate algal cells, thereby facilitating sedimentation out of the cultivation media;

Table 3. Baseline feasibility assessment of harvesting and extraction technologies.

Technology	Energy Input (kWh/kg)	Chemical Cost (USD/Kg)	Electricity Cost (USD/kg)	OPEX (USD/kg)	OPEX (USD/Gal)	PEL
Baseline Harvesting Technologies						
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
NAABB Harvesting Technologies						
Chitosan Flocculation	0.005	0.055	0.000	0.055	0.377	0.093
$AlCl_3$ 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
Baseline Extraction Technologies						
Pulsed Electric Field	11.52	0.000	0.922	0.922	6.280	198.9
Wet Hexane Extraction	0.110	0.001	0.009	0.010	0.068	1.904
NAABB Extraction Technologies						
Solvent Phase Algal Migration	1.648	0.947	0.132	1.079	7.352	28.45
Ultrasonic Extraction	0.384	0.000	0.031	0.031	0.209	6.630
Nanoparticle Mesoporous	0.008	54.35	0.001	54.36	370.5	0.137
Supercritical	1.174	0.000	0.094	0.094	0.640	20.27

**The highlighted harvesting technologies were selected for scale-up.*

- Cross-flow membrane filtration, a process using novel ceramic-coated membrane sheets with pore structures and surface properties engineered for algal harvesting;
- Electrocoagulation or electrolytic aggregation, a process applying a charge to algal cells forcing them to aggregate and sediment; and
- Wet hexane extraction via the Valicor process, which is able to capture a high percentage of lipids from algal biomass and was demonstrated at large scale in the Pecos testbeds. This was used to provide oil to the Conversion Team for jet/diesel and biodiesel production.

Scaled up three innovative harvesting technologies

Ultrasonic Harvesting

A pilot-scale ultrasonic harvester was assembled and tested outdoors with *N. oculata* feedstock provided by Solix Biosystems from their Coyote Gulch, Colorado algae cultivation facility. The scaled-up unit operated at 45–225 L/h (Figure 16). The system achieved a typical concentration factor of 6X averaged over trial periods and a peak concentration factor of 18X above the feedstock concentration.

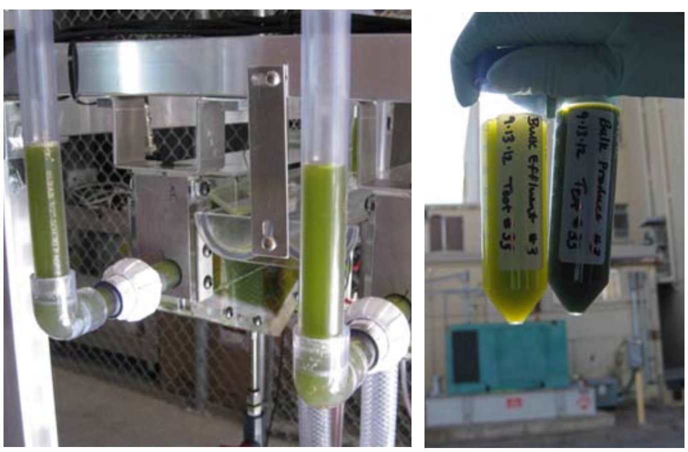


Figure 16. Ultrasonic harvester. Left panel, side view of two 2 L modules attached to a customized cart containing twelve modules. The dilute feedstock is fed into the modules by gravity flow and the concentrate is collected from the bottoms of the modules. Right panel, visual comparison of the dilute feedstock (left tube) and concentrated product (right tube).

Cross-Flow Membrane Filtration Harvesting

For the scaled-up membrane filtration field test, we developed a thin porous Ni alloy metal sheet membrane (Figure 17). A cross-flow membrane module was assembled on a mobile unit that was tested at the Texas AgriLife Research Station, Pecos, Texas testbed facility using active cultures of *N. salina* and *Chlorella* sp. *DOE1412*.

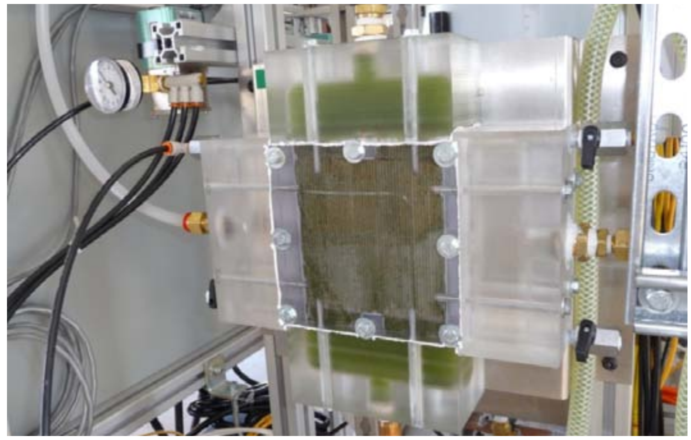


Figure 17. Cross-flow membrane modules assembled for field tests (0.26 m^2 of membrane area, 1 mm feed flow channel opening).

Electrocoagulation Harvesting

The team conducted field tests of the electrocoagulation (EC) process using a commercial electrocoagulation unit from Kaselco (Figure 18), traditionally used for wastewater treatment. The field tests were conducted at the Texas AgriLife Research Station, Pecos, Texas testbed facility using active cultures of *N. salina*. The tests achieved a 50X concentration factor and 95% recovery of algae using only 25% of the energy used by the baseline centrifuge technology. Data from the EC process collected with the commercial Kaselco EC unit were used by the Sustainability Team in their financial model in place of the conventional centrifuge.

All three NAABB harvesting technologies showed promise as primary harvesting techniques. In addition, cross-flow filtration was demonstrated for further dewatering to 24% solids. All showed large energy savings at the demonstrated scales compared to the baseline technology of centrifugation, and may be

Figure 18. Kaselco reactor test bed at NAABB's Pecos, Texas testbed facility.

used in combination with each other to achieve higher concentration factors or higher throughput. Moreover, the Sustainability Team calculated that these three NAABB harvesting methods presented significant GHG emission reduction compared to the centrifuge baseline.

Overall, the field tests served to: (1) demonstrate the feasibility of the technology at the target scale; (2) identify technical gaps needing further research and development, particularly with efficient operation of the scaled-up devices; and (3) introduce potentially game-changing harvesting technologies to industry.

Harvesting and Extraction—Next Steps

Continued refinement and expanded demonstration of the innovative NAABB harvesting technologies at scale would serve to reduce the financial risk to industry, thereby encouraging the acceptance and transfer of these technologies to industrial use. However, further development is needed to maintain consistent high performance over long durations of operation.

A wide variety of real-life microalgal feedstocks need to be tested in each device in order to better understand how broadly each approach can be applied. Additionally, the quality of the feedstock input (e.g., ash content) and its effect on harvesting performance needs to be determined.

Not withstanding these uncertainties and room for improvement, incorporation of NAABB's energy-efficient harvesting technologies into algae biofuel production processes would ultimately lower the cost of algal biofuels and lower the carbon footprint of algal biofuels production.

Conversion

At the start of the NAABB project, the vision for producing fuel was to cultivate and dewater algae, use a wet extraction technique to separate the lipid fraction from the algae, convert the algal oil directly to fuel, and investigate options for the leftover LEA. However, a gap analysis late into the NAABB program identified the need for improved extraction/conversion processes that: (1) significantly improved the overall yield of fuel from algal biomass; (2) could combine extraction and conversion steps, thereby simplifying unit operation; and (3) could convert the whole algal biomass directly into a bio-crude oil that can be upgraded directly to fuels. As a result, a direct conversion process route was evaluated (Figure 19). This direct processing route does not require high lipid-containing algal feedstock and therefore allows for large-scale algae cultivation to focus purely on achieving high algal biomass productivity rather than lipid production.

NAABB utilized its cultivation testbeds to produce algal biomass as previously described and distributed it throughout the consortium to investigators working on lipid extraction and various conversion pathways. Sufficient quantities of crude lipid, LEA, and whole algal biomass materials were produced to perform all fuel conversion experiments at the bench and pilot scale using actual algal production strains instead of surrogate oils and/or algal biomass. Along the way, NAABB obtained an unprecedented amount of characterization data of the biomass, lipid extracts, crude oil, LEA, and upgraded fuel products from various algal strains and processes.

Developed detailed algal biomass and biofuel characterization methods

A Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry method was developed for comprehensive characterization of neutral, polar, and membrane lipid components from many algal, crude lipid extracts, bio-oil, and fuel samples produced by NAABB consortium partners. This new analytical technique provides significantly more information than the traditional methods and allowed NAABB researchers to study changes in lipid composition in greater detail. Using this approach, NAABB researchers monitored lipid profiles during growth cycles and identified a novel class of sulfate lipids in several marine species. After the algal fuels were produced through various conversion processes, the fuels were analyzed to compare the yield and composition.

Produced jet/diesel fuel that met ASTM specifications using NAABB strains and production pathways

The majority of the lipid conversion to fuel was done using UOP's Ecofining™ process, a commercialized catalytic hydrotreating technology similar to that used by the petroleum industry, to produce jet fuel, diesel fuel, and naphtha (gasoline). The NAABB Conversion Team realized early on that crude algal oil is not of sufficient quality to process directly; hence, as shown in Figure 19, a pretreatment step was developed to remove problematic metals, corrosive ions, and organic contaminants.

A significant accomplishment was production of algal fuels that met ASTM standards. This demonstrated that algae-based fuels produced using NAABB strains and processing technologies were of sufficient quality for use in diesel and jet engines. The majority of this work was done with four different algal species: two marine strains, *N. salina* and *N. oceanica*; and two freshwater strains, *A. protothecoides*, and *Chlorella* sp. *DOE1412*. Table 4

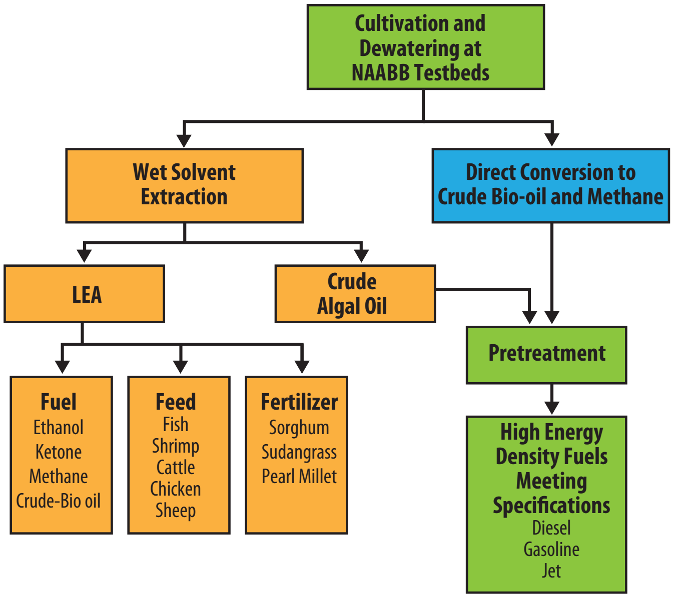


Figure 19. Pathways investigated by NAABB for producing fuel: The traditional method via extraction and separation of lipid material from LEA (left path on chart) and the direct method (right).

provides the jet fuel specifications and how NAABB-generated algal fuels met these standards from the two marine strains. The fuel specifications for diesel were also met for these strains and selected freshwater strains as well. The consortium was able to cultivate multiple algae strains in outside testbeds; and harvest, extract, and convert the algal oils and or biomass to fuel using a variety of different conversion processes.

In addition to the Ecofining process, fatty acids from *Nannochloropsis* sp. and *Chlorella* sp. algae were converted to fuel using two other industrial processes: (1) the Centia process that uses catalytic decarboxylation, for production of jet fuel and diesel fuel; and (2) the Albemarle process, that uses a solid acid catalyst to produce fatty acid methyl esters (FAME), commonly called biodiesel.

Developed processes and economic models for eight fuel production pathways

Techno-economic models and Aspen process models were completed for the majority of the fuel conversion processes using algae thermophysical property data. Previously, the majority of the models were based on surrogate property data from soy or corn, which can lead to inaccurate predictions. The advantages to having modeled all these processes include the ability to:

- Compute CAPEX and OPEX to compare processes at a variety of scales;
- Include costs of ancillary equipment (pumps, heat exchangers) in all estimates;
- Estimate value of co-locating a process within an existing facility (petrochemical, power plant, wastewater treatment, etc.);
- Model the effects of contaminants or residual upstream processing compounds (metal ions, inorganics) on the downstream process;

Table 4. NAABB algal biofuels met the jet fuel specification.

Algal Biomass Source				Cellana *N. oceanica* (High Lipid)	Solix *N. salina* (High Lipid)	TAMU Pecos *N. salina* (Low Lipid)	Cellana *N. oceanica* (High Lipid)	Cellana *N. oceanica* (Low Lipid)
Extraction Process				Inventure FAME	Valicor Wet Solvent	Valicor Wet Solvent	Valicor Wet Solvent	PNNL HTL
Crude Oil Type				Distilled FAME	Crude Lipid Extract	Crude Lipid Extract	Crude Lipid Extract	HTL Bio-Oil
Parameter	**D7566 HEFA Specification**	**Jet A**	**Jet A1**					
Density (g/L)	730 - 770	775 - 840	775 - 840	755	753	756	749	780
Freeze Point (°C) max	-47	-40	-47	-49	-63	-62	<-80	-57
Flash Point (°C) min	38	38	38	43	40	45	40	59
Distillation								
10% Recovered Temp (T10) °C max	205	205	205	156	160	150	152	167
Final Boiling Point (°C) max	300	300	300	279	271	284	264	272
T50-T10 min	15	–	–	36	34	39	28	37
T90-T10 min	40	–	–	92	85	84	70	75

- Optimize production and "right-size" a facility (i.e., determine if one large process stream is desirable running at partial capacity in winter months or if it is better to have multiple smaller process streams to adjust to varying feedstocks); and
- Understand the ability to integrate production of fuel from algae into existing petrochemical plant infrastructure through blending of feedstock oil.

Demonstrated a high yield, direct conversion HTL process

Direct conversion of the wet whole algae biomass to bio-oil was investigated using the thermochemical processing method of hydrothermal liquefaction. In addition, a catalytic hydrothermal gasification process was investigated for the conversion of wet LEA to methane and as companion waste water treatment for HTL processing. The combined HTL-CHG processing route resulted in the best oil yields, process economics, and life cycle assessment. A simplified process flow diagram for the combined HTL-CHG process with pictures of resulting process streams is shown in Figure 20.

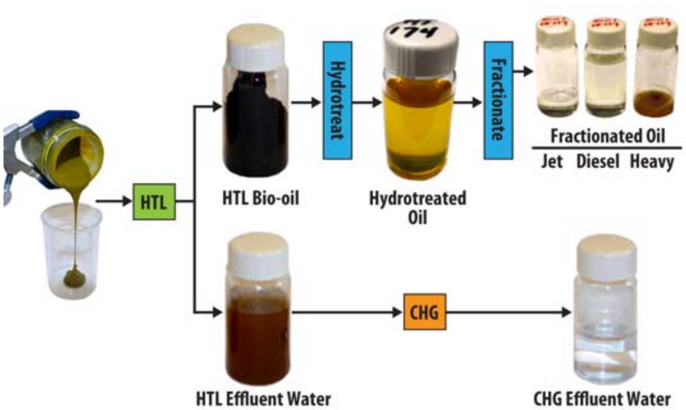


Figure 20. The process flows of HTL and HTL with CHG.

