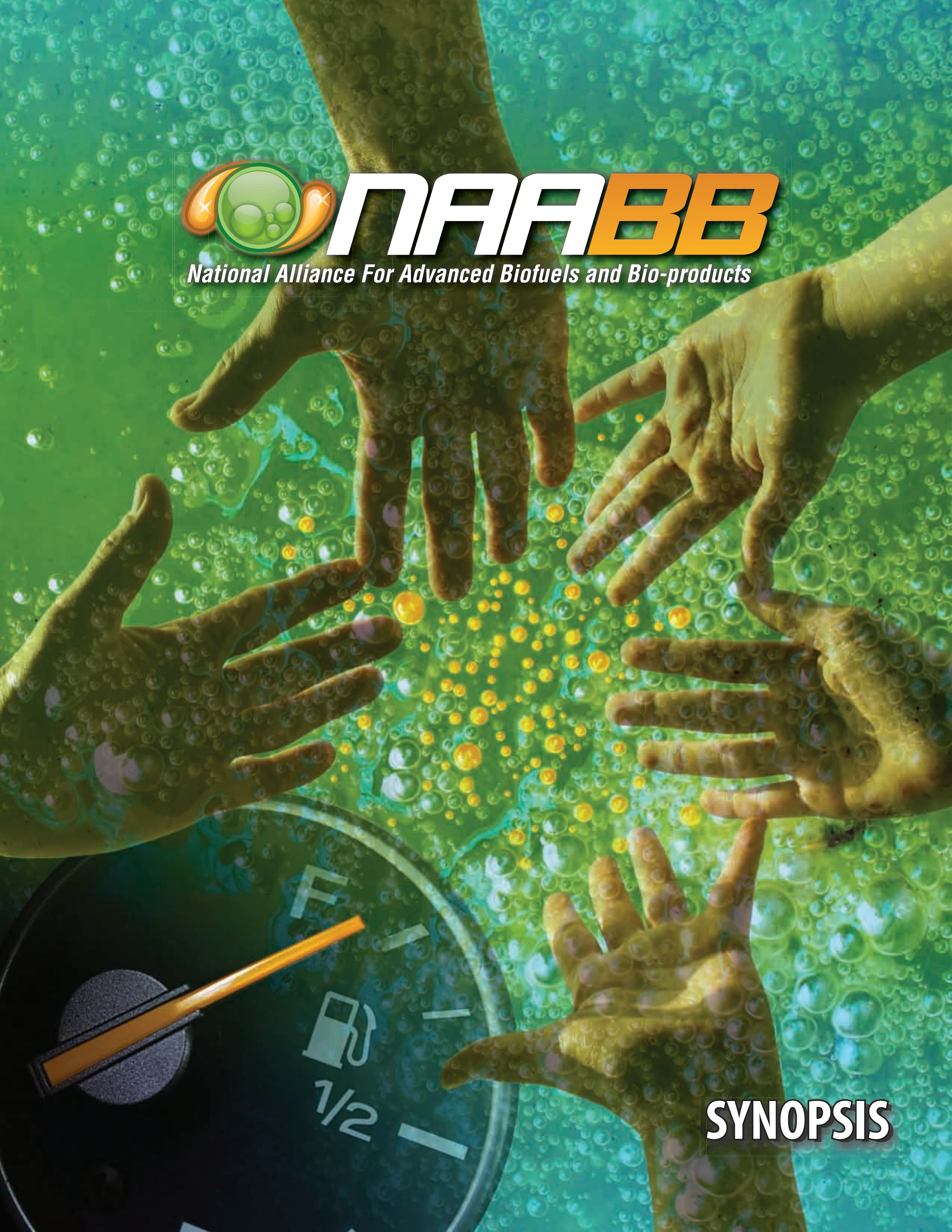


NAABB

National Alliance For Advanced Biofuels and Bio-products

SYNOPSIS

Table of Contents

Preface

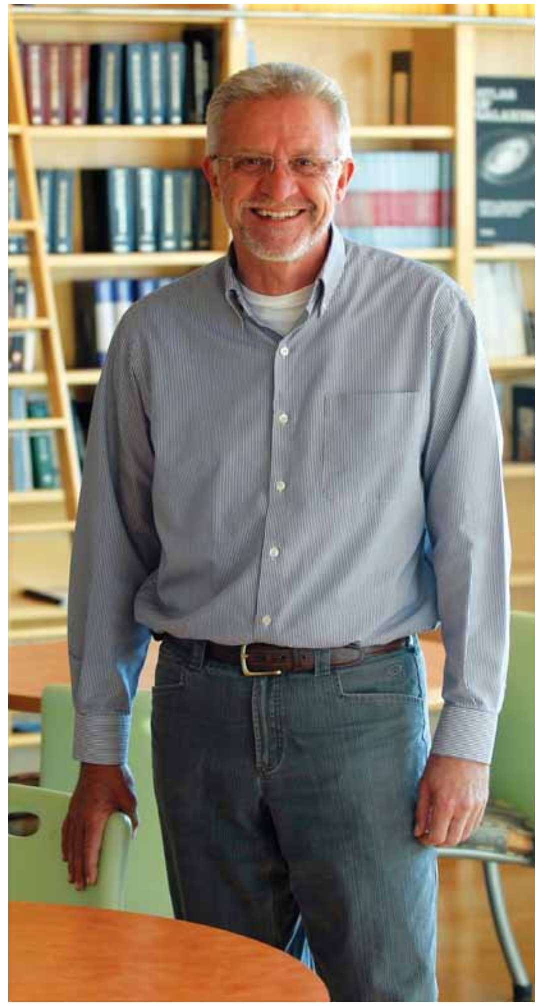
The National Alliance for Advanced Biofuels and Bioproducts (NAABB), an algal biofuels research consortium, was formed to specifically address the objectives set forth by the U.S. Department of Energy, Energy Efficiency and Renewable Energy (DOE-EERE), Office of Biomass Programs (now called the Bioenergy 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

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. Department of Energy [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

