

Microalgae Grown in Photobioreactors for Mass
Production of Biofuel

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In the wake of rising energy costs resulting from the depletion of readily available fossil fuels, the economic favorability of developing alternative forms of energy has increased substantially. A rapidly growing world economy, and thus demand for oil, coupled with a peak in the production capacity of major oil suppliers has led to a sharp rise in the market price of crude oil, which will likely persist and continue to rise. As the market price of oil rises, alternative forms of energy and fuel have a much greater potential to become serious competitors.

The implementation of alternative energies is also a matter of national security, considering that the U.S. imports more than 2/3 of the oil it consumes. Especially because many of the countries whom the oil is imported from have questionable humanitarian policies, and harbor extremists with radical anti-American ideologies, it is even more important that a method is developed to end the countries dependence on foreign resources. While there may be untapped domestic resources that could provide the nation with energy for several decades, long term sustainability in the transportation energy market after production capacity can no longer meet the demand needs to be considered.

Furthermore, enhanced awareness of the potential for greenhouse gases to impact the global climate in unforeseen ways gives another reason for researching sustainable fuel sources that do not emit greenhouse gases. Carbon dioxide, although a relatively weak greenhouse gas, is the main perpetuator of anthropogenic climate change due to the immensity of the magnitude of the gas emitted by fossil fuel combustion. Thus, another

motivation for the design of alternative energy systems is the minimizing of carbon dioxide emissions.

The most abundant source of energy available, even more abundant than all fossil fuels combined, is the radiation we receive from the sun. The difficulty of course, is converting solar energy into a practical form of usable energy, and doing this efficiently. A number of technologies have been developed to access this immense source of energy, but human beings were certainly not the first organisms to use the sun's energy for their own purposes. Nature spent billions of years perfecting a method for utilizing the sun's energy, and it's arrogant to think that we could do better on our own.

There are two possible types of liquid fuels that may be produced from organic plant matter, ethanol and biodiesel. Both of these fuels are compatible with existing technologies, as ethanol will power a spark-ignition engine and biodiesel a diesel engine. This is important because it makes implementation of these technologies into society is made much more practical, as there is currently a public demand for these fuels. However, since ethanol has roughly 64% the energy content of biodiesel, it would require substantially more ethanol to replace petroleum than it would biodiesel.³ For ethanol to replace all transportation fuels, there would require 61% of available cropping land to be devoted to growing sugar cane, which produces more ethanol than any other plant.³

As shown in table 1, terrestrial crops used for the production of biodiesel are not any more promising than sugar cane. Take soybean, for example, which is the most commonly recognized source for biodiesel in the U.S. For soybean to replace 50% of transportation fuel needs in America, it would require more than 3 times the existing US

cropping area. However, the oil yield for microalgae is remarkably high compared to all other crops, such that the land requirement actually seems feasible.

Table 1: Comparison of Oil Yield for Various Crops

Crop	Oil yield (L/ha)	Land area needed (M ha)^a	Percent of existing US cropping area^a
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae^b	136,900	2	1.1
Microalgae^c	58,700	4.5	2.5

a For meeting 50% of all transport fuel needs of the United States.

b 70% oil (by wt) in biomass.

c 30% oil (by wt) in biomass.

Thus, the main subject of this paper will be to address the design considerations implicit in obtaining and refining the algal oil yields shown above into usable fuel, in a manner that is economical and energy efficient. There are three primary aspects of producing algal biodiesel, they are: the design of the growth system, harvesting and conversion to biodiesel, and selection along with possible genetic modification of the algae strain. Growth systems for photosynthetic microalgae may be separated into two major categories, open and closed. Open systems are most often in the form of “raceway” style ponds, and have been in use since the 1950s. Although inexpensive in terms of the capital investment, open systems are subject to contamination, poor light utilization, evaporative losses, diffusion of CO₂ to the atmosphere, and requirement of larger areas of land. More importantly, the extent of improving the design of open

raceway ponds is limited, whereas engineering theory of photobioreactors has only just begun.

