



## Overview: Algae Oil and Biofuels

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Microalgae were already considered a potential biofuel source when mass cultures were first scaled-up in a small pilot plant consisting of plastic bag-type photobioreactors on a rooftop at MIT (Burlew, 1953). Methane production from algal biomass was studied at U.C. Berkeley during the 1950s, and an initial systems analysis for open ponds published (Oswald and Golueke, 1960). The energy shocks of the 1970s led to renewed interest in microalgae biofuels, particularly hydrogen and methane, in combination with wastewater treatment (Benemann et al., 1980). From 1980 to 1995, the US Dept. of Energy through SERI (now NREL) supported the Aquatic Species Program (ASP), for production of vegetable oils with microalgae (Sheehan et al., 1998). This concept was based on findings, starting in the 1940s, that some microalgae can accumulate a high content of such oils. A major issue addressed by the ASP was open ponds vs. closed photobioreactors for algae mass culture, with open ponds selected due to much lower costs (Benemann et al., 1982; Weissman et al., 1987). The ASP program culminated in an open pond pilot plant at Roswell, New Mexico (Weissman et al., 1988). Algae oil production is still a long-term R&D goal (Benemann and Oswald, 1996; Benemann, 2002), and the ASP provides an excellent blueprint for a future program: an open collaboration by researchers from academia, national laboratories and industry, not inhibited by concerns about IP or commercial interests.

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## **Aquatic Species Program (ASP): Lessons Learned**

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The US Department of Energy's Aquatic Species Program (ASP) represents an 18 year, >\$25 million investment in developing fuels-from-algae technology. The program began in 1978 and supported work both at the National Renewable Energy Laboratory (NREL; previously the Solar Energy Research Institute) and at other institutions through subcontracts. Early ASP work focused on isolation of over 3,000 microalgal strains from the wild, characterization and screening of the strains for stress tolerance and neutral lipid production, and examination of various process steps such as de-watering and oil extraction. As the project advanced, physiology and biochemistry studies relating to lipid accumulation were carried out. Tools were developed to allow the metabolic engineering of microalgae with the goal of enhancing lipid production. Various demonstration-scale microalgal mass culture experiments were carried out, including the operation of a test facility in Roswell, NM. Technoeconomic analyses and resource assessments were also important aspects of the program. The program ended in 1996 due to budget cuts and the determination that the technology was unlikely to be cost-competitive with petroleum, which was trading at under \$20/bbl at the time. This presentation will provide an overview of some of the research conducted under the ASP and a discussion of the important lessons learned and technical/cost barriers that were identified.



## Bioprospecting for Algae

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The generic term “microalgae” refers to a large group of very diverse photosynthetic organisms of microscopic size. Due to the vast number of microalgae and their occurrence from moderate to any kind of extreme environments, it is expected that they display great diversity in their metabolisms. In general, bioprospecting refers to the process of exploring biodiversity of the metabolism of organisms for use in biotechnology applications. Until recently efforts of general microbial bioprospecting focused on habitats, such as marine environments and rain forests, mainly for exploration of diversity in microbes production of compounds useful in pharmaceutical industry. The specific process of bioprospecting with regards to biofuels from microalgae includes the efforts of isolation, identification, and screening of microalgae strains for their use in biofuels production. Systematic collection and identification of microalgae as well as early efforts to use microalgae oils for generation of diesel go back at least to the 1930’s. The presentation will include a historical perspective on microalgae bioprospecting and then report on current efforts of isolation of novel microalgae strains for biofuels research. Discussed will be for example the selection of habitats to be explored. Recent sampling included a diversity of habitats including freshwater, brackish, marine, and hypersaline environments. Further, types of microalgae that may be expected to result from isolation efforts will be discussed. Finally, some of the major challenges faced in the process of bioprospecting for novel strains of microalgae will be discussed.

