DOE/DE-FG02-08ER64622

Sustainable Biofuels Development Center

Final Technical Report

Period Covered: August 2008 – September 2013

March 2015

Kenneth F. Reardon
Colorado State University
Fort Collins, Colorado

DOE/DE-FG02-08ER64622

PREPARED FOR THE U.S. DEPARTMENT OF ENERGY
OFFICE OF SCIENCE
UNDER CONTRACT NO. DOE/DE-FG02-08ER64622

DOE/DE-FG02-08ER64622

Sustainable Biofuels Development Center

Final Technical Report

Period Covered: August 2008 – September 2013

March 2015

Kenneth F. Reardon
Colorado State University
Fort Collins, Colorado

PREPARED FOR THE U.S. DEPARTMENT OF ENERGY
OFFICE OF SCIENCE
UNDER CONTRACT NO. DOE/DE-FG02-08ER64622

Abstract

The mission of the Sustainable Bioenergy Development Center (SBDC) is to enhance the capability of America's bioenergy industry to produce transportation fuels and chemical feedstocks on a large scale, with significant energy yields, at competitive cost, through sustainable production techniques. Research within the SBDC is organized in five areas: (1) Development of Sustainable Crops and Agricultural Strategies, (2) Improvement of Biomass Processing Technologies, (3) Biofuel Characterization and Engine Adaptation, (4) Production of Byproducts for Sustainable Biorefining, and (5) Sustainability Assessment, including evaluation of the ecosystem/climate change implication of center research and evaluation of the policy implications of widespread production and utilization of bioenergy. The overall goal of this project is to develop new sustainable bioenergy-related technologies. To achieve that goal, three specific activities were supported with DOE funds: bioenergy-related research initiation projects, bioenergy research and education via support of undergraduate and graduate students, and Research Support Activities (equipment purchases, travel to attend bioenergy conferences, and seminars). Numerous research findings in diverse fields related to bioenergy were produced from these activities and are summarized in this report.

All portions of this report are Unclassified.

Acknowledgements

The many technical contributions of the researchers involved in the activities of the Sustainable Bioenergy Development Center are acknowledged, especially the faculty members, postdoctoral researchers, and students involved in the research projects described here.

Table of Contents

List of Figures

List of Tables

1. Introduction

Since the 1970s, several factors have driven interest in developing biofuels as alternatives to liquid transportation fuels derived from petroleum. The first factor, dominant in the 1970s and again with the rapid rise of oil prices in the late 2000s, is about the confluence of declining petroleum production and increasing global demand. While discussion of this factor has changed in recent years owing to the expansion of fracking, the increase of oil supply from Iran, and the oil pricing strategies of OPEC, one should note that the petroleum production rate is not solely a matter of what is possible (the amount of total reserves) but also what markets and societies deem feasible and environmentally acceptable.

The second factor pertains to economic and political issues. In the late 2000s, the US imported as much as 60% of its petroleum needs. While that level has decreased significantly, the fraction of oil and petroleum products imported in 2014 was still 48%. Thus, a large fraction of the country's energy economy relies on imports, and a concomitant amount of money is spent outside the US. Furthermore, this dependence on imported petroleum links the US economy more tightly to certain international policy discussions than many find desirable.

Finally, there is the factor of environmental sustainability, and particularly of global climate change driven by greenhouse gas production. The consumption of fossil fuels, no matter the price or the level of domestic production, exacerbates climate change. While solar, wind, and geothermal energy are critical sources of renewable electricity, the only source of renewable liquid transportation fuels is from biomass. Furthermore, only biofuel production involves removal of carbon dioxide from the atmosphere and has the potential for net negative carbon emissions.

Biofuels are an attractive option to address the all of these factors. Biofuels are produced from plant-based (and thus renewable) materials that are transformed by chemical, thermal, and/or biological processes (Hill 2006; Vertès et al. 2008; Wackett 2008). Ethanol and biodiesel are the best-known biofuels. Additionally, biofuels such as butanol, "bio-gasoline", and hydrogen may become important. Biomass can also be used to produce energy in the form of heating fuels (methane) or electricity. Bioenergy can be produced domestically, potentially providing a buffer against price fluctuations and geopolitical uncertainty. Finally, the carbon dioxide produced by the combustion of biofuels is offset by the photosynthetic uptake of carbon for plant growth, suggesting the possibility of fuel production with a carbon footprint that is much smaller than current alternatives (Hill 2006; Vertès et al. 2008). However, there is increasing evidence that production of biofuels using traditional processes (cornstarch or sugarcane to ethanol by fermentation, and lipids from oilseed crops to biodiesel by transesterification) are not as sustainable as would be desired (Hill 2006; Weng et al. 2008). Moreover, it is critical to maintain US leadership in the generation of new bioenergy technologies.

Many challenges, including agricultural issues, efficient processing, and engine optimization, must be addressed before the benefits of biofuels can be realized. An important challenge for agricultural and plant scientists is the fact that essentially all of highest productivity farmland in America is utilized for food or fiber production. There is the capability to increase production on marginal croplands, but the high energy (for fuel and fertilizer) and water requirements raise questions about the sustainability of biofuel production on a large enough scale to supply any significant portion of America's future liquid fuel needs. The largest source of liquid biofuel is ethanol, which is currently produced in the US by fermenting starch from corn (Gray et al. 2006; Vertès et al. 2008). Efforts are underway to address the issues of scale and sustainability by developing the capacity to produce ethanol from cellulosic feedstocks, which are much more widely available than starch-based feedstocks and require less water and energy (Vertès et al. 2008; Weng et al. 2008).

Another general challenge is presented by the need for cost-effective processing technologies. For example, the resource advantages of using cellulosic feedstocks are limited by the high cost of the chemical and biological processing used prior to fermentation to ethanol and other biofuels, and improvements to the fermentation process itself are required (Vertès et al. 2008; Wackett 2008). In addition, there is a need for engine technology that will allow efficient, clean utilization of biofuels.

While biomass-derived energy has tremendous potential, it is also clear that all aspects of the bioenergy industry – including agricultural practices, biomass processing, and engine design – must be developed in an integrated and careful manner so that the goals of economic, environmental, and sociological sustainability are achieved.

2. Overview of the Sustainable Bioenergy Development Center

2.1. Mission

The Sustainable Biofuels Development Center was established in 2008 and approved as a Center at Colorado State University in 2010. In 2012, the SBDC was designated as a Program of Research and Scholarly Excellence at the University. In 2010, the name was changed to Sustainable Bioenergy Development Center to indicate a scope that is broader than biofuels.

The mission of the SBDC is to enhance the capability of the US bioenergy industry to produce transportation fuels, chemical feedstocks, and energy on a large scale, at competitive cost, through sustainable production techniques. The SBDC accomplishes this mission through a combination of research, technology transfer, and training of students and postdoctoral researchers.

2.2. Organization and Management

Activities in the SBDC are organized in five focus areas:

- Development of Sustainable Crops and Agricultural Strategies, including crops better suited for marginal lands and alternative biomass sources such as algae;
- Improvement of Biomass Processing Technologies, including improved pretreatment of lignocellulosic materials, novel bioconversion processes, and more efficient process engineering approaches;
- Biofuel Characterization and Engine Adaptation, including methods to relate engine performance to biofuel chemistry, methods to predict engine performance, and technologies to modify engines for cleaner use of biofuels;

- Production of Byproducts for Sustainable Biorefining, including technologies to convert waste products from biofuels production into valuable products or formation of non-fuels products through extraction or conversion, thereby improving process economics; and
- Sustainability Assessment, including evaluation of the environmental (water, greenhouse gas, etc.) implications of bioenergy production and use, evaluation of the policy implications of widespread production and utilization of bioenergy, and evaluation of the commercial implications of center efforts.

Recently, research in these five focus areas has been oriented to emphasize two sustainable feedstocks for the production of advanced biofuels: (a) lignocellulosic biomass from terrestrial energy crops, and (b) algae and other photosynthetic microorganisms.

The activities of the Sustainable Bioenergy Development Center are coordinated by Dr. Kenneth F. Reardon, the Director of the Sustainable Bioenergy Development Center, with assistance from the SBDC Steering Committee and the Office of Sponsored Programs (for financial and contractual matters). The members of the SBDC Steering Committee are Dr. Daniel Bush, Professor of Biology; Dr. Jan Leach, Professor of Bioagricultural Science and Pest Management, and Research Associate Dean of the College of Agricultural Sciences; and Dr. Keith Paustian, Professor of Soil and Crop Sciences. Individual research projects are directed by faculty members at Colorado State University who are experts in the topic of the project.

3. Project Objective

The objective of this project was to develop new sustainable biofuels-related technologies by supporting technology-oriented research projects that emphasize sustainable bioenergy production processes across the breadth of relevant fields (plant biotechnology to engine technology).

To achieve the project objective, the Sustainable Bioenergy Development Center at Colorado State University performed three sets of activities:

- Bioenergy research projects: Short proposals were sought from Colorado State University faculty members in the five SBDC focus areas, and nine projects were selected by the SBDC Steering Committee. Distribution among the focus areas was a criterion in the selection of projects.
- Student Research and Education Support: Selected graduate students were provided with fellowships to support their research and education in sustainable bioenergy production. In addition, summer research experiences in bioenergy were funded for undergraduate researchers.
- Research support activities: To enhance the capabilities of SBDC bioenergy researchers to achieve the project objective, the project supported acquisition of equipment, conference and meeting travel, and visits and seminar presentations by experts in this field.

4. DOE-Funded Research in the Sustainable Bioenergy Development Center

4.1. Research Initiation Project 1: *Rapid Evaluation of Algal Strains using a MicroBioGenerator* (PI: Charles S. Henry, Co-PIs: June I. Medford, Department of Biology, and Kenneth F. Reardon, Department of Chemical and Biological Engineering).