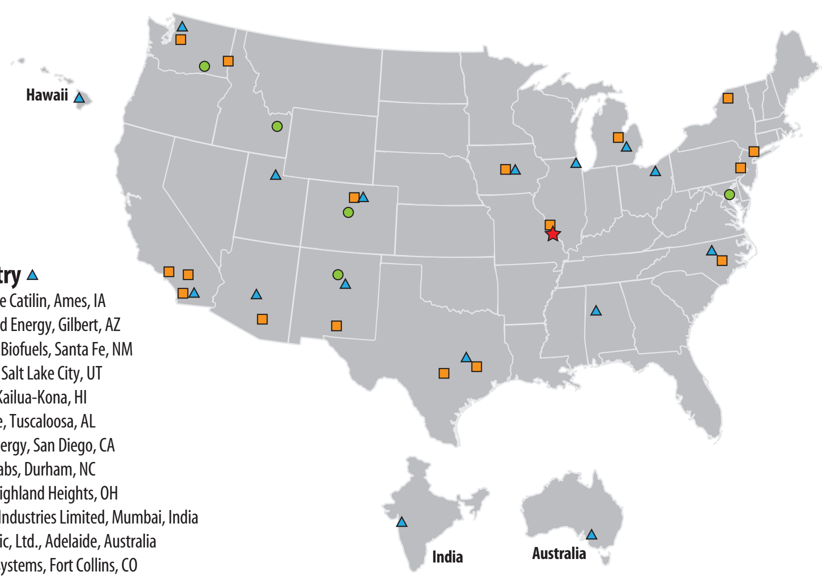


Figure 1. Map showing the locations of the thirty-nine NAABB consortium partners.

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).

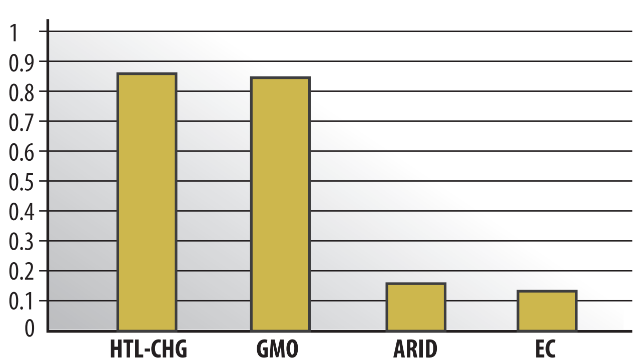


Figure 2. Potential cost reductions (y-axis) that can be achieved with four main NAABB innovations, hydrothermal liquefaction with catalytic hydrothermal gasification (HTL-CHG), a novel genetically modified algae strain (GMO), a unique design open pond cultivation system (ARID), and electrocoagulation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

