MILESTONE 1 REPORT

Executive Summary:

Although there is much debate concerning global warming, atmospheric carbon dioxide accumulation and the burning of fossil fuels, there is a general consensus that the United States needs to find ways to use domestic sources of energy to decrease our dependence on foreign energy supplies. Coal is our most abundant energy resource and it is used extensively for generating electricity even though it produces large quantities of carbon dioxide when burned. The energy industry is interested in technology that can capture carbon dioxide from coal combustion in an economical and sustainable manner. The industry is also interested in renewable fuels that can be used to displace fossil fuels.

In this research project we will investigate a process for using solar energy, photosynthesis and rapid growth algae to capture carbon dioxide (as is contained in flue gas) and produce lipids that can be transformed into the renewable fuel biodiesel. This fuel can in turn generate revenue for the power industry. The algae cultures used in this research will be selected for their ability to fix carbon dioxide and we will demonstrate their capacity for carbon dioxide capture when grown in photo bioreactors. This algal biomass can then be used as a slurry in place of other agents to control coal dust and can serve as a renewable energy fuel as defined under the Minnesota Renewable Energy Standards. Alternatively, the lipids (oil) in the algal biomass can be extracted and converted to biodiesel (for use in combustion turbine plants or commercial sale) using our new biodiesel catalyst technology called the Mcgyan® process. The Mcgyan® process uses a fixed bed metal-oxide reactor to rapidly produce biodiesel fuel from lipid feedstocks such as algae oil. The biodiesel can be sold on the liquid fuels market to produce revenue to support the costs associated with CO₂-capture. It also has the potential to create revenue for the power company from internally generated carbon credits. Overall this CO₂-capture/biodiesel process will partially close the carbon cycle at coal-fired plants and reduce fossil carbon emissions.
Technical Progress:

M1 Goal 1: Review Literature on Existing Methodology for Algae Cultures

A general literature review of algal production systems has been performed and while there are a variety of methods for mass culturing algae outdoors, very few designs have been successful on a large scale for long periods of time. Open systems are simple, have low capital and maintenance costs and are the most prolific design to date. However, the small surface area to volume, large area required, and high risk of contamination limits the areal productivity. Open systems are also typically low-density cultures, which increases the downstream processing costs such as harvesting and dewatering. Closed systems have demonstrated an increase in the areal productivity and volumetric density over open systems, but the initial capital and maintenance costs are often too great for the system to be economically viable on a large-scale. Two-stage systems attempt to combine the cost benefits of an open system with the high productivity and ability to maintain a monoculture of closed system. Although the two-stage system shows great promise, it has not been demonstrated as economically viable as a commercial system (please see appendix A for the complete literature review).

M1 Goal 2: Design and Construct a Lab-scale Photo Bioreactor

SarTec has researched and developed a photo bioreactor (PBR) that allows one to test different growth parameters and algae species independently. Several PBR designs were constructed and tested. The final design was both relatively inexpensive and fully functional. Eight “final design” photo bioreactors in all have been constructed; they are made from clear, thin-wall polycarbonate tubes mounted in a PVC base.

The poly-carbonate tube is 3.5 inches in diameter and 12 inches in length; holding a volume of 1.5 liters. The thin walled (0.03 inch thickness) polycarbonate material allows for high light penetration and still provides adequate containment. There is a HDPE, semi transparent cover on top with a siphon tube for removal of algae and an airline going into vessel to supply an air and carbon dioxide mixture. The airline runs internally to the bottom of the reactor and a fine pore ceramic diffuser is attached to the end of the line to disperse the carbon dioxide and air mixture into the solution and thus achieve greater solubility per volume of gas used. There is a small opening on top through which culture and nutrient media can be added to the PBR. A 6 inch diameter by 12 inch length sheet metal pipe surrounds the polycarbonate tube in a concentric manner and an opaque top cover in conjunction with the opaque PVC base allow one to minimize the amount of external light reaching the PBR. Cool white LED strip lights are mounted on the internal side of the metal pipe tubular shield in a spiral fashion. The PBR has been certified operational by Professional Engineer Joel Schumacher (please see appendix B for illustrations of the PBRs).