## The Role of Culture Collections

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The algae represent huge genetic diversity, including bacteria and members of every major clade of eukaryotes except animals. Thus, it is not surprising that the breadth of their biochemical potential is as yet largely unknown. Large publicly accessible collections of living algae serve as repositories for this diversity. Many individuals and institutions throughout the world maintain collections of various sizes and diversities (see World Federation of Culture Collections <WFCC> site). The largest and most diverse of these in North America are <UTEX> at the University of Texas in Austin and the <CCMP> at the Bigelow Laboratories in Boothbay Harbor, ME, which house primarily freshwater microalgae and marine phytoplankton, respectively. The personnel who manage large culture collections of algae are trained to isolate strains from mixed cultures and biological contaminants, develop suitable media and environmental conditions for culturing previously uncultured algae, and identify field samples of algae. The large service culture collections in the U.S. are subsidized by the NSF, which expects these collection to accession potentially valuable recent isolates and provide healthy cultures to the community of scientists engaged in basic research. Although well managed collections serve those functions well, user needs are changing and demands on culture collections are becoming more diverse. For example, modern collections should (1) provide structured training for scientists, technicians and other users who wish to learn culturing techniques, (2) maintain stock cultures under cryopreservation whenever possible, (3) authenticate strains by DNA sequence analysis correlated with traditional methods, and (4) isolate and archive genomic DNA. Culture collections are also best equipped for certain kinds of strain characterization and screening such as measurements of growth kinetics under specified conditions, nutrient/light/temperature requirements and tolerances, and DNA sequence information. They are also well equipped to provide to the user community culture media and samples of algal DNA. Systematic application of these and other services will require a reassessment of user needs and funding levels by the agencies that support culture collections, but will make it much easier for those wishing to exploit algae for applied research and commercial applications to rationally select off-the-shelf algae.



Genome sequencing of eukaryotic algae  
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Whole genome sequence information is now publicly available for about a dozen eukaryotic algae, with perhaps another dozen or so algal genomes at various stages of completion. A majority of projects are with marine algae. All algae fall into two general categories – those that possess plastids derived from a primary endosymbiosis in which a eukaryotic heterotroph engulfed a cyanobacterium and those that possess plastids derived from a secondary endosymbiosis in which a eukaryotic heterotroph engulfed a eukaryotic autotroph. Comparative genomic studies have provided evidence for distinguishing features between these two major algal groupings. With advances in next generation sequencing technologies, it is now becoming possible to sequence different isolates from a single species and to conduct metagenomic and metatranscriptomic studies on environmental samples dominated by eukaryotic microbes. I will provide an overview of current genomic studies with marine eukaryotic algae and will provide specific examples from our work with marine diatoms.



## Algal Model Systems

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The molecular understanding of algae lags behind almost all other major groups of organisms including plants, bacteria and fungi. This is somewhat surprising given the history of early scientific investigations using algae and the potential of algae as food source, and as a biotechnological platform. Algae have been grown commercially at large scale, and use only sunlight as an energy source while sequestering CO<sub>2</sub> during the production of biomass, making algae a particularly attractive system for the production of biofuels. In order to fully exploit the biological advantages of algae we need to develop the knowledge base, as well as the genetic and molecular tools necessary to enable algal oil production at industrial scale. Key to developing the tools and knowledge base required to achieve these goal will be identifying model algal species that can act to focus national research and development efforts in a concerted manner. The choice of algal species will depend upon a number of factors including growth rates, desired products, and biochemical and genetic characteristics. Characteristics of existing algal model species will be presented as well as a discussion of the attributes that need to be considered in choosing additional algal species for investigation as biofuel platforms.



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## Algal Physiology

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Microalgae have a broad range of growth and oil yield potentials. In addition, the ability to utilize various organic substrates to supplement growth differs between various species. Photoheterotrophic growth also impacts the efficiency of solar energy conversion in many algae. Using *Chlorella* as a model, we will focus on how to optimize oil production in a closed loop continuous harvesting system (patent pending) utilizing both solar energy and reduced carbon sources.



## Metabolic Pathways Involved in Storage Lipid Accumulation

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Fatty acids and derivative compounds (triglycerides, fatty alcohols, wax esters, etc.) produced by photosynthetic organisms are the most abundant renewable source of reduced carbon chains for use as fuels and chemical feedstocks. As such, a thorough understanding of the basic biochemistry of fatty acid and glycerolipid synthesis is critical for the engineering of high-yielding oleaginous algae and oilseed-plants. In this talk, I will give a review of the metabolic pathways in plants and algae leading to production of triacylglycerol, the primary storage lipid in plants. I will begin with an overview of the pathways for production of acetyl-CoA, the precursor for all lipid syntheses, and review recent information on the regulation of fatty acid synthesis. I will then highlight the assembly of fatty acids into glycerophospholipids and betaine lipid, fatty acid desaturation, and final assembly of triacylglycerol. I will also present a reconstruction of algal lipid metabolism based on the recently published *Chlamydomonas* genome, and will compare and contrast algal lipid metabolism with that of seed plants.