Wet algal biomass (15 -20% solids) is fed directly to the HTL system, which produces bio-oil and an effluent water stream that phase separates without the need of solvent extraction. The bio-oil stream is readily upgraded via hydrotreating to hydrocarbon fuel. The hydrotreated oil can then be fractionated into jet, diesel, and naphtha fractions. The effluent water stream is then processed with CHG to recover additional fuel in the form of a methane gas/carbon dioxide mixture, and the water stream is recycled to a pond. Advantages of the HTL-CHG processing pathway include: (1) capture of 85% of the carbon in algae as fuel-grade components (bio-oil that can be upgraded to diesel, jet, gasoline, and syngas); (2) production of a bio-oil that can be readily converted to meet diesel and Jet A fuel standards; (3) effective wastewater treatment to reduce the organic content and provide methane for process energy; (4) recycle of water and nutrients (nitrogen, phosphorous, and other trace minerals for algal cultivation; and (5) significant decrease in capital and operating costs compared to processes requiring high lipid-yielding algal biomass and extraction of the lipid from the biomass. As part of the NAABB effort a pilot-scale system that can be used for both HTL and CHG process development was designed and is being built by one of the NAABB industrial partners. The Sustainability Team used data from the HTL-CHG process in their financial model, in place of the baseline lipid extraction process.

The NAABB consortium also investigated biological conversion processes for LEA including:

- Hydrolysis followed by ethanol fermentation; and
- Mixed organic acid fermentation followed by ketonization and catalytic upgrading to aromatics.

Conversion — Next Steps

NAABB demonstrated that a variety of algal strains could be converted into high quality fuels that met ASTM standards. Several different conversion processes were shown to be effective. Next steps include understanding long-term catalyst performance and materials of construction. This will include developing low-cost methods that purify intermediate crude oil streams.

A major NAABB advancement was the development and demonstration of a combined HTL-CHG process that uses algae concentrated from the pond. This new method produces a high yield of algal bio-crude that can be readily upgraded to hydrocarbon fuels. Further optimization, integration, and scale-up are needed for full integration of these combined technologies that can be broadly deployed while significantly reducing processing costs, simplifying operations, and providing means to recycle water and nutrients.

Finally, techno-economic and life-cycle models should be continually updated as new data become available and applied to guide future research as algal conversion processes are developed, scaled-up, and demonstrated in industrial settings.

Agricultural Coproducts

The traditional vision to produce biofuel from microalgae is to cultivate the algae, separate the biomass from the spent culture media, extract the lipids, and convert the lipids to fuel. Since an industry must effectively use every part of the raw material to be environmentally and financially sustainable, this team addressed the question: Can the LEA, which is high in protein, be used as feed or fertilizer?

Extensively characterized LEA for feed value and contaminant concentration

Having a consistent and high quality feed that is well characterized is required if the LEA will be used as animal feed. Prior to NAABB this information was scant in the literature. Hence, NAABB extensively characterized LEA from several sources. The feed value of LEA is largely driven by:

- **Organic matter**—The organic matter content ranged from 40% to 76%. Lower organic matter contents are a result of higher ash content in the LEA. This dilutes the valuable components of LEA (protein, lipids, and energy), which decreases the price per ton.
- **Crude protein**—The protein content ranged from 12% to 38%. A high ash content dilutes the crude protein; however, on a protein basis, there are no data to suggest a discount relative to other feedstuffs.
- **Residual lipid**—The residual lipid content ranged from 1% to 10%. Any lipid remaining in the LEA increases the energy content of the LEA and translates into increased value.
- **Mineral content**—The effects of growth and harvesting strategies, such as the presence of heavy metals in the water supply or use of flocculants to aid in harvesting, significantly impact the mineral profile of the LEA and can increase the concentrations of divalent cations (calcium, aluminum, iron, and manganese) that are regulated by the feed industry. This highlights the potential influence that upstream processes for harvesting can have on the value of LEA and the potential for toxicity.

Demonstrated palatability of LEA in animal studies

Feeding studies using LEA were performed with sheep, swine, shrimp, chicken, fish, and cattle (Figure 21). This is the first set of data on use of LEA in both animal and mariculture feed. Table 5 provides a summary of the findings. The major issue is that mineral content must be closely monitored and upstream processes standardized to produce a more consistent biomass. NAABB valued LEA as a feed supplement for animals at $160/ton and for mariculture at $200/ton. Whole algae for mariculture is valued at closer to $400/ton.

Evaluated the use of LEA as a fertilizer

NAABB fertilizer studies were primarily completed in greenhouses using pearl millet and sorghum-Sudangrass (Figure 21). Based on the results of the fertilizer evaluation experiments, LEA:

- Is labile and highly mineralizable, compared to wheat straw;

Table 5. Summary of the feeding studies conducted on animals and mariculture.

Type of Animal Tested	Performance	Digestibility/Palatability
Fish (red drum and hybrid striped bass)	Replaced up to 10% of crude protein from fishmeal and soy protein concentrate with LEA without causing substantial reductions in fish performance.	Excellent
Shrimp	At least a 20% inclusion level of LEA could replace the expensive soybean and/or fish meals in shrimp feed.	Excellent
Cattle	Supplementation of LEA stimulated forage utilization to a similar extent as cottonseed meal in cattle (100 mg N/kg body weight).	Blends of LEA and conventional protein supplements will minimize concerns of palatability. Does not impair fiber digestion.
Sheep	LEA may be a viable protein and mineral supplement for sheep; however, caution is advised for diets containing greater than 20% LEA due to slight reductions in performance.	Good
Pigs	Use of LEA is not recommended at this time. Supplementation with 5–20% LEA was tested and reductions in growth and weight gain were noted.	Not palatable
Chicken	Inclusion of 5% LEA in young broiler chicken and laying hens diets may be viable.	Good

Figure 21. (Top) LEA was made into pellets for use in some of the animal feed studies. (Middle) LEA feed studies were conducted in cattle. (Bottom) LEA was evaluated as a fertilizer.

- Provides a source of available N with residual nirate-nitrogen after the growing season; and
- Provides sufficient nutrients to produce greater yield than inorganic fertilizer for at least two growth cycles.

Based on analysis of the current prices for N, P, K, and char, the value of LEA is about $30/ton.

Agricultural Coproducts—Next Steps

LEA contains protein and minerals and is palatable for many animal species. However, the wide variability of feedstocks is a challenge for consistency.

The value of LEA for feed markets did not offset the cost of separations of lipids from algal biomass. Use of fertilizer has an even lower value.

In summary, while NAABB extended the science foundation for agricultural coproduct use, we do not recommend further research in this application area for DOE.

Sustainability

Given the complexity of the NAABB project, the Sustainability Team broke the modeling process into a series of subtasks. NAABB partners developed estimates of the energy, economic, and environmental impacts of algae-based biofuels, using multiple platforms to address sustainability. A unique aspect of the NAABB effort was the integration of several modeling platforms to address sustainability based on the same set of assumptions and operational scales.

As an extension of DOE's sustainability-model harmonization effort, experimental data from NAABB were used to update or modify the models in the harmonization series. The NAABB Sustainability Team helped bring together the life cycle assessment (LCA) and techno-economic analysis (TEA) communities to develop a consistent set of assumptions and agree on a baseline for comparison of NAABB-developed technologies to the NRC report and to the DOE harmonized models for algae-based biofuels.

Evaluated the economic and environment impacts of fifteen different technologies

Observations from Field Cultivation Data

To measure the environmental, economic, and energy characteristics of algal fuel production, it is important to have accurate estimates of biomass production. One significant limitation of the current models based on the existing literature on algae cultivation is the extrapolation of productivity and yield from lab-based experiments. It is well known that the productivity values measured in the lab do not translate into production in the field. NAABB was able to collect first-hand production data from five different outdoor algal cultivation facilities over a multi-year time period, thereby gaining a unique understanding of production of algae in the field and addressing this significant limitation of the literature on the economic and environmental profile of algal biofuels. One of the most interesting aspects of the data is the variance in productivity by season. As expected, the changes in solar irradiance and temperature affect algal productivity. The data show that a simple average is not an appropriate assumption for productivity measures. Thus, economic and life cycle analyses should explicitly incorporate the seasonal risk of biomass production.

Analyzed production data from outdoor algal cultivation facilities

Regional Feasibility of Algae Production

NAABB analyzed a large number of resource-feasible algae production sites in the United States selected from the Biomass Assessment Tool (BAT) analysis (Figure 22) for the production of 5 billion gallons per vyear (BGY) of algae biofuel in three different organism-based scenarios: a generic freshwater strain, a specific freshwater strain *(Chlorella)*, and a saltwater strain *(N. salina)*.

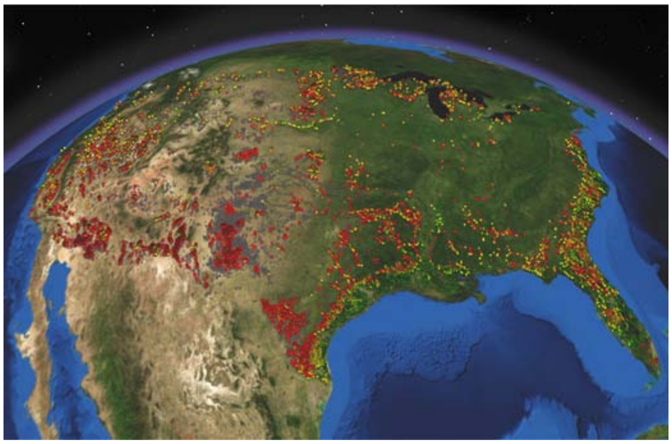


Figure 22. Resource-feasible algae production sites in the United States were selected from the BAT analysis

Overall, the site-selection results show remarkable similarity between the freshwater scenarios. The productivity values and number of sites required to meet the 5 BGY target are essentially the same in the states along the Gulf of Mexico and South Atlantic. However, the organism chosen for the saltwater scenario *(N. salina)* had a much lower biomass production rate and, despite the higher lipid content, required nearly twice the number of farm sites to reach the 5 BGY target. In addition, the average cost for providing saltwater to a site is over $1 million. It is doubtful that there are enough economical saltwater sites in the study area to meet the 5 BGY target based on *N. salina*. The results show that it is especially important to maximize production when utilizing saline waters to offset the added supply costs.

LCA Analyses

NAABB examined the energy and material results of twenty-four different growth scenarios published in the literature. The GHG emissions for these scenarios ranged between 0.1–4.4 kg CO_2eq/kg biomass, with the fossil energy demand of 1–48 MJ/kg biomass. Based on this large variation, the potential for algal biofuels to reduce GHG emissions depends on the design of the complete algal fuel production pathway. NAABB further used a resource assessment model that provided productivity and water demand month by month for several thousand locations over a simulated period of 30 years for the generic freshwater, freshwater *Chlorella*, and saltwater *N. salina* cases. These data were studied with regard to seasonal, monthly, and yearly variability. The GHG emissions during winter months were both highly variable over the thirty-year period and large in absolute value compared to emissions associated with petroleum diesel, which has 99,900 gCO_2 MMBTU. GHG emissions averaged annually, and over various seasons are presented in Table 6. A key result of this analysis is that a robust growth regime over the entire year may be more important to algae GHG emissions and energy use than choosing the highest peak value. Overcoming low winter biomass productivity, which leads to large winter emissions and highly variable fall emissions, remains a challenge.

Table 6. GHG emissions by algae strain with multiple-season averages (g CO_2eq/MMBTU).

	Generic Algae Strain	*Chlorella*	*Nannochloropsis*
Annual	82,800 ± 10,500	134,800 ± 34,500	176,400 ± 46,300
Spring, Summer, Fall	65,100 ± 1700	76,100 ± 6000	106,400 ± 9100
Spring, Summer	62,500 ± 1100	65,500 ± 2700	94,800 ± 4700

Determined that a robust growth regime over the entire year is more important in lowering GHG emissions than selecting for peak productivity

Cultivation and Fuel-Production Pathways

Two NAABB pathways were selected for LCA analysis: the ARID pond design and the hydrothermal liquefaction of LEA. ARID was considered because pond-mixing energy is one of the largest contributors to energy demand in the baseline process, accounting for roughly a quarter of the life-cycle fossil energy inputs to produce renewable diesel. The ARID, when using pumps with 60% total efficiency, reduces mixing energy from 48 kWh/ha/d (baseline raceway) to 24 kWh/ha/d and decreases the lifecycle GHG emissions by about 30%.

NAABB investigated operations that converted lipid extracted residuals and whole biomass from *N. salina* to diesel blend stock by HTL and subsequent upgrading by hydrotreating. The associated process model was used for anaerobic digestion. These two scenarios showed that improvements in GHG emissions compared to the baseline harmonization process can be made for both scenarios and lower fossil energy use is possible by processing whole biomass with HTL.

Further, different hydrotreated renewable fuel conversion systems were analyzed using the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation (GREET) model, harmonized inputs, and proprietary UOP jet fuel inputs. The life-cycle GHG emissions for jet fuel were 89,150 g CO_2 eq/MMBTU compared to 67,630 g CO_2 eq/MMBTU for renewable diesel.

Scaling Algae Systems for Seasonal Production

The Sustainability Team assessed the economic variability of algal systems considering spatial and temporal constraints. Figure 23 shows the average cost per gallon of triacylglycerides (TAGs) for the harmonized baseline design for six sites across the Southeast and Southwest.

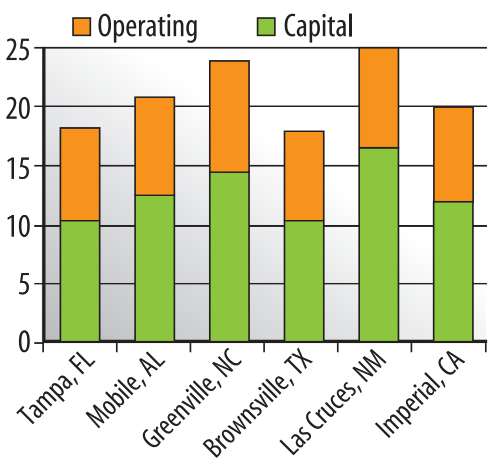


Figure 23. Cost assessment ($/gallon TAG) of the harmonized baseline design in several locations across the southern United States using respective BAT algal biomass productivity.

Regardless of the region, biological factors play a significant role in making algae a viable feedstock for biofuel production. To drive costs below $5/gal TAG, the biomass productivity will need to be increased 2.5X over the baseline with a corresponding lipid content to 45%. Engineering advancement to existing technologies or development of new, innovative technologies will also be needed to decrease the cost below $5/gal.

Energy-Limited Model of Algal Biofuel Production

The absence of thermodynamic and kinetic rate data for algae has previously limited the validity of computer-aided models. Therefore, NAABB integrated algae-specific data into TEA models to better understand the traditional pathway of producing biodiesel from crude lipid. The important outcomes of the energy-limited model and areas for additional research include (1) the importance of recycling water, carbon, and debris and investigating the effects on algal growth; (2) the impact of pumping large amounts of water and developing methods to minimize this; (3) the importance of photosynthetic efficiency and continuing algal biology studies to improve this through genetic engineering; and (4) the value of using an integrated systems approach and computer-aided simulation. Overall, these models demonstrate that, for the algae to-biodiesel industry, improvements in cultivation to increase productivity (either through biological or pond improvements), better harvesting and extraction methods tested at large scales, and maximizing recycle in the production process are required.

Developed a rigorous approach for assessing technologies to fully evaluate seven scenarios

Financial Feasibility Analysis

The Sustainability Team analyzed the economic feasibility of alternative NAABB technologies for the production of algal biofuels. The technologies were all demonstrated at sufficient scale to provide adequate information, showed promise for reducing costs over baseline, decreased overall energy utilization, and showed potential for scalability. In addition, an evaluation was included for GMO data from a laboratory strain of algae showing the potential for substantial productivity improvements (Table 7). The technologies selected for the financial analysis show significant gains in lowering costs, reducing energy

Table 7. 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	Crude HTL oil & methane	Crude HTL oil & methane	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/m²/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	13,510,000	42,320,000	20,330,000	51,570,000
Location	Pecos, TX	Pecos, TX	Tucson, AZ	Pecos, TX	Pecos, TX	Tucson, AZ	Tucson, AZ

input requirements, increasing production, and increasing receipts. For example, HTL-CHG reduces energy consumption 98% over wet solvent extraction by eliminating unit operations and the use of solvents; the GMO strain increases production 250%; and ARID cultivation increases receipts 27%.