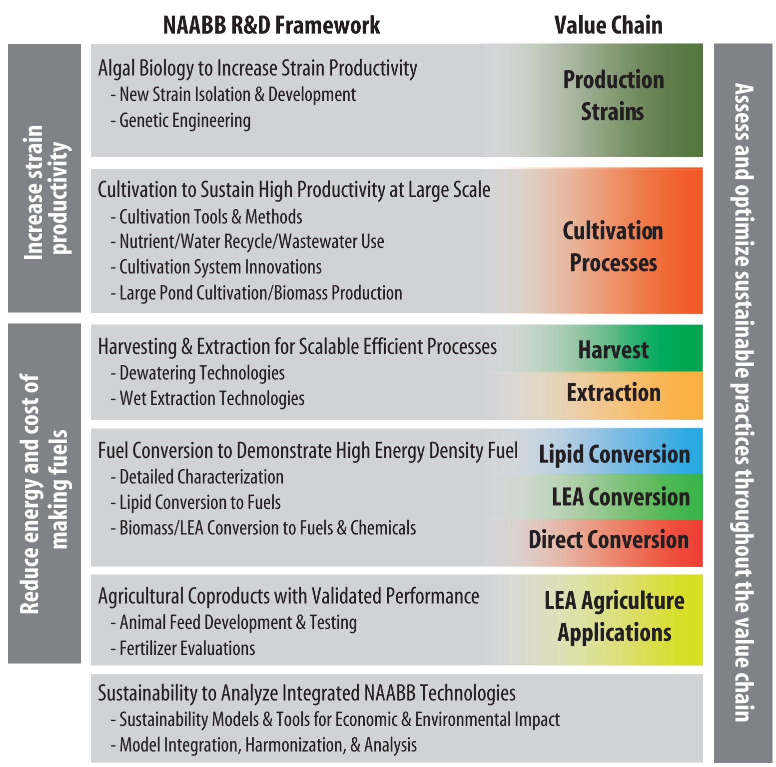


Figure 3. The NAABB R&D Framework.

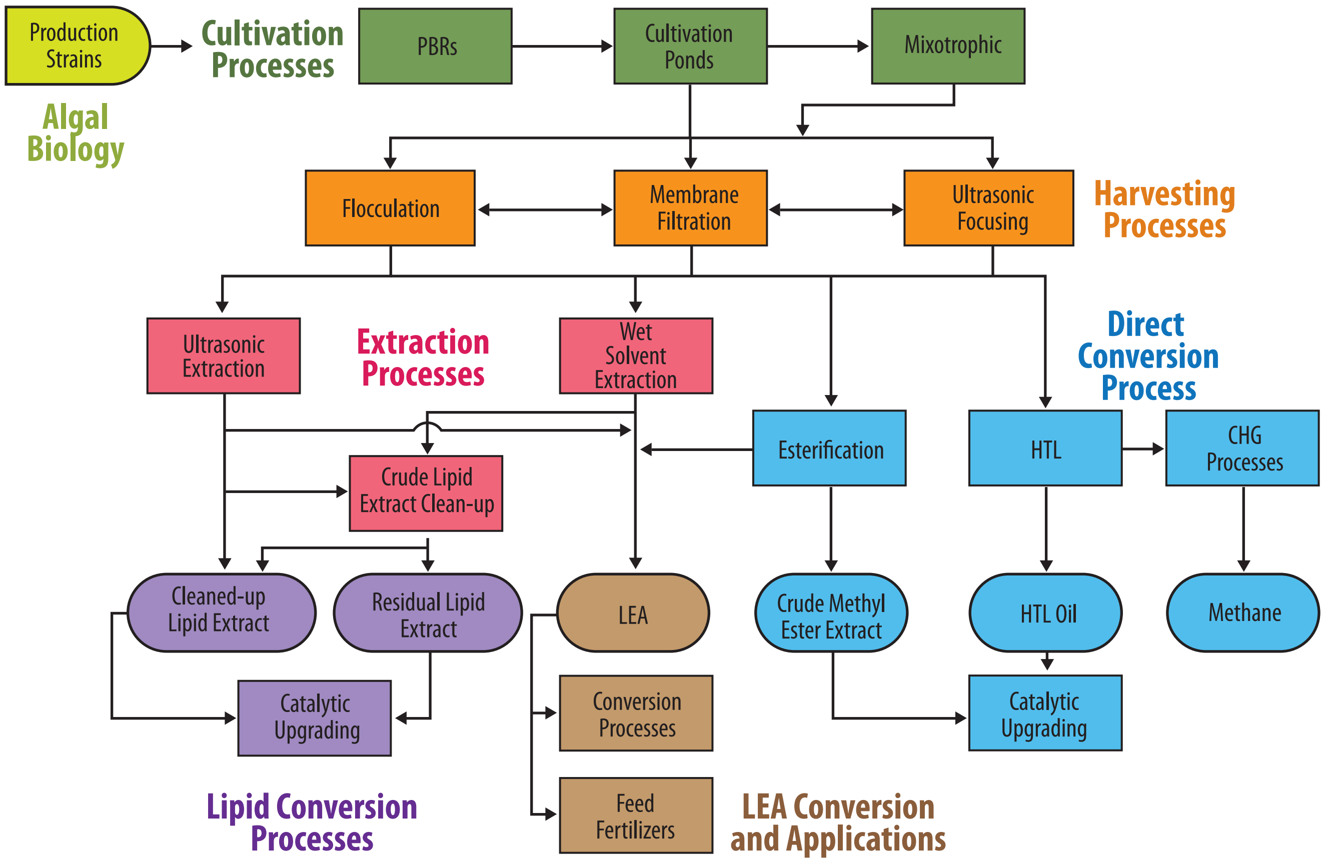


Figure 4. The NAABB Process Matrix.