The goal of this project was to develop a microfluidic device termed the MicroBioGenerator that permits the rapid characterization of algal strains and the environmental conditions associated with optimal lipid production for biofuel generation. This microfluidic device permits high-throughput growth of algal strains under a variety of stress conditions that are likely to induce lipid accumulation. The project was motivated by the need to better understand and control conditions under which algae generate high concentrations of lipids, and to rapidly assess environmental isolates that may yield either higher amounts of lipid or lipids with more desirable chemical properties. Current methods for each of these steps utilize traditional culturing techniques that involve large solution volumes (L scale) and long culture times. The solution volumes make culturing large numbers of species challenging, especially since each species should be tested with multiple stress conditions for varying durations. Microfluidics offers an attractive alternative to this problem.

The project aims were: (1) Develop a multi-chamber microfluidic cell culture device for algae; (2) Demonstrate the ability to culture multiple algal strains in the same device under varying stress conditions; and (3) Use the MicroBioGenerator to screen multiple algal strains. A microfluidic device was developed that permits high-throughput growth of algal strains under a variety of stress conditions that are likely to induce lipid accumulation (Figure 1). This device was tested with four strains of algae and was shown to be capable of yielding results similar to those obtained in larger-scale cultures, including lipid accumulation under nutrient stress conditions (Figure 2). This microfluidic platform could replace current methods for the screening of culture collections, which require large solution volumes (L scale) and long culture times. This research was published as:

> Holcomb, R.E., L.J. Mason, K.F. Reardon, D.M. Cropek, and C.S. Henry. 2011. Culturing and Investigation of Stress-Induced Lipid Accumulation in Microalgae Using a Microfluidic Device. *Analytical and Bioanalytical Chemistry*, 400, 245–253.

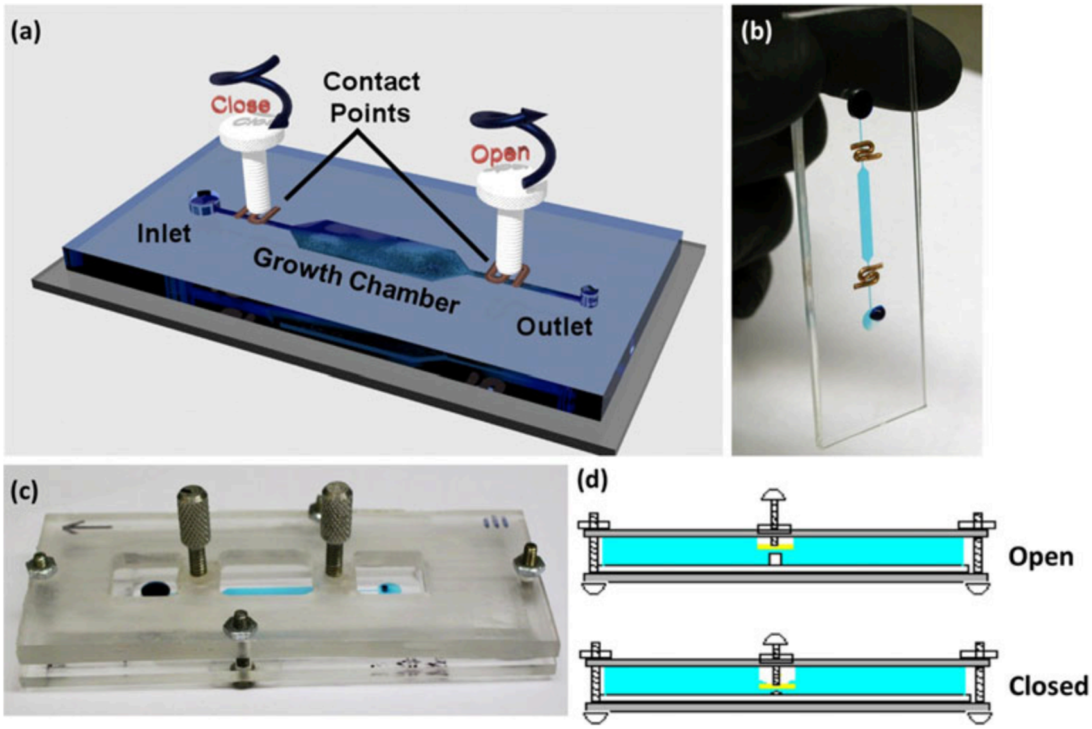

Figure 1. Illustrations of the MicroBioGenerator. (a) Schematic of the microfluidic system used in microalgal culturing and environmental stress studies. (b) Microchip showing microfluidic features and imbedded copper wire contact points. (c) Photograph of the microchip in the PMMA construct containing actuating screws. (d) Schematic showing valve operation for the assembled device. From Holcomb et al. 2011.

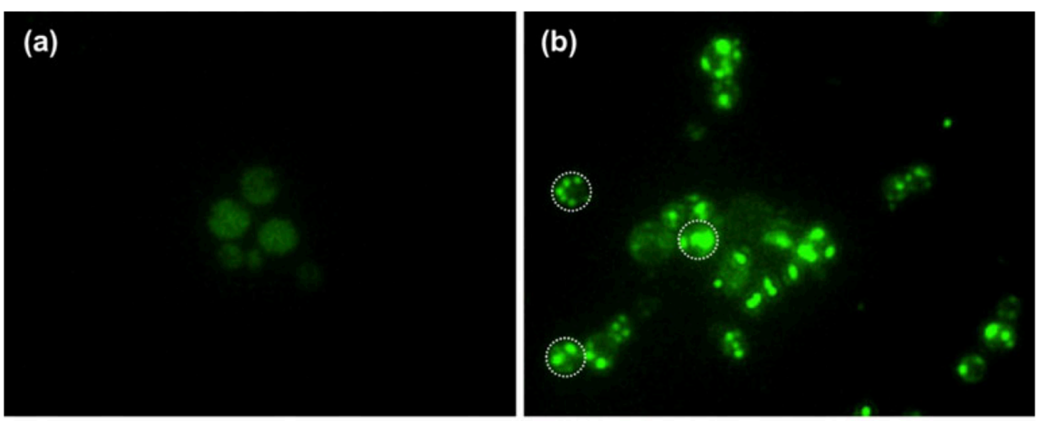

Figure 2. Images from the MicroBioGenerator. (a) Image of nonstressed *N. oleabundans* microalgae cultured in nitrate-replete medium in the microfluidic system. Microalgae were lipid stained with BODIPY 493/503. (b) Image of a stressed, microfluidic culture of *N. oleabundans* microalgae cultured in nitrate-depleted medium and stained with BODIPY 493/503. Lipid bodies are clearly evident as the higher intensity fluorescent regions inside the cells. Dashed white circles are drawn around selected cells as an aid to help discern cell boundaries. From Holcomb et al. 2011.

***4.2. Research Initiation Project 2:** In Situ Examination of the Potential Role of Supercritical-Fluid Treatments in Conversions of Agricultural Biomass* (PI: Gary E. Maciel, Department of Chemistry)

The conversion of various forms of renewable biomass into fuels, chemicals and/or other useful materials typically involves several steps or processes. A crucial step, and typically a problematic one, is the pretreatment step, in which a thermochemical procedure attempts to 'separate', to some degree, the major components of the lignocellulosic material, so that subsequent steps are more efficient. The goal of the proposed project was to examine and demonstrate the advantageous utility of supercritical fluids (SCFs) in the pretreatment of representative agricultural biomass (poplar, corn stover, switchgrass). The first phase of this project focused primarily on the development and refinement of NMR apparatus and techniques that make possible the *in situ* NMR measurements on biomass while under SCF treatment. A suitable apparatus and technique for interfacing the NMR probe with the SCF syringe pump was developed, despite the challenge of the high sample pressures (ca. 85 atm) required.

In addition, preliminary NMR measurements, including relaxation measurements, were made on poplar wood (sawdust) and poplar-derived lignin. Largely on the basis of the results of those preliminary NMR measurements, the very difficult S/N challenges facing this study were recognized, especially for ^{13}C-based relaxation measurements (valuable for elucidating the state of motion/mobility). Two parallel pathways for overcoming the technical difficulties of making NMR measurements under SCF conditions were evaluated. The first was in-situ SCF CO_2 examination using static-sample NMR and ^{13}C-enriched poplar wood, and the second is treating samples with supercritical trifluoromethane in sealed glass ampoules under magic angle spinning. Results from the first approach are shown in Figure 3. This research was published as:

Kohn, B., Davis, M., and Maciel, G. 2011. In situ Study of Dilute H2SO4 Pretreatment of C-13-Enriched Poplar Wood, Using C-13 NMR. *Energy and Fuels,* 25(5), 2301 - 2313.

Kobayashi, T., Kohn, B., Holmes, L., Faulkner, R., Davis, M., and Maciel, G. 2011. Molecular-Level Consequences of Biomass Pretreatment by Dilute Sulfuric Acid at Various Temperatures. *Energy and Fuels*, Volume 25(4), 1790 - 1797.

