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# Report from Workshop on Biological Capture and Utilization of $CO_2$, Charles F. Knight Center, Washington University in St. Louis, September 1-2, 2009

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This workshop brought together experts in photosynthesis, bioenergy, microalgae, coal and carbon sequestration to discuss opportunities and challenges in biological $CO_2$ sequestration. Photo courtesy of Michelle Liberton.

# ABSTRACT

This report is an assessment of a workshop that was sponsored jointly by the Global Climate and Energy Project (GCEP) at Stanford University and the International Center for Advanced Research in Energy and Sustainability (I-CARES) at Washington University in St Louis. Experts in biology, engineering, and combustion were invited to Saint Louis to participate in the two day discussion that was aimed at identifying fundamental research necessary for realization of technologies that will enable efficient photobiological capture of $CO_2$ emissions from fossil fuel power sources on a global scale.

Researchers studying fossil-fuel based power generation emphasized the immense scale of carbon capture necessary to slow the release of carbon dioxide into the atmosphere. To fully offset the carbon emitted from anthropogenic sources, taking into account the 55% that are captured by enhanced biological and physical processes in the global carbon cycle, an additional 4 Gigatons (Gt) of carbon must be captured per year, and that number is likely to increase.

Options for technology that can capture such an immense amount of carbon in the near term are limited. There are real opportunities for achieving significant reductions in $CO_2$ emissions in the algal field but the current state of research is still very much in the realm of basic science and much is needed to be done before we can think about it in technological terms. Biological capture by photosynthetic microbes is an attractive technology because it is renewable, scalable, and may be used to produce fuels and chemicals cheaply. Identification of novel, robust strains, and breakthroughs in bioreactor design and harvesting/extraction technology, are necessary to realize this goal. This workshop set out to assess the productivity and carbon capture capacity of photosynthetic microbes and determine the areas in which there are opportunities to achieve significant reductions in $CO_2$ emissions.

# INTRODUCTION

The estimated anthropogenic contribution to the carbon cycle in the form of $CO_2$ released into the atmosphere is approximately 9 Gigatons (Gt) per year. Approximately 7.6 Gt of this is from fossil fuels and 1.4 Gt from land-use change. While as much as 55% of this carbon is absorbed by natural processes, up to 4 Gt are deposited in the atmosphere every year (Lal, 2008).

As much as 65% of this anthropogenic contribution to atmospheric carbon comes from large stationary sources globally, many of these being coal fired-power plants that provide the base-load of electricity demand. In the US, these sources supply 60% of electricity. With the demand for electricity expected to grow at a rate of 1.8% annually over the next 20 years, should coal remain the primary resource for electricity generation in the US, these emissions are set to grow.

By the year 2025, 100GW of new coal-fired steam electricity is expected to be online in the US alone. Although new plants are expected to be more efficient, making use of integrated gasification combined cycle (IGCC) technology, the US will still likely rely on the existing fleet of pulverized coal (PC) fired power plants as well. These currently supply the base-load electricity, 320 GW capacity and 1,900 billion Kilo-watt hours per year, which is difficult at this time to replace completely by renewable resources. Having carbon capture technologies that are affordable, sustainable and that might produce carbon neutral fuels is highly attractive and necessary for overall emissions reductions.

Carbon capture and storage (CCS) technologies can be used to mitigate carbon emissions that would otherwise be released to the atmosphere. $CO_2$ generated in concentrated streams by the combustion of fossil fuels, as in the flue gas from power plants and exhaust gas from cement and steel manufacturing processes, can be captured and sequestered. Additionally, some CCS technologies can capture and sequester atmospheric $CO_2$. The prevalent use of coal combustion for electricity generation is driving much of the demand for CCS technologies. However some estimates predict the costs of non-biological CCS technology deployment to be economically attractive only after the year 2030, making implementation at a large scale unlikely in the near term. (Herzog, 2009)

Biological systems could potentially make a significant contribution to CCS technology, as they can be deployed in a sustainable, renewable manner. Photosynthetic microbes are an attractive option for biological CCS because they have the ability to capture sunlight and use that energy to store carbon in forms useful to humans such as fuels, food additives, and medicines. The fact that many algae can have a doubling time of as little as 4 hours makes accumulation of biomass and production of useful molecules realistic on an industrial scale. To use algae as a

carbon capture technology however, a number of important limitations need to be overcome.

Much basic research has been carried out on algae as a production system for fossil fuel alternatives including diesel-like polymers, methane and hydrogen. The US government funded 25 years of research under the Aquatic Species Program (ASP) at the National Renewable Energy Laboratories (NREL), a program that was shut down in 1996, due to funding constraints. This research effort led to the isolation of roughly 3000 species of algae that might be useful in this regard (Sheehan *et al.,* 1998). Most of these have been lost over the years due to the absence of culturing necessary to propagate these species. Approximately 300 of these strains have survived and NREL is revisiting the project. Additionally, recent financial investments from the private sector into research towards deployment of algae as a fuel production system indicate that this technology is near the point of profitability. The success stories so far have come mostly from companies producing both fuels and high value products.

The use of algae cultured in non-oceanic environments to capture emissions directly from fossil fuel sources could be a technology that aids in the inexpensive reduction of $CO_2$ emissions from the energy sector over the coming decades. The ultimate carbon emissions associated with deployment of such a technology would depend on the capacity and efficiency of the algae to capture the carbon and on the use of the stored carbon after capture.

This report discusses the material presented at the workshop held on September 2009 at Washington University in St. Louis, which set out to identify opportunities for fundamental research that would help to increase the capacity and efficiency of algae to capture and use this carbon. References to outside and existing literature are made where appropriate to support and help further explain some of the findings.

## Game-changing technological improvements

Improvements in delivery, capture, and the metabolic transformation of carbon dioxide into industrially relevant molecules are necessary before algae can be used on a globally significant scale. We feel that the nine topics below are where the largest gains can be made in the near future towards technologies that biologically capture greenhouse gases from fossil fuel sources and contribute to the global energy system.

1. Bioprospecting and Development of Robust Strains
2. Bioreactor Design
3. Harvesting and Extraction
4. Carbon Dioxide Uptake and Utilization
5. Light Harvesting Efficiency
6. Maximize Biomass Production
7. Biofuel/Bioproduct Production

8. Water Use Efficiency
9. Integrated Power Plant Design

The key limiting factors that can be targeted within each of the above topics are discussed in the following sections.

## Overview of photosynthetic carbon capture and fixation

Algae capture $CO_2$ and fix it into carbon molecules using photosynthetic processes similar to land plants. Photosynthesis uses the energy from light to reduce carbon from $CO_2$ to complex carbon-based molecules that act as stored energy. These molecules are often fuels, fuel precursors, or high value chemicals.

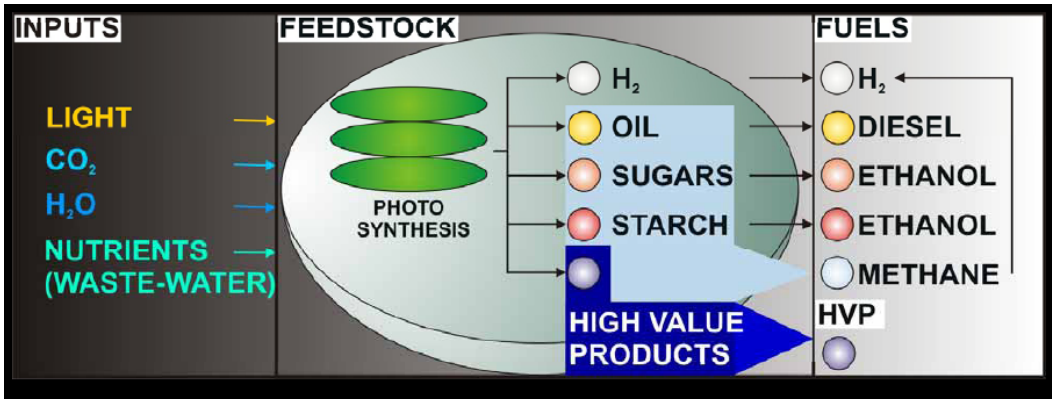



Figure 1. Overview of inputs and outputs of photosynthesis in algae. Light, $CO_2$ and water are utilized by the photosynthetic reactions to produce valuable products such as hydrogen ($H_2$), oil (including triacylglycerols; TAG's), sugars and starch. $H_2$ can be utilized as a fuel or light energy carrier in fuel cells. Oils, sugars and starches can be converted into fuels such as diesel, alcohols or methane. High value products (HVP) can be made to supplement costs and include products such as carotenoids, antibodies or commodities such as organic acids (Hankamer presentation).

Photosynthesis begins with absorption of particular wavelengths of light by specialized pigment-protein complexes native to the organism (Figure 2). This absorbed light energy is quickly converted into chemical energy carriers such as NADH and ATP, which fuel subsequent steps in biological carbon capture and fixation.

Many factors influence the rate of photosynthesis including light intensity, $CO_2$ concentration, mass transfer of $CO_2$ into liquid, temperature, and availability of nutrients. Additionally, the amount of ribulose 1, 5-bisphosphate carboxylase oxygenase (RuBisCO) present in a cell represents an intrinsic limit in the rate of carbon fixation. Other less energy intensive carbon fixation pathways exist in biological organisms and possibly can be used to make the process more efficient.

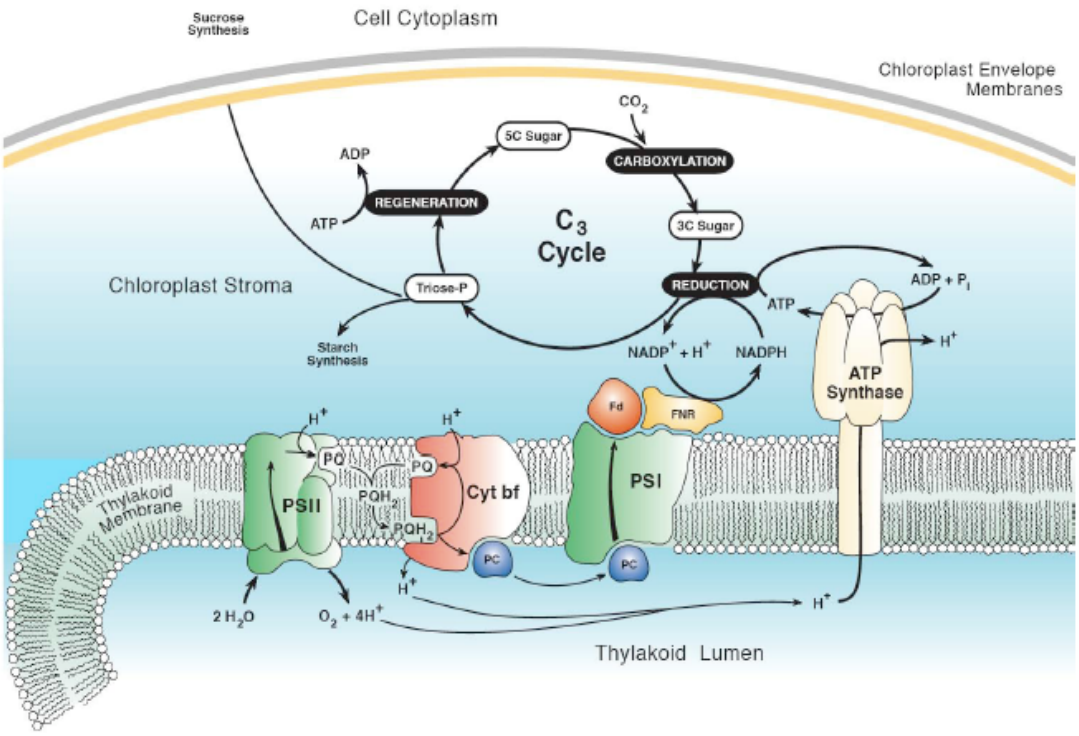



Figure 2. Overview of oxygenic photosynthesis and carbon fixation by the Calvin Cycle. Light driven water oxidation by photosystem II (PSII) releases protons into the lumen and generates $O_2$, electrons and protons. The protons are utilized by ATP synthase for ATP production. The electrons are shuttled through the electron transfer chain through the light driven protein complex, photosystem I (PSI), to ferredoxin (Fd). Finally, ferredoxin-NADP reductase (FNR) mediates reduction NADP to form NADPH, a soluble reducing equivalent. The Calvin Cycle (C3 cycle) involves three phases: $CO_2$ fixation by RuBisCO, followed by reduction using NADPH and ATP and finally regeneration of precursors (Don Ort).