## Regulation of Oil Biosynthesis in Algae

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The widely recognized need for the development of biomass-based domestic production systems for high energy liquid transportation fuels can potentially be addressed by exploring oil (triacylglycerol) biosynthesis in microalgae. Many algae including *Chlamydomonas* accumulate triacylglycerols when cultures enter stationary phase leading to nutrient limitation. The identification of microalgae genes encoding the enzymes and regulatory factors required for the induction of triacylglycerol biosynthesis is the immediate goal of the current work conducted in my lab. Genetic mutant screens are currently underway for loss of triacylglycerol accumulation under induced conditions, and for gain of triacylglycerol biosynthesis under non-induced conditions. Mutants have been identified and their biochemical and physiological characterization has begun. The affected genes will be identified and characterized based on a gene tagging approach. In addition, a high-throughput cDNA pyrosequencing experiment has been conducted under induced and non-induced conditions to generate a deep set of expressed sequence tags for comparative transcriptional profiling. This set has provided a rich complement of potential target genes for the genetic engineering of algae producing strains. Already, a more targeted effort focuses on candidate genes encoding diacylglycerol acyltransferases (DGATs), the predicted key enzymes of triacylglycerol biosynthesis. The newly identified genes and the functional genomic information will provide novel targets for future engineering approaches towards optimizing microalgae oil production strains.



## PRODUCTION OF MICROALGAE IN OPEN PONDS

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When grown rapidly, microalgae can fix solar energy in the chemical bonds of their cells with an efficiency approaching 5 percent of the energy in the visible portion of the solar spectrum. This efficiency is year round about 10 times as great as that attainable by the major plant crops such as corn, rice, sugar cane and wheat.

Cultivation methods for microalgae in open ponds have been developed to a point where media specification and unit designs are fairly standard, but there remain many technical, engineering and economic questions that stay between the desired productivity and the present available data to produce algal bio-fuels.

In this presentation we will deal with a few basic practical design requirements for open ponds to produce freshwater and marine microalgae at max. We will cover the significant progress in algal biomass production since the first large scale engineering design and trial of open raceways by Prof. W. J. Oswald and associates in Imperial Valley, the *Dunaliella* project 1983. The original layout system design passed significant technological changes over the last 25 years which make it attractive today for feasible algal bio-energy production.

The major issues which will be reviewed and discussed within the frame of algal cultivation design in open system are: area layout, pond size and design, ground infrastructure, depth, pond lining and liners, paddle wheels, flow velocity and turbulence, head loss and depth, carbonation and pH control, system control and accessories.

Our aim is to focus on the major technological requirements to reach the target of 3 to 5 percent photosynthetic efficiency in open ponds as equivalent to yearly average productivity of 25-42 grams algae/m<sup>2</sup>/day (222 - 374 pounds algae/acre/day).



**Wind, Sea and Algae**  
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There are considerable efforts underway worldwide to establish methods for cultivating large amounts of algal biomass to be used in the production of alternative fuels. To date, these efforts have focused on growing algae in enormous shallow ponds excavated in deserts or in extensive networks of tubing in elaborate bioreactors. While both these cultivation systems are effective for growing algae, they are both restricted by the availability, associated costs, and competition for other uses of the land and water. Both the land and water problems could be overcome by relocating the algal production facilities to some location offshore. While this idea has been considered, the problems of access, infrastructure, maintenance, and processing are regarded as too formidable to justify serious attempts to develop offshore cultivation systems. Indeed, for most offshore locations this may be true, but then there is Lolland.

With the world's largest offshore windmill farm and a commitment to innovation for alternative energy, Lolland has access, infrastructure, energy and the will to become a leader in establishing the world's first offshore algal energy research facility. This raises the question: What is the feasibility, scalability, and impact of building such an algal energy facility using the windmill infrastructure as the foundation for a containment system and excess wind energy for processes such as mixing, pumping, lighting, and harvesting the algae?

We will discuss the critical biological, materials, logistical, and economical challenges of locating a center for offshore algal energy research in Lolland, Denmark.

## Strategic Road to Commercialization: Food and Fuel from Algae

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Sufficient food and fuel for our planet is no longer assured. No amount of solar, wind, or nuclear generation can supply the vast amounts of chemical energy needed for our future food, industrial feedstock, and transportation sectors. As scientists and engineers, we must conceive, and provide for the foundation of a vast new photosynthesis industry that can draw upon the same capitalistic zeal that built our great industries in the past.