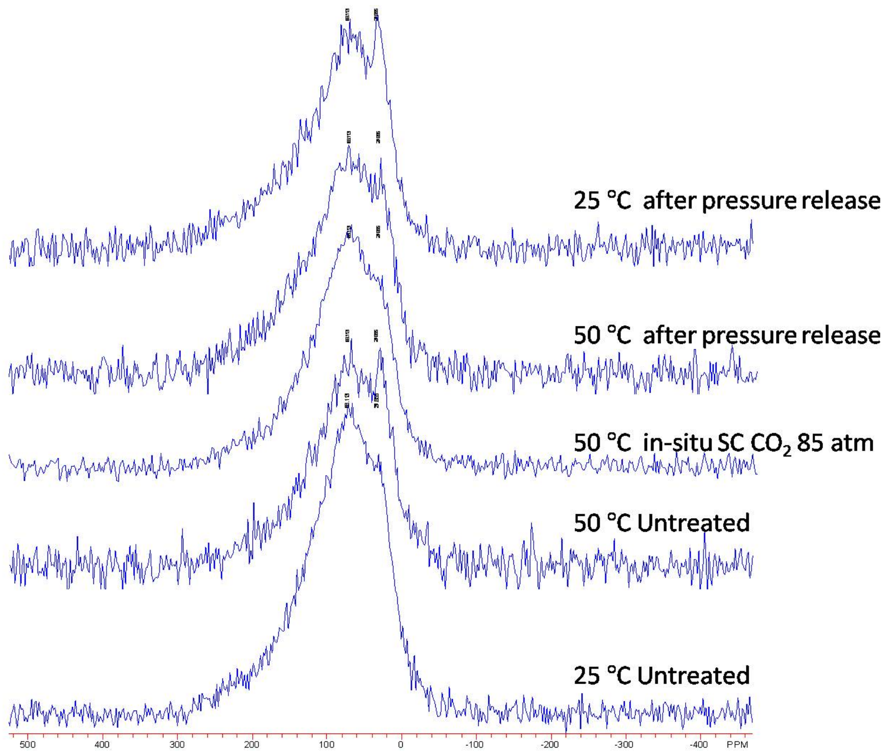

Figure 3. ^{13}C CP static spectra of ^{13}C enriched *Populus angustifolia* before, during and after in-situ treatment at 85 atm and 50 °C with super-critical carbon dioxide. Number of acquisitions = 32, contact time = 1 ms, recovery time = 1500 ms to suppress background signal.

4.3. Research Initiation Project 3: *Generation of a Lipid-Accumulating Cyanobacterium* (PI: Christie A.M. Peebles, Department of Chemical and Biological Engineering; Co-PI: Kenneth F. Reardon, Department of Chemical and Biological Engineering)

The goal of this project was to develop a lipid-accumulating strain of *Synechocystis* sp. PCC 6803 by targeting the accumulation of triacylglyerides (TAGs) and or fatty acids (FAs). Two strategies were investigated:

(a) Since thioesterases can cleave the acyl-ACP linkage releasing free FAs and reducing the accumulation of long-chain acyl-ACPs that inhibit *acc* and reduce FA synthesis, a plant thioesterases that targets 18:0 or 18:1 (Δ9) FAs was overexpressed.

(b) To slow the degradation of free FAs, the second target was to inhibit β-oxidation. The first gene in the β-oxidation pathway, *fadD*, was knocked out and the effect on FA accumulation evaluated.

This project was the starting point of a research endeavor that has the potential to lead to photosynthetic, advanced biofuel production that is both economically and environmentally sustainable. The potential commercial impact of this research is the development of an inexpensive, high-energy alternative biofuel that has a low carbon footprint. In addition, lipid-producing cyanobacteria could serve as a platform to engineer the sustainable production of a variety of biochemicals, including nutraceuticals (omega-3 fatty acids), polymers, high-value lubricants, adhesives, cosmetics, and detergents. In a photobiorefinery, the co-production of high value products can improve the economics of fuel production.

Initial results of the second strategy formed the basis for a successful proposal to the National Science Foundation (C. Peebles, PI). Results from this research were published in:

> Y.E. Cheah, S.C. Albers, C.A.M. Peebles. 2013. A novel counter-selection method for markerless genetic modification in *Synechocystis* sp. PCC 6803. *Biotechnol. Prog.* 29 (1), 23-30.

4.4. Research Initiation Project 4: *Nanoparticle Catalysts for Biomass Conversion into Platform Chemicals and Biofuels* (PI: Eugene Chen, Department of Chemistry)

The goal of this project was to develop novel catalyst systems based on zero-valent metal nanoparticles (NPs) for highly efficient, homogeneous biomass conversion into platform chemicals such as 5-hydroxymethyl furfural (HMF) and biofuels such as 2,5-dimethylfuran (DMF). Two zero-valent metal NP types were investigated, one type preformed from zero-valent molecular complexes such as $Cr(CO)_6$ by microwave irradiation or thermal decomposition in ILs, and the second type generated in situ under the real biomass conversion reaction conditions that employ either the zero-valent $Cr(CO)_6$ or the divalent $CrCl_2$. Zero-valent $Cr(CO)_6$ was shown to be a very effective catalyst for the glucose-to-HMF conversion, producing HMF in 49.4% yield ([EMIM]Cl, 10 mol% catalyst, 120 °C, 6 h), which is higher than that (45.3%) achieved by the benchmark divalent chromium catalyst $CrCl_2$ under identical conditions. These NP catalysts have potential to aid in the production of biofuels by at least three different pathways (Figure 4). Results from this research were published in:

> He, J.; Zhang, Y.; Chen, E. Y.-X. 2013. Chromium(0) Nanoparticles as Effective Catalysts for the Conversion of Glucose into 5-Hydroxymethylfurfural", *ChemSusChem*, 6, 61–64.

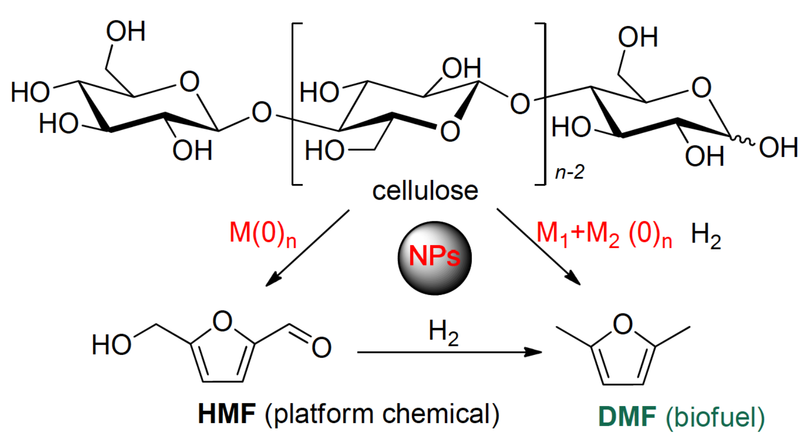

Figure 4. Potential pathways for biomass conversion by nanoparticle catalysts.

4.5. Research Initiation Project 5: *Development of a Terpenoid Platform for the Production of Biofuels in Cyanobacteria* (PI: Christie A.M. Peebles, Department of Chemical and Biological Engineering)

The overall goal of this project was to produce terpenoid hydrocarbons such as botryococcene in the cyanobacterium *Synechocystis* sp. PCC 6803. To demonstrate the feasibility of this approach, the short-term goal of the project was to increase the metabolic flux to the terpenoid pathway by increasing the production of the terpenoid precursors IPP and DMAPP in *Synechocystis*. In nature, there are two independent pathways that lead to the production of IPP and DMAPP, the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. *Synechocystis* uses the MEP pathway to produce IPP and DMAPP.

In this project, metabolic engineering tools were used to begin to engineer both the MEP and MVA pathways in *Synechocystis*. As a starting point, a suite of control elements capable of gene control at a variety of expression strengths was created in the form of a library of 10 promoter constructs. These constructs were developed and built via rational design techniques by adding individual nucleotides in a step-wise manner within the −10 and −35 cis-acting regions of the *tac* promoter. The resulting library (Figure 5) provides cyanobacterial metabolic engineers with an important new tool.

The preliminary data from this project contributed to the funding of an NSF grant on cyanobacterial photobiorefineries (K. Reardon, PI) and a USDA graduate fellowship to S. Albers. Results from this research were published in:

Albers SC, Gallegos VA, Peebles CA. 2015. Engineering of genetic control tools in *Synechocystis* sp. PCC 6803 using rational design techniques. *J. Biotechnol.* 216, 36-46.

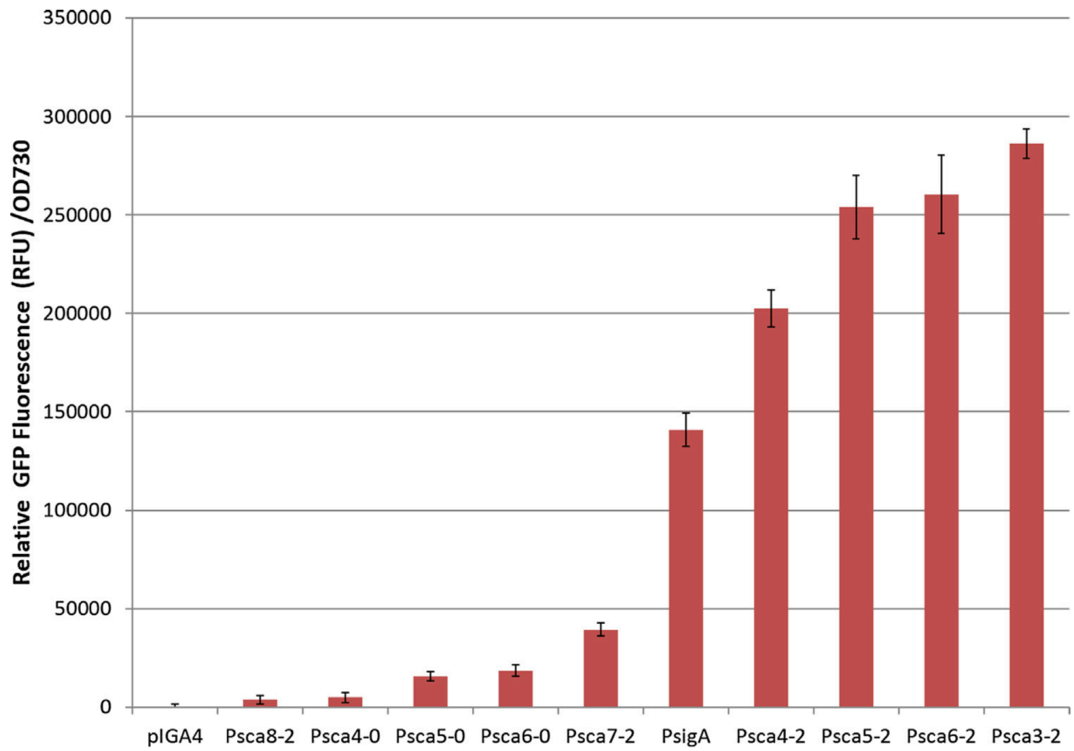

Figure 5. Promoter suite showing dynamic expression control within *Synechocystis*. The data represent the various promoters and their expression strength based on the promoter strength of the baseline promoter $P_{sca8\text{-}2}$. From Albers et al. 2015.