## Bio Prospecting

As mentioned above, the DOE ASP identified 3000 strains of algal species that were interesting from a basic research perspective. Recently, there has been a major push towards similar research identifying strains of microalgae that are well-suited for use in industrial processes. The isolation of novel strains that are tolerant to unique conditions present in industrial processes is an effective way to bring down up-front costs when designing a process, as a well-suited organism will allow for major input reduction.

Isolating strains with faster growth rates than strains currently available would improve carbon capture and biomass accumulation abilities without the need for genetic modification. Additionally, isolation and study of strains that grow in hypersaline environments may lead to significant water-use savings. Alternatively, considering alternatives to monocultures might mitigate costs required to prevent or deal with contamination. Finally, finding strains that are well suited to an

environment with vast and rapid temperature changes may prove useful for minimizing the amount of heating and cooling necessary to keep a culture alive. In all, the opportunity for bio-prospecting right now is immense, and large-scale efforts have a very good chance of finding strains that are naturally suited for bioenergy production.

## Bioreactor Design

The physical location in which microalgae are grown has a dramatic effect on the type of system that is the best for productivity. Typically, there are two competing growth systems for microalgal culture: open ponds and enclosed photobioreactors. The strengths and weaknesses of both have been discussed in great detail in recent years, and will only briefly be mentioned here.

The major limiting factor to both open pond and enclosed photobioreactor operation is water usage. Typically, sites considered the best for algal production have warm temperatures and high average irradiance throughout the calendar year. In locations with these properties, evaporation from open ponds, and gradual heating of photobioreactors become a problem. The solution to both of these problems is to use more water, either to replace the water lost through evaporation, or to evaporatively cool the photobioreactor. In either situation, total water usage for production processes inflates dramatically, and sometimes reaches the point at which the cost and availability of water renders the process useless.

This presents a major opportunity to the scientific community. Major advances in technology for the efficient cultivation of microalgae needs to be made before implementation can occur in many regions. Raceway pond design needs to become more resistant to contamination, and resistant to evaporation. Additionally, a low-cost gas delivery technique needs to be designed if algae are to ever capture carbon from power plants.

## Harvesting and Extraction

Though algae are very useful for production of high value products through genetic engineering, the harvesting of the desired products can introduce significant costs into the production process. Currently, algae are harvested by very energetically expensive means, most commonly centrifugation. When producing renewable fuels from algae, up to 50% of the cost comes from harvesting and extraction, and there is a major opportunity for cost savings with innovative harvesting technologies (Richard Sayre). Improvements in harvesting and extraction technologies will have the biggest effect on renewable fuel prices, as their value per unit volume is relatively low (as compared to other chemicals), and thus so are profit margins.

Research into harvesting technologies has yielded some interesting insight into the most cost-effective techniques. The biological process of flocculation, in which

microalgae clump together and settle out of the media may offer a low-cost method for harvest, as the organisms will self-separate from the media. Flocculation is still poorly understood, however, so current research in that area seeks to understand the molecular factors that trigger flocculation, and identify strains that naturally flocculate.

Another interesting harvesting technique takes advantage of the chemical properties of the chemical being produced. Often, fuel molecules are non-polar, and thus will separate from water on their own. By engineering algae to excrete the molecules or by harvesting the molecules while leaving the cell intact would make harvesting trivial, as the molecules will naturally separate from the culture. This process would also make continuous production easier to achieve, as the living cells will continue to produce the desired product instead of being harvested along with the chemical of interest.

The particular harvesting method that will yield the lowest cost will likely be unique to each production process. The fuel produced, algal strain used, and production technique, are the upstream factors that dictate the cheapest harvesting method. As a result, scientists need to develop an array of low cost, energy-efficient harvesting technologies that can be used in the wide variety of harvesting conditions that will be present in the future.

## Carbon Dioxide Uptake and Utilization

Current designs for amine scrubbing for removal of $CO_2$ from flue stream of coal-fired power plants assume that 90% of the $CO_2$ is removed (Rochelle, 2009). Algae would need to have the ability to capture as much $CO_2$ as current CCS technologies are projected to, at similar costs, or if less $CO_2$ is captured the costs must be significantly lower. The production of biofuels and high value bioproducts would offset the costs of implementing biological organisms as a carbon capture technology as it would in the case of other CCS technologies when fuels are synthesized from the captured $CO_2$.

As oxygenic photosynthetic organisms, algae are well-adapted to capturing ambient $CO_2$. Growing algae to capture ambient $CO_2$ will remove carbon dioxide and sequester it in the form of biomass. Depending on the use of the accumulated biomass, products derived from this process are at best carbon-neutral. Some studies suggest that a 1MW plant facility producing 8323 metric tons of $CO_2$, or 2269 metric tons C would require between a 16 hectare algal bioreactor facility yielding 80 g dry weight $m^{-2}$ $day^{-1}$ or a 64 hectare algal facility yielding 20 g dry weight $m^{-2}$ $day^{-1}$ (Ben Hankamer, presentation).

Bringing a concentrated source of $CO_2$, such as the flue gas from a power plant, into contact with algae to increase capture efficiency and productivity has its challenges. Efficiently capturing carbon dioxide from an elevated $CO_2$ source depends on many

factors, but one of the most limiting at present is the ability of the algae to capture and fix carbon at a sufficient rate to avoid acidification of the medium (and thus crash of the culture). Due to this, research is under way to isolate and engineer strains that are tolerant to high $CO_2$ levels, and are effective at removing large quantities of $CO_2$ in one pass.

## *Biological CCS Technologies*

Historically, establishing the limits of carbon uptake and fixation in microalgae represented a major challenge. Green algae can grow at $CO_2$ concentrations ranging from <=0.01% to > 0.05%. Recent findings in *Chlamydomonas* indicate that there are typically three tiers of $CO_2$ concentrations within this range that have distinct carbon concentrating mechanisms (CCM): very low (< = 0.01%), low (0.03 – 0.4%), and high (> or = 0.5%) (Martin Spalding, presentation). Ambient $CO_2$ is at 0.038%. Understanding the differences in CCM that allow strains to flourish at these three levels is needed in order to improve $CO_2$ fixation.

In addition to the efficiency losses due to carbon delivery to the cell, another well-documented efficiency loss occurs at the cellular site of carbon fixation: RuBisCO. This is due to the fact that there is a secondary oxygenase activity in RuBisCO, which represents a major waste of cellular resources. Ongoing efforts to improve algal carbon uptake efficiency at various $CO_2$ concentrations are discussed.

### Ambient $CO_2$ concentrations

At ambient (385 ppm) $CO_2$ concentrations, microalgae growing in full sunlight can become carbon-limited. In this scenario the rate of carbon uptake and utilization in the culture exceeds the mass transfer of $CO_2$ from gas into the media. Significant cellular energy in microalgae is devoted to concentrating and importing $CO_2$. In addition, many enzymatic steps are needed to complete the Calvin Cycle leading to reduced carbon building blocks utilized by downstream processes. In order to improve algal $CO_2$ absorption, ongoing research seeks to grow microalgae with modified carbon concentrating mechanisms and alternative $CO_2$ utilization pathways.

### Elevated Carbon Dioxide Concentrations

From a technological point of view it is possible for microalgae to capture carbon from the flue gas emitted from stationary fossil fuel powered sources. Some of the limiting factors identified by various studies are the relatively large land area required, the ability to capture only 25 to 30% of $CO_2$ in one pass from a flue stream, the cost of pumping the flue gas, and the undeveloped state of this technology (Benemann, 2003).

There are many fundamental questions that still need to be answered regarding microalgal growth at elevated $CO_2$ concentrations. The most critical determination is the maximum amount of $CO_2$ sequestered from a given concentration of input gas. There was, however, debate as to the actual amount of $CO_2$ that can be removed from the input stream. Data presented by Martin Spalding suggested that less than 5% of the $CO_2$ can be removed from a stream containing >1% $CO_2$ if the cells are

only at a modest density (Figure 3). However, others suggested that as much as 70% uptake from a 2% $CO_2$ stream could be captured in cyanobacteria. The pH of the media and the tolerance of the organism to high $CO_2$ will play important roles in the amount taken up. Further results indicate that the maximum amount of $CO_2$ sequestered from a given concentration of input gas is all of it. The determination of this limit depends on many factors including the design of the photobioreactor, bubble diameter, bubble lifetime, and culture density (Lada Nedbal, presentation). The wide range of values necessitates further research into this key component of carbon capture.

At both ambient and elevated $CO_2$ concentrations there are important issues to consider when growing algae for the purpose of $CO_2$ capture and high productivity. The following sections discuss some of these aspects and avenues for potential research opportunities that may lead to increased efficiencies.

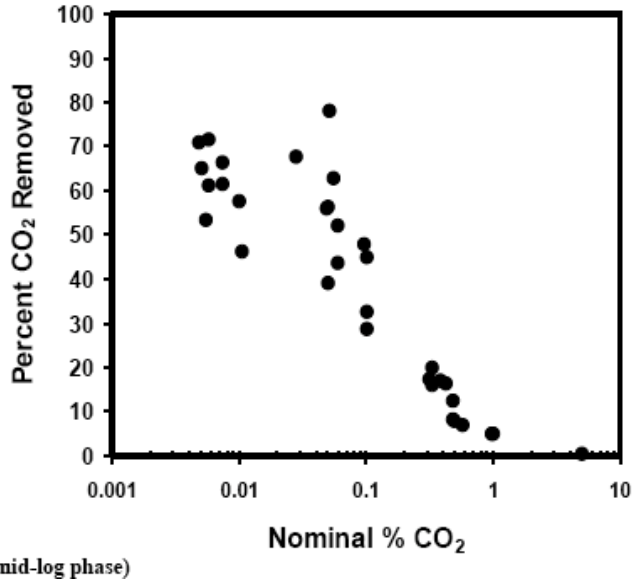



Figure 3. The percentage of $CO_2$ removed from the incoming bioreactor gas stream by a mid-log phase (~1-2x$10^6$ cells/ml) Chlamydomonas culture over a range of incoming $CO_2$ concentrations (nominal % $CO_2$). Each data point represents an independent cell culture. (Martin Spalding presentation; Vance and Spalding, 2005).

## Mass transfer

The mass transfer of carbon dioxide from air into the media can be growth-limiting in dense algal cultures. The transfer of $CO_2$ from a gas to a liquid depends on many parameters. Physical parameters such as gas flow rate, $CO_2$ partial pressure, bubble diameter and lifetime can have large influences on the rate of transfer.

The water chemistry also influences the solubility of $CO_2$ and therefore the transfer capacity. $CO_2$ can be dissolved in water according to Henry's law and, in a small extent, reacts with water to carbonic acid ($H_2CO_3$). The equilibrium shifts towards $HCO_3^-$ (bicarbonate) as the pH increases to a neutral range. $HCO_3^-$ is actively

transported into microalgae while $CO_2$ enters the cell by passive diffusion (see figures 4 and 5). The pH of the media plays a major role in mass transfer and can drastically alter growth dynamics of the organism. Controlling pH by the addition of buffering agents can therefore affect mass transfer of $CO_2$ and carbon uptake by the organism.

### *Carbon Concentrating Mechanisms*

Genetic modification of CCMs may improve the energetic efficiency and rate of carbon uptake in oxygenic photosynthetic organisms. Green algae and cyanobacteria have evolved mechanisms to uptake and concentrate inorganic carbon from the environment (Figures 4 and 5). The strategy utilized depends on the form of carbon encountered. Conversion of $CO_2$ to $HCO_3^-$ in an aqueous environment is pH dependent, with basic environments promoting formation of $HCO_3^-$. Within the cell, enzymatic interconversion takes place in order to transport and concentrate $CO_2$ at the place of carbon fixation in the chloroplast pyrenoid in green algae or carboxysome in cyanobacteria. Elucidation of the components of both prokaryotic and eukaryotic carbon concentrating systems is underway as reviewed in Lou Sherman's presentation. This will allow for the generation and isolation of mutants with enhanced uptake capacity. There is, however, an energetic cost to operate CCMs. This can be circumvented by growing the algae at high $CO_2$ concentrations, where a CCM is likely unnecessary.