From a system engineering perspective this monumental effort cannot ignore the complexities of profitability in commodities, massive scalability, environmental sustainability, diminishing water supplies, vulnerability to terrorism, CO<sub>2</sub> pricing, mitigation, and regulation, global climate change, patent pooling, patent trolls, bio-prospecting, workforce development, environmentalism, system ecology, the energy industry, regulatory agencies, the inevitable politics, and a rational record that justifies vast private investment over time. Only by facing these existent system constraints squarely can we focus our science on the salient fundamentals and provide the directionality that is required for this new industry to lift off on the necessary time scale.

This talk will paint a candidate vision for an early industrial algae scenario related to scaleable domestic production of carbon neutral jet fuel and discuss some of the many system considerations required to enable and accelerate this specific outcome.

# Production of Algae in Conjunction with Wastewater Treatment

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Algae-based wastewater treatment is one approach to algae biofuels that can decrease overall costs while also providing a needed service. In northern California, several algae wastewater treatment facilities, each covering several hundred acres, are tangible examples of large-scale algae cultivation. However, these exhibit very low productivities and require expensive chemical flocculation methods to harvest the algae. High productivity wastewater ponds were introduced by Oswald and colleagues at the University of California, Berkeley starting in the 1950s (Oswald 1988), but these raceway-style, mechanically mixed “high rate” ponds still required chemical flocculation. The most plausible approach to inexpensive harvesting is bioflocculation followed by sedimentation, which has been demonstrated at pilot scale (Benemann et al. 1980). A major change since this early work is that wastewater treatment regulations now often include nutrient removal (tertiary treatment), which was not a design objective for past pond technologies. Thus, our research is now aimed at nutrient removal using high rate ponds. Addition of CO<sub>2</sub> to the ponds is required to allow the algae to assimilate nearly all of the soluble nitrogen and phosphorus present in wastewater. The low nitrogen concentrations are expected to promote both a high lipid content in the algae biomass and also improve the bioflocculation-sedimentation. The source of the CO<sub>2</sub> would be combusted biogas from the anaerobic digestion of primary sludge and the algal residues after oil extraction. CO<sub>2</sub> fertilization is used in algae farms producing food products, but has not been used in wastewater treatment. We report here on laboratory studies demonstrating the effect of CO<sub>2</sub> additions on nutrient removal and algae biomass production from wastewaters (Woertz et al. 2008). Although limited by terrain, climate, and resource availability, the potential of wastewater/biofuel technology is substantial both in the U.S. and worldwide (Van Harmelen and Oonk, 2006).

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# Closed Photobioreactors: Design Considerations for Sustainable High-Yield Algal Oil Production

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Microalgae have long been considered a promising alternative source of feedstock for biofuel. An oil yield as high as 440 barrels per hectare of algal mass culture per year has been projected and has been perceived as an achievable goal by the industrial and business worlds as well as the public. Unfortunately, this oil yield figure has never been achieved, even on a small bench-scale and under well-controlled laboratory conditions. The failure stems from two major reasons. One relates to the inherent limitation of naturally occurring oleaginous algal strains that only express or accelerate the synthesis and accumulation of oil/neutral lipid when the cells grow slowly or stop dividing, which makes the high oil yield projection questionable. Another is due to the technical limitations associated with the existing algal mass culture systems and processes which fail to facilitate high biomass and oil production. In this presentation, several biological questions will be addressed: why and when do algae make oil? What are the key environmental factors that affect oil synthesis and how they function at the molecular and cellular level? The major design considerations for an efficient algal oil production system will be described. Finally, a hybrid algal culture system concept will be introduced that integrates an open pond and a closed photobioreactor. The systems integration and process configuration are designed to enable the key biological and engineering variables and their interactions to be better controlled and to facilitate optimal, sustainable growth and production of oil-rich algal biomass.



## Power Plant Emissions to Biofuels

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Under joint sponsorship of the Coal to Hydrogen Program and the Gasification Program, the U.S.

Department of Energy, National Energy Technology Laboratory (USDOE-NETL) awarded a Cooperative Agreement with Arizona Public Service (APS) to develop and demonstrate a process for co-producing Substitute Natural Gas (SNG) and electric power via a coal gasification pathway. While the co-production of SNG and electricity is the foremost objective of the Cooperative Agreement, a required feature for the process was inclusion of a methodology for management of carbon dioxide (CO<sub>2</sub>) emissions. After analysis of several more traditional options for capture of carbon dioxide, the APS team has undertaken evaluation of a concept that “recycles” the carbon dioxide emissions from fossil fueled plants. In this recycle concept, CO<sub>2</sub> is fixated by the photosynthesis of microalgae using the emissions from fossil-fueled power plants and resulting algal oil is converted to various products including jet fuel. The presentation will discuss aspects of the project related to a one-quarter acre feasibility field test where emissions from the APS Red Hawk power plant were utilized to feed algae growth and efforts being undertaken to produce a within-spec JP8 fuel.