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.

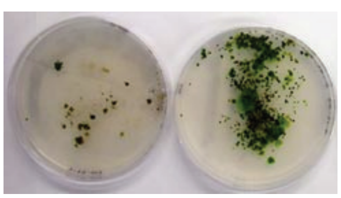
Completed genome sequencing on eight new algae strains

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

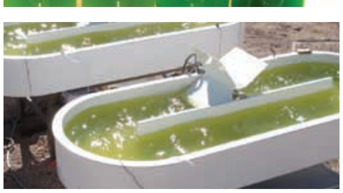

5. Validate

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.

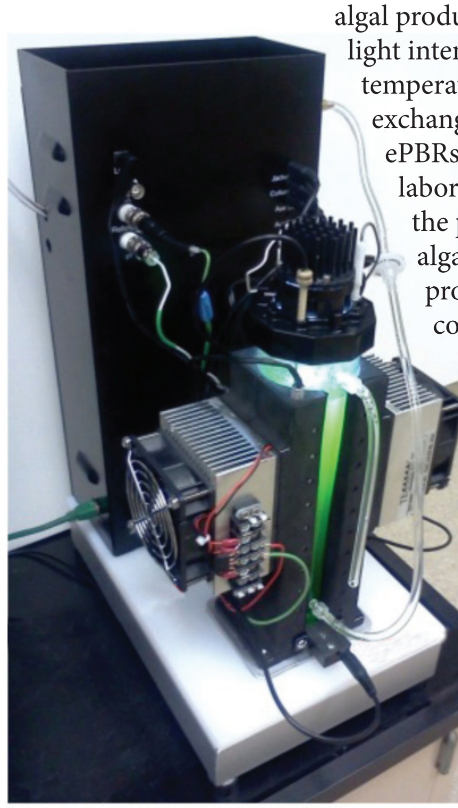


Figure 7. The Environmental Photobioreactor (ePBR).

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.

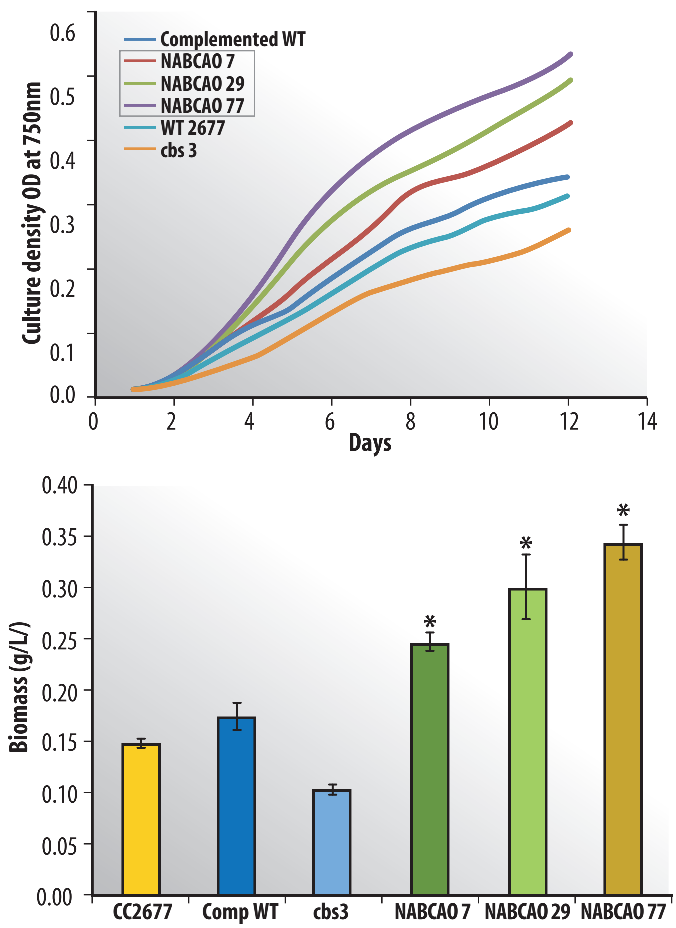


Figure 8. Cellular (top) and dry weight (bottom) productivity of wild type (WT) and transgenic algae with self-adjusting light-harvesting antenna. The NABCAO lines have been engineered to self-adjust the ratios of their chlorophyll binding proteins, and hence peripheral light-harvesting antenna size,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