The results from simulating a large algae farm with technologies developed by NAABB suggest that algal crude oil could be financially feasible if CAPEX and OPEX can be reduced further. However, the NAABB innovations remain untested in large outdoor raceways. Great strides have been made by the NAABB consortium, but continued enhancements are needed in algal biology and would be further useful in the areas of cultivation, harvesting, and extraction. Further research to improve algal biology and crop protection is a pathway to reduced costs of production for algae crude oils. The total costs that may be expected from improved biology are summarized in Figure 24, assuming no reductions in CAPEX and OPEX. These costs start with the combination of NAABB technologies (Scenario 7) and then decrease in a nonlinear fashion as we increase the biomass productivity to drive costs of algal crude oil to $7.50/gallon. Further analysis shows that with the NAABB technologies, biomass productivity will need to be increased along with further reductions in CAPEX and OPEX through new approaches in cultivation and decreases in water and other nutrient utilization in order to hit a $2/gallon biocrude target.

Sustainability–Next Steps

Based on the research conducted by NAABB, the following broad research areas are important to the sustainability of algal biofuels and are in need of further evaluation:

- Reduction of water in the entire production system;
- Robust cultivation, harvesting, and extraction systems;
- Improved production strains;
- Cost-effective sourcing of CO_2, water, and nutrients; and
- Improvements in industrial design and logistics.

The work completed by NAABB highlights the need for innovative research into cultivation technologies and the conclusion that this research must be closely linked to the extraction technologies. By considering water in cultivation conjointly with extraction, nonlinear reductions in the environmental and economic impacts of algal-based biofuels can be realized, which will push algal fuels onto a more sustainable pathway.

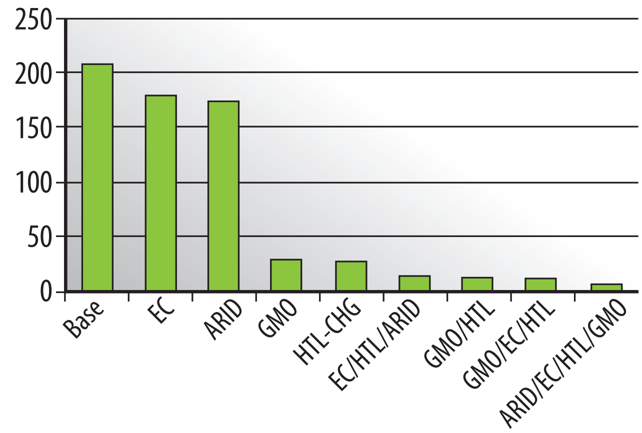


Figure 24. Total economic plus financial costs of algal crude oil production for pre-NAABB technology and for alternative technologies developed by NAABB ($/gallon).

NAABB Management, Organization, and Approach

Effectively capturing the value of consortium research requires a strong and efficient leadership and team structure (Figure 25).

- The Donald Danforth Plant Science Center was the Lead Institution and provided project and finance management and administrative support;
- The Board of Directors assured the overall strategy of the consortium met DOE and member needs and oversaw the business and other affairs of the consortium;
- The External Advisory Board assessed progress towards scope, developed strategy for outlying years, and provided advice on future initiatives to the Board of Directors and Executive Management Team;

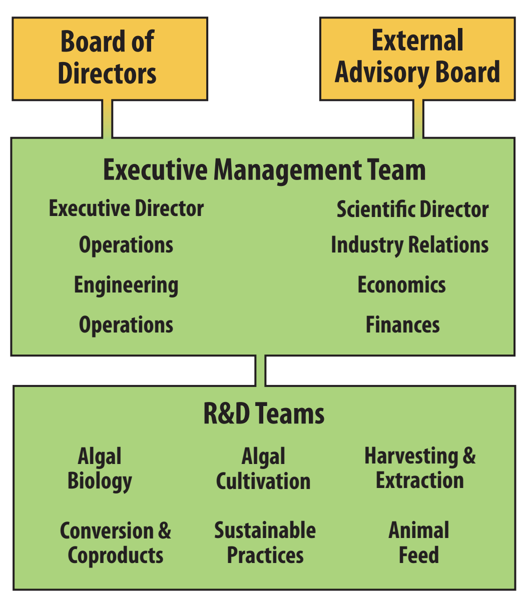


Figure 25. NAABB's management, organization, and approach structure.

- The Executive Management Team established goals and objectives and executed the program;
- The Operations Team oversaw the integration of over eightyv individual projects, both within each major task area and between the major task areas; and
- Team Leads oversaw the science focus of the individual projects and the overall science and technology within their respective teams.

NAABB operated as a dynamic consortium. As needs were identified, new projects and team members were added. As projects finished, institutions and teams were deactivated. Hence, at the initiation of the program twenty-eight institutions were part of NAABB. At the conclusion the number of organizations who had played a role grew to thirty-nine. Additions were made based on specific needs of the consortium and expertise of the performing institution. Collaborations included two international partners.

Major Deliverables

NAABB expanded the state of technology for algae-based advanced biofuels through the following major accomplishments:

- Screened over 2000 algal strains from nature;
- Discovered a new high-performing strain, *Chlorella* sp. *DOE1412* (Figure 26).

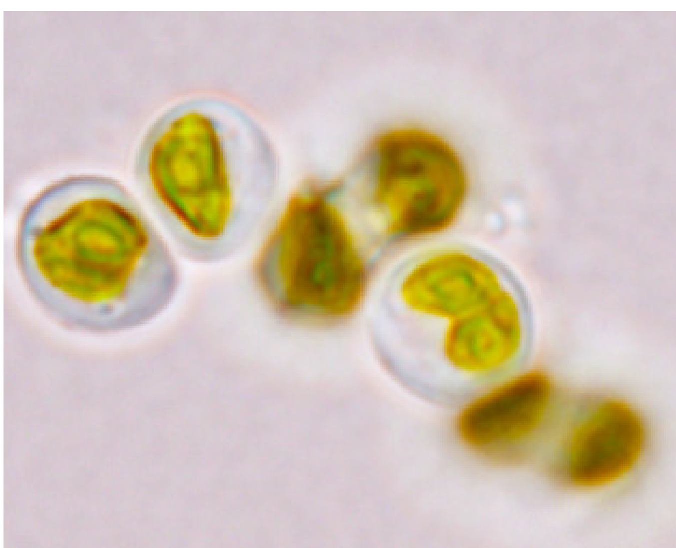


Figure 26. Photomicrograph of the NABBB-discovered strain *Chlorella* sp. *DOE1412.*

- Developed an algal biology toolbox for new strain transformation with 50 gene targets and 8 new sequenced strains;
- Demonstrated new strains in large outdoor ponds and took the biomass through the entire process to produce green diesel/jet fuel, biodiesel, and various products;
- Validated the use of lower-cost media and impaired water in cultivation;
- Improved cultivation methods with improved heat management, CO_2 utilization, and low-energy mixing;
- Demonstrated 3 innovative harvesting technologies at larger scale;
- Converted NAABB–derived algae to fuels that met standard specifications for quality;
- Demonstrated strong cost savings by combining unit operations for wet extraction and conversion;
- Developed the most comprehensive data set available on agricultural coproducts; and
- Completed 7 scenario models that carefully examined the algal enterprise.

In addition, NAABB outreach made the following contributions to the algae technology community:

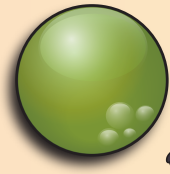
- Deposited 30 new algae strains into the UTEX culture collection;
- Started a new peer reviewed journal, *Algal Research* (by Elsevier);
- Initiated a new conference series: *International Conference on Algal Biomass, Biofuels, and Bioproducts*;
- Contributed over 100 peer-reviewed publications. NAABB publications have an overall Impact Factor of 4.6, and an H-Index of 11 as of February 2014;
- Compiled 5 advanced-degree theses;
- Filed 37 intellectual property disclosers; and
- Formed one new company, Phenometrics.

Conclusion: The Road to $7.50 per Gallon

In the following section we summarize NAABB's impact on the algal biofuel production process in a storyboard format. A more detailed discussion of all aspects of the NAABB consortion are available in the Full Final Report.

DISCOVERY

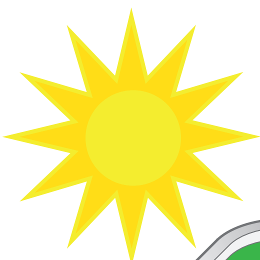
The Road to $7.50/Gallon

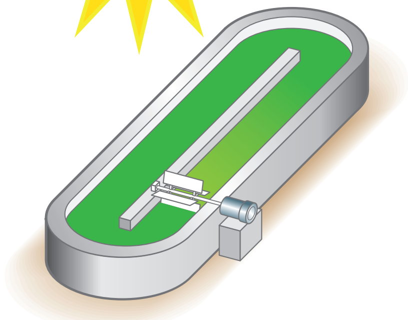


Isolation of New Algae Strains

To make algal biofuels more cost competitive, NAABB set out to find new strains of algae better suited for industrial production than known strains. NAABB developed innovative strategies for discovering strains that grow fast with high lipid yields over a wide range of temperature, light, water, and culture conditions. Over two years, NAABB screened over 2200 wild type strains and deposited 30 strains in the UTEX culture collection. *Chlorella* sp. *DOE1412*— discovered in a ditch in West Texas—showed tremendous potential for use in biofuel production and was selected in 2011 to be the NAABB production platform strain. *Chlorella* sp. *DOE1412* was fully sequenced and the sequence information will be used to guide genetic engineering of it in the future to increase photosynthetic efficiency and productivity.

CULTIVATION

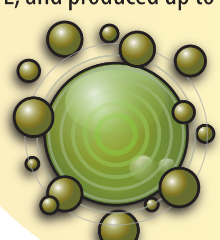




Cultivation

To fully characterize *Chlorella* sp. *DOE1412*, it was grown first in a climate-controlled system that simulated sunlight and the data were modeled using the Biomass Assessment Tool to predict optimal annual biomass productivity. Since the simulation results looked favorable, *Chlorella* sp. *DOE1412* was moved from the laboratory to the field. It was grown outdoors in 23,000 L NAABB testbeds that have a traditional raceway design with paddlewheels. It was also grown in newly developed systems including the Aquaculture Raceway Integrated Design (ARID) temperature control system. *Chlorella* sp. *DOE1412* performed well outdoors, growing at up to 30 g/m^2/day. Furthermore, it tolerated temperatures from 40°–110°F, withstood a range of salinity from freshwater to 25 g/L, and produced up to 25% lipid in open systems.

HARVESTING



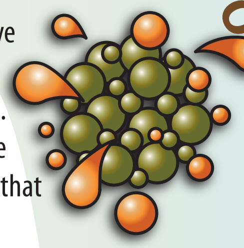
Harvesting

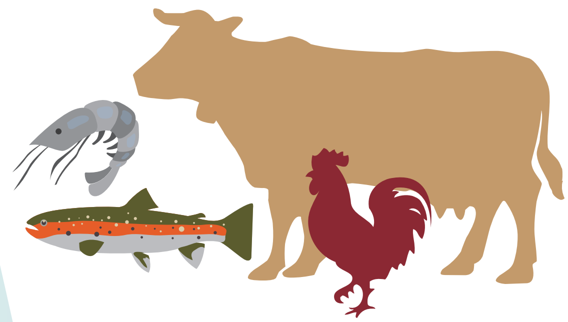
After a strain is cultivated in an open pond, the dilute algal biomass must be separated from the water. The traditional harvesting method is centrifugation, which NAABB employed extensively to harvest *Chlorella* sp. *DOE1412*. NAABB also successfully demonstrated the ability to harvest *Chlorella* sp. *DOE1412* in a higher-efficiency, high-flux cross-flow filtration system with new lower-fouling membranes and in an electrocoagulation (EC) process with 95% recovery efficiency.

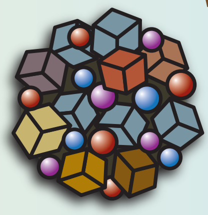
CONVERSION TO FUEL OR COPRODUCT

Fuel and Feed or Fuel Alone?

Traditionally, the steps following harvesting involve extracting the lipids from dried biomass for conversion into fuel and capturing the residual biomass or lipid extracted algae as a coproduct. NAABB employed a wet extraction process to achieve lipid separation, thus avoiding a costly drying step. The wet-extracted *Chlorella* sp. *DOE1412* was successfully converted by the NAABB team into Jet A and biodiesel fuels that met ASTM specifications. In addition, the *Chlorella* sp. *DOE1412* LEA was evaluated for digestibility and nutrient value as animal feed and successfully met criteria to be used as a feed supplement. Finally, NAABB also used a hydrothermal liquefaction (HTL) process to convert whole wet *Chlorella* sp. *DOE1412* biomass into high quality fuel.





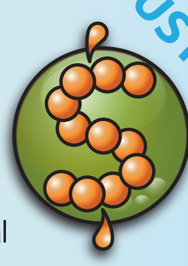


Is it Sustainable?

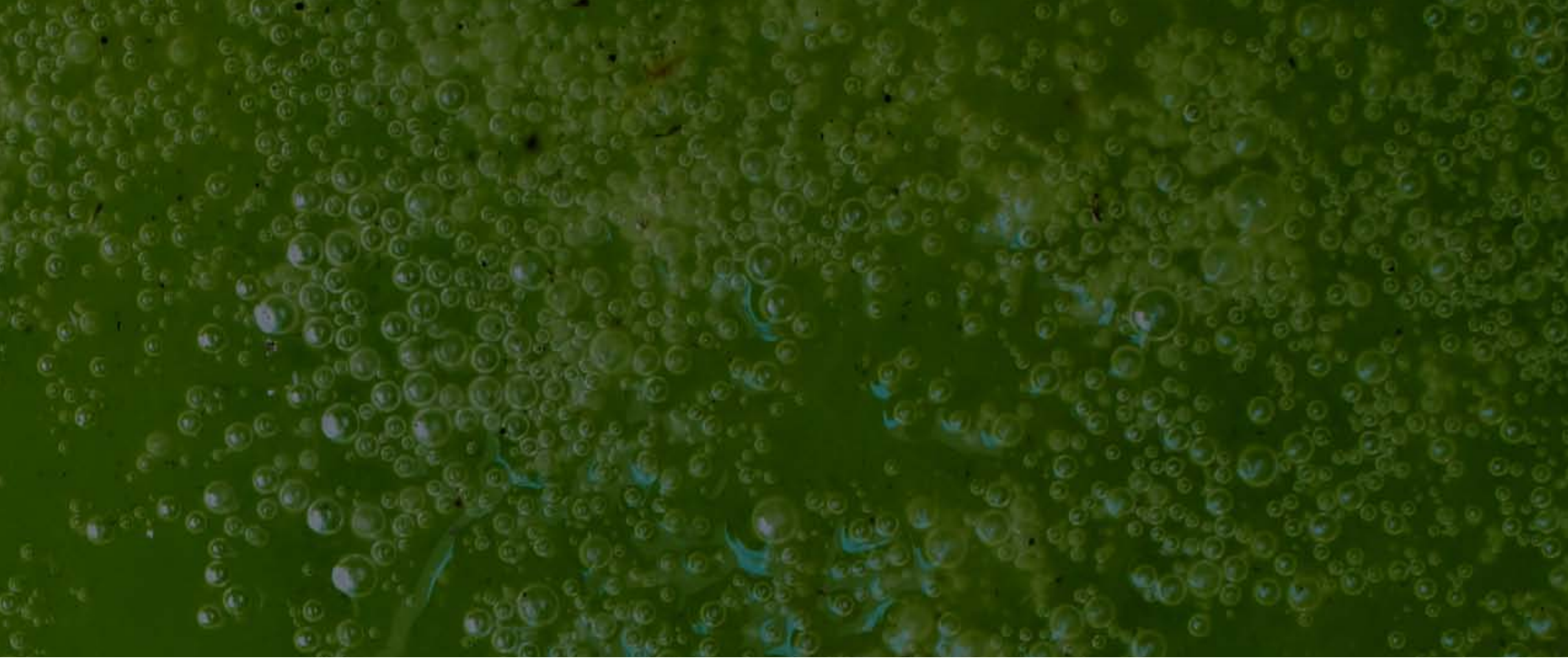
Within a three-year time span, NAABB researchers isolated a strain, grew it in large-scale outdoor ponds, harvested it using innovative technologies, and converted it to fuel via two energy efficient extraction and conversion pathways.