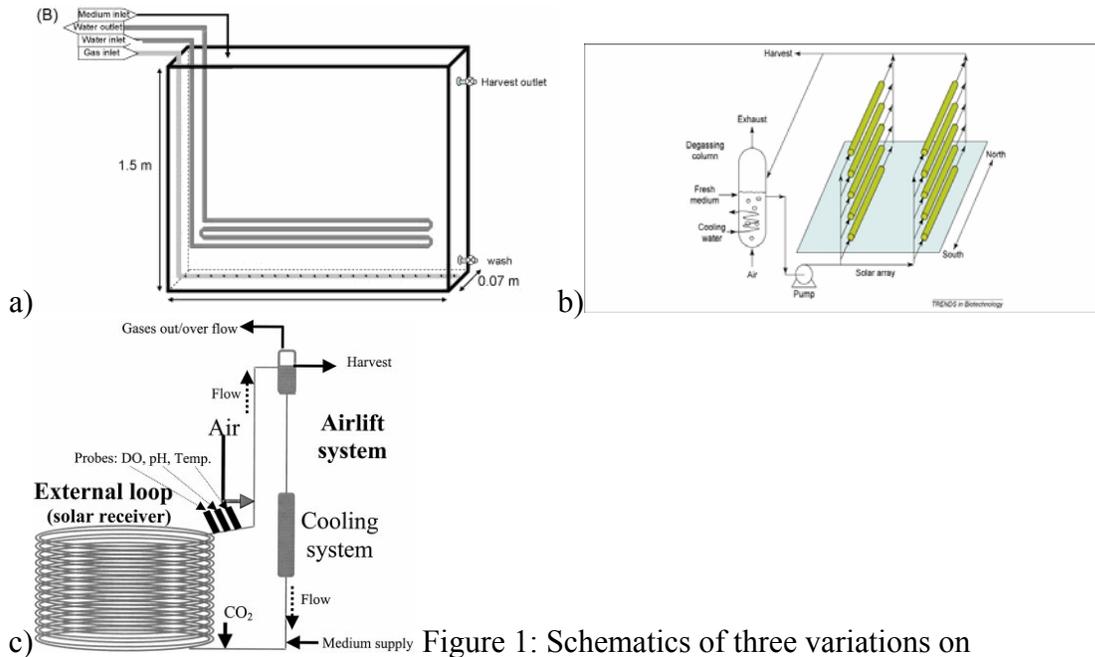


Figure 1: Schematics of three variations on photobioreactor design, as follows: (a) a vertical flat plate reactor, (b) horizontal tubes arranged vertically, (c) tubes forming a helical coil.

Photobioreactors are capable of achieving the remarkably high production rates necessary to make petroleum replacement feasible, with a reasonable amount of land. There are several different photobioreactor designs, and some examples of existing reactors are shown in figure 1. Each of these reactors has some benefits and drawbacks, but regardless of which reactor design is chosen, there are similar technical requirements that must be met in order to have maximized algae growth. However, further discussion will focus primarily on tubular reactors because present research is much more extensive on these compared to the flat plate reactors.

When designing a photobioreactor, the ultimate goal is to maximize the specific growth rate, μ , which is defined as the “increase in cell mass per unit time per unit cell mass.” For any culture, it may be calculated as

Eq 1: specific growth rate = $[\mu] = \ln(N_2 / N_1) / (t_2 - t_1)$ Where N_1 and N_2 = biomass at time1 (t_1) and time2 (t_2) respectively; Levasseur et al (1993).¹⁰ Its units are inverse hours.

Only by maximizing the average specific growth rate will it be possible to obtain a high enough production rate of algal biomass to make the system economical. There are many variables and constraints that need to be considered for optimal design: mass transfer of carbon dioxide and oxygen, prevention of oxygen poisoning, solar irradiation received by the reactor, solar irradiation inside the reactor, prevention of solar inhibition, sufficient mixing of the suspension, control of light-zone dark-zone cycling, as well as pH control and sufficient addition of nutrients or necessary growth media. Furthermore, the effect of scaling up the reactor size for mass production needs to be considered in order to ensure that all of these variables remain at acceptable levels.