4.6. Research Initiation Project 6: *Conversion and Extraction of 5-Hydroxymethylfurfural from Glucose* (PI: Ranil Wickramasinghe, Department of Chemical and Biological Engineering; Co PI: Xianghong Qian, Department of Mechanical Engineering)

The overall goal of this project was to use combined modeling and experimental techniques to develop a cost effective technology to produce 5-hydroxymethylfurfural (HMF), a critical intermediate in the production of liquid biofuels from glucose, the most abundant monomer sugar derived from biomass. HMF is a critical and versatile intermediate in the conversion of biomass to liquid biofuels such as dimethylfuran (DMF), liquid alkanes and many other value-added products. Biofuels derived from lignocellulosic biomass are one of the leading renewable energy candidates to replace fossil based transportation fuels. The key bottleneck for lignocellulosic-derived biofuels is the lack of technology for the efficient and cost-effective conversion of the biomass to liquid fuels.

Dumesic and his group have developed several technologies to convert biomass carbohydrates to DMF and liquid alkanes via a hexose dehydration to HMF in the liquid phase. However, the starting material for these conversion routes is fructose, an isomer of glucose. Fructose dehydration to HMF has a much higher conversion and selectivity than glucose (Roman-Leshkov et al. 2006). Since glucose is the major hydrolysis product from biomass, an additional isomerization step is needed to convert glucose to fructose. This additional step will increase the cost and limit the overall biomass to HMF conversion yield. It is therefore critical to develop methods that can directly convert glucose to HMF with high yields. Earlier studies show that HMF yields are strongly dependent on reaction conditions, particularly the media composition (Chheda et al. 2007).

In this project, two approaches were pursued to improve the conversion of glucose to HMF: molecular modeling to determine the best solvents and reaction conditions, and development of a membrane extraction system to improve HMF conversion by preferentially removing the reaction product. The research program was organized into four specific objectives: (1) Determine equilibrium β-D-glucose/solvent structures using Car-Parrinello based *ab initio* molecular dynamics (CPMD) simulations; (2) Elucidate glucose to HMF conversion mechanism(s) and rate-limiting step(s), reaction free energies, and barriers using CPMD combined with metadynamics (MTD) simulations; (3) Verify theoretical predictions by conducting glucose to HMF conversion reactions; and (4) Develop a membrane extraction system for HMF extraction from the reaction medium.

The modeling component of the project focused on understanding the fundamental processes governing the conversion and selectivity for HMF production from glucose and improving HMF yield. In particular, the solvent effects on the mechanism(s), rate-limiting step(s), free energies and reaction barriers for the catalytic conversion of glucose to HMF were investigated in water, organic solvent, and their mixtures. CPMD simulations were used to simulate equilibrium glucose/solvent structures as well as conversion intermediates (Figure 6).

The experimental component of the project focused on validating the theoretical results and developing a unique membrane extraction technique to extract HMF from the aqueous reaction media with improved yield and selectivity. Preliminary data were obtained for this membrane extraction process (Figure 7, Table 1). The rate of extraction was found to be unaffected by the aqueous or organic phase flow rate, indicating that the membrane mass transfer coefficient dominates. However, transfer of HMF into DMSO was limited by a relatively low octanol-water partition coefficient.

The preliminary data from this project contributed to the funding of a subcontract from the National Renewable Energy Laboratory (R. Wickramasinghe, PI). Results from this research contributed to an article that focuses on the use of membrane extraction in future biorefinery separations:

Grzenia DL, Qian X, Silvério da Silva S, Wang X, Wickramasinghe R. 2011. Membrane Extraction for Biofuel Production, *Membrane Science and Technology*, 14, 213 -233.

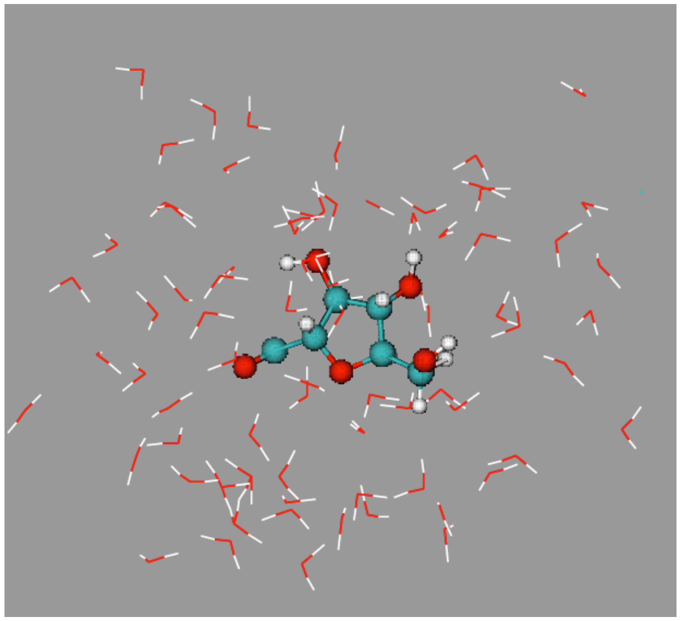

Figure 6. HMF intermediate formed during glucose to HMF conversion in water. This intermediate was produced via protonation of C2-OH and the subsequent breaking of the C2-O2 bond and the formation of the C2-O5 bond.

Table 1. Experimental conditions tested for membrane extraction of HMF (DMSO as the extractant).

Experiment	Molar Ratio Water:DMSO	Aqueous Flow Rate (g/s)	Organic Flow Rate (g/s)	Initial HMF Concentration (g/mL)
1	1:0	8.8	8.8	4.61E-03
2	1:0	17.7	4.4	4.36E-03
3	1:0	13.3	6.6	4.46E-03
4	1:1	8.8	4.4	4.17E-03
5	10:1	8.8	6.6	5.30E-03
6	10:1	13.3	4.4	5.27E-03
7	15:1	8.8	4.4	4.16E-03

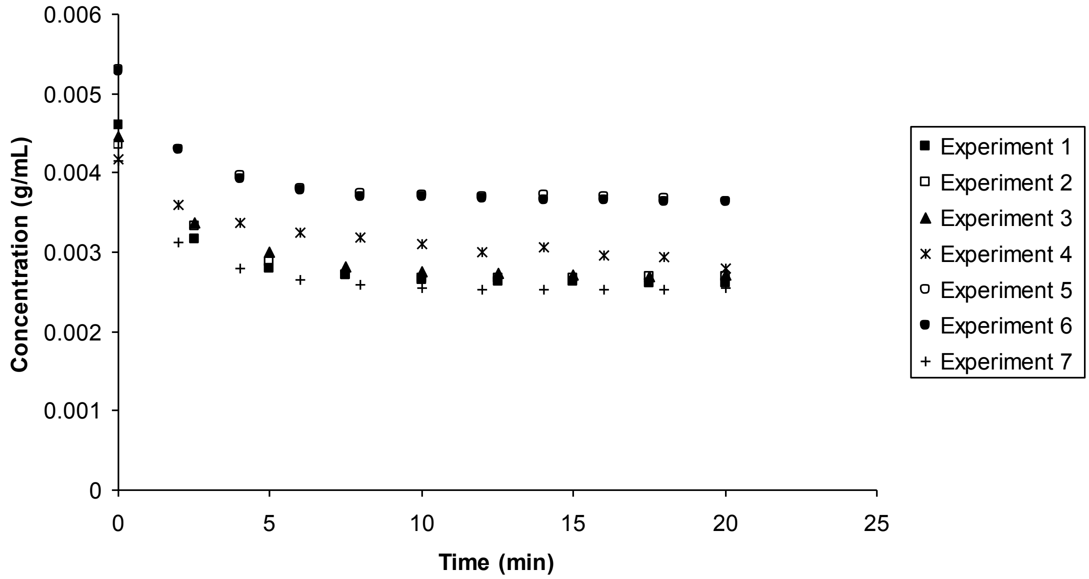

Figure 7. Decrease in HMF concentration during membrane extraction (experimental conditions correspond to those in Table 1).

4.7. Research Initiation Project 7: *Product Effect of Chemical Structure on NOx and PM Emissions from Algae-Based Biodiesel FAME* (PI: Anthony J. Marchese, Department of Mechanical Engineering; Co-PIs: Daniel B. Olsen, Department of Mechanical Engineering, and John Volkens and Jeffrey L. Collett, Department of Atmospheric Science)

Studies have shown that the magnitude of pollutant emissions (e.g. NO_x and PM) from diesel engines operating on fatty acid methyl esters (i.e., FAME biodiesel) are directly linked to the chemical structure of the triglycerides present in the bio feedstock. Specifically, NO_x emissions have been shown to increase with increasing number of double bonds in the hydrocarbon chain and decrease with increasing carbon chain length; and PM emissions have been shown to decrease with increasing carbon chain length (McCormick et al., 2001; Schonborn et al., 2008). However, studies that have been done to date to characterize the pollutant emissions of algae-derived FAME have used materials with far different fatty acid composition than FAME derived from existing vegetable or animal fat feedstocks. The goal of this project was to examine the effect of the chemical structure of algal bio-crude feedstocks on diesel pollutant formation in a 56 HP John Deere 4024T diesel engine operating on algae-derived FAME biodiesel.