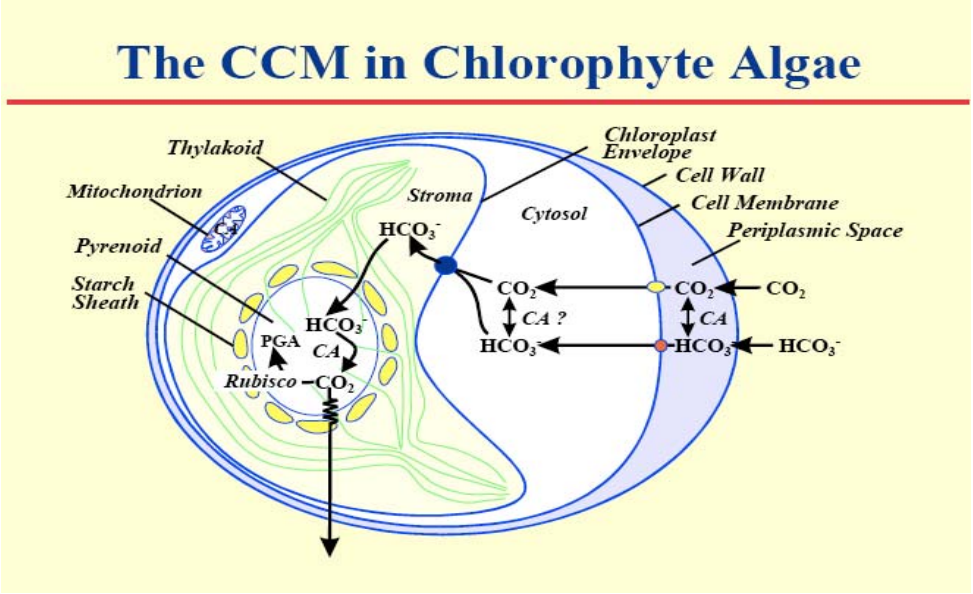



Figure 4. $CO_2$ concentrating mechanism in a green alga. Bicarbonate ($HCO_3^-$) is transported into the chloroplast and converted into $CO_2$ by carbonic anhydrase (CA) to provide substrate $CO_2$ for RuBisCO in the pyrenoid, the site of carbon fixation. (Martin Spalding presentation; Badger and Spalding, 2000).

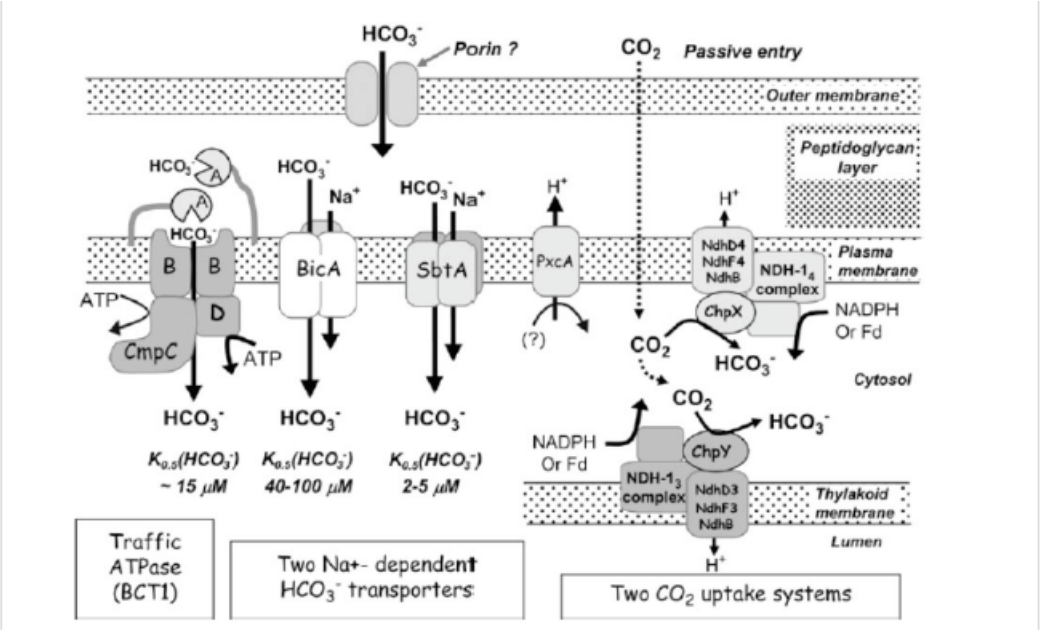



Figure 5. Carbon transport in Cyanobacteria. Bicarbonate ($HCO_3^-$) is actively transported across the membrane using multiple mechanisms tuned to substrate availability. Once internalized, $CO_2$ is then concentrated in the carboxysome, a bacterial subcompartment housing carbon fixation enzymes. (From Lou Sherman presentation; figure from Price *et al.*, 2007)

### *Increasing efficiency of Calvin Cycle*

In oxygenic photosynthetic organisms, $CO_2$ is fixed in the Calvin Cycle by RuBisCO. Substantial losses to photosynthetic efficiency lie between initial charge transfer reactions of photosynthesis and downstream carbohydrate biosynthesis. Depending on the mechanism utilized to fix carbon and the amount of ATP and NADPH utilized, and assuming total incident radiation including infra-red, the maximal theoretical efficiency at this stage (including light capture and energy transduction) is between 8 and 13% before losses due to photorespiration and respiration (Don Ort presentation, Zhu et al, 2008).

#### Photorespiration and modification of RuBisCO

$CO_2$ and $O_2$ are both substrates of RuBisCO. Fixation of $CO_2$ results in 2 molecules of 3-phosphoglycerate (3-PGA), while fixation of $O_2$ results in the production of 3-PGA and 2-phosphoglycolate (2-PG) (Figure 6). 3-PGA is an intermediate in the reductive $C_3$ cycle for production of intermediates in biosynthesis and energy production and also for regeneration of Calvin Cycle intermediates. The byproduct of $O_2$ fixation, 2-PG cannot be utilized by the reductive $C_3$ pathway and therefore must be recycled to recover the carbon through the photorespiratory C2 cycle (Figure 6). Photorespiratory metabolism inherently decreases carbon fixation efficiency, and estimates are that at current atmospheric $CO_2$ concentrations, for every three carbons fixed, one oxygen molecule is fixed. To minimize photorespiration, plants have evolved mechanisms to increase $CO_2$ concentrations

by spatially separating primary $CO_2$ fixation and RuBisCO activity (C4 plants) or by temporally separating photosynthesis and carbon fixation (CAM plants).

Algae and cyanobacteria have evolved efficient carbon concentrating mechanisms in order to reduce the oxygenation reaction (Figures 4 and 5). By actively transporting carbon to the site of carbon fixation, photorespiration is reduced due to the increased ratio of carbon to oxygen. Cyanobacteria do not contain organelles and must complete the photorespiratory C2 cycle within their cytoplasmic space. Green algae have evolved several C2 pathways to efficiently recycle 2-PG and recover $CO_2$ (Figure 8).

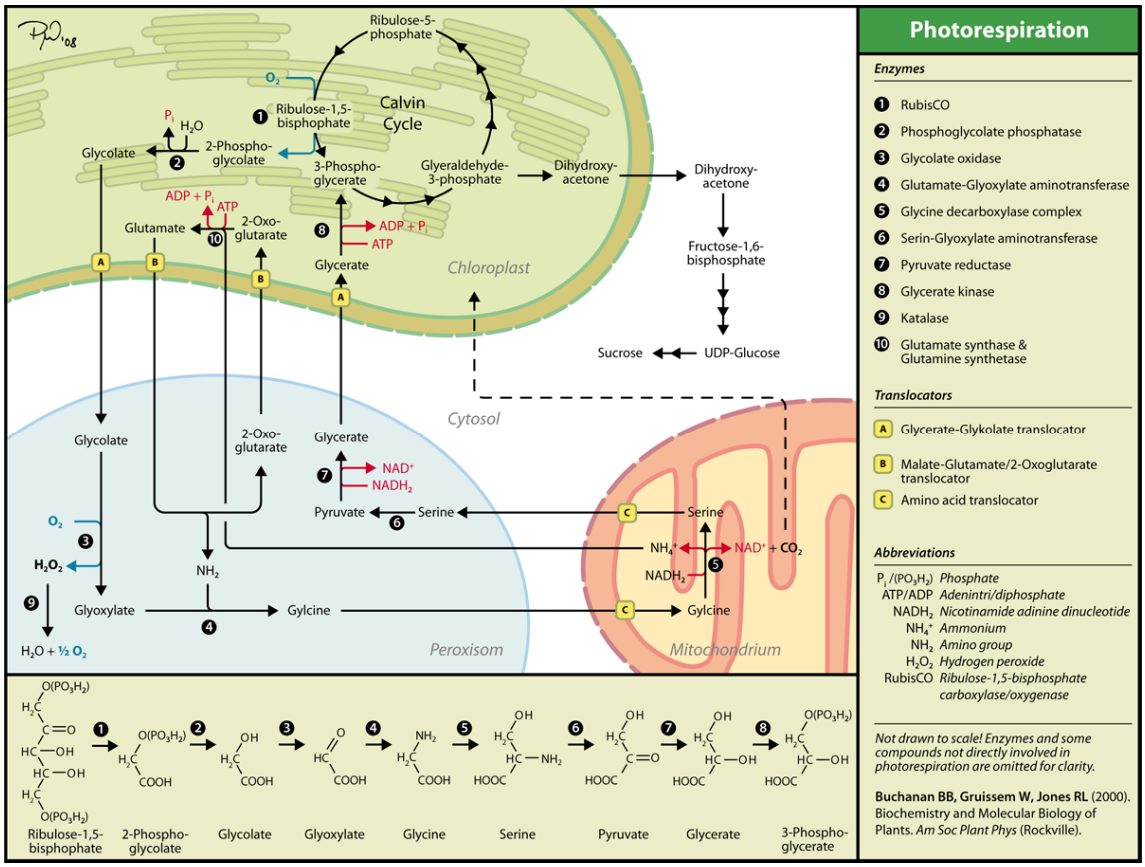



Figure 6. Photorespiratory $C_2$ cycle in plants. Oxygenase activity of RuBisCO leads to the formation of 3-phosphoglycerate and 2-phosphoglycolate (2-PG). Because 2-PG cannot be used in reductive C3 carbon metabolic pathways, the carbon is recovered through the C2 cycle. This process is divided among the chloroplast, mitochondria and peroxisome. This pathway also generates the amino acids glycine and serine, (modified from Buchanan *et al.*, 2000).

Because the oxygenase activity of RuBisCO leads to decreased productivity, there has been interest in modifying the enzyme's catalytic properties. Simultaneous enhancement of RuBisCO specificity and catalytic rate has been a scientific goal for a long period of time because of implications for yield in crop-producing plants. However, active site modification of the RuBisCO enzyme has led to the discovery that catalytic rate and specificity are inversely related (Don Ort presentation; Figure 7). RuBisCO may already be optimized and further modifications may not improve function (Tcherkez *et al.*, 2006). While increasing enzymatic catalysis may be difficult, there has been interest in modifying Calvin cycle protein levels to increase recycling of intermediates and $CO_2$ incorporation. This is an active area of investigation.