The specific growth rate of an algal culture is directly related to the amount of solar irradiance received by the cells. Several functional relationships have been derived for determining the specific growth rate from the expected levels of irradiance and an experimentally obtained maximum that is unique to the specific species being grown. While several relationships have been developed, the equation shown here should be sufficient:¹

$$\mu = \mu_{\max} * I_{av} / (I_k + I_{av})$$

Where I_{av} is the average irradiance inside the reactor and I_k is an organism-specific constant.

For *P. tricornutum*, values of 0.063 and 114.67 $\mu\text{E}/(\text{m}^2\text{-s})$ for μ_{max} and I_k , respectively. Although this species does not have particularly high lipid content, only 20-30%, it is useful to have an idea of the order of magnitude of growth rates that may be expected.

A major constraint on specific growth is the solar inhibition phenomena, for which algae growth slows upon reaching a certain level of solar irradiance. The effects of photoinhibition are shown in figure 2. These measurements were taken for an 80 m long tubular reactor, with carbon dioxide rich gas flowing through the length of the tube, acting as the mechanism for both mixing and deoxygenation. Dissolved oxygen at the outlet of the reactor is indicative of the amount of photosynthetic activity. Thus, for values smaller than the optimal fluid velocity of 0.50 m/s, photoinhibition clearly lowers the photosynthetic activity as a clear decline in dissolved oxygen is noticeable at the higher irradiance levels, which reaches a maximum shortly after the 12th solar hour as one might intuitively expect.

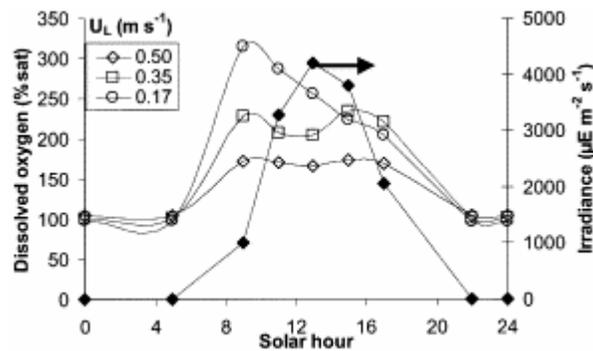


Figure 2: photosynthetic activity dependence on solar irradiance.¹

A partial explanation for the relative consistency of the DO peak during midday for the highest fluid velocity examined is the increased radial velocity, which ultimately leads to an increase in the cycle frequency between light and dark zones in a tubular reactor. A depiction of the light and dark zones in a tubular reactor is shown in figure 3.

The relationship between the fluid velocity and cycle frequency is described as follows.¹

If ϕ is defined as the fractional culture volume that is illuminated, then the cycle

frequency may be expressed as:

$$\text{Eq 3 } v = (1 - \phi) / t_d, \text{ where } t_d \text{ is the time spent in the dark zone.}$$

The volumetric rate of flow out of the dark zone may be defined as:

$$\text{Eq 4 } Q_R = \text{Dark Zone Volume} / t_d$$

And the radial velocity is related to Q_R by the following:

$$\text{Eq 5 } U_R = Q_R / s \quad (s \text{ is defined in figure 3})$$

The radial velocity is also related to the fluid velocity by:

$$\text{Eq 6 } U_R = 0.2 * \{(U_L^7 \mu / (d_t \rho))\}^{1/8}$$

Where U_R , the radial velocity, is a function of the superficial liquid velocity (U_L), the tube diameter (d_t), and the density (ρ) and viscosity (μ) of the culture broth.

Thus, there is a clear connection between the fluid velocity and cycling frequency, which must be sufficiently large enough to overcome the effects of solar inhibition. A

minimum cycle frequency of 1/s has been determined to be necessary for this purpose.