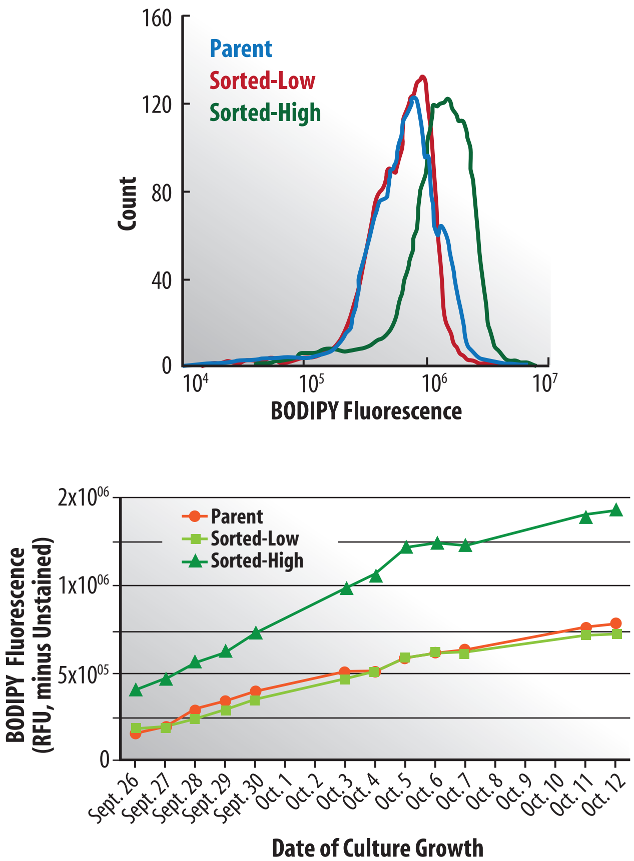


Figure 9. (Top) Histograms of BODIPY fluorescence of parent and sorted populations. (Bottom) During nitrogen starvation, all cultures accumulated lipids, with the sorted-high population outperforming the parent on all days (average 2X improvement).

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.

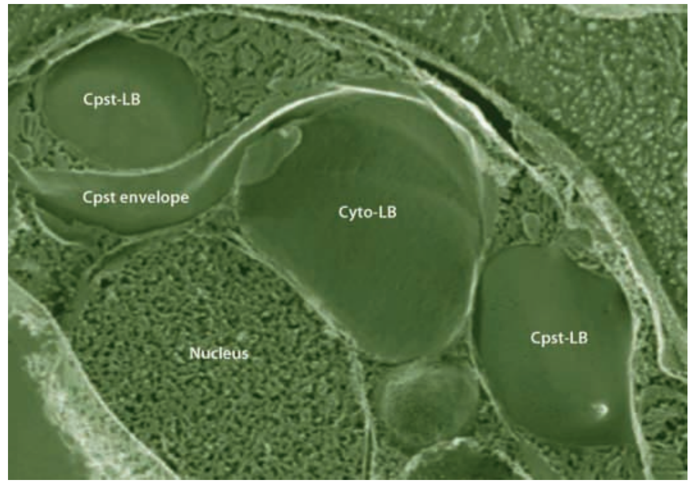


Figure 10. Cytoplasmic and chloroplast lipid bodies in the *sta6* strain of *C. reinhardtii*.

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.

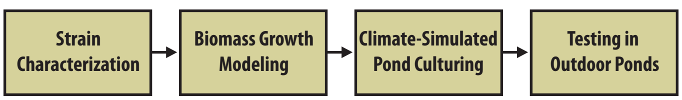


Figure 11. NAABB stepwise strain screening strategy.

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.

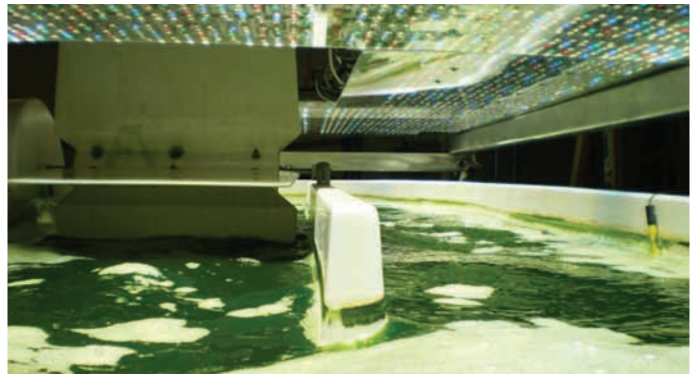


Figure 12. Environmental Simulated Culture System: Indoor raceway pond with temperature control and LED lighting to simulate sunlight spectrum and intensity and climate for any potential production site.

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.