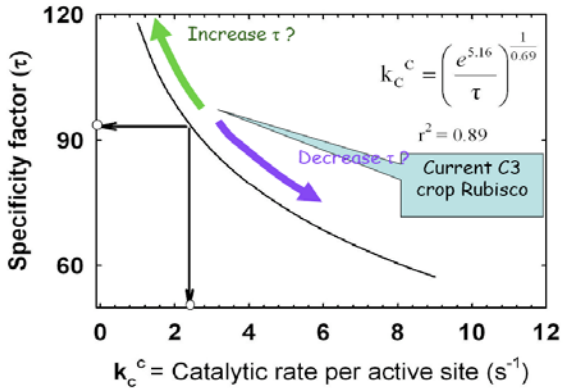



Figure 7. Trade-off between RuBisCO specificity and catalytic rate. Carbon fixation competes with photorespiration because $CO_2$ and $O_2$ are both substrates for RuBisCO. The oxygenase activity is not desirable as it leads to losses in carbon fixation. Analysis of the natural genetic variation in the kinetic properties of RuBisCO from divergent photosynthetic organisms reveals that forms with higher specifity factors have lower maximum catalytic rates of carboxylation per active site , and vice versa. This inverse relationship implies that higher specifity factors would increase light-limited photosynthesis, while the associated decrease in catalytic rate would lower the light-saturated rate of photosynthesis. The daily integral of $CO_2$ uptake by a crop canopy is determined by a dynamic combination of light-limited and light-saturated photosynthesis. At current atmospheric $CO_2$ levels the average specifity factor of current C3 crops exceeds the level that would be optimal for the present atmospheric [$CO_2$] of >380 ppm but would be optimal for ~220 ppm, which is close to the average of the last 400,000 years prior to the Industrial Revolution. Canopy simulations reveal that 10% more carbon could be assimilated by C3 crops if they were operating with a C4 RuBisCO and this advantage would grow as atmospheric $CO_2$ levels continue to increase (Zhu, *et al.*, 2010).

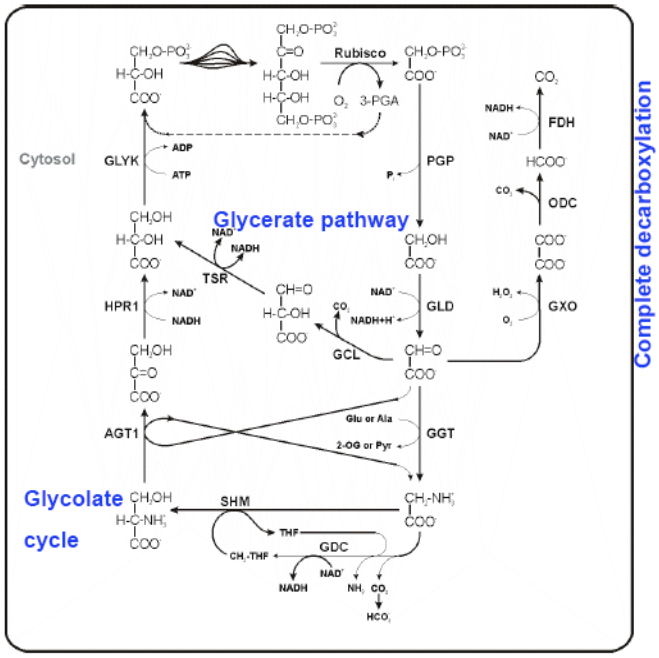



Figure 8. Cyanobacteria use a plant/bacterial-like photorespiratory pathway. Phosphoglycolate generated as a byproduct of RuBisCO oxygenase activity is metabolized using several pathways. Schematic drawing of the complete photorespiratory 2-PG metabolism in cells of Synechocystis sp. strain PCC 6803. 2-PG metabolism is branched into three routes: plant-like glycolate cycle, bacterial-like glycerate pathway, and complete decarboxylation branch (PGP – 2-PG phosphatase, GLD – glycolate dehydrogenase, GGT – glycine/glutamate aminotransferase, GDC – glycine decarboxylase, SHM – serine hydroxymethyltransferase, AGT1 – alanine/glyoxylate aminotransferase, HPR1 – hydroxypyruvate reductase, GLYK – glycerate kinase, GCL – glyoxylate carboligase, TSR – tartronic semi-aldehyde reductase, GXO – glyoxylate oxidase, ODC – oxalate decarboxylase, FDH – formate dehydrogenase), (Hagemann *et al*., 2010).

### *Secondary Pathways*

Diverse carbon capturing pathways have evolved to sustain biomass production in a variety of environments. We have already discussed several of the limits to carbon fixation using the Calvin cycle native to microalgae. In addition to the Calvin cycle, four additional $CO_2$ fixation routes have been identified (Figure 9 and Bob Blankenship presentation). While several of these pathways require anoxic conditions due to the $O_2$ sensitivity of some of the enzymes, others can occur during aerobic metabolism. Of particular interest, the 3-hydroxypropionate pathway utilizes the enzymes acetyl-CoA carboxylase and propionyl-CoA carboxylase to fix $CO_2$ into glyoxylate, an intermediate in carbon metabolism. Genetically engineering alternative $CO_2$ fixation strategies might be advantageous because they may avoid the regulatory constraints and substrate limitations of native pathways.

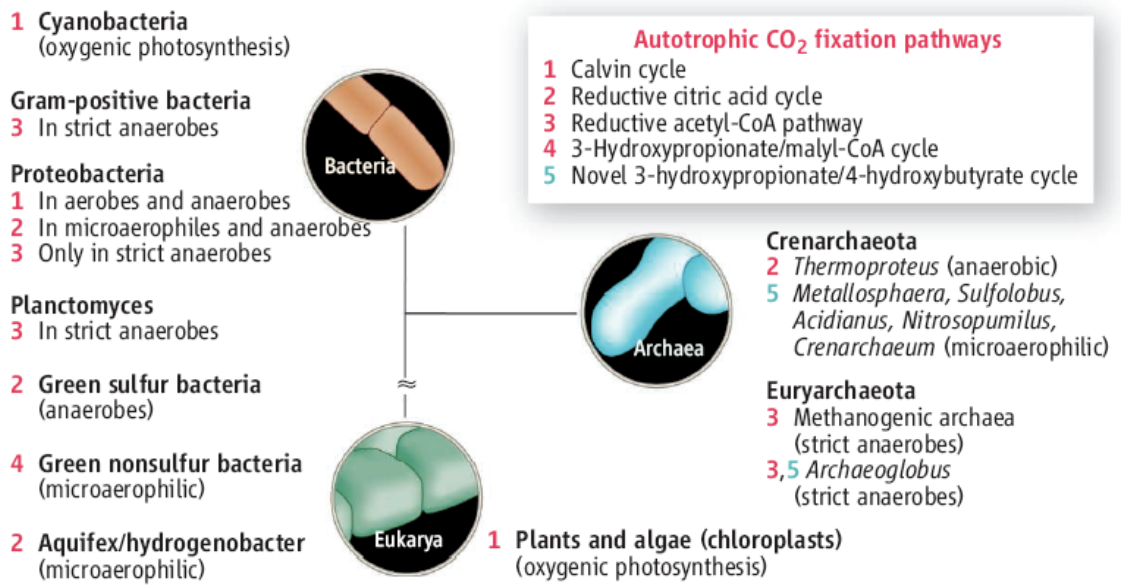



Figure 9. Autotrophic Carbon Fixation Pathways. Most photosynthetic organisms can grow autotrophically using only $CO_2$ as a carbon source. Plants, algae, cyanobacteria and photosynthetic proteobacteria use the Calvin cycle (pathway 1) to fix $CO_2$. The green sulfur bacteria use the reductive citric acid cycle (pathway 2), while green non-sulfur bacteria use the 3-hydroxypropionate cycle (pathway 3). The other pathways shown are known only in non-photosynthetic organisms. (Figure from Thauer, 2007; legend, Bob Blankenship).

## Light Harvesting Efficiency

The amount of solar energy reaching the earth far exceeds current human energy demand. Photosynthetic organisms have the capacity to harvest a portion of this energy and store it in the form of reduced carbon that can be utilized for both energy and be converted into useful products. There are, however, significant thermodynamic limits imposed on the photosynthetic conversion of sunlight to reduced carbon. The numerous reactions needed to facilitate this process inevitably lead to losses in efficiency. Therefore, this workshop spent a substantial amount of time discussing the theoretical limits of photosynthetic efficiency in an effort to determine what can be done to reach these goals.

Photosynthetic efficiency is the fraction of total solar radiation that is converted into chemical energy during photosynthesis (Equation 1).

$$2H_2O + CO_2 + \text{energy} \rightarrow CH_2O + H_2O + O_2 \qquad (1)$$

Photosynthetic efficiency is affected by several physical parameters: light intensity, partial pressure of oxygen and $CO_2$, temperature, pH and nutrients. However, the

degree that each affects a system varies and in some cases will be different in aquatic versus terrestrial species.

When all of the losses are summed, the maximal theoretical limit at this stage for plants is between 4.6% and 6% for C3 and C4 plants, respectively, and 8% for microalgae (Figure 10). The highest reported efficiencies for C3 and C4 plants is about 40% and 60% of the maximum, however, average crop yields fall far below this number (Don Ort). Assuming a C3-like metabolism for photosynthetic microbes and minimization of photorespiration by enriching the atmosphere with $CO_2$ from flue gas, the theoretical efficiency of photosynthesis is 10.6% (Marcel Janssen). In the short term, increasing maximum efficiency may be difficult. During the workshop several interesting ways to increase the maximum efficiencies were discussed.

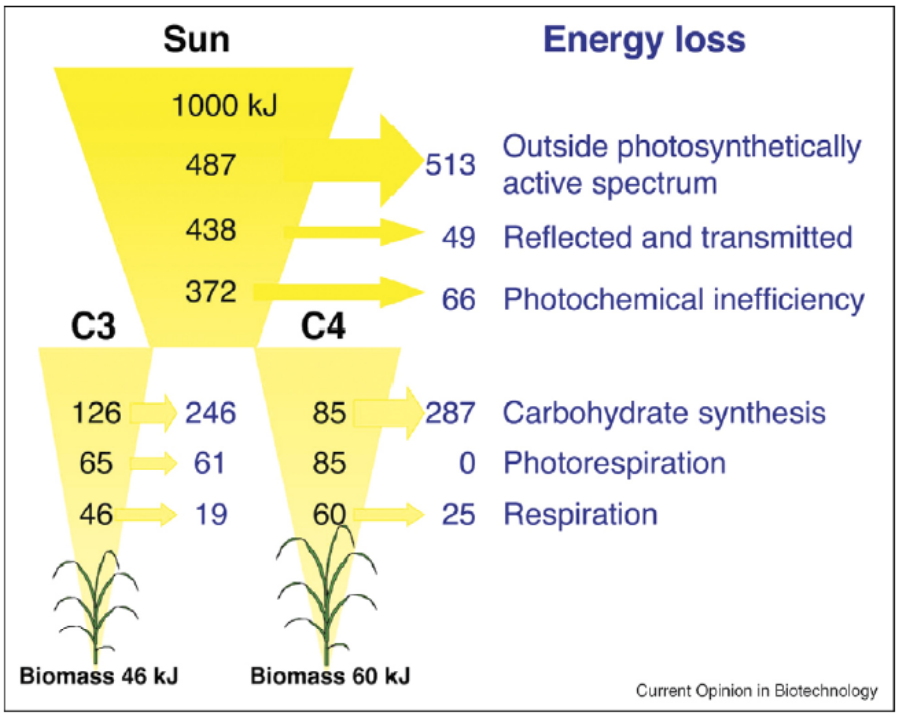



Figure 10. Maximum theoretical photosynthetic efficiency in plants and microalgae. C3 and C4 plants have a maximum theoretical yield of 4.6 and 6.0%. Maximum reported yields are 50-60% of theoretical yields, but average yield is 1% (Figure from Don Ort Presentation; Zhu et al., 2008).

## *Modification of Antenna Complexes*

One major limitation to photosynthesis in full sunlight is that photosynthetic reaction centers quickly become saturated (Figure 11). At low light intensities, in the morning and evening, and in shaded environments, photosynthetic activity increases linearly with light intensities. However in the middle of sunny day, for photosynthetic organs or organisms exposed to full sunlight become saturated and must dissipate this excess energy by non-photosynthetic means.