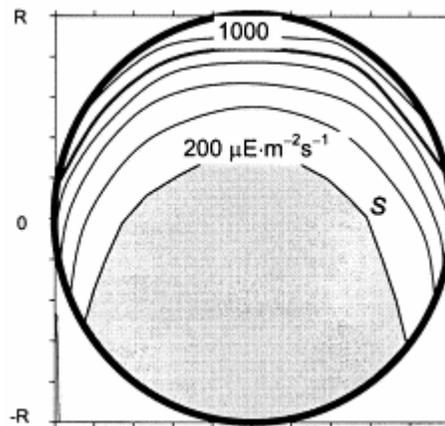


Figure 3. Irradiance profiles inside the solar collector tube at midday and for a dilution rate of 0.04 h^{-1} . The tube diameter was 0.06 m.

Because a high cycling frequency is required for maintaining high production rates, and thus a relatively high fluid velocity as well, it is necessary to limit the tube diameter. For large diameter tubes, the turbulence required to produce an adequate frequency gets to be too great. Limitations arise from the sensitivity of the cells to shear damage from microeddies below a certain size, as well as limitations in the structural

strength of the plastic used for construction of the reactor. Thus, it has been determined for *P. tricornutum* that the maximum tube diameter for large scale production should not exceed 0.1m.¹⁶

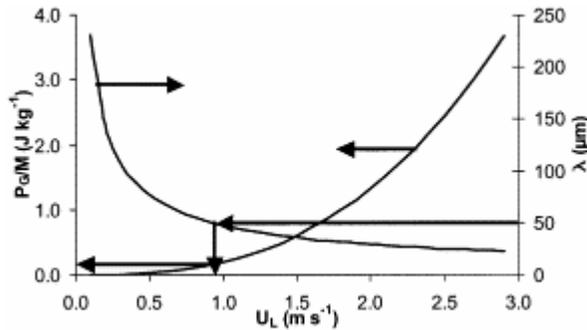


Figure 4: Influence of liquid velocity on the length λ of the microeddies and power dissipation (consumption) for a 0.06 m diameter tube and a fluid with waterlike properties.¹¹

Oxygen poisoning may also occur when the level of dissolved oxygen in the algal solution, produced by the algae during photosynthesis, gets to be great enough that the algae growth slows, and the cells may be damaged by photooxidation as well.¹ It has thus been reported that the maximum length for a continuous tube should be no greater than 80 m, and the oxygen concentration should not be greater than 3 times atmospheric conditions.^{1, 4, 5, 6, 7} The 80 m value was calculated from the equation:

$$\text{Eq 7: } L = \{U_L * ([O_2]_{\text{out}} - [O_2]_{\text{in}})\} / R_{O_2} \quad ^1$$

Where L is the tube length, U_L is the maximum permissible fluid velocity without damaging the cells = 0.5 m/s, $[O_2]_{\text{out}}$ is the maximum oxygen concentration (300% atm conditions), and R_{O_2} is the volumetric photosynthesis rate value estimated at $0.003 \text{ mol O}_2 \text{ m}^{-3} \text{ s}^{-1}$.

This means that for large reactors, air bubbling zones may be required to strip the oxygen out of the solution.^{1, 11, 5}

It has been shown that bubbling carbon dioxide rich gas through a tubular photobioreactor not only provides CO_2 to the algae but also aids in deoxygenation of the suspension, provides mixing to increase the cycle frequency thereby limiting solar inhibition, and is even used for pH control. As carbon dioxide moves through the culture

broth, it is consumed by the algae and the pH increases as a result. CO₂ may be consumed at a rate as high as 26 g CO₂/m³-h.²⁰ Optimal pH values for photosynthetic algae have been reported at values between 7.7 and 8.5.^{1, 3, 9, 11}

Nutrients such as phosphates, nitrates, and ammonia need to be added to the culture media in concentrations suitable for meeting the demand of the algae. In fact, algae have been proposed as a treatment method for removing these nutrients from wastewater,¹³ and the possibility of using secondary treated wastewater as a nutrient source in photobioreactors has been investigated.¹² However, it has been determined that fertilizer production would still be necessary for large scale algae growth.