M1 Goal 3: Determine Optimal Operating Parameters for the Lab-scale Photo Bioreactor

The PBRs operate by inputting a gas mixture into the algae solution contained in each PBR. The gas solution consist of 1 part carbon dioxide and 9 parts air; giving it a carbon dioxide concentration of approximately 10% (v/v). Approximately 1.6 ml per minute of this mixture will be needed per PBR to sustain algae growth with an initial setup flow of 5 ml per minute to each PBR (pumped through a fine bubble diffuser located at the bottom of each PBR) to ensure good circulation and prevent clumping of algae cells. The algae will be grown indoors under artificial LED light with a daily cycle of 15 hours light and 9 hours dark. The temperature of
the algae solution will be maintained between 70 – 90 degrees F. After a PBR is established with an algae culture, algae cells will be harvested at a rate of approximately 50% of the PBR volume per day. Nutrient growth media will replace the volume harvested. A sample growth media formulation is contained in appendix C for algae species Dunaliella.

**Milestones:**
We have completed Milestone 1 and have made significant progress on Milestone 2. The primary goals for Milestone 2 are:

- **M2 Goal 1:** Select and collect algae samples
- **M2 Goal 2:** Establish algae cultures and cultivate algae in the laboratory
- **M2 Goal 3:** Optimize algae growth parameters
- **M2 Goal 4:** Analyze and profile algae composition

**Project Status:**
A fair amount of time was spent designing the photo bioreactors and determining the ideal operating conditions for them. The literature review was helpful in this regard and relatively straightforward. Algae samples have been collected and culture cultivation has been ongoing in the photo bioreactors. Additionally, we have begun investigating the optimization of the growth parameters for the algae species. Equipment has been ordered to analyze and profile the algae composition and to collect and separate the algae from the growth media fluid. At this point, the project is ahead of schedule.

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Appendix A

Literature Review of Algal Production Systems

Introduction

Although there is much debate concerning global warming, atmospheric carbon dioxide accumulation and the burning of fossil fuels, there is mounting international pressure to reduce the global accumulation of carbon dioxide in the atmosphere.\(^1\) In the United States this is also a growing concern and there is also a general consensus that the U.S. needs to find ways to use domestic sources of energy to decrease our dependence on foreign energy supplies. Coal is our most abundant energy resource and is used extensively for generating electricity but produces large quantities of carbon dioxide in the process. Currently, the energy industry has had an increased interest in technology that can capture carbon dioxide from coal combustion in an economical and sustainable manner.

Microalgae are photosynthetic microbes that show great CO\(_2\) mitigation potential by biologically fixing carbon through photosynthesis at rates up to fifty times greater than the fastest growing terrestrial plant.\(^2-4\) While the theoretical productivity of algae is impressive, practical large-scale algae production systems and techniques are still being developed. The wide variety of mass algae cultivation systems can be categorized into either open, closed, or two-phase systems each with its own benefits and limitations. This review will summarize the various outdoor naturally illuminated mass culturing systems that have been attempted.

In microalgal farming the key parameter in a system’s viability is its areal productivity, dry biomass production per unit area and time, typically reported as g m\(^{-2}\) d\(^{-1}\). Every algal production system strives to increase the areal productivity while reducing the capital and processing costs. The illuminated surface area ultimately limits productivity as long as the inorganic nutrients, nitrogen, phosphorus, potassium, carbon, and trace metals are sufficiently supplied. Open
systems, such as ponds, lagoons, stirred raceways and circular ponds maximize the illuminated surface area to volume ratio by keeping the water depth shallow – 20 cm or less. To further increase the illuminated surface area closed systems such as tubular, airlift coils and columns, and flat plate photo bioreactors (PBR) were developed.