The big picture questions are: When will fuel made from algae be available for wide-scale use? Is it economically and environmentally sustainable? Although a definitive timeline is difficult to provide, the *Chlorella* sp. *DOE1412* story demonstrates how much can be accomplished through consortium research. Experimental results from field studies with *Chlorella* sp. *DOE1412* have been incorporated into economic models, which demonstrated that the cost of producing fuel from this organism through the ARID, EC, and HTL pathway can be decreased from more than $200 per gallon to less than $8 per gallon biocrude compared to the traditional raceway, centrifuge, wet extraction, and conversion pathway. Life cycle assessment models incorporating data from processes that used *Chlorella* sp. *DOE1412* also found favorable results, provided that nutrients can be recycled during processing and growth can be smoothed throughout the annual production cycle. Under those circumstances, our models showed that algal fuels can qualify as advanced biofuels and produce fewer GHG emissions relative to petroleum fuels.

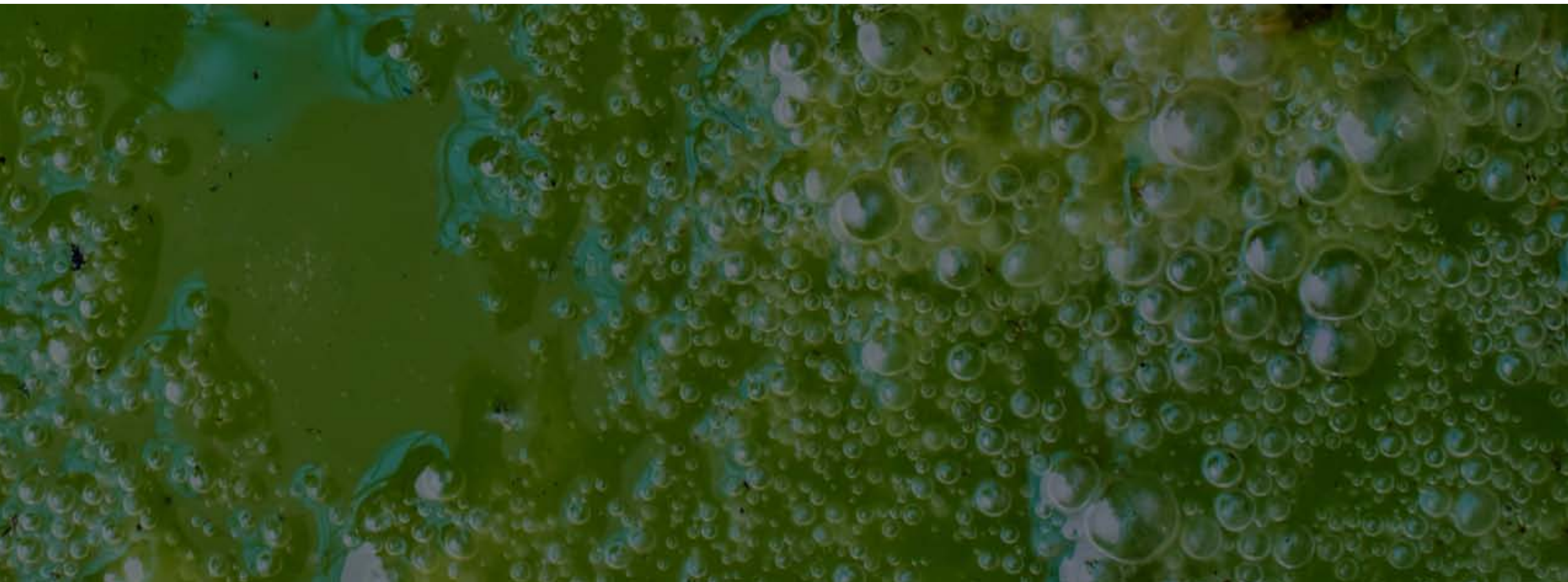
SUSTAINABILITY



The three years of integrated research produced by NAABB demonstrates that a sustainable algal biofuels industry is possible and that further interdisciplinary research can produce both the incremental improvements necessary to be sustainable and the breakthrough advancements that can revolutionize the production of advanced biofuels.







Perspective 1. NAABB was Preceded by the Aquatic Species Program

The genesis of the U.S. DOE's algal biofuels program was the Aquatic Species Program (ASP). From 1978 to 1996, the DOE Office of Fuels Development funded a program to develop renewable transportation fuels from algae. The main focus of the program was producing biodiesel from high-lipid-content algae grown in ponds, utilizing waste CO_2 from coal-fired power plants. Over almost two decades, this program made significant advances in the science of algal biology for manipulating lipid content of microalgae and the engineering of microalgae production systems. The total investment made in the ASP was $25.05 million over an approximate 20-year period.

While the ASP clearly served as an important precursor to NAABB and other ongoing R&D efforts for the development of algal biofuels, there are also some important distinctions between these programs. In contrast to the ASP, the NAABB consortium was a much more intensive effort with a budget of $67.7 million ($48.6 million DOE and $19.1 million cost share) spread over only a three-year period from 2010 to 2013. Whereas the entire ASP budget focused exclusively on upstream processes for algal biology and cultivation methods to produce algal lipids, the NAABB program budget was integrated more evenly across both upstream and downstream processes with a very significant modeling component. Certainly the large investment of stimulus dollars made available for the NAABB consortium provided the kind of funding levels needed to establish a critical mass of integrated R&D to aggressively tackle the entire algal fuel cycle. At the same time, the relatively short three-year project period did not really allow enough time to take full advantage of the momentum of the very effective R&D consortium.

While the ASP stated goal was producing fuel from algae, there was no significant effort focused on the extraction of lipids or the conversion of biomass or lipids to fuels. In contrast, NAABB was a fully integrated program where selected algal species were scaled up in cultivation and then harvested, extracted, and converted to fuel products using a variety of different processes. Various algal feedstocks, algal extracts, and resultant fuel products were thoroughly characterized and detailed process models were developed. This has resulted in a one-of-a-kind data set and process models for evaluating techno-economic and life-cycle implications for the entire algal fuel cycle. These kinds of data along with the analysis and insights that can be derived from detailed integrated process evaluations conducted within NAABB were not available from the ASP.

Applied Biology

The ASP focused its algal biology efforts on a fairly specific aspect of algae and their ability to produce natural oils. This involved bioprospecting to find microalgal species that produced a lot of oil but could also grow under severe conditions. Although a number of algal strains were investigated for growth and lipid-production properties, the best candidates were found in two classes, the Chlorophyceae (green algae) and the Bacillariophyceae (diatoms). The ASP bioprospecting efforts resulted in a large culture collection containing over 3000 strains of organisms. After screening, isolation, and characterization efforts, the collection was eventually winnowed down to around 300 species that were housed at the University of Hawaii. The current status of this culture collection has been reported as mostly lost due to lack of support for ongoing preservation efforts.

NAABB also had an aggressive bioprospecting component where 2000 independent algal isolates were collected across the United States. More than 60 strains were identified that outperformed existing benchmark production algal strains. The top strains with high biomass productivities and lipid yields went on to cultivation trials and the genomes of two of the top performers have been sequenced. Thirty of the best-performing strains have been deposited within the University of Texas (UTEX) Culture Collection of Algae. NAABB strain prospecting and screening also found Chlorophyceae to be the most productive strains. Diatom species were tested but the high ash content precluded them from consideration as suitable feedstocks for downstream processing into fuels.

Much of the ASP biology program's research focused attention on understanding and manipulating the elusive "lipid trigger." In the closing days of the program, researchers initiated the first experiments in metabolic engineering as a means of increasing oil production. Researchers demonstrated an ability to make algae overexpress the Acetyl CoA carboxylase (*ACCase*) gene, a major milestone for the research, with the hope that increasing the level of *ACCase* activity in the cells would lead to higher oil production. These early experiments did not, however, demonstrate increased oil production in the cells.

In contrast, the NAABB program was positioned to really leverage the "omics" revolution that had emerged over the past 14 years since the end of the ASP, leading to new powerful tools for sequencing and analysis. These tools enabled tremendous advancements that were not even thought possible during the ASP era. While the ASP began developing an understanding of some very limited genomic data, biosynthetic pathways, and nascent genetic engineering efforts, NAABB exploded this base of information by providing new genomic sequences, new gene targets for enhancing productivity of biomass and lipids, and new genetic engineering tools for improved strains. In just three short years NAABB completed the sequencing of eight new algal genomes for target species and identified multiple gene targets associated with light capture, photosynthetic electron transfer, carbon dioxide fixation, and carbon metabolism for increasing productivity. Using these targets, a transgenic pipeline platform was developed and over 50 independent transgene constructs were expressed in the model strain, *Chlamydomonas reinharditii*. Several of these transgenic strains have demonstrated 2-fold increases in biomass productivity over the wild-type strains in laboratory benchmarking studies.

The ASP recommended the issue of productivity, in its various guises and aspects from species control to lipid (oil) yield and harvesting, as a central subject for any future U.S. R&D program in microalgal biodiesel production. Essentially, the ASP concluded that the focus should be on developing the microbial catalysts that can convert solar energy to a liquid fuel at high overall efficiency. They believed that this effort would require a relatively long-term R&D effort, which would, at least initially, be focused on the fundamental and early-stage applied research required for such a biocatalyst development effort.

NAABB's focus transitioned from an early focus on lipid productivity to maximizing fixed carbon productivity into fuel. This transition was driven by experimental data showing that lipid extraction and conversion processes resulted in significant losses in carbon to fuels. This led to a parallel effort to look at direct conversion routes with processing by hydrothermal liquefaction. This method could extract the lipid and convert protein and carbohydrate fractions to fuel, resulting in a significant increase in fuel yield. Once this processing route was proven effective, this also decoupled the need for the production of high-lipid-containing biomass, which always results in a significant productivity penalty associated with the cultivation of algal biomass under stressed conditions. This process route also has the potential to provide for nearly complete nutrient recycle. These relationships (cause and effect) between strain selection, cultivation strategies, and downstream extraction and conversion processes were brought to light within NAABB. In particular NAABB could begin to look at biology improvements in the context of downstream processing drivers.

Algae Production Systems

In studies conducted in California, Hawaii, and New Mexico, the ASP proved the concept of long-term, reliable production of algae. California and Hawaii served as early testbed sites. Based on results from six years of tests run in parallel in California and Hawaii, 1000 m^2 pond systems were built and tested in Roswell, New Mexico. The conclusion from these cultivation studies at the New Mexico testbed location indicated that if such geographical locations are to be used in the future, some form of temperature control with enclosure of the ponds may well be required.

NAABB also had multiple cultivation testbeds located across the Southwest, Gulf Coast, and Hawaii. Clearly severe seasonal effects were observed during winter months at all locations except Hawaii. NAABB researchers were able to develop and validate a new pond design that retains heat in the culture ponds during cool winter-month operation, greatly improving productivity. This Aquaculture Raceway Integrated Design pond system could be a major engineering improvement that addresses this challenge associated with large-scale cultivation at most locations in the continental United States.

An important lesson from the ASP outdoor testing of algae production systems was the inability to maintain laboratory organisms in the field. Algal species that looked very promising when tested in the laboratory were not robust under conditions encountered in the field. In fact, the best approach for successful cultivation of a consistent species of algae was to allow a contaminant native to the area to take over the ponds.

NAABB recognized that predicting the performance of algal strains in an outdoor, large-scale cultivation system was a big challenge based on this previous work by the ASP, others, and our own cultivation studies at outdoor cultivation testbeds. NAABB researchers developed and validated an environmental simulated culturing screening process that provides a method to evaluate the production potential of strains in the laboratory prior to conducting expensive large-scale cultivation trials at specific production sites.

The ASP conducted some of the first biomass resource assessment efforts for microalgae production. These assessments indicated that although the technology faces many R&D hurdles before it can be practicable, it was clear that resource limitations were not an argument against the technology.

While this general statement may be true, NAABB made use of a new GIS-based Biomass Assessment Tool to look at resource assessment in a much more comprehensive fashion. These analyses have further defined the preferential land areas by screening all the resources and attributes needed to maximize productivity, economics, and life cycle considerations including climatic conditions, land suitability, consumptive and nutrient water requirements, access to downstream infrastructure across a range of local to national scales, saline water availability and transport (seawater, saline groundwater, and produced water), growth characteristics, and resource requirements for alternative algae strains.

Future Directions

The ASP provided a very good summary in its recommendations for future directions that are still useful and relevant today for the development of algal biofuels. These recommendations from the ASP and a summary of NAABB's actions are discussed below.

ASP Recommendation: Put less emphasis on outdoor field demonstrations and more on basic biology.

The ASP concluded that much work remains to be done on a fundamental level to maximize the overall productivity of algal mass culture systems and the bulk of this work is probably best done in the laboratory.

NAABB followed this recommendation, dedicating 25% of its budget to laboratory efforts in algal biology for making more productive strains. Increasing the productivity of algal biomass through continued advancement in biology is still the single highest priority in terms of impacting the viable production of algal biofuels.

ASP Recommendation: Start with what works in the field.

The ASP concluded that it was best to select strains that work well at the specific site where the cultivation technology is to be used. These native strains are the most likely to be successful. Then, research should focus on optimizing the production of these native strains and use them as starting points for genetic engineering work.

Selection of production host strains is a critical starting point for developing genetically enhanced strains and this is the approach that was taken by NAABB. More than 2000 strains were screened for improved productivity and 30 strains were identified and deposited at UTEX for continued development as potential production strains. Several of these strains that showed the greatest potential for scale-up and high productivities in outdoor cultures were selected for sequencing and development of genetic tools to enable the development of genetically modified strains.

ASP Recommendation: Maximize photosynthetic efficiency.

The ASP concluded that not enough was understood about the theoretical limits of solar energy conversion. Recent advances at the time in understanding photosynthetic mechanisms at a molecular level, in conjunction with the advances being made in genetic engineering tools for plant systems, offered exciting opportunities for constructing algae that do not suffer the limitations of light-saturation photoinhibition.

This was the major emphasis of the algal biology efforts within NAABB where over 50 gene targets were discovered and screened for increased yield and oil production in transgenic strains. One transgenic strain developed with enhanced photosynthetic electron transfer properties resulted in a 2.5-fold increase in growth over the wild type. This effort clearly demonstrates the potential of biological enhancement in maximizing photosynthetic efficiency. NAABB has developed a number of tools and strategies to further maximize photosynthetic efficiency and aerial productivities.

ASP Recommendation: Set realistic expectations for the technology.

The ASP concluded that the projections for future costs of petroleum were a moving target and expected that petroleum costs would remain relatively flat over the next 20 years. They expected that it would be unrealistic for algal biodiesel to compete with such cheap petroleum prices and predicted that without some mechanism for monetizing its environmental benefits (such as carbon taxes), algal biodiesel would not get off the ground.

NAABB was able to quantify the life cycle benefits associated with algal biofuels production and model specific R&D enhancements that can enable competitive production economics with continued development. This is still a longer-term pathway to profitability but NAABB has clarified the required approach.

ASP Recommendation: Look for near-term, intermediate technology deployment opportunities such as wastewater treatment.

The ASP concluded that excessive focus on long-term energy displacement goals will slow down development of the technology. They believed that a more balanced approach is needed in which more near-term opportunities can be used to launch the technology in the commercial arena. Several such opportunities exist. Wastewater treatment is a prime example. The economics of algae technology can be much more favorable when it is used as a waste treatment process and as a source of fuel. This harks back to the early days of DOE's research.