Temperature control is also of great importance for growing algae. At 35 C, cultures of *P. tricornutum* were photoinhibited, with the collapse of the culture occurring at higher temperatures.¹⁴ Heat exchangers using cold water have been shown to be effective for maintaining an optimal temperature range, which is between 20 and 30 C, but further add to the cost of building the reactors.^{1, 3, 5, 6, 15}

One final control parameter needs to be discussed, and that is the dilution rate. The dilution rate is defined as the ratio of the incoming flow rate to the reactor volume. This value is not dependent on the superficial fluid velocity, as the fluid velocity may be altered by adjusting the superficial gas velocity. At steady state, however, the dilution rate should be equal to the specific growth rate.⁶

The biomass productivity rate, which is ultimately what needs to be maximized, is the product of the dilution rate and the concentration of biomass at the effluent of the reactor. Thus, it is necessary to maximize the biomass concentration while maintaining a high rate of dilution. In table 2, the highest recorded biomass production rate in terms of

mass/time has been presented. A maximum rate of 2.04 g/L-d was achieved in a coiled tubular bioreactor. Other values were included to show the effect of solar irradiance on yield, as well as the reduction in productivity resulting from a non-ideal selection of the dilution rate.

Table 2¹⁶: Comparison of hourly production rates of biomass for tubular reactors with varying dilution rates and solar irradiance. The tube diameter is 0.06 m in all cases.

Volume L	U_L m/s	D, h^{-1}	I_{wm}	$C_b, g/L$	$P_b, g/L-d$	v, Hz	Source
220	0.3	0.025	2366	6.6	1.66	0.684	16
220	0.3	0.04	2319	4.4	1.76	0.0628	16
220	0.3	0.04	2860	5.1	2.04	0.0638	16
220	0.3	0.04	1211	2.7	1.08	0.651	16
200	0.5	0.05	1289	2.38	1.19	-	11

Once design specifications have been determined for algae production, the next step is to select a method for converting the algal suspension into biodiesel. The most commonly recognized method for converting any oil to biodiesel is known as transesterification using alcohol and a catalyst.

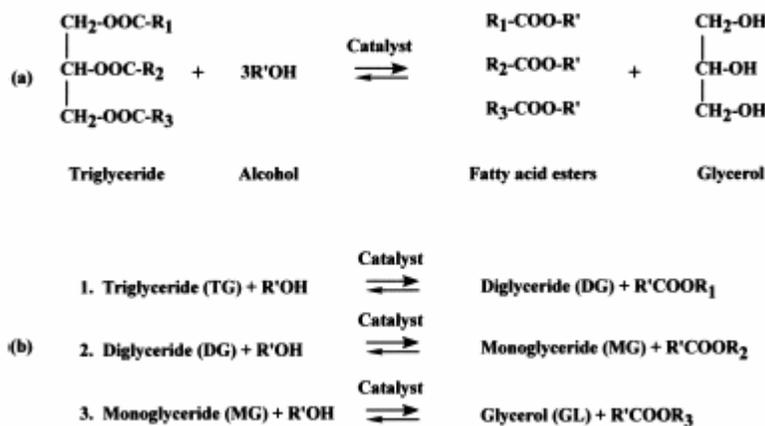


Figure 4: transesterification to biodiesel¹⁷

To ensure that the triglycerides are fully reacted to glycerol and methyl esters, the reaction described in figure 4 is usually performed with excess methanol (because it is cheap), with molar ratios of 6:1 for methanol to triglycerides being common. Common catalysts are sodium and potassium hydroxide, and are generally added at 1% by weight of oil. The transesterification process then occurs at 60 C, and takes about 90 minutes. Since oil and alcohol do not mix, there are two liquid phases during the reaction, and biodiesel must ultimately be “recovered by repeated washing with water to remove glycerol and methanol.”⁵ The final yield of methyl esters is often higher than 98% on a weight basis.