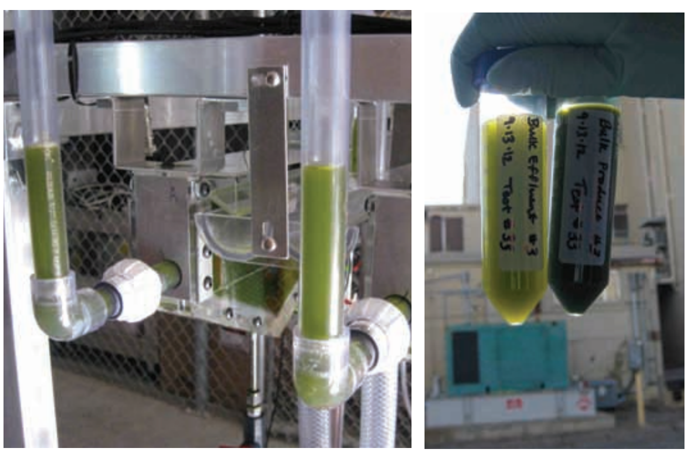


Figure 16. Ultrasonic harvester. Left panel, side view of two 2 L modules attached to a customized cart containing twelve modules. The dilute feedstock is fed into the modules by gravity flow and the concentrate is collected from the bottoms of the modules. Right panel, visual comparison of the dilute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

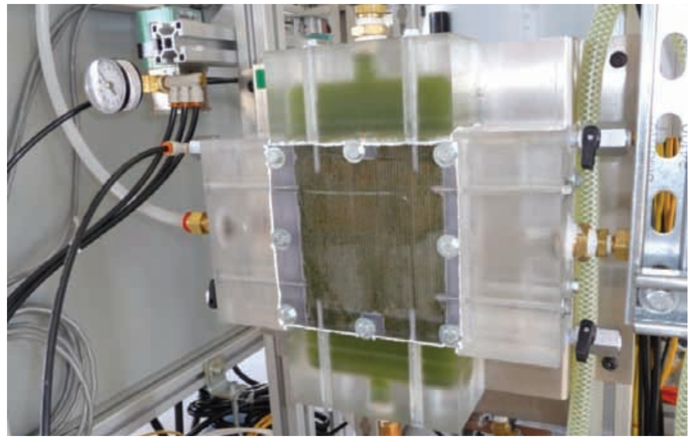


Figure 17. Cross-flow membrane modules assembled for field tests (0.26 m^2 of membrane area, 1 mm feed flow channel opening).

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

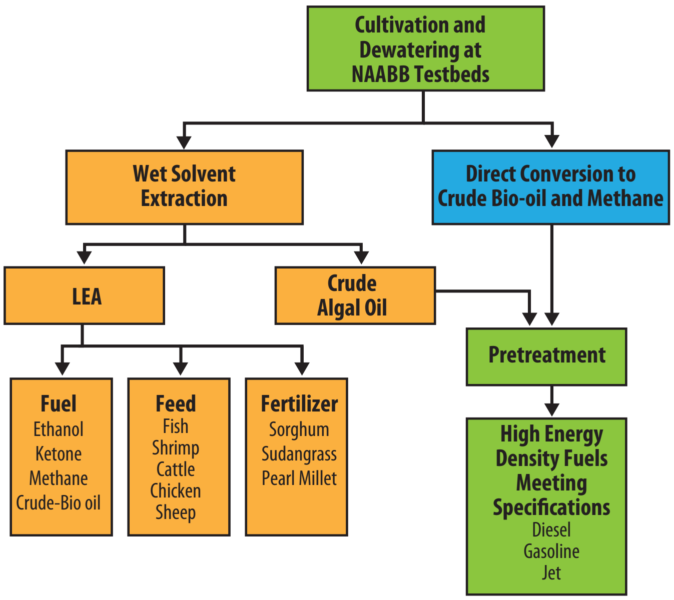


Figure 19. Pathways investigated by NAABB for producing fuel: The traditional method via extraction and separation of lipid material from LEA (left path on chart) and the direct method (right).

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.

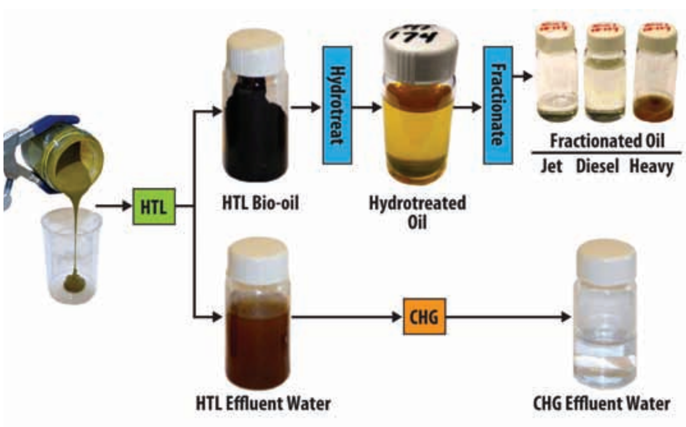


Figure 20. The process flows of HTL and HTL with CHG.