Task 1. Identify and obtain pure FAME components based on the algae FAME profiles. Table 2 presents the results of gas chromatography of FAME derived from the transesterification of *Nannochloropsis oculata* and *Nannochloropsis* sp., two of the many microalgae species currently under consideration for biofuel production. Algal oil is unique in that it tends to contain a significant quantity (~5-20% by volume) of long highly unsaturated oils, which are rarely observed in more traditional biodiesel feedstocks, such as soy and rapeseed (canola) oil (Graboski and McCormick, 1998). The two most common types of long and highly unsaturated oils found in algae oil tested to date are eicosapentaeonic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are omega-3 oils found in fish oil and commonly used as nutritional supplements. In fact, the use of algae as an alternative source for these fatty acids has been the focus of many research studies, but there has been little research on the combustion properties. The specific fatty acid content in the algal oil varies widely depending on the species and growing conditions such as water salinity, pH, light intensity, and temperature (Brown et al., 1993; James et al., 1989; Guckert and Cooksey, 1990; Kessler, 1980). Variation in these conditions will also affect the growth rate and amount of oil produced, properties that currently may be a higher priority for companies developing algae for biofuel purposes. If emissions are added to the requirements when selecting an algal species/growing condition, there may be a candidate that is an efficient producer of lipid oil that is favorable from an emissions standpoint. Most likely, the best oil from an emissions standpoint would contain as little EPA and DHA as possible, but the goal of this project is to provide evidence that can help algae-for-biofuels companies optimize their algae.

Numerous start-up companies are competing globally to develop proprietary systems and processes for maximizing algae growth for biofuels, including the CSU spinoff company Solix Biofuels. However, no such companies are currently at full-scale production and algae-derived oil is currently a scarce commodity. Therefore, it is currently cost prohibitive to obtain samples large enough to accommodate the necessary emissions test matrix. Accordingly, “synthetic” algal oil methyl ester formulations were produced by transesterifying pharmaceutical grade fish oil with pure methyl esters obtained through Procter and Gamble. Using this technique, fatty acid composition of the algal oil fatty acid was reproduced with reasonable accuracy to make two synthetic algal-based FAME fuels. The first fuel had approximately 20% by volume omega-3 fatty acids (with >4 double bonds) and the second fuel contained approximately 10% omega-3 fatty acids.

Task 2. Develop engine/dynamometer test set up and design and build ultra low volume fuel injection system. To determine the effect of the algal oil composition on engine emissions, two different algae-based oils with different amounts of EPA and DHA were transesterified to biodiesel FAME and their emissions compared against that of soy biodiesel, rapeseed biodiesel, petroleum diesel and blends thereof. Emissions testing was performed using a John Deere 4024T, non-road diesel engine meeting USEPA Tier 2 emissions regulations. This 4-cylinder engine has 2.4 L displacement, turbocharged aspiration and is rated at 56 BHP. Tests were performed using the steady-state C1 Test Cycle as specified in ISO 8178-4 guidelines. Even though fish oil was a major component in the simulated algal oil, running all four cylinders on this specialty fuel would be very costly. As such, the injection and exhaust systems were modified to reduce the need for the synthetic algal oil. The three remaining cylinders operated on petroleum diesel during all tests. The injection system is capable of delivering petroleum diesel fuel to all four cylinders during engine warmup and in between all tests and switches to supplying specialty fuel to one cylinder during exhaust sampling, while keeping the other three cylinders running on diesel. The cylinder used for testing was one of the outside ports, enabling easier access. A LabView-based control system was used to control the fuel delivery system that controls the fuel type, pumps the fuel to the engine head, and measures the mass flow rate of the fuel. Two Micro Motion CMF010 coriolis mass flow meters were used to measure the diesel and specialty fuel flow rate. A schematic diagram of the injection system is shown in Figure 8.

Task 3. Characterize gaseous and particular emissions in algae-derived diesel exhaust. Engine emissions tests were performed on the John Deere 4024T, non-road diesel engine meeting USEPA Tier 2 emissions regulations. The emissions tests focused on NO_x, PM and VOC emissions from a diesel engine operating on FAME blends representative of those derived from algal feedstocks to determine which chemical structures (i.e., carbon chain length and degree of unsaturation) will minimize these emissions. Tests were performed on 9 different fuel blends at 2 different engine loading conditions. Each fuel/load condition was repeated twice.

Gaseous Exhaust Emissions. Exhaust gas measurements were made using 5-gas emissions analysis system that includes chemiluminescence measurement of NO, NO_2 and total NO_x, flame ionization detection of total hydrocarbons, paramagnetic detection of oxygen and non-dispersive infrared detection of CO and CO_2. In addition, a Nicolet Magna 560 Fourier Transform Infrared (FTIR) spectrometer was used to obtain speciated measurement of hydrocarbons through C4, NO_x compounds (NO, NO_2, N_2O, N_2O_5, NH_3, etc.), and a variety of hazardous air pollutants/VOC's such as formaldehyde, acetaldehyde, acrolein, benzene, toluene, xylene, ethylbenzene, and 1,3,butadiene. It has been well established for methyl esters produced from vegetable oil that NO_x emissions typical increase with increasing levels of unsaturation in the hydrocarbon chains of the fatty acid methyl esters (McCormick et al., 2001). Accordingly, it was hypothesized that the algae-based methyl esters, which contained over 10% highly unsaturated compounds DHA (C22:6) and EPA (C20:5), would result in increased NOx with respect to the baseline ULDS. However, the results (see Fig. 9) showed brake specific NO_x emissions decreases (g/kW-hr) of 10% and 2% for 50% and 75% load conditions, respectively. This is a surprising result that will require additional testing at a more comprehensive set of loading conditions.

Rapid NO_x Measurements. Instantaneous NO_x measurements were accomplished using a Cambustion CLD500 Fast NO_x chemiluminescence analyzer. This unique device enables measurement of NO and NO_2 with an extremely high response time of 2 ms by locating the chemiluminescence detector in a remote sample head directly at the source. Two sample heads were used to measure the instantaneous NO emissions from the petroleum diesel cylinder and special fuel cylinder, respectively.

Diesel Particulate Matter Characterization. To characterize the particle size distribution and organic carbon (OC) composition from algae-derived biofuel combustion, engine-out particulate matter was characterized on-line, using an Aerodyne Aerosol Mass Spectrometer (AMS). The CSU AMS, acquired in 2007, is one of the first units to incorporate high-resolution mass measurements using a time-of-flight (ToF) mass spectrometer. The AMS is capable of direct measurement of both particle size (between approximately 50 and 1000 nm) and composition. The high mass resolution and accuracy of this instrument enables information to be obtained about particular compounds (such as polycyclic aromatic hydrocarbons, PAH) present in sampled aerosols. In our experiments we will use the AMS to directly sample and characterize particles produced by combustion from each fuel type. The high resolution ToF-AMS data will be examined to look for chemical “signatures” of particles produced during combustion of each fuel type.

In addition to the AMS, additional particulate matter size distributions were measured using a Scanning Mobility Particle Analyzer (SPMS). Total PM mass emissions were measured using gravimetric sampling and the ratio of elemental carbon to organic carbon (EC/OC) was quantified via chemical analysis of PM collected onto another set of filters. Analysis of all the gaseous emissions data, total particulate matter and SMPS data is complete. Analysis of the AMS data and EC/OC analysis will be completed shortly. The results show increased number of smaller particles for the canola methyl ester (RME), SME and A1ME as compared to the ULSD. The A2ME fuel produced similar particulate size distribution to that of the ULSD.

In-Cylinder Pressure Data. To determine the effect of the fuel type on the combustion processes within the engine cylinder, two of the four cylinders were instrumented with high-speed Kistler pressure transducers, which were integrated with a Hi-Techniques data acquisition system. Instantaneous pressure, crank angle and cylinder volume data were then processed to calculate the apparent heat release rate (J/degCA) for the engine cylinders operating on petroleum diesel and specialty fuels, respectively. Preliminary results of heat release rates (J/deg) suggested that the algae methyl ester blends result in an advance in the start of combustion, but a decreased premixed burn fraction (% of heat release that occurs during the premixed autoignition phase of combustion). The reduction in premixed burn fraction might explain the observed decreased NO_x emissions.

The preliminary data from this project contributed to the award of four grants to this project team, and to several publications:

“The Effect of Chemical Structure on Pollutant Formation Kinetics in Algae-Derived Biofuel Combustion”, Co:PI: Azer Yalin, National Science Foundation, $324,268, 2009-2012.

“National Alliance for Advanced Biofuels and Bio-products: CSU Component”, (Co-PIs: Kenneth Reardon, Shawn Archibeque). Department of Energy, $1,259,248, 2010 -2013.

“Evaluation of Renewable Aviation Jet Fuel from Alternative Sources”, The Boeing Company, $25,000, 2011-2012.

“Evaluation of Cellulosic Biomass Derived Oxygenates as Drop-In Blend Components”, Department of Energy, $500,000, 2013 – 2015.