Although this method is the most common for vegetable oil conversion to biodiesel, it may not be ideal for producing algal biodiesel because of the necessity to first harvest and dry the algae. This process can be a major operating expense, or add substantial time to overall production rate, depending on whether filtration or centrifugation is chosen. However, there is a process that can treat wet material directly to produce biodiesel.

Thermochemical liquefaction has received some attention as a possible alternative to convert algal biomass directly to biodiesel, without prior drying. This process involves the application of pressurized N₂ at 2-3 MPa for 1 hour, and at high temperature ranging from 250 – 400 C.¹⁸ The nitrogen is used to control the evaporation of water, but the mechanism for this is unclear. The mixture is then treated with CH₂Cl₂ catalyst, which causes the separation of biodiesel from an aqueous phase. For the algae strains *Botryococcus braunii* and *Dunaliella tertiolecta*, addition of 5% by weight of the catalyst is necessary.¹²

The heating energy for liquefaction of *B. braunii* was reported to be 6.69 MJ/kg and compares favorably to the heating value of the oil produced at 45.9 MJ/kg. This value was based on a culture concentration of 0.5 kg biomass/m³, indicative of what may be expected from the effluent of a raceway pond. However, the concentration of biomass produced in photobioreactors was shown to be more than ten times the concentration used in their calculation. Since water evaporation is likely the key source of energy consumption for liquefaction, a ten fold increase in concentration can be expected to result in a ten fold decrease in energy required per unit mass of biomass. Although the truth of that statement needs to be determined experimentally, thermochemical liquefaction appears to be the ideal method for converting high concentration algae suspensions to biodiesel.

The final aspect of designing an algae production system is selection of the algal strain to be utilized. In future operations, this selection will likely be coupled with genetic modification of the parent strain to further optimize the organism for maximized oil production. However, several existing species of microalga have exceptionally high oil content; examples include *B. braunii*, *Neochloris oleoabundans*, and species of *Nannochloropsis* and *Schizochytrium*, which have oil contents of 25-75%, 35-54%, 31-68%, and 50-77%, respectively.⁵ Of course, oil content is not the only important aspect of algae characteristics, but it is certainly the most useful for determining potential oil yield.

Sustainable Development

So for implementation into a sustainable development design, the per capita energy usage and population size need to be known in order to determine how much

algae needs to be produced, and how much land that will be required. If a modest decrease in energy usage is assumed for simplicity to be 8 TOE (tons of oil equivalent)⁷, than for a community of 100,000 people, 800,000 TOE would be required, which is equal to 2.345×10^{10} MJ. Using a heating value of 45 MJ/kg for microalgal oil, this corresponds to $2.345 \times 10^{10} \text{ MJ} / 45 \text{ MJ/kg} = 52.21 \times 10^8 \text{ kg}$ of oil. If oil yield is valued at 50% of the biomass by weight, than $1.042 \times 10^9 \text{ kg}$ of algal biomass need to be produced annually. At a production rate of $2 \text{ g/L-d} = 730 \text{ g/L-yr}$, $1.427 \times 10^9 \text{ L}$ or 1.427 million m^3 of reactor volume would be necessary.

One 80 m section of tubing with a diameter of 0.1 m, the maximum constraints outlined previously, would have a volume of 0.6283 m^3 . Thus, 2.272 million lengths of 80 m tubular reactor will satisfy the total energy demand of 100,000 people. If these reactors could be arranged as shown in figure 1b with 2 m spacing, then ten tubes may fit in $150 \text{ m}^2 = 0.037 \text{ acres}$, or 270.27 tubes/acre, of land and still operate effectively. This corresponds to $(270.27 \text{ tubes/acre}) * (0.6283 \text{ m}^3/\text{tube}) * (1 \text{ kg oil/m}^3\text{-d}) * (365 \text{ d/yr}) = 61980.88 \text{ kg oil/acre}$, or $(*45 \text{ MJ/kg}) 2.789 * 10^6 \text{ MJ/acre-yr}$ produced. Thus, $(2.345 \times 10^{10} \text{ MJ/yr}) / (2.789 \times 10^6 \text{ MJ/acre-yr}) =$ approximately 8,400 acres of land that would be required to satisfy the TOTAL energy demand of 100,000 people. This is more ambitious than the original intent, which was to only replace transport fuels. Regardless, it shows the feasibility of using algae for large scale energy production.