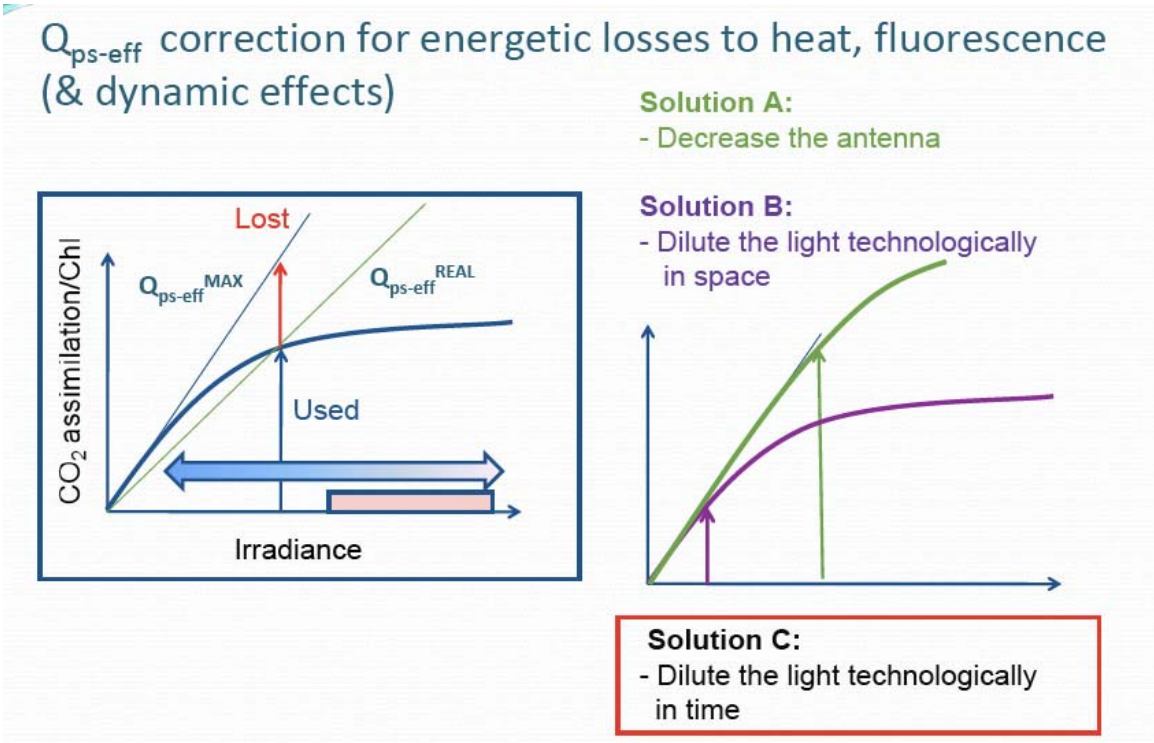



Figure 11. Losses in photosynthesis with increasing light intensity and possible ways to overcome the loss. At high light intensity, photosynthetic antennae complexes dissipate excess energy as heat and fluorescence, leading to less light utilization. To overcome this limitation, one could decrease antennae size, or dilute the light spatially or temporally (Ladislav Nedbal presentation).

In aquatic photosynthetic organisms, light saturation in full sunlight is made worse by the large antennae complexes that are used to harvest light at low intensity for example due to shading in high density cultures. At high light intensity, light harvesting ability exceeds photosynthetic electron transport capacity. Instead of direct transfer to reaction centers, excess energy is dissipated in the form of heat. These mechanisms have evolved to reduce the formation of reactive oxygen species generated as a byproduct of photosynthesis. Research suggests that fine-tuning the antenna size in aquatic photosynthetic organisms can increase overall biomass yield when grown in high density, which will likely be necessary for carbon capture (Figure 11 and 12).

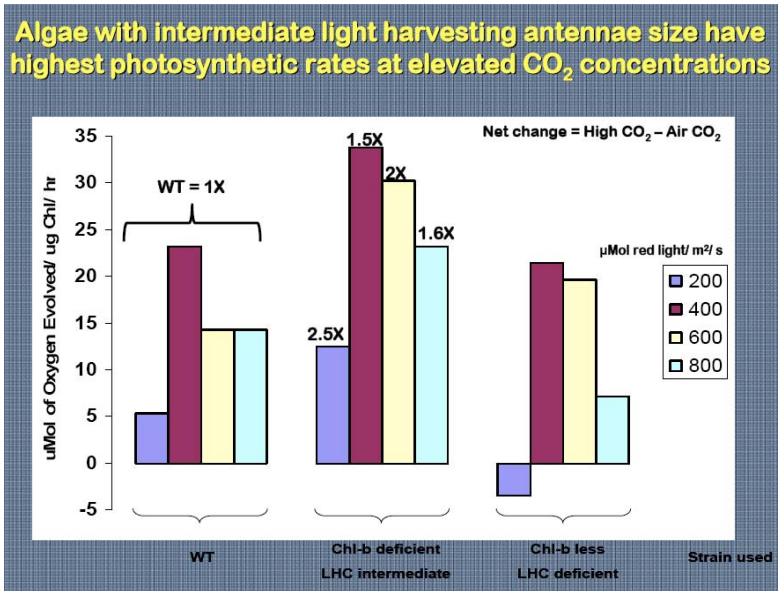



Figure 12. Photosynthetic rates in algae with varying antennae size. Photosynthetic activity was measured in algae strains with reduced antennae or lacking antennae in air and in high $CO_2$ at various light intensities. Photosynthetic rates are expressed as the rate in the presence of 10 mM sodium bicarbonate minus the rate in air (Richard Sayre presentation).

### *Increasing Photosynthetically Active Radiation (PAR)*

Another major loss in efficiency is due to the amount of the solar radiation that can be absorbed by photosynthetic pigments. Over half of the solar radiation impacting the earth is outside the range of photosynthetically active radiation (PAR) (400-740 nm) (Don Ort). There are additional losses within this spectrum due to reflectance and transmittance of green light. Furthermore, losses due to photochemical inefficiency in the form of heat also represent a substantial fraction. Therefore, even before carbon fixation takes place, approximately 60% of the energy available in total solar radiation is not harvested.

Increasing the spectrum of solar radiation captured by photosynthesis increases the potential maximal efficiency. Biotechnology and physical science each have promising solutions to this problem. First, it is important to note that the capacity to increase the amount of harvestable sunlight has already evolved in one particular organism, *Acaryochloris marina*. This organism contains a novel chlorophyll molecule, chl-d, that exhibits a red shifted absorption spectra, extending the photosynthetically active radiation for this organism approximately 40 nm compared to plants, microalgae and related cyanobacteria. Transferring enzymes capable of producing chl-d into other organisms may have the ability to increase the amount of usable wavelengths in the solar spectrum, potentially increasing the maximum efficiency by as much as 5% compared to organisms with only chl-a.

Another way to increase the amount of sunlight that can be utilized is to use materials or chemicals with the ability to shift the wavelength of light from a non-usable wavelength to one that the reaction centers can use. There are many materials capable of shifting light from a short wavelength, high-energy light to a

longer wavelength, lower energy light. In fact, this Stokes shift is commonly seen in fluorescent molecules. One target would be to shift green light to red light. This could significantly increase the total amount of photosynthetically active radiation and therefore increase efficiency.

## Maximize Biomass Production

Microalgae are attractive for biofuel production because for some species the biomass doubling times are in the range of 4 to 24 hours. Additionally, there are strains that contain up to 80 percent oil by dry weight (Banerjee *et al*., 2002; Chisti, 2007, 2008). The accumulation of high biomass over a short time period is desirable and indeed may be essential for making algal culture a viable option for contributing to the energy supply. Currently, ongoing research seeks to confer short doubling times upon strains, and increase yield of high value products from the resulting biomass.

Algae can grow in the absence or presence of light. In the absence of light some algae can grow heterotrophically using reduced carbon skeletons, such as glucose, as substrate. In this mode of growth, the growth rate is much higher than it can be when algae grow in the presence of light and photosynthetically or photoautotrophically. Under optimal conditions, the maximum photoautotrophic growth rate ($\mu_{max}$) is only half that of heterotrophic bacteria because of major differences in the allocation of cellular resources (John Raven, presentation).

During photoautotrophic growth, as much as 30% of the total cellular protein is allocated to the processes of photosynthesis and carbon fixation. Typically, RuBisCO accounts for 10% of total protein content of these cells and the apoproteins in the photosynthetic apparatus accounts for up to 20% (Beardall and Raven, 2010). Additionally, in the same size cell, compared to heterotrophs, photoautotrophs have only about half as much of the machinery necessary to make monomers for DNA, RNA, and protein synthesis, and for polymerizing the resulting monomers. A generalized equation for the specific growth rate of an alga can be expressed in terms of the maximum specific reaction rate $R$ of a catalyst $i$ (e.g. enzyme, transporter, redox agent, pigment-protein complex), and a factor $F$ for the fraction of this reaction rate needed to account for the observed growth rate, with $F$ varying as a function of environmental factors such as inorganic carbon and light supply. The growth rate hypothesis resulting from this observation is valid for about half of algal species, and says: $\mu$ (specific growth rate) is a linear function of rRNA content, with a constant specific reaction rate of rRNA at all rRNA contents (Equation 2).

**Relation of specific growth rate μ to content and reaction rate of catalysts**

$\mu = B_i \cdot C_i \cdot R_i \cdot F_i$

where

μ = specific growth rate (mol C assimilated • mol C in cell$^{-1}$ • s$^{-1}$)

$B_i$ = mol of catalyst of essential reaction *i* • mol C in catalyst

$C_i$ = mol C in catalyst • mol C in cell$^{-1}$

$R_i$ = maximum specific reaction rate of the catalyst of reaction *i* with the reaction product scaled to units of mol C from mol C of product per mol cell C (mol C transformed • mol catalyst$^{-1}$ • s$^{-1}$)

$F_i$ = fraction of potential $R_i$ in cell needed to account for observed μ

Equation 2 (John Raven, presentation).

Algae grown photomixotrophically, where they use not only endogenous but exogenous carbohydrates as an energy source, show a higher $\mu_{max}$ than when grown photoautotrophically, but the cost of the resulting fuel is increased because of the added cost of reduced carbon sources. Additionally, photomixotrophic growth has many implications for greenhouse gas emissions depending on how the feedstock that provides the reduced carbon was grown, obtained and processed. Growing cultures solely under heterotrophic conditions would also preclude the direct capture of carbon dioxide from fossil fuels sources. Aside from growth on waste carbon sources or in a two-stage production method (see Biofuel/Bioproduct Production), heterotrophic algal growth will likely be prohibitively expensive.

Compared to heterotrophic organisms, photosynthetic organisms require substantially more metal ions for growth due to their important role as redox active cofactors in photosynthetic electron transfer (Shcolnick and Keren, 2006). Among these, iron homeostasis was identified as being critical for optimal growth as it is often a limiting factor under both natural and artificial growth conditions (Nir Keren, presentation). Additionally, many algae are auxotrophic for certain vitamins such as vitamin $B_{12}$ which they must obtain from the environment. The need to include high value compounds in algal media would increase the cost of production considerably. However, algae can obtain vitamin $B_{12}$ by direct association with bacteria, which obtain fixed carbon from the photosynthetic algae in return. Mixed cultures such as these might reduce the risk of contamination from other adventitious micro-organisms (Alison Smith, presentation). Maximization of productivity will depend on identification of other factors required for optimal growth.

## Biofuel/Bioproduct Production

A large number of products can potentially be made from microalgae ranging from fuels to herbicides, and polymers with desirable biophysical or bioactive properties.

The ability to genetically engineer microalgae by the addition of genes encoding enzymes of alternative biosynthetic pathways allow for the production of a wide array of chemicals.

## *Fuels from microalgae*

A number of fuels can be made by microalgae, including fermentation byproducts, long-chain hydrocarbons, and hydrogen. The specific properties of the molecules being produced will dictate the harvesting strategy to be used (see Harvesting and Extraction) and thus the overall energy balance of the process. Methane, ethane, long chain alcohols, oils, fatty acid esters, and isoprenes can all be made using algae. While chemically diverse, they are biosynthetically derived from acetyl-CoA or related small molecule intermediates, with the exception of methane. The following sections discuss in more detail some of these products and their uses.

### Ethanol and other fermentative alcohols

Despite its energy density being less than that of gasoline and most biodiesels, ethanol is an attractive transportation fuel because of its use in the existing fuel infrastructure. Ethanol can be blended into gasoline at various concentrations and used in conventional internal combustion engines, which are reported to perform just as well as those with conventional gasoline. At present however, the production of ethanol and other products of fermentation by algae that can be produced naturally are currently at yields too low to be economically viable. Ongoing research such as strain optimization of species that already produce these molecules seeks to increase the yield of fermentative alcohols.