Fuel production from microalgae is a long-term proposition and there was a significant shakeout in the number of microalgae-based fuel companies during the three years of NAABB, bearing out this fact. Most small algae-based companies found it necessary to shift focus away from fuels toward higher-value products in order to generate near-term revenues. Water treatment options along with the production of higher-value products both remain potential viable pathways to bridge the gap between now and the time when the production of algal biofuels becomes economically viable.

Conclusions

The overall conclusion of the ASP from the review of two decades of DOE-funded R & D in microalgal mass culture for biodiesel and other renewable fuels was that the technology still requires relatively long-term R&D for practical realization. The ASP's initial, rather optimistic cost and performance projections were not met and could only be met with significant productivity enhancements. At the time, this conclusion was largely due to the following factors.

The expectations for the future costs of fossil fuels have declined.

Fuel costs have roughly doubled between the end of the ASP in 1996 and today. It is unlikely going forward from today that we will see trends for declining fossil fuel prices.

The value of byproduct credits for waste treatment, greenhouse gas mitigation, or higher-value coproducts are either uncertain or relatively low.

NAABB has clearly better quantified the value of agricultural coproducts and their potential impact on process economics and life cycle. The value of LEA for feeds and fertilizers was shown to be too low to drive favorable economics for the production of algal biofuels alone. The need and value of nutrient recycle was also recognized by NAABB but actual demonstration of nutrient recycle was not accomplished as part of the NAABB legacy.

The recent engineering designs and economic analyses have projected higher costs than earlier estimates, partly because of greater detail and realism, thus requiring higher productivities to achieve cost goals.

NAABB's economic models clearly determined that major reductions in capital and operating cost associated with the production of algal biomass will be required. The best way to reduce these capital and operating costs is through increased productivity, which is now possible using biological improvements, as early experimental results indicate.

The actual productivity results of the outdoor experimental work were well below the projections on which the economic analyses are based.

NAABB has developed new methods for screening strains to better predict their performance in large-scale open outdoor ponds at any geographical location at any time of the year. These tools can be used to validate improved strains including GMOs going forward so that sustainable high-productivity strains are developed that can be scaled-up more reliably.

Sustained R&D

The ASP's general conclusion regarding the need for additional long term R&D remains true today and there is really no substitute for a sustained R&D effort funded by DOE and industry specifically focused on developing the drop-in fuels to make this a reality. NAABB has clearly helped move the bar in this regard. The real difference is that NAABB has provided significant advancements along the development timeline that go beyond where the ASP left off in 1996 in terms of new baseline technologies, know-how, and process understanding. These advancements will be vital for developing viable pathways and approaches for producing cost-competitive drop-in fuel from microalgae. NAABB was able to do this by developing and demonstrating upstream and downstream process technologies and collecting data across the entire process matrix. In doing so, NAABB developed detailed models (TEA and LCA) to evaluate fully integrated process options that can guide critical R&D pathways informed by sensitivity analyses.

Perhaps most importantly, NAABB developed a set of powerful new algal biology tools including algal genomes, gene and pathway targets, transformation tools, and potential production strains that can significantly enhance algal biomass productivity. Application of these biology tools will be the number one enabling factor for establishing a cost-competitive algal biofuels industry. Other enabling NAABB technological advancements also have tremendous potential to further improve process economics and LCA implications, including biomass growth models and environmentally simulated culture systems for strain screening; new harvesting, extraction and conversion process technologies; and detail models for resource assessment, LCA, TEA, and profitability analyses. Collectively, these NAABB developments have significantly advanced the state-of-technology baseline from the ASP and provide new technologies and pathways that can lead to a sustainable biofuels industry from microalgae with sustained investments.

Perspective 2. NAABB and the National Research Council Report on Sustainable Development of Algal Biofuels in the United States

Scope and Summary

During execution of the NAABB project, the NRC convened a committee of fifteen experts to examine the sustainable development of algal biofuels at the request of DOE-EERE's Biomass Program. The committee produced its report in 2012. The purpose of this study was to identify and anticipate potential sustainability concerns associated with a selected number of pathways for large-scale deployment of algal biofuels, discuss potential strategies for mitigating those concerns, and suggest indicators and metrics that could be used and data that could be collected for assessing sustainability across the biofuel supply chain to monitor progress as the industry develops. The study relied on available published literature at the time but did not consider the emerging results from R&D efforts within NAABB or other ongoing DOE-funded efforts. The committee concluded that the scale-up of algal biofuel production sufficient to meet at least 5% of the U.S. demand for transportation fuels would place *unsustainable demands* on energy, water, and nutrients with current technologies and knowledge. However, the potential to shift this dynamic through improvements in biological and engineering variables exists.

The committee found that sustainable development of algal biofuels would require research, development, and demonstration of the following:

- Algal strain selection and improvement to enhance desired characteristics and biofuel productivity.
- An energy return on investment (EROI) that is comparable to other transportation fuels, or at least improving and approaching the EROIs of other transportation fuels.
- The use of wastewater for cultivating algae for fuels or the recycling of harvest water, particularly if freshwater algae are used.
- Recycling of nutrients in algal biofuel pathways that require harvesting unless coproducts that meet an equivalent nutrient need are produced.

In addition, the committee concluded that a national assessment of land requirements for algae cultivation is needed that takes into account climatic conditions; freshwater, inland and coastal saline water and wastewater resources; sources of CO_2; and land prices to inform the potential amount of algal biofuels that could be produced economically in the United States.

The committee did not consider any one of these sustainability concerns a definitive barrier to sustainable development of algal biofuels because mitigation strategies for each of those concerns had been proposed and were being developed. However, they believed that all of the key sustainability concerns needed to be addressed in an integrative manner. To do this they concluded that research, development, and demonstration were needed to test and refine the production systems and the mitigation strategies for sustainability concerns and to evaluate the systems and strategies based on the sustainability goals.

Independently, NAABB was conducting research, development, and demonstrations focused on new strains, processes, systems, and strategies in an integrative manner as suggested by the NRC committee. As a result many of the sustainability concerns raised by the NRC report for sustainable algal biofuels were addressed by NAABB and progress was made toward some potential technical solutions.

Algal Biofuel Supply Chain

The NRC report addressed the algal biofuel supply chain and concluded that an integrated coordination of biological processes (for example, selection of algal strains and development of algae cultivation) and engineering processes (for example, reactor design, harvesting and dewatering methods, and processing) was needed to realize the potential of algal biofuels. NAABB took this approach to address the entire spectrum of upstream and downstream processes. This included technology development efforts across the algal biofuel supply chain that provided integrated data sets and the development of TEA and life-cycle–assessment models for those processes.

For algal biology, the NRC report underscored a critical need for basic and applied research to expand the spectrum of germplasm available for the enterprise. The committee specifically recommended research on expanding the light spectrum useful for photosynthesis, improving the distribution of incident light to various aquatic photosynthetic scale-up processes, and enhancing the efficiency of RuBisCO or other basic physiological processes to better utilize carbon that could lead to dramatic improvements in productivity. Many of NAABB's algal biology efforts were directed toward developing genetic-engineering methods to improve photosynthetic efficiencies and increase productivities. In fact, NAABB was able to demonstrate up to 2.5-fold improvement in growth rate through the development of new GMO production strains.

The NRC report also stressed the importance of crop-protection research that focuses on reducing biomass losses to pathogens and grazers for increasing productivity. They concluded that because contamination by other algal species is largely unavoidable, especially in open-pond algal cultures, improving the existing understanding of how algal biomass production systems can be managed as complex bioengineered systems would be helpful. NAABB algal biology efforts included activities to understand the principles of population, community, and ecosystem ecology. Some important ecophysiological parameters that can be exploited to provide protection against grazers and pathogens were identified. In addition, antimicrobial peptides were identified that kill bacteria and rotifers without harming algae.

The NRC report also found that improvements in algae cultivation methods and the physical processes used to harvest, dewater, and convert algal biomass into fuels are as important to the sustainable development of algal biofuels as improvements in algal strains. New ways should be explored to reduce the energy requirements for converting cultivated algae in an aqueous solution into a dewatered state that can then be processed into fuel. NAABB focused a significant effort on algal cultivation through directed research activities at six cultivation testbeds located in Texas, New Mexico, Arizona, Washington, and Hawaii. Cultivation methods to improve energy inputs and improve productivity were developed as well as methods to make use of wastewaters and nutrients. NAABB also had a focused effort to develop and evaluate several new promising harvesting methods to improve energy efficiency and cost. Three of these novel methods (electrocoagulation, crossflow filtration, and ultrasonic focusing) were scaled-up for integrated testing at the algal testbeds and process models were developed for further evaluations.

The NRC report concluded that research and development in understanding how dewatered algae can be processed into a fuel and whether algae can produce a useful hydrocarbon directly without the need for harvest and dewatering and with minimal processing could be an important contributor to reducing production costs. While NAABB did not evaluate methods to produce hydrocarbons without harvesting the algae (e.g., lipid secretion and recovery), NAABB did investigate several different routes to process algal biomass into hydrocarbon fuels. Initial efforts focused on wet-solvent–extraction technologies and multiple conversion technologies of the crude-lipid extracts and LEA to hydrocarbon fuels and coproducts. NAABB also evaluated direct conversion methods of wet algae to hydrocarbon fuels by HTL and catalytic upgrading. These efforts demonstrated that the direct processing route via HTL has the potential to provide higher fuel yields at lower production costs with the potential for nutrient recycle (N and P).

Pathways for Algal Biofuel Production

The NRC committee looked at a number of different combinations of cultivation and processing options resulting in more than 60 different proposed pathways for producing algal biofuels. They selected a small number of the most likely designs to use as a framework for the analysis of sustainability. The pathways help illustrate the resource requirements and potential impacts associated with greatly scaling up various approaches to produce algal biofuels. These pathways also allow different approaches to be compared and contrasted directionally, enabling conclusions to be drawn, pitfalls identified, and potential solutions drafted. The reference pathway is drawn from the recent National Renewable Energy Laboratory techno-economic analysis of algal biofuel production (Davis, R. et al., *Applied Energy* 10:3524-3531, 2011). NAABB also conducted pathway analysis for several different pathways using models developed from experimental data. Several of the pathways evaluated by NAABB were very similar to those selected and evaluated within the NRC report.

Reference Pathway: Raceway Pond Producing Drop-in Hydrocarbon

The reference pathway assumes that microalgae are cultivated in saline water in an open raceway pond. Algae are harvested and lysed to release lipids, which are collected for further processing into a drop-in hydrocarbon fuel. The residual mass is treated by anaerobic digestion to generate biogas for power and recycle nutrients.

NAABB was part of the DOE Harmonized Modeling Team and participated in developing an updated version of the reference pathway that was used as a baseline to build and compare alternative pathways. NAABB R&D activities focused on all the major elements (saline species, open cultivation ponds, various harvesting methods, various extraction methods, and various conversion methods) of the reference pathway with the exception of anaerobic digestion. NAABB evaluated a thermochemical process alternative to anaerobic digestion called catalytic hydrothermal gasification where residuals are gasified to generate methane for power and nutrients for recycle without significant waste sludge.

Alternative Pathway 1: Raceway Pond Producing Drop-in Hydrocarbon and Coproducts

This pathway is identical to the reference pathway except that the LEA biomass is sold as a feed byproduct so there is no anaerobic digestion or nutrient recycle. That change could affect energy requirements, GHG emissions, and nutrient requirements depending on the high-quality coproduct. Coproducts also can affect economic viability. This pathway results in increased nutrient requirements for algae cultivation compared to the reference pathway because of the loss of biomass nutrients from direct coproduct sales. The scale of biofuel production has a large impact on the volume of coproduct streams and therefore their value. The committee believed that coproducing high-value products, such as chemical feedstocks, with biofuels would be viable only on a small scale.

NAABB intensively evaluated agricultural coproduct values for LEA as animal feeds, maricultural feeds, and fertilizers. Feeding trials were conducted using LEA in a variety of feed formulations for cattle, swine, chicken, fin fish, and shrimp. In addition, the LEA was evaluated as a fertilizer. The results of these studies showed that the LEA can be used as a low-value feed supplement and fertilizer. Economic models indicated that the value of these coproducts was too low to impact the profitability of algal biofuel production. In addition, NAABB sustainability analysis confirmed the findings and concerns of the NRC committee that this pathway will have a large negative impact on nutrient use and that the scale of potential operations of fuel production would saturate the coproducts market and cause a collapse in the values of those products. NAABB also believes that coproducing high-value products, such as chemical feedstocks, with biofuels would be viable only on a small scale.

Alternative Pathway 2: Open Raceway Pond Producing Esterified Biodiesel (Fatty Acid Methyl Esters)

This pathway is identical to the reference pathway with the exception that the lipid extract is converted to fatty acid methyl esters (FAMEs) instead of hydrocarbon fuel with a glycerol coproduct. The process still maintains the anaerobic digestion step.

NAABB focused several efforts on converting crude lipid extracts and wet algal biomass to FAMEs. These included technologies for catalytic conversion using a solid acid catalyst and direct supercritical processing of crude lipids and whole biomass. These FAME conversion pathways were compared to those focused on producing drop-in fuels or using the crude FAME as a feedstock for conversion to a drop-in fuel. In general, NAABB found there was no economic or sustainability benefit in converting algal biomass or crude lipid extracts to FAMEs versus drop-in hydrocarbons. In fact, many of the long-chain unsaturated fatty acids were problematic for FAME fuels.

Alternative Pathway 3: Algenol Direct-synthesis Pathway Uses a Closed Reactor with an Organism that Directly Produces Alcohols

Growing microalgae in photobioreactors (PBRs) can avoid a number of the sustainability concerns associated with open-pond cultivation, but it may require substantial energy input for pumping and mixing water and for temperature control. The approach would likely reduce incidents of contamination by algae and other microorganisms and evaporative loss of water. Other than using a different cultivation system from the other pathways discussed above, this pathway does not require harvesting, drying, and rupturing the algal cells to extract algal oil because the cyanobacteria secrete alcohol into the medium continuously. The direct synthesis of ethanol reduces downstream processing and could result in substantial energy savings and associated cost savings. In addition, some members of the public might find cultivation of genetically modified algae in enclosed reactors more acceptable than in open ponds.

NAABB did not evaluate this pathway but did have cultivation efforts to evaluate the best use of PBR systems. In general, through limited PBR research efforts, NAABB concluded that PBRs are likely most useful as inoculum production systems for large-scale open ponds. Several of the conversion technologies developed and evaluated by NAABB may also have application in the treatment and conversion of residual GMO algal biomass from the PBR systems used in this pathway.

Alternative Pathway 4: Pyrolysis of Whole Biomass and Hydrotreating to Yield Hydrocarbon Blendstock

The NRC committee concluded that thermochemical technologies that can convert the whole algal biomass may have advantages in that they can accept any type of biomass, including biomass of aquatic microalgal and macroalgal species. In addition, lipid-producing microalgae are not required for fuel production in this pathway. Algal strains or even mixed cultures can be selected for their high biomass productivity and ability to fix carbon. Algae would have to be harvested, dewatered, and likely dried for use as feedstock.

NAABB evaluated several direct conversion technologies including pyrolysis, direct supercritical FAME production, and hydrothermal liquefaction. Pyrolysis requires that the algal biomass be dried prior to processing at a significant energy penalty. Direct supercritical FAME production could readily utilize wet algal biomass but relies on high lipid content for high yields of crude FAME. HTL utilizes wet algal biomass and converts the lipid, protein, and carbohydrate fractions into hydrocarbon fuels. This technology allows for using low-lipid algal feedstocks from strains selected and cultivated for maximum biomass productivity; it also provides very high yields of hydrocarbon biocrude easily upgraded to drop-in fuels. Over all, NAABB data and modeling efforts indicate that the whole-biomass HTL pathway results in better process economics and sustainability than the pathways that rely on lipid production and extraction (baseline pathway and alternative pathways 1 and 2).