References:

1. Molina, E. et al., 2001. Tubular photobioreactor design for algal cultures. Journal of Biotechnology. Vol. 92, # 2. Pg 113-131
2. Xu, Han. et al., 2006. High Quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. Journal of Biotechnology. Vol 126, # 4. Pg 499-507

3. Chisti, Y. 2008. Biodiesel from microalgae beats bioethanol. Trends in Biotechnology. Vol 26, # 3. Pg 126-131
4. Scragg, A.H. 2002. Growth of microalgae with increased calorific values in a tubular bioreactor. Biomass and Bioenergy. Vol 23, # 1.
5. Chisti, Y. 2007. Biodiesel from microalgae. Biotechnology Advances. Vol 25, # 3. Pg 294-306
6. Molina Grima, E. Adien Fernandez, F.G. Garcia Camacho, F. Chisti, Y. 1999. Photobioreactors: light regime, mass transfer, and scaleup. Journal of Biotechnology. Vol 70, #1-3. Pg 231-247
7. http://www.nationmaster.com/graph/ene_usa_per_per-energy-usage-per-person
8. Dufreche, S. Hernandez, R. French, T. et al. Extraction of Lipids from Municipal Wastewater Plant Microorganisms for Production of Biodiesel. 2006. AmerOil Chem Soc. 84:181-187
9. Janssen, M. Tramper, J. Mur, L. Wijffels, R. 2002. Enclosed Outdoor Photobioreactors: Light Regime, Photosynthetic Efficiency, Scale-Up, and Future Prospects. Biotechnology and Bioengineering, Vol 81, # 2. Pg 193-210
10. Wikipedia.com
11. Fernandez, F.G. et al. 2001. Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. Chemical Engineering Science. Vol 56, #8. Pg 2721-2732
12. Sawayama, S. 1999. Possibility of renewable energy production and CO₂ mitigation by thermochemical liquefaction of microalgae. Biomass and Bioenergy. Vol 17. #1 Pg 33-39.
13. Shi, J., Podola, B. 2006. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. Journal of Applied Phycology.
14. Fernandez, F.G. et al. 2003. Outdoor production of *Phaeodactylum tricornutum* biomass in a helical reactor. Journal of Biotechnology. Vol 103. # 2. Pg 137-152
15. Molina Grima, E. et al. 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology Advances. Vol 20, # 7-8. Pg 491-515
16. Molina Grima, E. et al. 2000. Scale-up of tubular photobioreactors. Journal of Applied Phycology 12: Pg 355-368
17. Fukuda, H., Kondo, A., Noda, H. 2001. Biodiesel Fuel Production by Transesterification of Oils. Journal of Bioscience and Bioengineering. Vol 92, No. 5. Pg 405-416
18. Aresta, M., Dibenedetto, A., et al. 2005. Production of biodiesel from macroalgae by supercritical CO₂ extraction and thermochemical liquefaction. Environmental Chemistry Letters
19. Hall, D.O., et al. 2002. Outdoor Helical Tubular Photobioreactors for Microalgal Production: Modeling of Fluid-Dynamics and Mass Transfer and Assessment of Biomass Productivity. Biotechnology and Bioengineering. Vol 82 # 1, Pg 62-73
20. Yun, Y., Lee, S.B., et al. 1997 Carbon Dioxide Fixation by Algal Cultivation Using Wastewater Nutrients. Chem. Tech. Biotechnology. Vol 69. Pg 451-455