Research also seeks to divert fermentative metabolism from ethanol production into higher-chain alcohols. These alcohols, such as isobutanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol, have more desirable fuel properties such as higher energy content, or hydrophobicities nearer to gasoline, leading to a fuel with combustion properties closer to current gasoline and that assists in purification of the fuel molecules from algal cultures (Atsumi *et al*., 2008). Simple genetic mechanisms can be used to directly convert ethanol into these other fuels. Though ethanol is a useful biofuel in the near-term, research that moves production to more complex alcohols will undoubtedly prove valuable. Many of these molecules are drop-in or direct replacement of gasoline and if produced by microalgae, these fuels are in principle carbon-neutral (assuming energy needs for growth, harvesting and processing could be reduced from current levels).

### Hydrocarbons and Biodiesel

Another promising route to algal fuel production is the harvest of oils for biodiesel (Chisti, 2007, 2008). Fatty acids, specifically triacylglycerols (TAG's), can be trans-esterified directly into biodiesel, producing glycerol as the side product. The production of non-polar lipids is in the range of 4-50% of total biomass of various algae strains (Hu *et al*., 2008). Strains that produce remarkably high levels of these oils are now being studied for other desirable characteristics. One example of productivity achieved to date in Fort Collins, Colorado consists of a 6,000 Liter

system that can produce 1.5 kg/day dry weight algae biomass with $CO_2$ enrichment (Pete Lammers, presentation).

### Hydrogen

Hydrogen production from microalgae is achievable now, although at low light-conversion efficiencies and under low irradiation (Ghirardi and Mohanty, 2010) but could reach relatively high efficiency values because the hydrogen production process is independent from the carbon metabolic pathways that account for many of the efficiency losses. The maximum theoretical efficiency is 10-13% (Ghirardi, *et al.*, 2009). With this in mind, a land area of about 4500 square miles (0.12% of U.S. land area) could effectively supply the transportation fuel demand, with estimated costs as low as $3/kg (Maria Ghirardi, presentation). The use of hydrogen as a transportation fuel is attractive because it is carbon-free. Its combustion does not produce $CO_2$ but instead only water.

In green, eukaryotic microalgae, hydrogen production is mediated by the activity of the algal [Fe-Fe] hydrogenase, which catalyzes the reaction in Equation 3:

$$2H^+ + 2e^- \rightarrow H_2 \quad (3)$$

There are some major hurdles to algal hydrogen production, however, the enzymes responsible for hydrogen production are sensitive to oxygen, and are not expressed in its presence. Ferredoxin, which supplies electrons to drive hydrogen production, is the major electron donor to other cellular redox processes as well, and if large numbers of electrons are diverted to hydrogen production, the pH gradient across the membrane that is established by electron transport from water is not dissipated through ATP production. This results in the down-regulation of rates of electron transport through the photosynthetic chain (Lee and Greenbaum, 2003). The isolation of natural strain variants that are better adapted at producing hydrogen has given some insights into methods towards alleviating these problems.

If algal hydrogen production is a realistic route to energy generation, there remain major technological limitations to using hydrogen on a global scale. Hydrogen has lower energy density than ethanol. Additionally, use of hydrogen as an energy carrier for transportation will depend a lot on safe storage and distribution technologies, as refueling stations must be constructed and hydrogen fuel cell technology still needs improvements before it is affordable.

#### *Methods to overcome losses in efficiency*

To address the problem of oxygen-sensitive hydrogenases, mutants that produce less intracellular oxygen have been generated. Interestingly, these mutants also show accumulation of more oil than wild-type strains (M. Posewitz, personal communication). Additionally, the oxygen tolerance of hydrogenases varies greatly, according to the amino acid sequence of the enzyme. Biochemical characterization of a variety of algal strains is under way in order to find hydrogenase homologs that have both high activities and increased oxygen tolerance (D. Sverdruzic and P. King:

Ghirardi slide 12). Additionally, a transformation system has been generated that expresses a tagged, active [FeFe] hydrogenase in *E. coli* that allows *in vitro* characterization of hydrogenase isoforms (P. King and M. Posewitz: Ghirardi slide 11). Computational studies of various hydrogenases indicate that two gas diffusion channels allow gas diffusion into the reaction center. Therefore, amino acid substitutions that affect the size of the gas channels are of particular interest. The goal here is to create a channel that allows free diffusion of hydrogen, but not oxygen (J. Cohen, P. King, and K. Schulten: Ghirardi, slide 15). The oxygen tolerance of the engineered enzymes is highly sensitive to the amount of reductant supplied during purification (Figure 13).

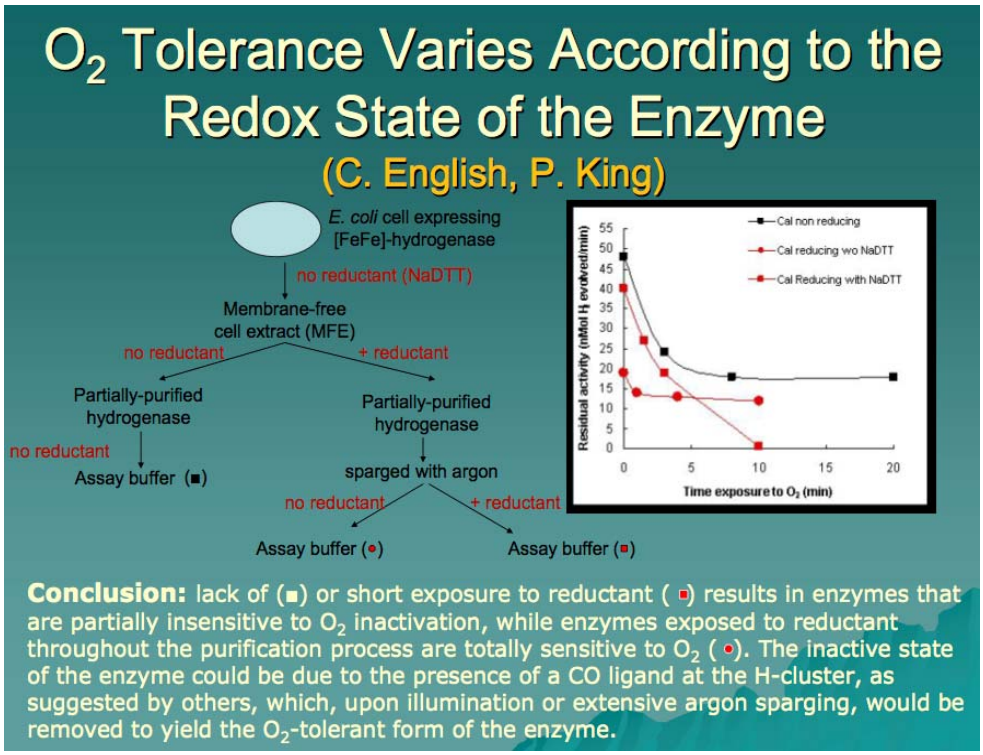



Figure 13. Oxygen tolerance of [Fe-Fe]-hydrogenase varies in response to enzyme redox state. Including reductant during enzyme purification leads to increased oxygen sensitivity. In the absence of reductant during purification, the hydrogenase is less sensitive to oxygen and maintains higher residual activity upon oxygen exposure. (Maria Ghirardi, presentation).

### *Optimization of production parameters*

Studies in several labs have identified mutants that produce more hydrogen than wild type strains. Kruse and Hankamer showed that the stm6 multi-phenotype mutant of *Chlamydomonas reinhardtii* (that cannot transition from linear to cyclic electron transfer), produces $H_2$ at higher rates and for longer periods of time than its parental strain. Sulfur deprivation, which is known in *Chlamydomonas reinhardtii* to increase hydrogen production, was tested on the *stm6* mutant. Hydrogen production was measured and results indicate the stm6 strain can

produce up to 490% more $H_2$ over a 300 hour period in sulfur-depleted media when compared to its parental strain, which is not a particularly high $H_2$-producer.

In an attempt to further boost production, researchers cloned the HUP1 (hexose uptake protein) hexose symporter from *Chlorella kessleri* into *stm6*, generating the strain known as Stm6Glc4. In the presence of 1mM glucose, $H_2$ production was seen to increase by 50% compared to the *stm6* strain without the transporter (Figure 14) (Doebbe *et al.,* 2007). This essentially created a mixed fermentative/ photosynthetic $H_2$ production system, and conversion efficiency of glucose to hydrogen was near 100%.

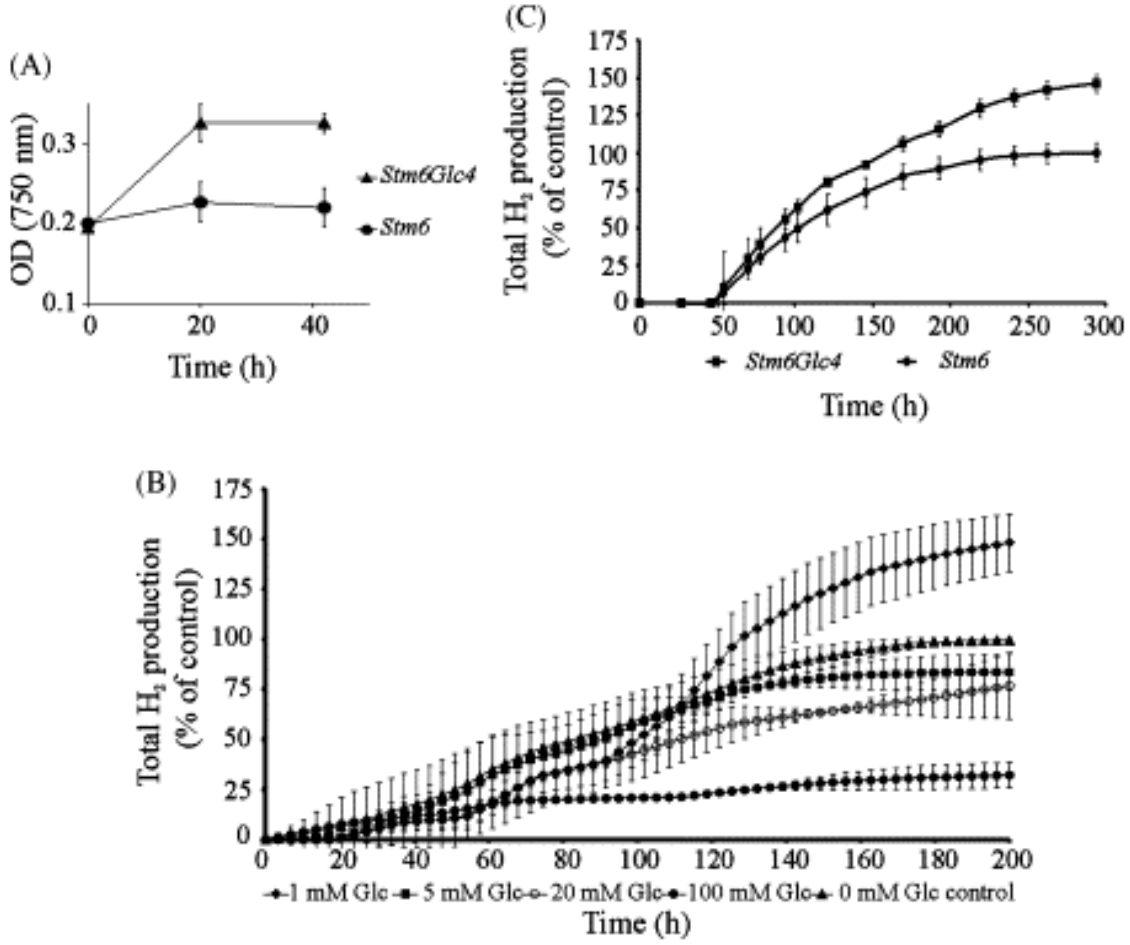



Figure 14: Influence of glucose on heterotrophic cell cultivation and $H_2$ production rates in the glucose transporting Stm6 transformant, Stm6Glc4. (A) Replication rates of Stm6 and the transformant Stm6Glc4 in HSM medium (lacks all carbon sources) supplemented with 100 mM glucose. Each data curve represents an average of at least three measurements. Error bars indicate standard error. (B) Total $H_2$ production of Stm6Glc4 as a function of glucose concentration during S-deprivation. Each data curve represents an average of at least three measurements. Error bars indicate standard error. The control measurement (Stm6 Glc4 + 0 mM glucose) was set to 100%. All cell cultures were adjusted to OD750nm = 1.5 (C) Total $H_2$ production of Stm6Glc4 compared to Stm6 in TAP medium containing 1mM glucose during S-deprivation. Each data curve represents an average of three measurements. Error bars indicate standard error. (Figure from Dobbe *et al*., 2007).