Natural Resource Use

Based on a review of published literature, the NRC committee concluded that the scale-up of algal biofuel production to yield 37.8 billion liters of algal oil (10 billion gallons) would place an unsustainable demand on energy, water, and nutrients with current technology and knowledge. The estimated consumptive use of freshwater for producing 1 liter of gasoline-equivalent of algal biofuel is 3.15–3650 liters. The estimated requirement for nitrogen and phosphorus needed to produce 37.8 billion liters of algal biofuels ranges from 6 million to 15 million metric tons of nitrogen and from 1 million to 2 million metric tons of phosphorus if the nutrients are not recycled or included and used in coproducts.

The NRC committee concluded that freshwater use for production of algal biofuel is inevitable because of two key drivers, evaporative loss in open-pond cultivation and discharge of harvest water from biofuel production systems that harvest the algae. Therefore, water use would be a serious concern in an algal biofuel production system that uses freshwater in open ponds without recycling harvest water.

NAABB research demonstrated that brackish water, produced water, and municipal wastewater are viable sources of water for algae cultivation. All of these water sources can significantly reduce the requirements for freshwater use. In addition, NAABB demonstrated the recycle of >90% of the water in cultivation and harvesting operations at its various test-bed locations. Information on the availability of inland fresh and saline water resources, produced water, and wastewater sources was investigated using the Biomass Assessment Tool (BAT), a GIS-based resource assessment tool showing the availability of these water sources to support large-scale cultivation at the most preferential locations in the United States.

Recycling of harvest water also is important in reducing nitrogen and phosphorus input to algae cultivation. Recycling nutrients through reuse of harvest water or the use of wastewater from agricultural or municipal sources provides an opportunity to reduce energy use, as synthetic fertilizer input contributes to energy input over the life cycle of algal biofuels. In addition, waste from concentrated animal feeding operations can provide enough fertilizers to meet the nutrient demand if these resources are available. NAABB research indicates the possibility of recycling 95% of the nitrogen and 90% of the phosphorus from the residual algal biomass back to cultivation by using direct processing technologies including HTL followed by CHG.

Assessment Framework

The NRC report concluded that key aspects of the sustainable development of algal biofuels are siting (for example, suitable climate and colocation of key resources) and recycling of key resources. Siting of algal biofuel production facilities needs to account for climate, topography, and proximity to water and nutrients. A national assessment of land requirements for algae cultivation is needed to inform the potential amount of algal biofuels that could be produced economically in the United States. NAABB was able to complete this important assessment using the BAT and then model TEA and LCA for algal biofuel production at those preferred sites.

Since each process in the production pathway could present sustainability challenges or opportunities to reduce resource use or mitigate environmental effects, all the sustainability challenges and opportunities have to be assessed from a systems perspective. Thus the committee reviewed LCAs performed to estimate resource use and environmental effects from cradle to grave for those parameters where published studies were available—for example, water use, net energy return, and net GHG emissions. Each pathway for producing algal biofuels combines cultivation, harvesting or product recovery, dewatering, and processing into a system. The abilities of different pathways to meet different aspects of sustainability vary, but, in all cases, improvements in productivity—for example, cell density in algae cultivation, algal product (oil or alcohol) yield, biomass yield, and processing yield of biomass to fuel—help reduce resource use and environmental effects.

NAABB modeled the performance of various strains and process technologies for cultivation, harvesting, extraction, and conversion to develop TEAs and LCAs. NAABB also developed and applied the very powerful GIS-based algal biomass resource assessment tool (BAT) to determine the best sites for algal biofuel production in the United States. Site-specific data was used to model production, cost, and life-cycle functions for these sites. These efforts confirmed the major findings from the NRC study that site selection and making improvements in algal biomass productivity and fuel yields are the most important factors in reducing resource use, environmental effects, and production costs. NAABB also demonstrated significant improvements in biomass productivity through the development of new GMO strains and improvements in fuel yields through the development of HTL as a direct processing technology. These technology improvements along with others that focused on improving cultivation and harvesting efficiency have the potential to greatly improve the energy balance, reduce GHG emissions, and enhance the overall sustainability of algal biofuels.

Program Objectives and Execution

Objectives

NAABB was formed to specifically address the objectives set forth by the DOE under the funding opportunity announcement (FOA) DE-FOA-0000123, Development of Algal/Advanced Biofuels Consortia.

In this announcement DOE sought consortia that would "synergistically use their unique capabilities to expedite the development of biomass-based fuel production pathways." The opportunity allowed and encouraged the participation of industry, academia, and government and/or nongovernment laboratories, including foreign entities, that could provide "best-in-class" technical approaches. NAABB specifically addressed Topic Area 1, Algal Biofuels Consortium/Consortia, addressing the pathways within the FOA:

Feedstock supply—Strain development and cultivation

Feedstock logistics—Harvesting and extraction

Conversion/production—ccumulation of intermediates and synthesis of fuels and coproducts

Governance and Oversight

NAABB was organized into teams (Figure 1) to address the technical objectives detailed in the R&D framework section of this report. NAABB's management philosophy evolved from the virtual consortium "without walls," a philosophy of openness, inclusiveness, efficiency, and effectiveness. Through effective communication at all levels, the management structure helped mitigate issues early and facilitate information transfer across the organization.

The teams housed multidisciplinary and multi-institutional components. Therefore, roles and responsibilities flowed down the organizational structure and into each team member across the institutions. Team leads had responsibility not just to their institutions but to all within their teams. Coordination of projects, equipment, and capabilities flowed through project/capability leads (not shown in the structure) that worked across teams and institutions.

All projects and data information were tracked to deliverables, milestones, and schedule through our Operations team responsible to the Operations Manager. The Scientific Director, Consortium Engineer, and Consortium Economist ensured that multidisciplinary approaches were considered when developing the objectives of the consortium and guided the consortium's strategic scientific and engineering approaches. A Director of Industry Relations was a member of the Operations team and was responsivble for managing NAABB's intellectual property.

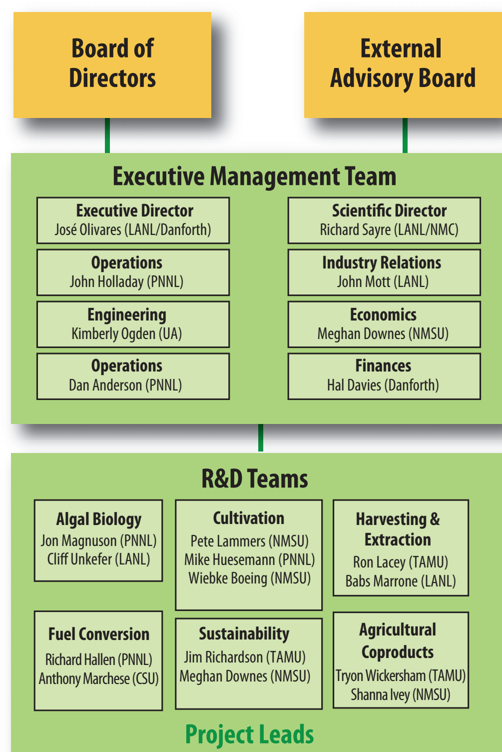


Figure 1. The Industrial Board was merged with the Board of Directors. Objectives became teams working on the tasks of Algal Biology, Cultivation, Harvesting and Extraction, Fuel Conversion, Agricultural Coproducts, and Sustainability.

The Executive Director was responsible for final implementation of the consortium's strategy and operational decisions and for resolution of conflicts. Originally an Industrial Board was formed but was later merged with the Board of Directors to help guide the consortium's overarching strategy and develop approaches to successful commercialization of our technologies, all within the goals of the DOE's intent for this consortium and with the direct involvement of the Bioenergy Technologies Office (BETO).

Danforth Plant Science Center, the consortium lead, was responsible for finance management, administrative support, project management, communication, and reporting. Danforth also provided support to the Executive Director and Scientific, Engineering, and Economic Directors through an Operations team that watched over all day-to-day operations of the consortium.

Board of Directors

Early in the formation of the consortium—and before the NAABB proposal was submitted to DOE—NAABB formed a Board of Directors to help set strategy and business affairs. This board, along with other strategic governance processes, was established through a set of bylaws approved by the whole membership.

The Board of Directors consisted of designated representatives from the following institutions:

- Three standing university representatives (New Mexico State University, Texas A&M/AgriLife, and University of Arizona).
- Three standing national laboratory representatives (Danforth/Los Alamos National Laboratory, and Pacific Northwest National Laboratory).
- Three standing industry representatives selected from the largest companies in the consortium (Cellana, Albermarle-Catilin, and UOP).
- Four rotating at-large representatives, elected annually—two elected by industry members and two elected by nonindustry members—with a one-year rotation period.
- One nonvoting representative from the DOE Office of Biomass Programs/BETO.

The NAABB Executive Director served as a nonvoting member of the board. The Chair of the board was elected annually. Richard Sayre (Danforth) served the first year and James Rekoske (UOP) served the second and third years.

The board met at least three times per year to chart the general course of the consortium, hear progress reports and ensure accountability, consider the process for addition of new members to the consortium and evaluate candidates, and be available to adjudicate disputes that might arise. All board meetings were open to all of the NAABB partners.

The board legitimized the governance, structure, and management of NAABB and was an indispensible component in managing NAABB affairs. It provided an oversight body for the full membership. The board's most important contributions included screening and approving new institutional members and vetting strategic direction for management to execute.

External Advisory Board

NAABB formed the External Advisory Board (EAB) to assess progress toward scope, set strategy for outlying years, and provide advice on future initiatives to the Board of Directors. The EAB included representation with a broad spectrum of expertise and experience including agriculture, energy, economics, and technology development. The EAB comprised members of academia, industry, government agencies, and national laboratories.

The EAB's charter was to provide advice and recommendations on technical progress and issues that are within the focus of the NAABB program. The contribution of the committee was to provide a broad peer review of NAABB's overall program and strategy. As a public-private partnership, NAABB devotes federal funds along with cost-share commitments from its partners to new research, development, and demonstration activities in algal biofuels. Therefore, the EAB provided invaluable assessments and recommendations on NAABB's scope, progress, and strategy toward developing technologies for cost-effective production of algal biomass and lipids, toward developing technologies for economically viable fuels and coproducts, and toward providing a framework for a sustainable biofuels industry. The board met for every NAABB internal review, which were held one to two times per year. The EAB members were not compensated for their time and effort; therefore, full participation in all of the meetings was spotty. The EAB members were:

- Chris Cassidy, USDA
- Tom Foust, The National Renewable Energy Laboratory
- Paul Gilna, Oak Ridge National Laboratory
- Mark Hildebrand, Scripps Oceanographic Institute
- William (Bill) Lyons, the Boeing Company
- Michael Lakeman, the Boeing Company
- Brent Massman, Monsanto
- Jeff Scheibel, Procter & Gamble
- Joyce Yang, DOE (*ex officio* advisory member, no voting rights)
- J. Alan Weber, MARC-IV
- Mary Rosenthal, the Algae Biomass Organization

The EAB was effective in providing the above assessments and did this directly to the NAABB Executive Team and DOE. This provided a degree of independent assessment within the program.

Role of DOE

DOE had substantial involvement in the consortium through project management oversight, participation in review and approval of projects, review of progress based on metrics, and participation in stage gate reviews (go/no go decision points) and peer reviews. This was achieved by having DOE fully integrated in the activities of the consortium through weekly meetings with the NAABB management team and through *ad hoc* nonvoting roles in the NAABB Board of Directors and EAB. In addition, DOE was involved in all major status meetings and reviews for the consortium. This substantial involvement allowed the NAABB leadership and DOE to work as a team by keeping a regular line of communication and dealing with issues quickly and effectively throughout the life of the program.

Team Members

NAABB members were chosen from a mix of academia, industry, and national laboratories. The complete list of partners is shown in Figure 2a and 2b. The consortium was managed through the Donald Danforth Plant Science Center. This management included all subcontracting to the partners (except to the national laboratories, which was done directly by DOE), project management, and reporting. The NAABB Principal Investigator, José Olivares, held an adjunct position at Danforth to help manage the consortium through the Center. Pacific Northwest National Laboratory provided additional project management for the consortium.

NAABB Algal Biofuels Consortium Partners

Lead Institution: The Donald Danforth Plant Center†*

* NAABB Team Management
† NAABB Board of Directors

National Laboratories

- Los Alamos National Laboratory / New Mexico Consotrtium*†
- 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

Slide 1

Figure 2a. List of all NAABB consortium partners.

Figure 2b. Map showing locations of NAABB partners.

The membership at the time of proposal submission included 28 of the members listed and grew to 39 within the life for the consortium. Each member organization—academic, national laboratory, and industry—brought a strategic capability to the consortium within the objectives of the program. A majority of the members held subcontracts that directly paid for all or part of their contributions. All of the industry partners and some of the academic institutions provided cost-share contributions to their projects. A small number of institutions became inactive in the program as their capabilities and projects ended within the award period. Within the second year of the consortium NAABB added two international partners, Reliance Industries Limited, India, and Pan Pacific Technologies, Australia. The consortium thus spread across the United States and extended to these two other countries, as shown in the map on Figure 3.

Communications

Due to the virtual nature of the consortium we understood early on that one of our major challenges would be effective communications. We attempted to achieve effectiveness through weekly, monthly, and yearly team and consortium meetings; site visits; highlight newsletters; and frequent emails and teleconferences. This effort was in addition to monthly technical reports, regular data sharing, reviews, and special briefings.

Team Meetings

The NAABB teams met regularly with their leadership, setting agendas, giving presentations, and tracking issues. Table 1 shows the breadth and frequency of these meetings.

Table 1. Frequency and topics of NAABB team meetings.

Team	Frequency	Topic
Executive Team	Biweekly	Overarching strategy, partnerships, science and engineering integration, overall coordination
Operations Team	Weekly	Project execution and tracking, issues tracking, financial status tracking, coordination of activities, subcontract management, consortium reporting, intellectual property management
Technical Teams	Monthly	Team and project status, technical presentations, scientific and engineering engagement by technical teams: Algal Biology, Cultivation, Harvesting and Extraction, Fuel Conversion, Agricultural Coproducts, Sustainability

In addition, project teams and institutions held local meetings more regularly. All of these efforts were achieved with the help of communications tools such as WebEx, GoToMeeting, regular teleconferencing, site visits, and staff interchanges between institutions.

Annual Meetings and Full Consortium Meetings

NAABB held several two- to three-day meetings as a consortium at a central location within the United States to review and establish strategic and tactical directions, review progress and status of projects, define gaps, and overcome major consortium issues. Table 2 summarizes the major consortium meetings held during this period. The full consortium, DOE, EAB, and Board of Directors were always invited to participate in what usually was a jam-packed and high-energy agenda. WebEx video teleconferencing was generally made available to members who were unable to attend.

Table 2. Schedule of major NAABB meetings with locations and topics.

Meeting Date	Location	Topic
April 15–16, 2010	St. Louis, MO	NAABB Algal Biofuels Consortium Kickoff Meeting
July 8–9, 2010	Ann Arbor, MI	NAABB Upstream Conference
Sept. 30–Oct.1, 2010	Tempe, AZ	NAABB Downstream Conference
Jan. 5–6, 2011	Denver, CO	NAABB Executive Team/Major Task Leads Program Review
April 7–8, 2011	Annapolis, MD	DOE Office of Biomass Programs Peer Review (Public Meeting)
May 11–13, 2011	Boulder, CO	NAABB EAB Program Review
Nov. 15–18, 2011	Tempe, AZ	NAABB Upstream/Downstream Meeting
Jan. 13, 2012	Los Angeles, CA	2012 NAABB Systems Biology of Algae Meeting
Feb. 8–10, 2012	Albuquerque, NM	NAABB EAB Program Review
Jan. 22–24, 2013	Tempe, AZ	Final NAABB Upstream/Downstream Meeting
May 20–24, 2013	Alexandria, VA	DOE Office of Biomass Programs Peer Review (Public Meeting)

Data Management, Sharing, and Archiving

The NAABB consortium implemented an integrated data management system for warehousing and utilizing data generated by the experiments and work conducted by NAABB researchers and partners. It was felt that adoption of an electronic laboratory notebook (ELN), database management system (DBMS), and inventory management system (IMS) was crucial for the success of NAABB.