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)*.

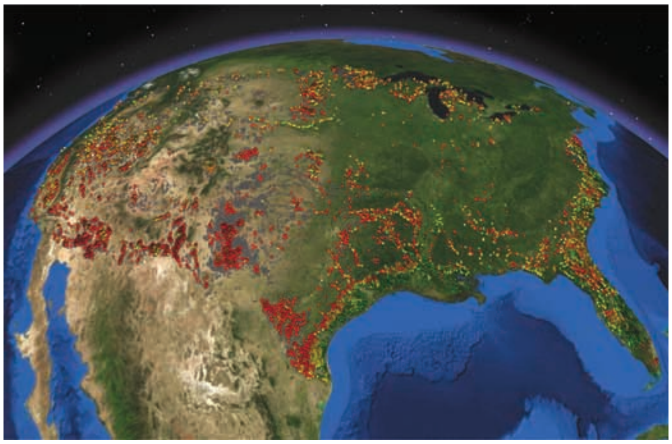


Figure 22. Resource-feasible algae production sites in the United States were selected from the BAT analysis

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.

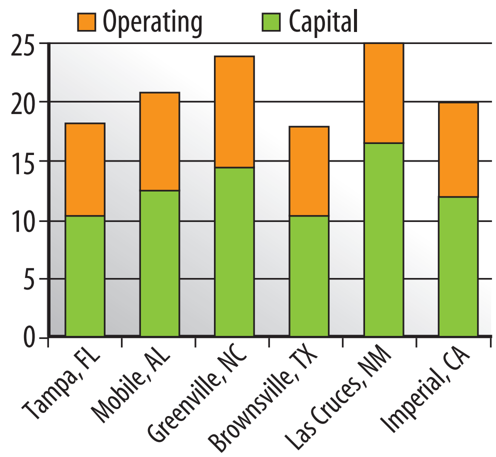


Figure 23. Cost assessment ($/gallon TAG) of the harmonized baseline design in several locations across the southern United States using respective BAT algal biomass productivity.

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.

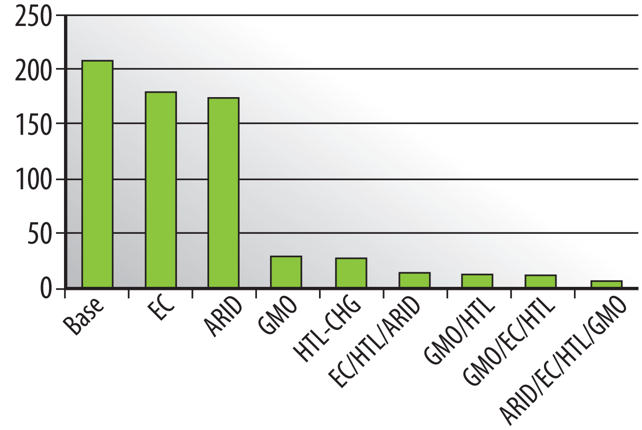


Figure 24. Total economic plus financial costs of algal crude oil production for pre-NAABB technology and for alternative technologies developed by NAABB ($/gallon).

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;

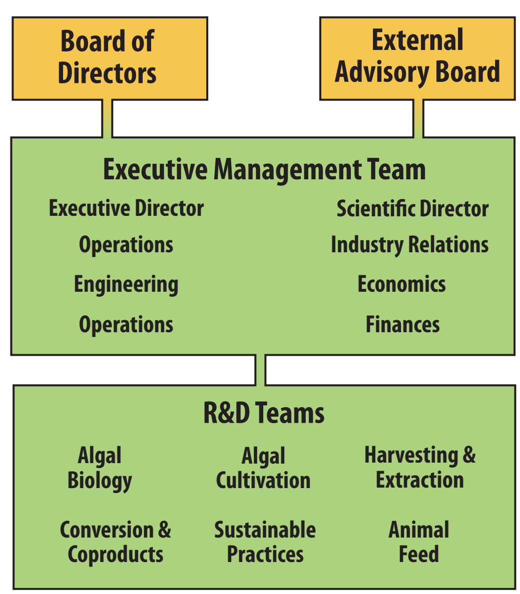


Figure 25. NAABB's management, organization, and approach structure.

- 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).