**Open Algae Production Systems**

Microalgae can be harvested from natural sources such as unmixed natural ponds, lakes, and lagoons. This approach is still practiced by a few health food companies around the world. In the U.S. and Asian countries *Aphanizomenon* and *Nostoc* are exclusively grown in natural waters for health food products. *Dunaliella*, which produces carotinoids as its primary product, is grown in 250 hectare and up to 50 cm deep lagoons in Australia and in smaller natural ponds in the Ukraine. Crater lakes in Myanmar (Burma) and Lake Texcoco in Mexico have been used to produce *Spirulina*. The Lake Texcoco system has since shut down due to urban and industrial pollution. While these systems have virtually no construction costs, the productivity and quality of the products are low and highly variable. Furthermore, feasible locations such as the alkaline crater lakes of Myanmar where villages can scoop out buckets of concentrated *Spirulina* are extremely rare. As a result, harvesting algae from natural sources is impractical for the mass production of most algal products.

To overcome the drawbacks of natural systems, man-made ponds were developed. *Dunaliella, Chlorella, and Spirulina* are cultured in man-made systems in the U.S., China, Israel and other countries. To date only these three species have been successfully mass produced in monoculture and marketed commercially because they can be cultured under extreme conditions (high salinity, high nutrients, and high alkalinity respectively), which limits contamination from competing organisms. Contamination from bacteria and other biological contaminants that reduce productivity or cause population crashes is a major shortcoming in open culture systems.

In the twenty-year U.S. DOE Auquatic Species program attempts at monocultures in open
raceway ponds were abandoned because of chronic contamination. The focus then shifted to wild native species that naturally took over the culture.  

Raceway ponds are currently the predominant commercial system but circular ponds and cascade ponds are also in operation. Raceway ponds are racetrack shaped ponds of with a typical depth of 20 cm and a paddle wheel to circulate the culture. Larger ponds are divided into channels and cover an area of up to 1 ha. The culture can be maintained at a cell density between 0.1 to 0.5 g L$^{-1}$ with average productivity of 25 g m$^{-2}$ d$^{-1}$ during summer irradiances. Lower production rates of 10 g m$^{-2}$ d$^{-1}$ are widely reported during less ideal conditions (e.g. winter months). Circular ponds, (used in Japan, Taiwan, and Indonesia) utilize a rotating radial scraper to stir a culture of less than 5 cm deep. Circular ponds are limited in size by the strain of the water on the rotating motor. Cascade ponds flow a thin film, less than 1 cm of culture, over a sloping glass surface. Operated in the Czech Republic and for several years in Western Australia, the cascade pond achieves a higher cell density of 10g L$^{-1}$ but has a comparable areal productivity of 25 g m$^{-2}$ d$^{-1}$.

**Closed Algae Production Systems**

In order to maintain a pure algae culture without using extreme environments closed systems, called photo bioreactors (PBRs), are necessary. Closed systems have been around since the 1950’s and are either tubular or flat-plate reactors. Tubular reactors come in a variety of designs: vertical airlift columns, horizontal tubes joined with U-bends, helical plastic tubing around a circular framework and α-shaped tubular reactors. Carvalho et al. provides helpful graphic diagrams of each system. The principles behind these designs are similar: a clear plastic or glass tube with a diameter of 2.5 to 40 cm increases the illuminated surface area to volume ratio allowing for high cell densities; turbulent gas is bubbled through the system to mix, and provide CO$_2$; a gas exchange or degasser system removes harmful O$_2$ build up; and the system is harvested and replenished with media continuously or semi-continuously. Flat plate reactors
utilize the same principles but the culture is contained between two clear panels a 2-4 cm apart.\textsuperscript{21} The panels may be vertically oriented or tilted to face south to maximize exposure to sunlight.\textsuperscript{10}