“Hydrothermal Processing of Biomass: Analysis and Testing of Upgraded HTL Product”, Pacific Northwest National Laboratories, $50,000, 2015 - 2016

Fisher, B. C., Marchese, A. J., Volckens, J., Lee, T. and Collett, J. 2010. Measurement of Gaseous and Particulate Emissions from Algae-Based Fatty Acid Methyl Esters. *SAE Int. J. Fuels Lubr.* 3, 292-321.

Marchese, A. J., Vaughn, T. L., Kroenlein, K. and Dryer, F. L. 2011. Ignition Delay of Fatty Acid Methyl Ester Fuel Droplets in Microgravity: Experiments and Detailed Numerical Modeling. *Proc. Combust. Inst.*, 33, 2021-2030.

Bucy, H., Baumgardner, M. and Marchese, A. J. 2012. Chemical and Physical Properties of Algal Methyl Ester Biodiesel Containing Varying Levels of Methyl Eicosapentaenoate and Methyl Docosahexaenoate. *Algal Research*, 1, 57–69.

Table 2. Weight percent of fatty acids in various vegetable oil feedstocks.

	Saturated Acids							Mono Unsaturated Acids			Total Poly Unsaturated Acids		
	8:0	*10:0*	*12:0*	*14:0*	*16:0*	*18:0*	*>18:0*	*16:1*	*18:1*	*22:1*	*n:2*	*n:3*	*n:4-6*
Coconut	7	7	47	15	8	2			6		2		
Rapeseed				3	2	1	1		12	55	15	8	
Soybean					9	4	8	1	26		55	6	
Nannochloropsis Oculata				4	29	1		24	9	1	4	2	28
Nannochloropsis sp.				3	14	11	3	19	6		7	3	20

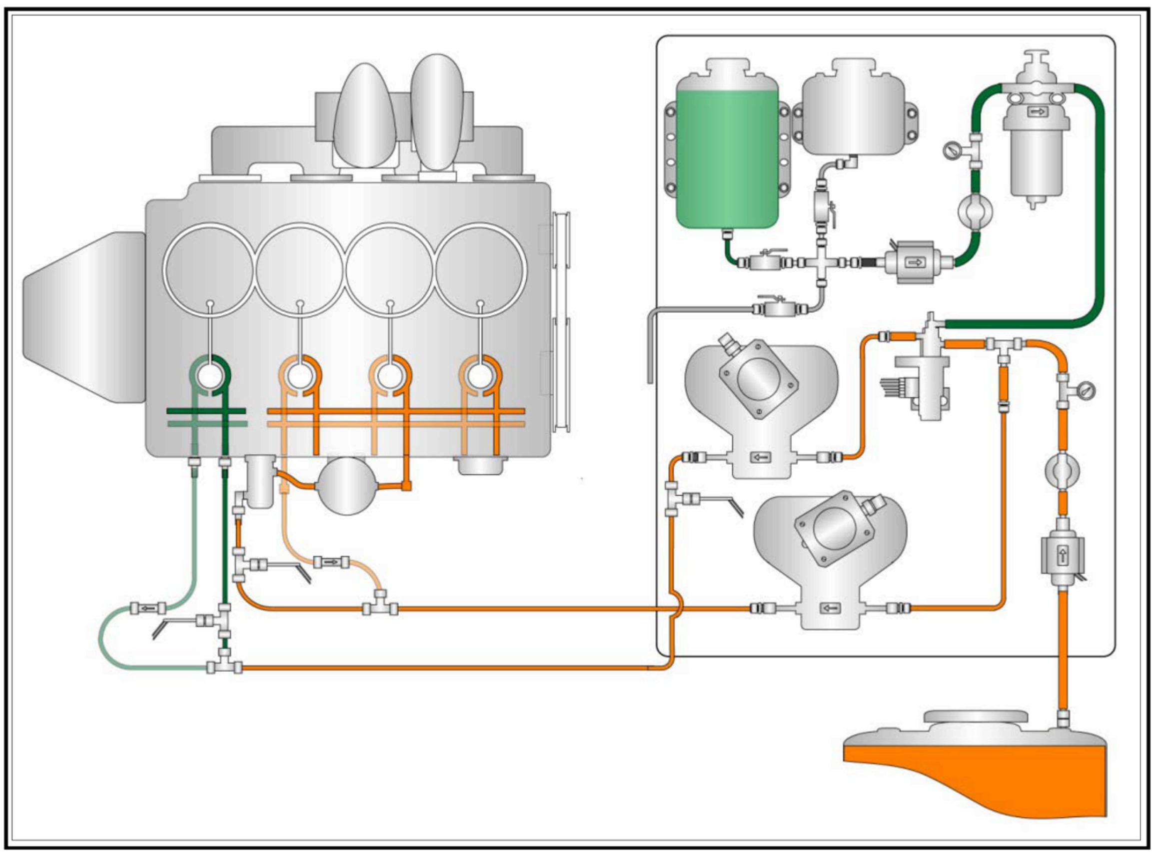

Figure 8. Low Volume Injection System, shown delivering diesel to all cylinders. Isolated cylinder is farthest to left.

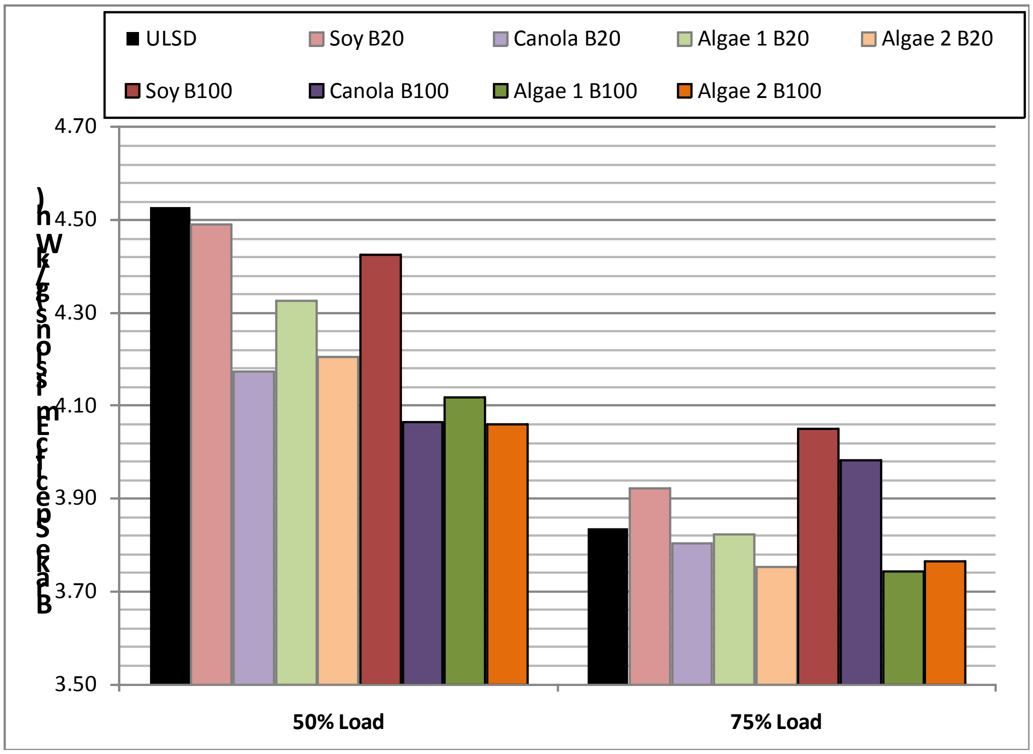

Figure 9. Brake specific NOx emissions for ULSD and biofuels (20% methyl ester and 100% methyl ester fuels) at two different engine loads at rated speed (1400 RPM).

4.8. Research Initiation Project 8: *Algal Photophysiology and Synthetic Biology* (PI: Graham Peers, Department of Biology)

The overall goals of this project were to investigate how algae thrive in the face of abiotic stress and environmental adversity and to translate these discoveries to the challenge of increasing algae crop production. The project used a combination of model organisms that are amenable to genetic manipulation (such as *Chlamydomonas*, *Synechocystis* and *Phaeodactylum*). DOE support was instrumental for collection of data that resulted in the awarding of two grants to Dr. Peers. The first grant is from the Genomic Sciences Program within the Biological and Environmental Science Division of the Department of Energy, Office of Science and is titled “Optimization of energy flow through synthetic metabolic modules and regulatory networks in a model photosynthetic eukaryotic microbe” with Andrew Allen (J. Craig Venter Institute as PI and Graham Peers as Co-PI ($1.64M to Peers). This work aims to increase biomass productivity and direct cellular material flow in marine diatoms (eukaryotic algae). The approach includes generating metabolic models, supported by cutting-edge –omics methods, to identify novel genetic engineering targets. These targets are manipulated using newly generated by molecular biology techniques for gene knockout (e.g. Weyman et al. 2015 doi: 10.1111/pbi.12254). The Peers lab has also focused on generating improved biomass composition data in environmentally relevant conditions for biofuel production.

The second grant was awarded to Peers as Co-PI and is an interdisciplinary effort led by Kenneth Reardon as PI. This grant is from NSF-EFRI and is titled “Integrated design of cyanobacterial biofineries” with $1.99M in total funding. This grant seeks to improve biofuel product yields in cyanobacteria based systems. The effort integrates life-cycle analysis, genetic engineering, photophysiology and –omics based inquiry to better understand the limitations of light energy transduction to biofuels in industrial conditions. DOE funds have also supported Dr. Peers’ research into the role of multiple energy dissipation pathways that responsible for regulating photosynthetic efficiencies in algae and cyanobacteria.

Results from this research contributed to one published article, others in press.