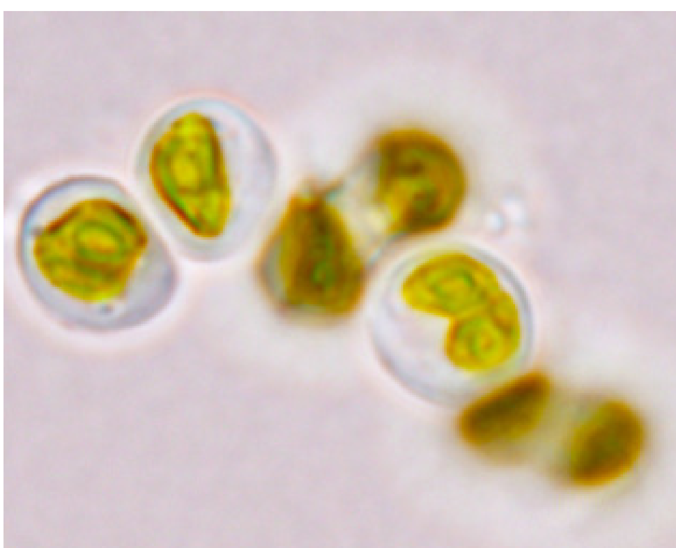


Figure 26. Photomicrograph of the NABBB-discovered strain *Chlorella* sp. *DOE1412.*

- 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:

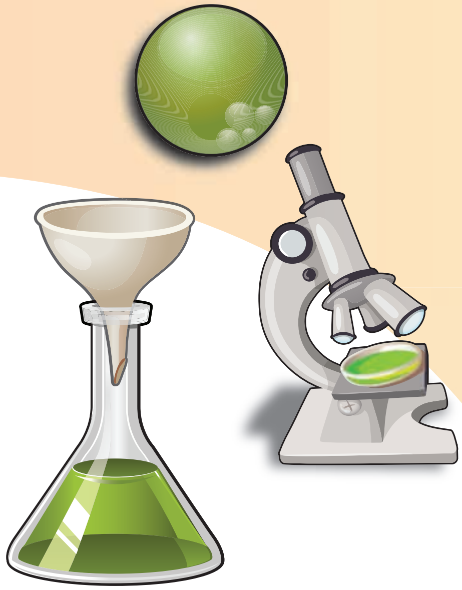
- Deposited 30 new algae strains into the UTEX culture collection;
- Started a new peer reviewed journal, *Algal Research* (by Elsevier);
- Initiated a new conference series: *International Conference on Algal Biomass, Biofuels, and Bioproducts*;
- Contributed over 100 peer-reviewed publications. NAABB 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

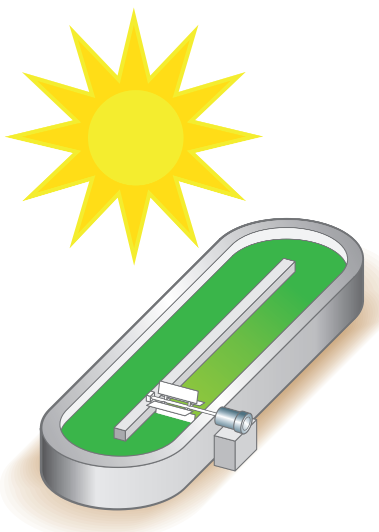
The Road to $7.50/Gallon



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.

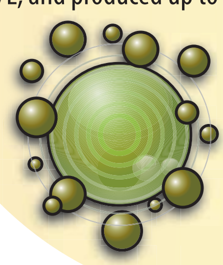
CULTIVATION



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 Recovery 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



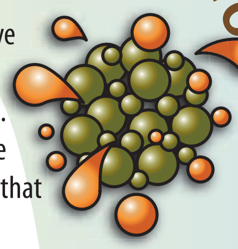
Harvesting

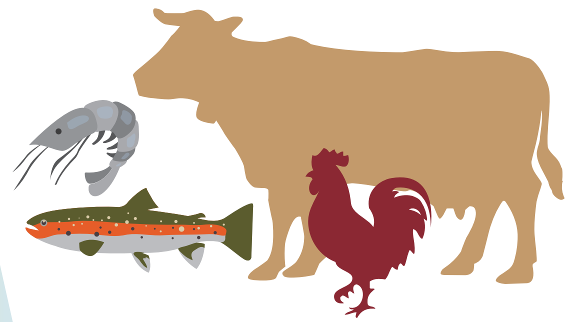
After a strain is cultivated in an open pond, the dilute algal biomass must be separated from the water. The traditional harvesting method is centrifugation, which NAABB employed extensively to harvest *Chlorella* sp. *DOE1412*. NAABB also successfully demonstrated the ability to harvest *Chlorella* 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.

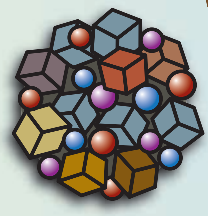
Fuel and Feed or Fuel Alone?

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.

CONVERSION TO FUEL OR COPRODUCT





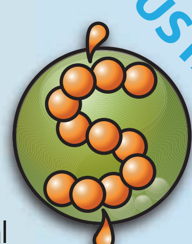


Is it Sustainable?

Within a three-year time span, NAABB researchers isolated a strain, grew it in large-scale outdoors 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.