To effectively meet project objectives and deliver critical information and data to all team members in a streamlined and time-appropriate manner with proper user-level controls and data security, NAABB evaluated, selected, and implemented an integrated data system that would deliver the following critical functions:

- An easy-to-use and easily customized graphical user interface for researchers and administrators;
- Advanced technical support available to all users throughout the life of the project;
- Ability to input/utilize Mac and PC productivity software applications within forms and experiments;
- Workflow tracking, creation, and handoffs;
- Inventory management with barcode and cost-tracking capability;
- SQL query functions and output to delimited file formats;
- Forms generation for customized data input;
- Prebuilt "ontologies" and/or access to existing scientific databases for chemistry, biology, and engineering to decrease implementation time;
- Integrated user-access controls; and
- Simple, quick implementation.

The ELN-DBMS system CERF by Rescentris Inc. was selected using the above criteria. The program implemented approximately licenses most partners in the consortium, with some partners holding several licenses. The CERF system served as a repository of data, but due to cultural and institutional barriers further functions were not implemented among the partnership. Most partners used their in-house methods for data collection and reporting and used the CERF system as a final repository of data to be shared with consortium members.

Lessons Learned

Lessons Learned from the EAB

- The EAB was effective in providing the above assessments and did this directly to the NAABB Executive Team and DOE.
- The EAB provided a degree of independent assessment within the program.
- EAB members were not compensated for their time and effort; therefore, full participation in all of the meetings was spotty.

Lessons Learned from the Board of Directors

- The board legitimized the governance, structure, and management of NAABB and was an indispensible component in managing NAABB affairs.
- The board provided an oversight body for the full membership.

Lessons Learned from the Membership Makeup

- Diversity was important due to the breadth of scope in the consortium.
- Project management was critical to help meet milestones and deliverables.

Lessons Learned from Communications

- Communications across the consortium was quite effective.
- Even with all of the communication media that NAABB had in place, it was at times felt that more was needed.
- Video teleconferencing as a tool was hampered across a few partners due to site restrictions and/or bandwidth issues.
- The ELN system served as a data and report repository, but most other functional components of the ELN were not implemented by investigators due to cultural and internal processes.

NAABB R&D Framework

NAABB was a large research consortium that required a large and complex work breakdown structure (WBS) to successfully manage and execute the project. The NAABB WBS contained six major research tasks, each with multiple subtasks that comprised more than 80 individual projects spread across more than 30 different institutions. The NAABB management team recognized that we needed to develop a management tool to communicate to our task leaders and principal investigators our aspiration to function as an integrated R&D consortium versus a collection of individual projects. With this objective in mind, the NAABB management team translated the complex WBS into an R&D framework focused on achieving NAABB's core objectives and helping to facilitate better horizontal integration of work within individual tasks and vertical integration of work between tasks.

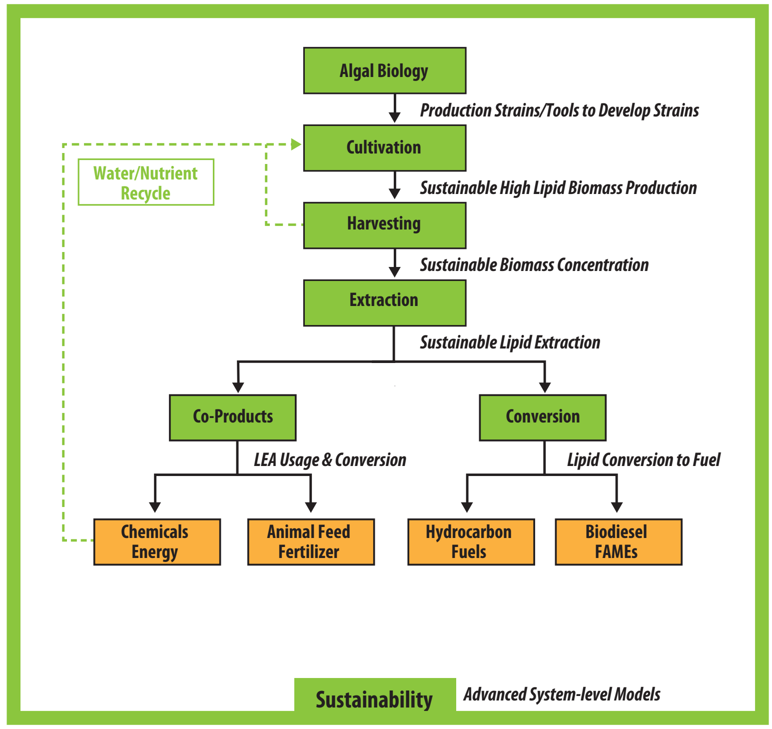


Figure 3. NAABB R&D framework.

We constructed the high level R&D framework (Figure 3) around the six major task areas of the WBS: Algal Biology, Cultivation, Harvesting and Extraction, Fuel Conversion, Agricultural Coproducts, and Sustainability. It shows the outcomes and connections between tasks. Below this level each task had a detailed R&D framework configured to capture key technical areas, work flows, and connections between projects and major tasks. In addition, we tied each task framework to milestones, deliverables, and decision points as a means to guide and manage R&D efforts across NAABB.

Algal Biology R&D Framework

The Algal Biology R&D framework (Figure 4) had two major technical thrusts: (1) genetic engineering to improve productivity and (2) mining the natural diversity. Within the first area significant R&D efforts in molecular biology focused on trait identification, phenotypic characterization, molecular biology toolbox development, crop protection, and assessing traits for improved production. Some of this work focused on understanding and mining key traits to improve lipid production or photosynthetic efficiencies from two selected research strains (*Chlamydomonas reinhardtii* and *Botryococcus braunii*). Other work focused directly on developing tools and methods to improve selected production development strains

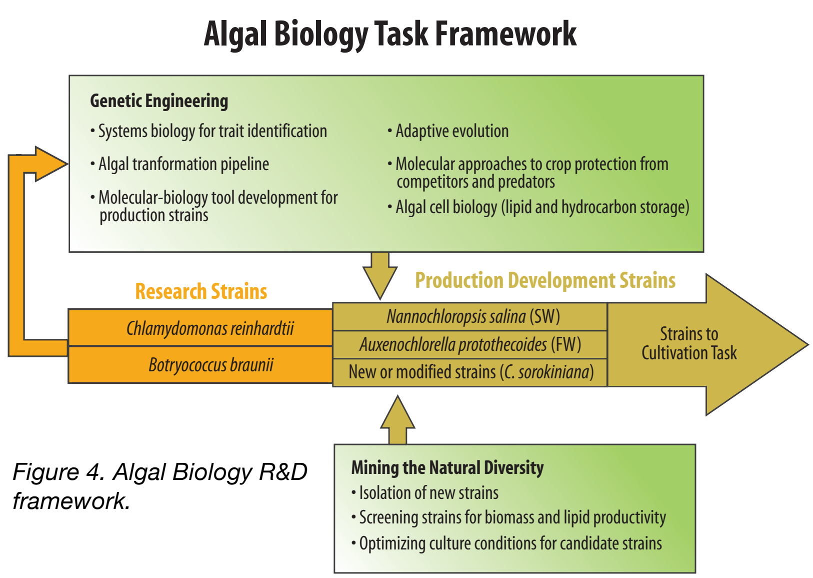


Figure 4. Algal Biology R&D framework.

(*Nannochloropsis salina*, *Auxenochlorella protothecoides*, and new isolates) that were selected for their potential as viable candidates for large-scale cultivation and the production of algal biofuels. The second thrust area focused on isolating and screening new potential production strains, optimizing their growth, and adaptive evolution of these natural strains. Collectively the objective of the Algal Biology framework was to develop new production strains and the molecular tools to continue to improve these strains. Production development strains from the Algal Biology task were provided to the Cultivation task for scale-up and production assessments. The following five major milestones and deliverables were completed as part of the Algal Biology task (Table 3).

Table 3. Algal Biology major milestones and deliverables.

Milestones (M) and (DL) Deliverables	Time Completed (months)
M1: 500 algal isolates screened, ≥ 10 promising high-lipid strains tested in culture	18
DL1: Genes for increased yield, productivity, nutrient utilization, or crop protection cataloged. Transgenic tools demonstrated for *C. reinhardtii, B. braunii,* and *Chlorella.*	18
DL2: First generation of *Nannochloropsis* and *Chlorella* strains obtained by adaptive evolution with demonstrated improvement in growth or lipid yield over parent strain.	18
M2: 1500 algal isolates screened, ≥ 30 best strains verified and deposited to UTEX.	36
M3: Transgenic strains incorporating best trait(s) demonstrated in culture.	36

Cultivation R&D Framework

The Cultivation R&D framework (Figure 5) had four major technical thrusts: (1) cultivation tools and methods, (2) cultivation-system innovations, (3) nutrient/water recycling and wastewater cultivation, and (4) large-pond cultivation and biomass production. This research was carried out in a combination of photobioreactors and small ponds as research tools and in a group of outdoor testbeds in Texas, New Mexico, Arizona, and Hawaii with both small- and large-scale cultivation systems. Within the first area, Cultivation Team research focused on environmental factors for predator control, climate simulation modeling, biomass growth models, and sensor development. The second thrust area focused on developing and testing novel cultivation systems to improve strain screening, cultivation efficiency, and productivity. The third area focused on using various wastewaters (produced water,

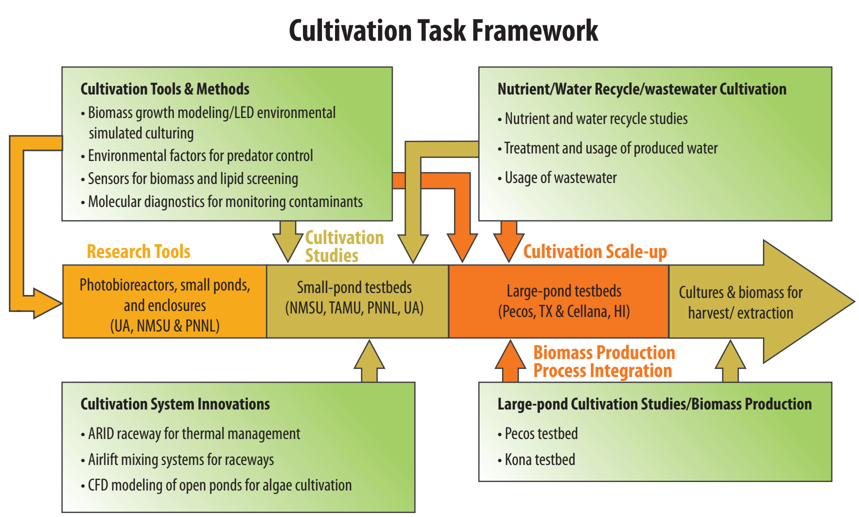


Figure 5. Cultivation R&D framework.

municipal wastewater, and brackish water) to support large-scale cultivation. The fourth thrust area focused on cultivation studies at the large-scale testbeds using production development strains from Algal Biology and Cultivation research that were selected for scale-up, productivity studies, and producing biomass for downstream processing tasks. Collectively the objective of the Cultivation framework was to develop systems and methods for sustainable high productivity, production of biomass from selected production strains, and providing a testbed for harvesting technologies. Biomass from NAABB production development strains was provided to the Harvesting and Extraction task for scale-up, production assessments, and the production of lipid extracts and LEA for Fuel Conversion team studies. The following three major milestones and deliverables were completed as part of the Cultivation task (Table 4).

Table 4. Cultivation major milestones and deliverables.

Milestones (M) and (DL) Deliverables	Time Completed (months)
DL1: Cultivation methods for greater than 5 gdw/L/day biomass at ≥ 50% lipid content with low nutrient consumption in a small-scale closed system demonstrated.	18
DL2: Test-bed facilities fully operational.	24
M1: Cultivation methods for approaching target growth rates and lipid yield with best strain and low nutrient consumption in a large-scale open pond system demonstrated.	36

Harvesting and Extraction R&D Framework

The Harvesting and Extraction R&D framework (Figure 6) had two major technical thrusts: (1) develop dewatering processes for harvesting and (2) develop wet-extraction processes for lipids. The harvesting/dewatering thrust focused on four different process technologies: chemical flocculation, electrolytic processing, cross-flow membrane filtration, and ultrasonic focusing. These harvesting technologies were evaluated for their application as primary harvesting methods from the cultivation pond to achieve 10X concentration and/or secondary dewatering of algae to achieve an additional concentration factor of 15–20X of the algal biomass. The extraction thrust area focused on four different wet-extraction processes for lipid extraction: amphiphilic solvent process, ultrasonic process, cavitation/separation, and wet solvent extraction from Valicor as a toll processor. One extraction technology, mesoporous extraction, focused on the purification of certain high-value products from lipid extracts. Each of the extraction technologies was evaluated for its application to recover lipids or fatty acid methyl esters (FAMEs) from wet algal biomass and to

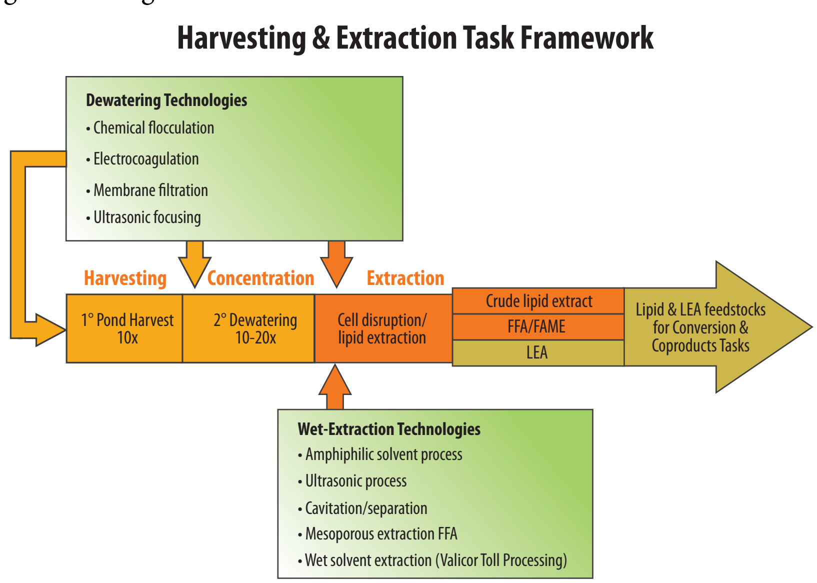


Figure 6.. Harvesting and Extraction R&D framework.

provide a high-quality LEA. In addition, the Valicor process was used to produce lipid extracts and LEA from NAABB strains produced in the large testbeds for Fuel Conversion and Agriculture Coproducts studies.

Collectively the objective of the Harvesting and Extraction framework was to develop and evaluate processes and methods for sustainable algal biomass harvesting and lipid extraction from algal biomass. Lipid extracts and LEA from NAABB production-development strains were provided to the Fuel Conversion task for production of fuels and to the Agricultural Coproducts task for evaluations as feed and fertilizer supplements. The following three major milestones and deliverables were completed as part of the Harvesting and Extraction task. In addition, this task had a major decision point and down-selection at 18 months to identify the most viable technologies for further scale-up and testing. Three harvesting technologies were selected for scale-up. At 18 months all extraction technologies other than the Valicor toll process were determined to be too early-stage (Table 5).

Table 5. Harvesting and Extraction major milestones and deliverables.