Due to the higher illuminated surface area the cultures in PBRs can reach much higher densities and reportedly higher areal productivity. The cellular density can reach up to 20\textit{g L}^{-1} with a volumetric productivity of 0.25-3.64 \textit{g L}^{-1} \textit{d}^{-1}.\textsuperscript{7} The areal productivity of a PBR system is calculated from the footprint of the facility. Since the reactors are often vertical or tilted much higher areal productivity, up to 130 \textit{g m}^{-2} \textit{d}^{-1}, has been reported.\textsuperscript{22} If the vertical systems are laid horizontally, the areal productivity is comparable to that achieved in open pond systems—25-27 \textit{g m}^{-2} \textit{d}^{-1}.\textsuperscript{7} The increase in areal productivity, however, is accompanied by a significant increase in capital and maintenance costs; so much so that only small-scale systems are producing algae. While there have been numerous pilot plant studies, any commercial facilities attempted were closed within a few years due to the high costs.

**Two-stage Algae Production Systems**

Recently, two-stage production systems that couple PBRs with open ponds have shown promise in increasing areal productivity while maintaining monocultures under non-extreme environments. A tubular or flat-plate PBR is used to cultivate a monoculture at high density. The culture is then used to inoculate an open pond where the algae rapidly grows and is harvested within a two days.\textsuperscript{23} The added benefit of the sudden change in culture conditions is that it stresses the algae, which can stimulate the production of oils and other valuable bioproducts.\textsuperscript{24} Huntley et al. demonstrated the system’s feasibility on an industrial scale for several years, producing 36.4 27 \textit{g m}^{-2} \textit{d}^{-1} of \textit{Haematococcus pluvialis}.

**Conclusion**

While there are a variety of methods for mass culturing algae outdoors, very few designs have been successful on a large scale for long periods of time. Open systems are simple, have
low capital and maintenance costs and are the most prolific design to date. However, the small
surface area to volume, large area required, and high risk of contamination limits the areal
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References

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Photo bioreactor (PBR) with LED light source and tubular photo shield (a) and with LED light source and tubular photo shield removed (b).
Photo bioreactor (PBR) showing LED light source in a spiral pattern on the internal side of the tubular photo shield.
Appendix C

1. **Dunaliella Media:** Make up a 10x macro nutrient stock solution in 1000mL of water. Then neutralize with HCl to pH 7.

<table>
<thead>
<tr>
<th>Salt</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂·6H₂O</td>
<td>15</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>5</td>
</tr>
<tr>
<td>KCl</td>
<td>2</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>2.6</td>
</tr>
<tr>
<td>KNO₃</td>
<td>5</td>
</tr>
<tr>
<td>Tris</td>
<td>12</td>
</tr>
</tbody>
</table>

2. Then make up a 1000x micro nutrient stock solution in 500 mL of water.

<table>
<thead>
<tr>
<th>Salt</th>
<th>mg/500 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA-Na</td>
<td>800</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>20</td>
</tr>
<tr>
<td>H₃BO₃ (Boric Acid)</td>
<td>300</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>7.1</td>
</tr>
<tr>
<td>CuCl₂·2H₂O</td>
<td>20</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>200</td>
</tr>
<tr>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>190</td>
</tr>
</tbody>
</table>

3. Separately make up 1% KH₂PO₄ (wt/v) and a 300mg/L FeCl₃, autoclave.

4. Mix media

<table>
<thead>
<tr>
<th>Dunaliella Media in 1000mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10x Macro nutrients</td>
<td>100mL</td>
</tr>
<tr>
<td>NaCl</td>
<td>10g</td>
</tr>
<tr>
<td>1000x micro</td>
<td>1mL</td>
</tr>
<tr>
<td>1% KH₂PO₄</td>
<td>10mL</td>
</tr>
<tr>
<td>300mg FeCl₃</td>
<td>1mL</td>
</tr>
</tbody>
</table>