Levering J, Broddrick J, Dupont CL, Peers G, Beeri K, Mayers J, et al. 2016. Genome-Scale Model Reveals Metabolic Basis of Biomass Partitioning in a Model Diatom. *PLoS ONE*, 11(5): e0155038. doi:10.1371/journal.pone.0155038

4.9. Research Initiation Project 9: *Molecular Mechanisms for Biomass Traits of Sorghum* (PI: Courtney Jahn, Department of Bioagricultural Sciences and Pest Management)

The general scope of this project was the investigation of physiological, morphological, and biochemical variation of *Sorghum bicolor* in the greenhouse and field. The project focused on understanding how plants grow and develop and how they respond to stress. The specific goal of the project was to determine the molecular mechanisms for how sorghum responds to environmental stress and how stress can affect sorghum quantity and quality of grain, sugar, and biomass production. A field-based component of the project included characterization of genetically diverse sorghum lines in irrigated and non-irrigated conditions. Results from this research contributed to a conference presentation, with a research journal article under review.

Jahn, C., M.F. Turner, J.S. Kirkwood, A.L. Heuberger, E.J. Wolfrum, C.D. Broeckling, and J.E. Prenni. 2014. The Relationship of Physiological, Morphological and Metabolic Traits to Biomass Accumulation and Drought Response in Sorghum. Presented at the ASA, CSSA, SSSA International Annual Meeting, November 5.

5. Student Research and Education Support

5.1. Graduate Student Fellowships

Ten PhD students (Table 3) were awarded fellowships for graduate education and research in sustainable bioenergy. The fellowship program included inter- and cross-disciplinary coursework, interdisciplinary research, and extra-university experiences (e.g., interactions with industry and non-governmental agencies), all of which promoted more effective research in sustainable bioenergy production.

Table 3. Graduate student fellowships awarded by the SBDC.

Recipient	Program	Research Area
Marc Baumgardner	Mechanical Engineering	Evaluation of biofuels in HCCI engines
Justin Sweeley	Chemical Engineering	Synthetic biology of algal metabolism
Guadalupe Aguirre	Biology	Woody biomass production
Trung Nguyen	Ecology	Ecosystem services and farm management optimization
Andrew Abeleira	Chemistry	Characterization of VOCs from biofuel combustion
Sarah Fulton-Smith	Ecology	Sustainability of sorghum bicolor varieties as bioenergy feedstock in semi-arid systems
Mike Angstadt	Political Science	Role of court structures in shaping environmental outcomes
Ann Kowalski	Chemical Engineering	Protein engineering of oxygenases for biofuel production
Michael Barich	Chemistry	Microfluidic devices for spectrochemical analysis of biofuels
Andrew Brandess	Agricultural and Resource Economics	Economic potential of bio-butanol on marginal lands

5.2. Undergraduate Research Project 1: *Bioconversion of Extracted Algal Biomass into Succinate* (student: Jennifer Lewis; mentor: Kenneth F. Reardon)

Algal biodiesel has the possibility to supply a large amount of fuel while requiring minimal land. The current process for algal biodiesel production is not economical and must be improved for the fuel to be reasonably priced. Converting lipid-extracted biomass, a waste stream from the biofuel production process, into a high value product such as succinic acid by fermentation is one way to increase the feasibility of algal biofuel. *Escherichia coli* afp111 has the capability to convert the sugars present in extracted microalgae into succinic acid when the biomass undergoes cellulase and protease pretreatments. This project demonstrated that the process has the potential to create valuable concentrations of succinic acid. More tests must be done in order to optimize the process and determine whether the protease step is necessary.

5.3. Undergraduate Research Project 2: *Molecular Cloning of a Laccase Gene from Phanerochaete chrysosporium* (student: Ms. Danielle Long; mentor: Dr. A.S.N. Reddy)

One of the obstacles in using lignocellulosic biomass is the presence of lignin, which limits access to cellulolytic enzymes. White rot fungus, *Phanerochaete chrysosporium,* and other fungi produce several potent lignin-degrading enzymes. The objective of this research is to clone one of the lignin degrading enzymes, laccase, from white rot fungus for future use for in in-planta degradation of lignin. Here we have induced the production of laccase from *Phanerochaete chrysosporium* using a nutrient deplete medium. The laccase mRNA was extracted and attempts made to amplify the laccase gene so that it can be expressed in energy crops to degrade lignin.

5.4. Undergraduate Research Project 3: *Over-Expression of a Sucrose Transporter Gene in Sugar Beet* (student: Ms. Carolyn Burek; mentor: Dr. Daniel Bush)

Sugar beet (*Beta vulgaris L.*) is a plant that can store a large concentration of sucrose, or sugar, in an underground tuber. Our objective is to determine if photosynthesis rates and plant growth can be altered if a sucrose-transporter gene is over-expressed. We have six genetically altered, or “transgenic”, lines of sugar beet that over-express a sucrose-transporter gene, which is hypothesized to increase the sucrose transporter (SUT) activity and increase sucrose loaded into the phloem. Sugar beet is a good model for this work because sugar beet can store high concentrations of sucrose in its tuber. This study aimed to compare variations in SUT transcript levels, alterations in photosynthesis rates, and variations in plant growth and tuber growth relative to the control. Reverse-transcriptase polymerase chain reaction (RT-PCR) confirmed that the transgenic lines all had expression of the SUT transcript. One transgenic line shows a much higher leaf number, as determined by counting the number of new leaves every week. In the future, we will use a Li-Cor photosynthesis instrument to measure rates of photosynthesis in the transgenic and control plants.

5.5. Undergraduate Research Project 4: *Engineering Hydrogen Production in Higher Plants* (student: Mr. Andre Hines; mentor: Dr. June Medford)

The hydrogenase protein is involved in fixing free hydrogen into hydrogen gas. To harness the effects of this protein, it must be transferred to a biological system, such as a legume plant, that can produce and store the gas for biofuel uses. This process begins with the construction of the hydrogenase gene into a plasmid. The work performed in this summer project involved the construction of the pCambid Hyd expression vector. Inserting the final part of the gene, the *hydEF* fragment, was the goal of the experiment. The results obtained were that the fragment was unfavorable for the bacteria cells used for the transformations. Therefore, completion of hydrogenase gene requires alteration of the *hydEF* fragment.

6. Research Support Activities in the Sustainable Bioenergy Development Center

6.1. Equipment Purchases

To enable high-quality research by SBDC researchers, major equipment items were purchased (Table 4). The majority of these items pertained to biofuels testing, and formed the basis of the new Advanced Biofuels Characterization Laboratory. It is well recognized that for any advanced biofuel to obtain widespread penetration, it must qualify as “fit-for-purpose”. A fuel is said to be fit-for-purpose if it meets regulatory and customer requirements when delivered to the refueling location. To determine if a fuel is fit-for-purpose, a set of physical and chemical properties should be considered, including energy density, oxidative/biological stability, lubricity, cold-weather performance, elastomer compatibility, corrosivity, emissions (regulated and unregulated), viscosity, distillation curve, autoignition characteristics, flash point, ad metal content. The Advanced Biofuel Characterization Laboratory was created to provide these capabilities for SBDC researchers, others at the University, and external partners, including National Laboratories.

Table 4. Equipment purchased in support of SBDC research.

Equipment	Purpose
Ignition quality tester	Biofuel testing
Semi-automatic vacuum distillation unit	Biofuel testing
Stabinger viscometer	Biofuel testing
Standard water cooled bronze flat flame burner	Biofuel testing
Cooper strip corrosion apparatus	Biofuel testing
ASTM D93 Automatic Flashpoint tester	Biofuel testing
ASTM D230 C200 Manual Calorimeter	Biofuel testing
Automated cold properties analyzer	Biofuel testing
4-position centrifuge	Biofuel testing
Liquid N2 cooled MCT-A detector	Biofuel testing
Standard purged system with regulator	Biofuel testing
Sidewell purge adaptors for non-smart accessories	Biofuel testing
TQ analyst professional chemometrics add-on	Biofuel testing
OMNIC 8 series	Biofuel testing
Nicolet 6700 FTIR spectrometer and software	Biofuel testing
Irrigation system	Biofuel crop management
Microscope	Algae characterization
Multitron with side cooler	Algae cultivation
Anaerobic chamber	Biofuel fermentation studies
Gas Chromatograph	Biofuel fermentation studies

6.2. Travel Grants

Attendance at technical conferences is an important means for learning about current research directions in the field and to exchange ideas with other leaders in the discipline. The competitive travel grants listed in Table 5 were provided from project funds for faculty, postdoctoral researchers, and graduate students. These travel grants enabled researchers to travel to national and international conferences or to attend a specialized workshops that enhanced their research capabilities. Priority was given to researchers who presented the results of their research at the conference or workshop.

Table 5. Travel grants awarded by the SBDC.

Recipient	Purpose
Faculty members	
Dan Bush	3rd GRISP Global Rice Phenotyping Network Workshop
Ranil Wickramasinghe	5th Sino-US Conference of Chemical Engineering
Graduate students	
Guadalupe Aguirre	Workshop on the Policy-Technology Interface for Bioenergy
Marc Baumgardner	Workshop at DOE Combustion Energy Frontier Research Center
Sam Evans	4th Berkeley Bioeconomy Conference
John Field	Collaborative research to perform an environmental assessment on an existing industrial scale rice husk gasification system in Cambodia
Margaret Fleming	2nd Pan-American Congress on Plants and Bioenergy
Scott Fulbright	4th Congress of the International Society for Applied Phycology
Torben Grumstrup	Workshop at DOE Combustion Energy Frontier Research Center
Christian L'Orange	Partnership for Clean Indoor Air meeting
Trung Nguyen	Workshop on the Policy-Technology Interface for Bioenergy
Eric Patterson	26th New Phytologist Symposium: BioEnergy Trees
Jason Prapas	Partnership for Clean Indoor Air meeting
Paul Tanger	Rice Research to Production Short Course, International Rice Research Institute
Timothy Vaughn	Workshop at DOE Combustion Energy Frontier Research Center
Wenlong Xu	Workshop on the Policy-Technology Interface for Bioenergy

6.3. Seminars

Visits by experts in the field, including seminar presentations, are important means for improving research outcomes by learning about the latest work in a specific area and having the opportunity to discuss research in detail. In addition, internal presentations by SBDC researchers are effective for cross-disciplinary learning. Both cases provide opportunities for collaboration. The project supported many researcher visits and seminar presentations (Table 6).