Milestones (M), Decision Points (DP), and Deliverables (DL)	Time Completed (months)
DL1: New harvesting/extraction technologies demonstrated at bench scale and scale-up defined (design specs, drawings, and performance reports).	15
DL2: Economic analysis establishing most-efficient technologies at liter scale complete.	18
DP2: (Go/No Go) Most viable technology(s) selected for large-scale field tests based on performance and scale-up viability criteria.	18
M1: Systems capable of 100–1000 L/hr feedstock processing demonstrated.	36

Fuel Conversion R&D Framework

The Fuel Conversion R&D framework (Figure 7) had three major technical thrusts: (1) lipid conversion to fuels, (2) biomass and LEA conversion to fuels and chemicals, and (3) detailed characterization of conversion feedstocks and products. The lipid conversion thrust focused on two main routes, one to produce FAME biodiesel by catalytic and sub-supercritical extraction and the other to produce hydrocarbon fuels by different catalytic upgrading methods. The biomass/LEA conversion thrust focused on processes to convert LEA to ethanol, mixed alcohols to gasoline, methane for power, and chemicals. In addition, whole biomass conversion by hydrothermal liquefaction and upgrading to hydrocarbon fuels was also a late addition to

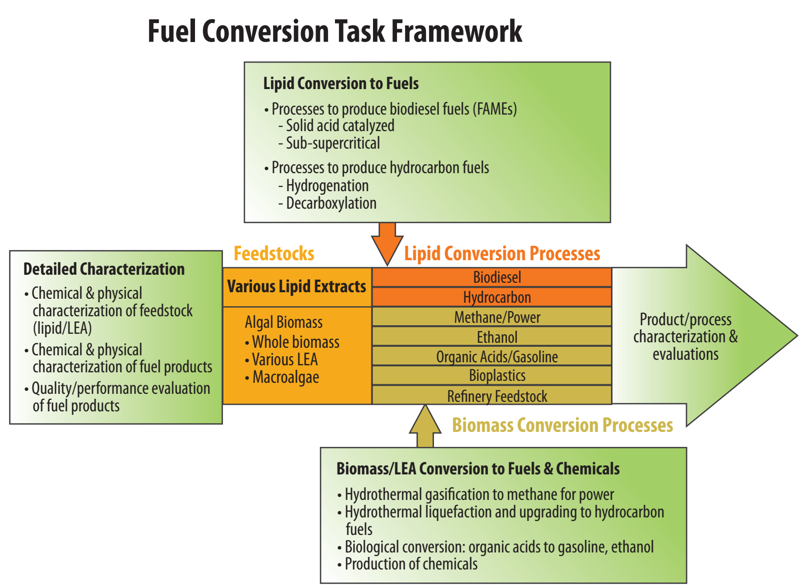


Figure 7. Fuel Conversion R& D framework.

this thrust area. The third thrust area focused on providing detailed physical and chemical characterization of the biomass, crude lipids, and LEA and of the resulting fuel and chemical products that were made. These efforts also included quality and performance evaluations of the fuel products. Collectively the objective of the Fuel Conversion framework was to develop and evaluate various processes and methods for converting algal biomass, lipid extracts, and LEA to fuels and chemicals. All the feedstocks (algal biomass, lipid extracts, and LEA) were derived from NAABB production-development strains and produced in the preceding task areas. The following four major milestones and deliverables were completed as part of the Fuel Conversion task (Table 6).

Table 6. Fuel Conversion major milestones and deliverables.

Milestones (M) and Deliverables (DL)	Time Completed (months)
DL1: Preliminary cost analysis, bench-scale rate data, and yield information obtained.	24
M1: Optimal conversion process selected for large-scale production and wide-scale use based on performance and scale-up requirements criteria.	24
DL2: Pollutant and greenhouse gas emissions from the combustion of algae-derived biofuels characterized.	30
DL3: Aspen process model of conversion technologies demonstrated.	30
DL4: Cost analysis, bench-scale rate data, and yield information obtained for production of chemical feedstock.	30

Agricultural Coproducts R&D Framework

The Agricultural Coproducts R&D framework (Figure 6) had two major technical thrusts: (1) animal-feed development and testing with LEA and (2) fertilizer evaluations of LEA .The LEA animal-feed thrust area focused on *in vitro* and *in vivo* testing for nutritional value, maricultural-feed studies (shrimp and fin fish), and large-animal feed studies (cattle, sheep, and swine). The LEA fertilizer thrust focused on small-greenhouse studies and limited field trials. Collectively the objective of the Agricultural Coproducts framework was to evaluate the performance and value of LEA coproducts for use in fertilizer and feed agricultural markets as a means to offset the production costs of algal biofuel. LEA from NAABB production development strains was provided to the Agricultural Coproducts Team for evaluations as feed and fertilizer supplements. Additional LEA was provided by General Atomics to support the quantities required for large animal trials. The following two major milestones and deliverables were completed as part of the Agricultural Coproducts task (Table 7).

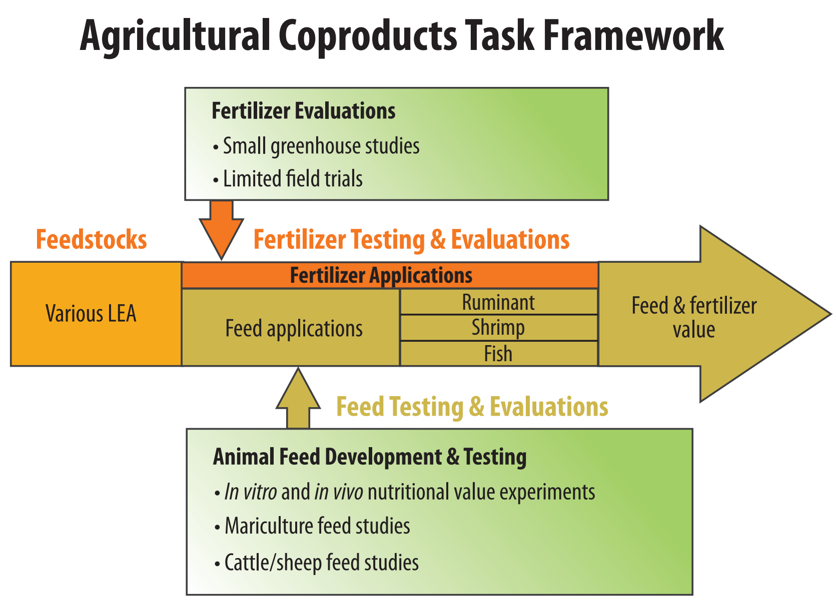


Figure 8. Agricultural Coproducts R & D framework.

Table 7. Agricultural Coproducts major milestones and deliverables.	
Milestones (M) and Deliverables (DL)	**Time Completed (months)**
M1: Feed value for LEA determined.	24
DL2: Best-performing feed formulations determined.	36

Sustainability R&D Framework

The Sustainability R&D framework (Figure 9) had three major technical thrusts: (1) sustainability models and tools for economic and environmental impact, (2) model integration, harmonization and analysis, and (3) data management and integration across NAABB via an electronic lab notebook. The development of economic and environmental models and tools was the central thrust of this task and focused on four major modeling efforts: TEA process models, LCA process models, GIS-based biomass resource assessment models, and comprehensive economic models for algae farms and global impacts. These modeling-development efforts and the various individual modeling components were harmonized and integrated into an Algal Integrated Simulation Model (AISIM) to perform scenario analysis using the data from NAABB-developed strains and process technologies. Collectively the objective of the Sustainability Team was to evaluate various processes and methods for producing algal biofuels using NAABB technologies and data through the integration of a diverse set of modeling tools. The following three major milestones and deliverables were completed as part of the Sustainability task (Table 8).

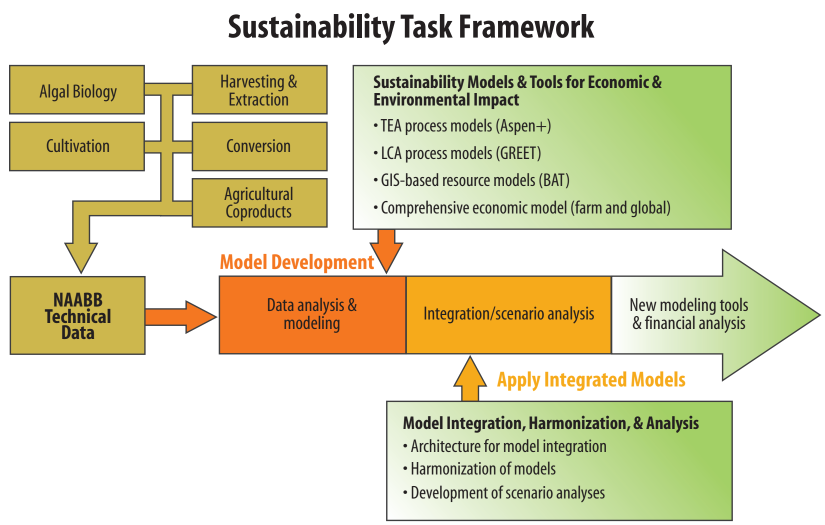


Figure 9. Sustainability R&D framework.

Table 8. Sustainability major milestones and deliverables.	
Milestones (M) and Deliverables (DL)	**Time Completed (months)**
DL1: Aspen process model for producing synthetic natural gas, liquid algal biofuel, and chemical feedstock completed.	12
DL2: AISIM data integration and standardization framework established.	24
M1: Web-based AISIM modeling and database system fully implemented.	36

Intellectual Property

Governance

Within the NAABB consortium, management of intellectual property (IP) was governed by a suite of agreements. IP comprises discoveries, innovations, biological materials such as new strains of algae, works of authorship, and inventions, along with derivative works, whether patentable or not, including computer software and code, patents and patent applications, trade secrets, mask works, copyrights, and copyrightable materials (i.e., technical data). The suite of agreements is composed of a memorandum of understanding, IP management plan, nondisclosure agreement, and material transfer agreement, along with a patent waiver issued by the U.S. Department of Energy.[1]

The suite recognizes two forms of IP:

- "Background IP" comprising IP produced outside of NAABB research and without using NAABB funding.
- "Program IP" comprising inventions, copyrightable works, and other IP first produced, e.g. conceived or reduced to practice or authored, in performance of NAABB research using NAABB funding. Under the patent waiver, the institution creating program IP is granted ownership of its program IP.

With regards to background IP, collectively the suite of agreements:

- Requests that NAABB members with background IP that may be pertinent to NAABB research disclose that IP to the NAABB Director of Industry Relations, who then discloses background IP to all NAABB members.
- Grants NAABB members that need to use background IP to perform NAABB research a license to use background IP only for purposes of doing NAABB research.
- Confirms that contribution of background IP to performance of NAABB research does not impact ownership of background IP.
- Requires that a NAABB member protects background IP that it receives from disclosure to any other party with the same degree of care that it protects its own IP.

With regards to program IP, collectively the suite of agreements:

- States that ownership of program IP will be determined by DOE's patent waiver and U.S. patent and copyright law.
- Stipulates that institutions that jointly invent program IP will jointly own that IP and will cooperate in securing protection of that IP.

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[1]Advance class waiver of patent rights for technology developed under the Office of Biomass program funding opportunity announcement, "Recovery Act: Development of Algal/Advanced Biofuels Consortia." DE-FOA-0000123, W(C)2009-012.

- Requires that NAABB members disclose their program IP to NAABB's Director of Industry Relations, who then will disclose program IP to all NAABB members.
- Requires that program IP be reported to the U.S. Department of Energy.
- Grants NAABB members that need to use program IP to perform NAABB research a license to use program IP only for purposes of doing NAABB research.
- Provides that a NAABB member that wants to use program IP for commercial purposes has a 30-day period after disclosure to contact the owner of program IP to request a commercial license.
- Requests that NAABB members make "best-efforts" to disclose strains to the "broad" research community.
- Requires that a NAABB member protects program IP that it receives from disclosure to any other party with the same degree of care that it protects its own IP.
- Sets the expectation that after obtaining patent or other IP protection, creators of program IP, including algal strains, will share their research results with the general research community through publications and conference presentations.

Results

At the time of this report, NAABB members had disclosed 33 new inventions, seven of which were associated with a U.S. or Patent Convention Treaty (PCT) patent application. These inventions are listed in the following table along with the inventing NAABB member. The inventions are categorized by the NAABB research program with which they are most closely associated.

Algal Biology		
	Donald Danforth Plant Science Center	Developing a transgenic photosynthetic organism that can autoregulate its light harvesting antenna size
	Los Alamos National Laboratory	Identification and creation of algal strains for the purpose of drop-in transportation fuels: *Tetraselmis* sp. *LANL1001* and others
	Los Alamos National Laboratory	Isolation of a high-lipid-content subpopulation of *Nannochloris* sp.
	Texas A&M University	Agrobacterium- and glass-beads–based transformation of different green algae strains
	Texas A&M University	Metabolic engineering of algae for aviation fuel production
	Targeted Growth	Modified photosynthetic microorganisms for continuous production of carbon-based products
	Targeted Growth	Modified photosynthetic microorganisms for continuous production of carbon-containing products
	Targeted Growth	Modified diacylglycerol acyltransferase proteins and methods of use thereof
	Washington University	Method for obtaining buoyant triacylglycerol-filled *Chlamydomonas reinhardtii*
Cultivation		
	Donald Danforth Plant Science Center	Exposure to decane leads to oil induction in algae at single cell and autospore stages
	Genifuel Corporation	Closed-loop system for growth of aquatic biomass and gasification thereof
	Los Alamos National Laboratory	Hydrogel-based integrated environments for microalgae cultivation
	Los Alamos National Laboratory	Reverse-flow submerged forward osmosis for clean water recharge to algae raceway systems
	Michigan State University	Photobioreactor/sensor array
	Eldorado Biofuels	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
Harvesting & Extraction		
	Los Alamos National Laboratory and Solix Biosystems	Selective acoustic collection of algae Method and apparatus for acoustically manipulating biological particles
	Los Alamos National Laboratory	Acoustic-driven emulsion destabilization
	Los Alamos National Laboratory	Optical-driven emulsion destabilization

	Texas A&M University	Solvent-phase extraction of algal oils via surface functionalized migration of algal cells from aqueous-phase to solvent-phase
	UOP	Comparison of metals and phosphorous removal of crude algae oil extracts using acid washing alone and in combination with base and water washing
	UOP	Removal of metals from algal oil by acid washing and combined base/acid washing
	UOP	Removal of chloride from triglyceride oils using a combination of hot base and acid washing
Fuel Conversion		
	Colorado State University	Bioconversion of extracted algal biomass into fuels and other chemicals
	New Mexico State University	Extractive conversion of wet algae to biodiesel under supercritical methanol conditions
	New Mexico State University	Extractive conversion of dry algae to biodiesel under microwave irradiation
	Pacific Northwest National Laboratory and Genifuel Corporation	Sulfate removal from hydrothermal environment
	Texas A&M University	Production of high-quality bio-oil, biochar, and synthesis gas from microalgae using pressure reactor and the TAMU fluidized-bed pyrolyzer
	UOP	Production of large molecular weight paraffinic waxes from hydroprocessing of algal oils
	UOP	Production of fully synthetic jet fuel from hydrothermally liquefied algal biomass
Other		
	Iowa State University	Selective absorption of tocopherol by pentafluorophenyl-functionalized mesoporous silica nanoparticles
	Los Alamos National Laboratory	Facile isotopic synthesis of isoprenoid precursors
	Los Alamos National Laboratory	Functionalization of sialic acid: odorant derivatives and adsorption/attachment to natural and/or manmade surfaces
	Eldorado Biofuels	Method and apparatus for greenhouse gas regulation using algae to create a strategic algae reserve energy supply

NAABB Publications

Algal Biology

Full-length, Peer-reviewed Publications

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Kim, H.S., T.L. Weiss, H.R. Thapa, T.P. Devarenne, and A.Han. A microfluidic photobioreactor array demonstrating high-throughput screening for microalgal oil production. *Lab Chip* 14 (2014): 1415.

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Cultivation

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Harvesting and Extraction

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Agricultural Coproducts

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Sustainability

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