Further strain optimization for enhanced light absorption characteristics was carried out by Oaf Kruse and Ben Hankamer, leading to generation of a truncated antennae mutant optimized for a bioreactor with a depth of 10 cm. With this mutant, in high light (700 μE), cell density reached 0.2g/l biomass in less than 5 days. This equates to a 50% improvement in mid-logarithmic growth rate (Beckmann *et al.,* 2009).

## *Value Added Products*

Value added products from algae have the potential to offset running costs when algae are used as a carbon capture technology. However, market prices and demand will determine the value of products from algae, so the ability to produce a wide array of products and to switch among these quickly may be necessary to make use of microalgae and its products economically viable. Advances in genetic engineering and synthetic biology currently underway, will make generation of strains tailored for production of a specific product faster and less expensive.

### Current Algal products

Currently, there are four major profitable products from microalgae: Agar, Alginic acid (used as a stabilizer and emulsifier in shampoos etc), Carrageenan (extracted from algal cell wall, used as a stabilizer and emulsifier in foods, and toothpastes), and diatomaceous earth. Microalgae are also a source of pigments that have a number of industrial uses. The carotenoids in particular are additives to food as coloring, vitamin supplements, health food products and livestock feed.

Some studies show the worldwide market value of carotenoids is projected to reach over one billion dollars by the end of the decade (Del Campo, 2007). The market value of one of these pigments, B-carotene, has been projected to reach $253 million by 2009. This pigment is of increasing demand in a variety of market applications including food coloring agent, pro-vitamin A (retinol) in food and feed, as an additive to cosmetics and as a health food product under the antioxidant label. This pigment is conventionally commercially produced from Dunaliella in open ponds (Del Campo *et al*., 2007). The market value for omega-3 fatty acids is presently $1.5 billion dollars, with a majority of this product presently extracted from fish oil. This market is expected to grow to over $10 billion in the next several years and with fish stocks rapidly depleting, production directly from algae may become a significant market.

### Therapeutic proteins

Protein-based therapeutics is one of the fastest-growing sectors of drug development (Manuell *et al.*, 2007). Microalgae have the potential to provide high yields of specific classes of recombinant proteins more rapidly and at much lower cost than traditional cell culture (Mayfield *et al*., 2007). For example, algal chloroplasts have proven to be a good system for the production of antibodies (Mayfield *et al*., 2003, Tran M., *et al*., 2009), bioactive mammalian protein, and others of pharmaceutical importance (Rasala *et al*., 2010, Manuell *et al*., 2007), and reporter proteins as tools in molecular biology (see Mayfied 2007, table 1, refs

therein). Advantages of microalgae over other expression systems would depend on the actual properties desired in the protein produced. Microalgae have proven successful at producing proteins with disulfide bonds, but that lack glycosylation. The time-scale from initial transformation of gene to production of protein of interest in microalgae is a relatively short two months. If edible strains are utilized for producing the protein of interest, expensive and often time-consuming purification steps are unnecessary (Manuell *et al.,* 2007).

### Other Products and Services

#### *Cosmetics*

Some companies are already producing ingredients for cosmetics from microalgae growing in fermentative mode. This means that the microalgae grow in the dark, with no requirement for a light source, making the growth and production much more efficient than microalgae grown under light conditions in photosynthetic mode. However, a feedstock in the form of glucose (or glycerol) needs to be supplied to the microalgae, in this case and does not allow/involve the direct capture of $CO_2$ from a fossil fuel source.

#### *Enzymes*

Many enzymes are required in the production processes of many industries including food, paper and pulp, cellulosic ethanol, etc. Isolation of these enzymes from naturally occurring sources can be time consuming and costly and sometimes requires many expensive purification steps where losses of enzyme are encountered. Heterologous expression systems for many enzymes are currently used and production using microalgae could make a contribution to the industry.

## Water Use Efficiency

Water requirements for large-scale culture of microalgae has been seen is a major hurdle in achieving sustainable deployment of these organisms for biological CCS. However, the possibility of incorporating microalgal growth and carbon capture with current water systems at coal-fired power plants may prove to make water use a lesser concern compared to non-integrated algae growth systems. Used mostly for cooling, freshwater use by power plants is only slightly behind irrigation (The largest use of freshwater), at 132 billion gallons per day in the US (Hutson *et al*., 2004).

Figure 15: The Wisconsin Power and Light Columbia Plant uses vast quantities of water per hour, and circulates that water in a large cooling lake located immediately next to the plant. Locations like this may present an opportunity for low cost retrofitting for algal carbon capture (Image: Louis J. Maher Jr., University of Wisconsin).

By coupling microalgal growth to existing water-cooling systems or using strains that grow in brackish or waste water, additional freshwater requirements for biological capture of $CO_2$ emissions from that same plant could be almost zero. A typical 500MW power plant uses 12 million gallons of fresh water per hour, consumes 250,000 gallons per hour, and often sits near a cooling lake (Figure 15, Richard Axelbaum presentation). The impacts on water use at power plants due to the deployment of microalgae can and should be done in a fashion that does not increase usage. However, the use of biocide in cooling water would likely be incompatible with algae production and so this idea may not be practical in systems other than once-through cooling systems.

## Integrated power plant design

To capture the carbon given off by coal-fired power plants, existing plants must be retrofitted, and newly designed plants must incorporate carbon capture into the exhaust scrubbing system. The cost of implementing such systems will be the major driving force behind changes, and for algae to be useful, they must be price competitive with non-biological forms of carbon capture (Richard Axelbaum presentation, Figure 16, reviewed in Buhre *et al.*, 2005). Furthermore, the type of coal utilized in the plant largely influences the cost of adding biological CCS, as SOx, NOx, and heavy metal concentration vary widely in coal deposits (Adel Sarofim,

presentation). The costs associated with CCS will depend on method of capture and employment of advanced separation technologies; whether it is post-combustion or precombustion (Rochelle, 2009). Predictions show these costs, in terms of energy penalties, can be approximately 13.5% for separation of $CO_2$ and another 9% for compression (Herzog, 2009). Storage costs are small in comparison (McCoy and Rubin, 2009). In economics terms, values in the region of $52 per tonne/$CO_2$ for capture and an additional $10 per tonne $CO_2$ for transport and storage have been calculated (Hamilton *et al*., 2009).

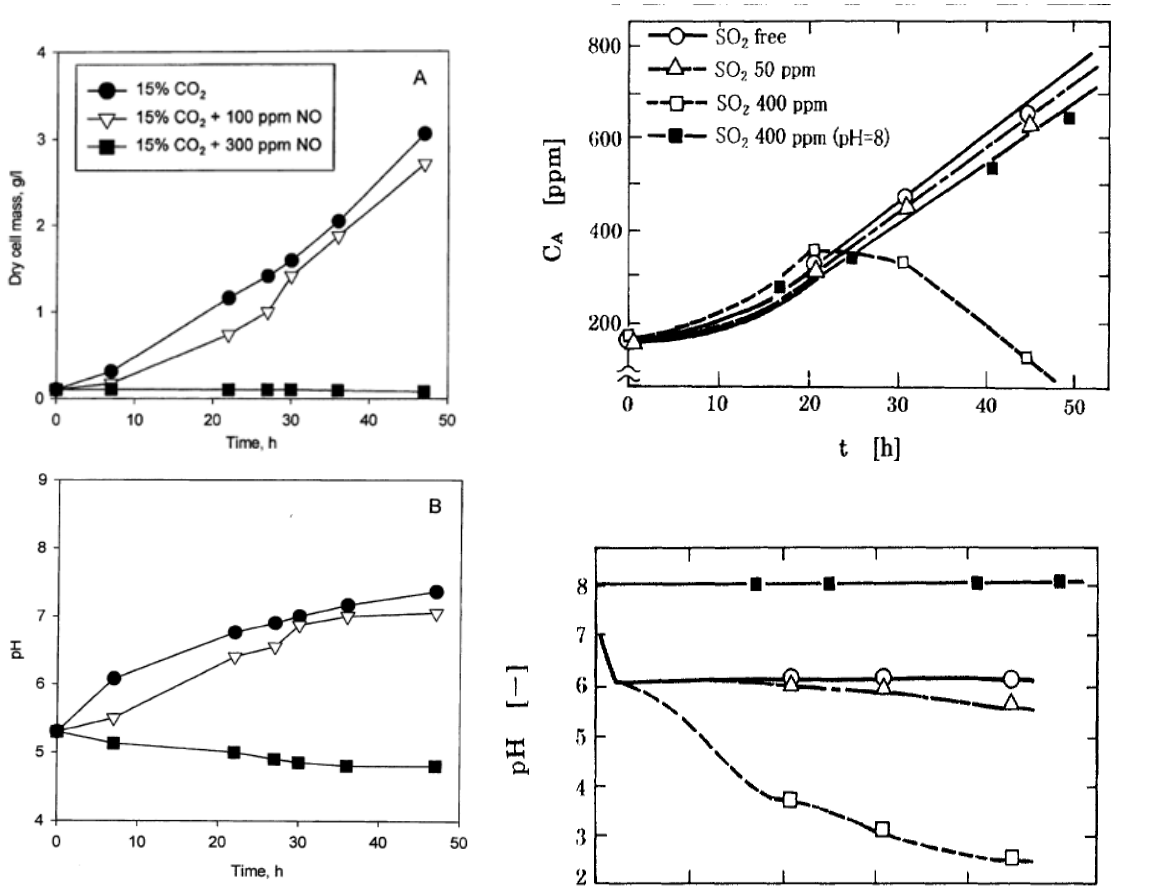
In comparison, costs of algae production could potentially be $250 per tonne $CO_2$ in a photobioreactor system (Chisti, 2007) and $55 per tonne $CO_2$ in a raceway pond system (Stepan *et al*, 2002). However the energy penalty associated with algae as a CCS technology would likely be zero or negative due to the production of large amounts of biomass that can be used as fuel.

### *De novo Design*

Integrating power plant design with algal carbon capture and remediation could be a means of controlling emissions and capturing SOx, NOx and heavy metals such as mercury (Hg) and perhaps additional contaminants from the flue stream. Currently, depending on the design of the power plant and emissions control measures that are in place, the energy penalty for controlling these emissions is in the region of 1% for NOx, 2% for SOx, and 0.4% for Hg (from Wilcox, J., 2010). However, at present only 30% of all US power plants have NOx/SOx scrubbing since the costs of implementing this are less attractive than the actual fines. Regulations for Hg emissions are in place in only 19 states and no other trace metals are regulated or scrubbed (from Wilcox, J., 2010).

The results below, presented by Richard Axelbaum, show that microalgae can tolerate $SO_2$ concentrations up to 400ppm, and NO concentrations up to 100 ppm (which are levels typically found in flue stream from coal-fired power plants) as long as the acidification of the medium in which the microalgae are growing is prevented (Figure 17, Lee *et al*., 2002, and Matsumto *et al*., 1997). While studies that establish the pollutant tolerance limits for algae are important, more research is needed to determine the amounts of contaminant actually removed by the algae.

If algae were deployed as a $CO_2$ capturing and storage technology having the ability to handle these components and to sequester them would be an added advantage since an additional technology need not be in place.




Lee *et al.*, 2002 Matsumoto *et al.*, 1996

Figure 17: Summary of studies of microalgae growth in simulated flue gas. Left-hand side: Productivity of Chlorella sp. KR-1 culture in the presence of NO (from Lee *et al*. 2002). Right-hand side: Productivity of Nannochloropsis salina culture in the presence of $SO_2$ (from Matsumoto *et al*., 1996). Results indicate that productivity is not significantly affected by NO and $SO_2$, at similar concentration levels to that of combustion flue gas, as long as pH is maintained in a favorable range.