Table 6. SBDC seminars.

Date	Speaker	Title
March 7, 2011	Steve Albers, PhD candidate in Cell and Molecular Biology, CSU	Increasing Terpenoid Production via Metabolic Engineering in *Synechocystis* sp. PCC 6803
March 7, 2011	Dan Bush, Dept of Biology, CSU	Enhancing Yield in Sugar Beet as a Model Energy Crop
August 21, 2011	Masaharu Komiyama, Graduate School of Medicine and Engineering, University of Yamanashi	Catalytic Supercritical Water Gasification of Ethanol Fermentation Residue.
Sep 16, 2011	J. Lucas Argueso, Dept of Environmental and Radiological Health Sciences, CSU	Functional Genomics of Bioenergy Yeast Strains
Sep 30, 2011	Chris Snow. Dept of Chemical and Biological Engr, CSU	Enzyme Engineering and Structure Determination via Recombination
Oct 14, 2011	Graham Peers, Dept of Biology, CSU	Looking for the Prize of Increased Crop Yields within the Diversity of Algal Photosynthesis
Nov 11, 2011	Maria Ghirardi, National Renewable Energy Laboratory	Microalgal Biofuel and Biohydrogen Research at NREL
Nov 29, 2011	Courtney Jahn, Dept. of Bioagricultural Sciences and Pest Management, CSU	Targets to Improve Quantity and Quality of Lignocellulosic Feedstocks
Jan 26, 2012	Adam Reed. Center for Energy and Environmental Security, University of Colorado	Conflicts in Implementing the Renewable Fuel Standard: an Analysis of EPA's GHG Emission Thresholds and Feedstock Tracking Requirements
Feb 23, 2012	Bill Parton, Natural Resource Ecology Laboratory, CSU	Impact of Second-Generation Biofuel Agriculture on

		Greenhouse Gas Emissions in the US
April 5, 2012	Peter Weigele, Staff Scientist, Chemical Biology, New England BioLabs	Microbes, Biomass, and Electricity
April 6, 2012	Robert McCormick, National Renewable Energy Laboratory	Biodiesel, Cold Weather, and Polymorphic Phase Transformation
March 5, 2012	Bruce Babcock, Cargill Chair of Energy Economics and Director, Biobased Industry Center, Iowa State U	Market Outlook for Advanced and Conventional Biofuels
April 27, 2012	Maureen McCann, Director of the Energy Center, Purdue University	Direct Catalytic Conversion of Biomass to Biofuels
Sep 13, 2012	Matt Posewitz, Dept of Chemistry and Geochemistry, Colorado School of Mines	Organism, Enzyme and Pathway Discovery for the Legos to Build Better Biofuel Phenotypes
Oct 16, 2012	Anthony Marchese, Dept of Mechanical Engineering, CSU	Fuel Properties and Pollutant Emissions from Algal Methyl Ester Biodiesel
Nov 13, 2012	Jan Leach and Dan Bush, Depts of Bioagricultural Sciences and Pest Management and Biology, CSU	Lessons from Rice Drive Feedstock Improvement in Switchgrass
Dec 4, 2012	Andrew Brandess, Dept of Agricultural and Resource Economics, CSU	The Economic Potential of Bio-butanol on Marginal Lands in a Buffered Rural Economy in Western Colorado
Dec 19, 2012	Laura Bartley, Dept of Microbiology and Plant Biology, University of Oklahoma	Molecular and Genetic Analysis of Cell Wall Cross-Linking in Rice for Biofuel and Food
April 15, 2013	Kenneth Street, International Center for Agricultural Research in the Dry Areas, Aleppo, Syrian Arab Republic, USDA-ARS Center for Genetic Resources Preservation	Swimming in the Gene Pool: a Rational Approach to Mining Genetic Resource Collections
April 19, 2013	David Zilberman, Dept of Agricultural and Resource Economics and Executive Committee Member, Energy Biosciences Institute	An Economist's View on Bioenergy and the Bioeconomy
April 23, 2013	Cathy Asleson, Director, Biocatalyst Development, Gevo, Inc.	Developing Catalysts for Renewable Chemicals and Advanced Biofuels

7. Conclusions

The research conducted for this project generated a wide range of findings relevant to the development of biofuels as a sustainable form of liquid transportation fuels. Some of these findings and conclusions are:

- A microfluidic device was created that is capable of supporting the phototrophic growth of algae. This device can be a tool in rapid screening of algal species and growth conditions for desired characteristics.
- Synthetic biology tools were developed for the manipulation of cyanobacteria for the production of hydrocarbons.
- Initial results were produced for an innovative means of understanding structural changes in lignocellulosic biomass undergoing supercritical carbon dioxide pretreatment.
- New catalyst systems based on zero-valent metal nanoparticles were developed and used to catalyze biomass-derived glucose to 5-hydroxymethyl furfural, a platform chemical.
- A novel method was piloted for liquid-phase conversion of glucose to 5-hydroxymethyl furfural, in conjunction with extraction of the HMF product.
- The characterization of algae-derived biodiesel fuels was performed under realistic engine conditions. Important new findings were obtained regarding engine performance and NOx emissions.
- Research into the systems biology of drought-tolerant sorghum was initiated.

8. Summary

The objective of this project was to develop new sustainable biofuels-related technologies by supporting technology-oriented research projects that emphasize sustainable bioenergy production processes across the breadth of relevant fields (plant biotechnology to engine technology). Nine new bioenergy research areas were initiated across a range of disciplines. These projects resulted, directly and indirectly, in many research publications and the acquisition of additional research funding. Sustainable biofuels technology-oriented outcomes were produced for algal biofuels (a device for rapid screening of new species and growth conditions, as well as tools for genetic modification to obtain desired properties), understanding of drought-tolerant sorghum (a bioenergy crop), pretreatment of lignocellulosic biomass, novel catalysts and processes for converting biomass-derived glucose to platform chemicals, and characterization of biofuel performance in engines. The project directly involved 16 faculty members, four postdoctoral researchers, 29 graduate students, and four undergraduate researchers.

8. References

Brown, M.; Garland, C.D.; Jeffrey, S. W.; Jameson, I. D.; Le Roi, J. M. 1993. The gross amino acid compositions of batch and semi-continuous cultures of *Isochrysis*, *Pavlova lutheri* and *Nannochloropsis oculata*. Journal of Applied Phycology, 5, 285-296.

Chheda, J. N.; Roman-Leshkov, Y.; Dumesic, J. A. 2007. Production of 5-hydroxymethylfurfural and furfural by dehydration of biomass-derived mono- and poly-saccharides. Green Chemistry, 9, 342-350.

Graboski, M.S. and McCormick, R. L. 1998. Combustion of Fat and Vegetable Oil Derived Fuels in Diesel Engines. Prog. Energy Combust. Sci. 24, 125-164.

Gray KA, Zhao L, Emptage M. 2006. Bioethanol. Current Opinion in Chemical Biology 10:141-146.

Guckert, J. B.; Cooksey, K. E. 1990. Triglyceride accumulation and fatty acid profile changes in *Chlorella* during high pH-induced cell cycle inhibition. Journal of Phycology, 26, 72-79.

Hill J, E. Nelson, D. Tilman, S. Polasky and D. Tiffany. 2006. Environmental, economic and energetic costs and benefits of biodiesel and ethanol biofuels. Proceedings of the National Academy of Sciences 103, 11206-11210.

Holcomb, R.E., L.J. Mason, K.F. Reardon, D.M. Cropek,.and C.S. Henry. 2011. Culturing and Investigation of Stress-Induced Lipid Accumulation in Microalgae Using a Microfluidic Device. Analytical and Bioanalytical Chemistry, 400, 245–253.

James, C. M.; Al-Hinty, S.; Salman, A. E. 1989. Growth and n-3 fatty acid and amino acid composition of microalgae under different temperature regimes. Aquaculture, 77, 337-351.

Kessler, E. 1980. Mass culture of *Chlorella* strains under conditions of high salinity, acidity and temperature. Archive Hydrobiology, 60, 80-86.

McCormick, R.L., Graboski, M.S., Alleman, T.L., Herring, A.M., Tyson, K.S. 2001. Impact of Biodiesel Source Material and Chemical Structure on Emissions of Criteria Pollutants from a Heavy-Duty Engine. Environmental Science and Technology . 35(9), 1742–1747.

Roman-Leshkov, Y.; Chheda, J. N.; Dumesic, J. A. 2006. Phase modifiers promote efficient production of hydroxymethylfurfural from fructose. Science, 312(5782), 1933-7.

Schonborn, A., Ladommatos, N., Allan, R., Williams, J. and Rogerson, J. 2008. Effect of molecular structure of individual fatty acid alcohol esters (Biodiesel) on the formation of NOx and particulate matter in the diesel combustion process. SAE 2008-01-1578.

Vertès A, Inui M, Yukawa H. 2008. Technological options for biological fuel ethanol. J Mol Microbiol Biotechnol. 15(1), 16-30.

Wackett L. 2008. Biomass to fuels via microbial transformations. Curr Opin Chem Biol. 12(2), 187-189.

Weng J, Li X, Bonawitz N, Chapple C. 2008. Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. Curr Opin Biotechnol. 19(2):166-172.