## Development of Algae Genetic Tools

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The development of algae as sources of lipids for biofuels will benefit from genetically-based approaches to optimize lipid yields and biomass accumulation. Genetic approaches are not limited to directed genetic manipulation, but can also include selection and classical genetics involving sexual crosses. Surprisingly little is known about lipid metabolism genes and their regulation in microalgae, and an investigation into gene expression pattern changes during lipid synthesis induction will facilitate rational genetic manipulation approaches. We have initiated such a project in *Cyclotella cryptica*, the best responding diatom species characterized in the Aquatic Species Program (ASP), coupled with a comparative analysis with *Thalassiosira pseudonana*. Understanding details of expression control are essential because an attempt in the ASP to increase lipid production in *C. cryptica* by overexpression of a key lipid synthesis gene was unsuccessful. In addition, basic genetic manipulation tools still need to be developed for diatoms and other microalgae to facilitate understanding the genetic basis of lipid induction, and to enable abundant lipid production. These tools include 1) new selectable markers, 2) “universal” transformation vectors capable of working in multiple species, 3) high efficiency transformation techniques, and 4) gene manipulation tools such as controllable promoters, homologous gene replacement, and knockout or knockdown approaches. Directed evolution/selection approaches to generate and isolate mutant strains for high lipid induction are also likely to be valuable tools, because an understanding of what genes are involved is not required. Sexual crossing approaches, which have been the mainstay of terrestrial crop plant development, may also be fruitful, and should be explored. The development of genetic manipulation, selection, and sexual crossing approaches should not only be considered important for the initial stages of development of algal biofuels technology, but will be of value throughout its continuing development, by enabling optimization of strains or species in response to changing engineering or production requirements.

## Factors that Limit the Efficiency of Photosynthesis for Biofuel Production

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Microalgae and cyanobacteria exhibit superior attributes to terrestrial plants as bioenergy sources, both as biomass feedstock and as cell factories for continuous fuel precursor production, while also serving as significant sinks for carbon dioxide uptake. Three persistent challenges for practical applications are to identify the best suitable genera/strains, optimal nutrients and controlling environmental variables for efficient growth. Many of these organisms rely upon multiple forms of metabolisms to produce energy for survival under natural conditions, including not only photosynthesis, but also respiration and fermentation. Selection of growth conditions is therefore critical to cell productivity and viability, as well as controlling lysis and contamination from other microbes. In this lecture I shall summarize our understanding of the bottlenecks in photosynthetic energy conversion efficiency from light absorption, charge separation and production of usable energy (ATP) and reductants (pyridine nucleotides) via the two photosystems. Published reports describing modifications of nutrients, environmental stresses and genetic transformants that alter the efficiency of biomass accumulation and its composition will be discussed. <http://www.princeton.edu/~catalase/>



## TECHNOECONOMIC ANALYSIS OF OIL PRODUCTION

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Microalgae hold the promise of higher oil yields per unit land area than any other biofuel feed stock. In recent months, a wide variety of photobioreactors and lipid extraction methods have cropped up with wildly varying forecasts of biodiesel and biomass production rates. This paper describes the general photobioreactor concept and the dominating factors that determine microalgal production. Using published theories and data, maximum attainable production rates of biodiesel are discussed along with what can reasonably be expected based on today's understanding. A comparison is made between open raceway ponds and enclosed photobioreactors. Given the production rate of a certain photobioreactor, it is valuable to know what capital investment would be permissible for such a photobioreactor to be economical. Using these microalgal production rates, an economic model is used to determine the revenue that can be generated from a biodiesel production facility. It is this revenue that governs what the maximum overall investment can be to break even.



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## **Bloomin' Government! – Environmental Laws and Regulations That May Impact Algae Production**

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Transformative technologies may not always fit perfectly within existing legal and regulatory structures. Misunderstandings of legal and regulatory requirements can result in costly delay and public mistrust of even the most promising technologies. Early and continuing communication with regulators may slow initial progress, but ignoring legal requirements can result in lawsuits, fines, and court orders. This presentation will address laws and regulations that could impact genetically engineered algae, as well as environmental laws and regulations that could impact all biofuel algal production, taking place indoors or out.