# Discussion

For algae to be deployed as a carbon capture technology and contributor to energy supply the process must be able to compete on a cost basis with other energy related technologies for carbon capture, production of fuels and other services. To be sustainable and deployed on a global scale the amount of water, energy, and land used must also be minimized to help preserve our natural ecosystems and avoid competition over these resources with other uses.

Other competing technologies include non-biological carbon capture and storage (CCS) of $CO_2$ from fossil fuel sources, hydrogen production from fossil fuels, and biomass, and production of hydrocarbon fuel molecules by micro-organisms (bacteria or yeast) using sugars derived from biomass feedstocks such as corn, sugarcane and lignocellulosic biomass.

Currently, CCS technologies are energy intensive and expensive, often requiring specific operating conditions of temperature, pressure, pH, and concentration in addition to specific citing where the properties of the subsurface allows $CO_2$ storage. Worldwide there are several carbon storage demonstrations, pilot and proposed projects underway to better understand the processes and challenges to carbon storage technologies (Haszeldine, 2009). There are many issues such as regeneration, low capacity, poor selectivity, poisoning that all incur significant energy and cost penalties. In sum, these projects, including $CO_2$ injected into oil reserves for enhanced oil recovery, represent only megatons of $CO_2$ that has been diverted from the atmosphere. It is a seemingly low volume in comparison to the quantities necessary to make a significant impact and indicates the need for additional CCS alternatives such as storage in geologic formations such as basalts and serpentines, deep ocean, and biological storage and carbon cycling.

Algae could be an alternative or additional technology to this if the overall running costs are less and implementation at scale can be achieved. However, considerations such as land availability, and water use may limit the potential for the sustainable deployment of algae as a means of capturing carbon from fossil fuel sources globally. The ability to couple algal growth to power plant water use and to use low-grade heat from the power plant, technological advances that bring harvesting and extraction costs down, and ultimately finding or engineering organisms that show an order of magnitude increase in efficiency will all impact the extent of deployment of this technology and its successful competition with other carbon capture technologies.

### Recommendations

Some of the key technological advancements, as identified by workshop participants, that need to be made in algae to make this an efficient, sustainable and competitive technology for carbon capture and fuel production, have been discussed in the previous sections.

The major findings suggest that focusing research on one area alone will not lead to the necessary improvements but that research focused on a few key aspects of algae

biology provides opportunities for increases in efficiency that together would lead to an order of magnitude improvement in the operating photosynthetic efficiency.

Improvements to the rate of carbon capture and fixation of algae can be made by targeting many aspects of algal photosynthesis. Alterations to the light harvesting part of photosynthesis by decreasing light saturation; increasing light capture; and increasing wavelength of light utilized; can all lead to an increase in photosynthetic efficiency.

In addition, exploring opportunities in engineering for coupling water use and heat from power plants to algal growth, complemented with the bio remediation capabilities of algae could further improve sustainability, and lower costs of deployment of algae as a carbon capture technology.

Economics are a key aspect to making algae a viable option for contribution to our energy supply and as a production system for high value products. The production of value added products could potentially offset running costs; however sustaining this with respect to the demands of the market place may be difficult. Having the ability to switch production from one commodity to another on a day to day basis may be important to avoid flooding the markets with a particular product.

The goal of achieving carbon capture and production of fuels or high value products at low cost of materials and high efficiency should be the primary goal and the realization of technologies to achieve this should be the focus of fundamental research.

## Acknowledgements

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# Glossary

Algae: eukaryotic photosynthetic organisms, which arose from endosymbiosis of a cyanobacterium that gave rise to chloroplasts. Algae are extremely diverse and inhabit all aquatic ecosystems. Land plants arose from one lineage in the green algae.

Auxotrophic: Requirement of a particular nutrient or compound not made by the organism.

Cyanobacteria: also called blue-green algae, these are the largest group of oxygenic photosynthetic prokaryotes.

C3 plant: $CO_2$ is fixed directly into the three-carbon intermediate 3-phosphoglycerate.

C4 plant: $CO_2$ is fixed into a four carbon organic acid that is concentrated in specialized carbon fixing tissues.

CAM plant: Primary $CO_2$ fixation and RuBisCO activity is separated diurnally.

CCM: Inorganic carbon concentrating mechanism.

Endogenous: Originating or produced within an organism, tissue, or cell.

Exogenous: Material that is present and active in an individual organism or living cell that originated outside of that organism

Green Algae: eukaryotic oxygenic photosynthetic organisms that are the evolutionary progenitors to land plants.

Heterotrophic: growth dependent on reduced carbon source obtained from environment.

$\mu_{max}$: The maximum specific growth rate in $(time)^{-1}$

Photoautotrophic: algal growth fueled by sunlight as the only energetic input.

Photobiological: light-driven energy accumulation by living organisms

Photomixotrophic: algal growth fueled by both electron-rich (reduced) carbon sources and sunlight as energetic inputs.

Photosynthetic efficiency: the fraction of light converted into chemical energy

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# Appendix

***Speakers***

Richard Axelbaum, Washington University in St. Louis
Bob Blankenship, *Washington University in St. Louis*
Yusuf Chisti, *Massey University, New Zealand*
Maria Ghirardi, *National Renewable Energy Laboratory*
Ben Hankamer, *The University of Queensland, Australia*
Marcel Janssen, *Wageningen University, Netherlands*
Nir Keren, *Hebrew University, Israel*
Olaf Kruse, *University of Bielefeld Center for Biotechnology, Germany*
Pete Lammers, *New Mexico State University*
Steve Mayfield, *The Scripps Research Institute*
Ladislav (Lada) Nedbal, *Institute of Systems Biology and Ecology, Academy of Sciences for the Czech Republic*
Don Ort, *U of Illinois, Urbana-Champaign*
John Raven, *University of Dundee, UK*
Adel Sarofim, *University of Utah*
Richard Sayre, *Donald Danforth Plant Science Center*
Lou Sherman, *Purdue University*

09-02-10

Alison Smith, *University of Cambridge, UK*
Martin Spalding, *Iowa State University*

***Workshop Agenda***

## Day 1: September 1, 2009

| | | |
|---|---|---|
| 8:00 – 8:30 | ***CONTINENTAL BREAKFAST*** | |
| **8:30 – 9:00** | Welcome and Introduction | |
| 8:30 | Welcome | Mark Wrighton, *WUSTL* |
| 8:40 | GCEP Introduction and Overview | Sally Benson, *GCEP, Stanford University* |
| 8:50 | I-CARES Introduction and Overview | Himadri Pakrasi, *I-CARES, WUSTL* |
| **9:00 – 11:30** | Plenary Session | ***Chair: Sally Benson*** |
| 9:00 | Biological Carbon Sequestration by Terrestrial Plants | Don Ort, *U of Illinois, Urbana-Champaign* |
| 9:45 | Limits to Algal Growth Rate and Their Alleviation | John Raven, *University of Dundee, UK* |
| 10:30 - 10:45 | ***BREAK*** | |
| 10:45 | Challenges Facing the Use of Coal in a Carbon Constrained World: What the Biologists Should be Thinking About | Adel Sarofim, *University of Utah* |
| 11:30 – 12:30 | ***LUNCH*** | |
| **12:30 – 2:45** | **$CO_2$ Capture by Algae** | ***Chair: Tuan-hua Ho*** |
| 12:30 | The Role of Inorganic Carbon Transport and Accumulation in the $CO_2$-Concentrating Mechanism and $CO_2$ Assimilation in Microalgae | Martin Spalding, *Iowa State University* |
| 1:00 | $CO_2$ Sequestration and Algal Bioenergy: What We Need is More Biology and Less Hype | Alison Smith, *University of Cambridge, UK* |
| 1:30-1:45 | ***BREAK*** | |
| 1:45 | $CO_2$ Fixation and Carbon Storage in Cyanobacteria | Lou Sherman, *Purdue University* |
| 2:15 | Alternative Pathways for $CO_2$ Assimilation in Photosynthetic Microorganisms | Bob Blankenship, *Washington University in St. Louis* |
| 2:45 – 3:15 | ***BREAK & PICTURE TAKING*** | |
| **3:15 – 5:00** | **Productivity and Utilization of Algae** | ***Chair: Jennifer Milne*** |
| 3:15 | Industrial Feasibility Studies of Microalgal Biofuel Systems and Their Targeted Improvement | Ben Hankamer, *The University of Queensland, Australia* |
| 3:45 | Critical Roles of Iron and Other Metals in Algal Productivity | Nir Keren, *Hebrew University, Israel* |
| 4:15 | Biomass and Bioproducts from Nannochloropsis sp. | Pete Lammers, New Mexico State University |
| 4:45 – 5:00 | ***CLOSING*** | Himadri Pakrasi, *I-CARES, WUSTL* |
| | **6:15** | **Bus Departs Knight Center for Dinner at Chase Park Plaza, 6:30-9:00** |

## Day 2: September 2, 2009

| | | |
|---|---|---|
| 7:30 – 8:00 | ***CONTINENTAL BREAKFAST*** | |
| **8:00 – 10:00** | Productivity and Utilization of Algae (contd.) | ***Chair: Ralph Quatrano*** |
| 8:00 | $CO_2$ Capture and Biofuel Production by Microalgae | Yusuf Chisti, *Massey University, New Zealand* |
| 8:30 | Algal Biohydrogen: Opportunities and Challenges in $H_2$ Photoproduction | Maria Ghirardi, *National Renewable Energy Laboratory* |
| 9:00 | Microalgae as a Platform for the Production of Biofuels and Bio-Product | Steve Mayfield, *The Scripps Research Institute* |
| 9:30 | Microalgal Biofuels; A Systems Approach | Richard Sayre, *Donald Danforth Plant Science Center* |
| 10:00 – 10:30 | ***BREAK*** | |
| **10:30– 12:30** | Coupling Emissions from Power Plants to Biological Capture and Fuel Production | ***Chair: Pratim Biswas*** |
| 10:30 | $CO_2$ Capture by Algae in Fluctuating Irradiance | Ladislav (Lada) Nedbal, *Institute of Systems Biology and Ecology, Academy of Sciences for the Czech Republic* |
| 11:00 | Molecular Engineering of Microalgae for Biomass and Bioenergy Production | Olaf Kruse, *University of Bielefeld Center for Biotechnology, Germany* |
| 11:30 | The maximal photosynthetic yield of microalgae in photobioreactors and the minimal requirement for gas transfer | Marcel Janssen, *Wageningen University, Netherlands* |
| 12:00 | Challenges and Opportunities for Biological Sequestration of $CO_2$ from Power Plant Flue Gases | Richard Axelbaum, *Washington University in St. Louis* |
| 12:30 – 1:30 | ***LUNCH*** | |
| **1:30 – 4:30** | **Roundtable Discussion** | ***Chair: Himadri Pakrasi*** |
| 4:30 | Depart | |

## *List of questions for roundtable*

1. How much carbon can be captured from the atmosphere and from fossil fuel sources by microalgae per year?

2. What is the potential contribution of microalgae to the current energy supply, in terms of production of fuel and chemicals?

3. What is the rate and efficiency of carbon fixation by microalgae in natural ecosystems and coupled to fossil fuel sources and what are the limiting factors in each case?

4. What are the factors that account for the major costs and environmental impacts of deploying microalgae at large scale to capture carbon from fossil fuel power plants?

5. How can the costs be reduced/off-set?

7. What are the land use requirements and water requirements for producing fuels and chemicals from microalgae? For example, tonnes of water per tonne of fuel? And m2/ tonne of fuel?

8. In what areas should fundamental research be focused to address the limiting factors of using microalgae for carbon capture from fossil fuel sources?

9. What would it take to reduce global anthropogenic CO2 emissions by 10%, through the use of microalgae? What is the net cost of emission reductions per tonne of CO2?

10. What are the key biological factors that need to be improved to increase algal carbon capture and utilization by an order of magnitude?

11. What are the engineering challenges to overcome to potentially increase algal carbon capture and utilization by an order of magnitude?

Percentage of $CO_2$ Emissions by fossil fuel source (Mt/year), globally