



McGill University
Department of Bioresource Engineering

Course
ENGINEERING DESIGN 2
(BREE 490)

Design Proposal

**Design of a Small Scale Algae Cultivation System to
Produce Biodiesel**

By:
Derick R.Poirier (260232967)
Edouard Michaux (260226690)
Megan Sumiko Fulleringer (260233808)

December 2nd, 2008

Executive Summary

Biodiesel is currently produced from crop feedstock, waste oil and animal fats which are not sufficient to cope with the growing demand for alternative fuel requirements. Cultivation of algae has the potential to provide for the need of producing renewable, affordable fuel without compromising food production. Algal cultivation systems such as photobioreactors or open ponds are the means used for algae production. Through this project an experimental small scale, low cost algae cultivation system will be designed. It will comprise selecting an algae strain and designing a cultivation system all the way from inputs to the biodiesel output.

Table of Contents

1.	Problem Statement.....	5
1.	Objectives and Scope	8
2.	Location and Context	9
3.	Literature Review	11
4.1	Biology concepts relating to algae	11
	A) <i>Algae in the environment</i>	11
	B) <i>Classification of Algae</i>	12
	C) <i>Growth Kinetic of Algae</i>	14
	D) <i>Conditions influencing growth</i>	17
	E) <i>Biomass productivity and lipid productivity</i>	19
	F) <i>Nutrient requirements</i>	20
	G) <i>Effect of nitrogen deprivation</i>	22
	H) <i>Light/Dark cycle</i>	23
	I) <i>Photoinhibition</i>	24
	J) <i>Likely algae candidates</i>	25
4.2	Methods for algae cultivation.....	26
	A) <i>Description of the Algal Cultivation System</i>	26
	B) <i>Open air systems</i>	28
	C) <i>Photobioreactors: description</i>	31
	D) <i>Airlift photobioreactor</i>	32
	E) <i>Tubular photobioreactor</i>	33
	F) <i>Vertical-Column photobioreactor</i>	34
	G) <i>Lighting in Photobioreactors</i>	35
	H) <i>Instrumentation and Control</i>	37
4.3	Harvesting techniques	37
5.	Revised Objectives and Scope	40
6.	Methodology	41
6.1	Design Approach.....	41
6.2	Expected Results.....	43
6.3	Cost Analysis	44

7. Work Schedule	45
8. Conclusion	46
Acknowledgments	47
Bibliography	48

List of Tables

Table 1 - Land area required to meet 50% of U.S. transport fuel needs	6
Table 2 - Classification of algae	13
Table 3: Biomass productivity, lipid content and lipid productivity of 30 algal strains	20
Table 4 - Algal group and culture collection of origin of 30 strains	25
Table 5 - Prospects and limitations of various culture systems for algae	27
Table 6 - Comparison between harvesting processes	39
Table 7 - Work Schedule	45

List of Figures

Figure 1 - Transesterification of oil to biodiesel	5
Figure 2 - Climate chart of Los Angeles	9
Figure 3 - Land suitable for agriculture and algae cultivation	10
Figure 4 - Interaction of main factors influencing algae growth	12
Figure 5 - Phases of a bacterial growth curve	15
Figure 6 - Growth rate as a function of temperature	18
Figure 7 - Schematic diagram on the use of nutrient ratios as indicator of nitrogen or phosphorus limitation.	21
Figure 8 - Effect of light intensity on the specific growth rate of microalgae	24
Figure 9 - Potentials of algal biomass: raw material, energy sources, products and applications ...	26
Figure 10 - Algal cultivation system using a two phase process.	27
Figure 11 - Raceway pond.	28
Figure 12 - Large scale raceway pond algae cultivation	28
Figure 13 - Airlift photobioreactor system	33
Figure 14 - Vertical column photobioreactors	35
Figure 15 - Penetration and dispersion of light in a photobioreactor tube	36

1. Problem Statement

It has long been known that the planet's fossil fuel stocks would not meet our energy requirements forever. As stocks diminish, prices will continue to rise. Furthermore, harmful emissions from the combustion of fossil fuels contribute to climate change (EIA). Both of these factors mean that there is great interest in finding a low-cost, renewable alternative to fossil fuels.

Biodiesel is a renewable, potentially carbon neutral transport fuel (OEE). The process occurs according to the following chemical reaction.

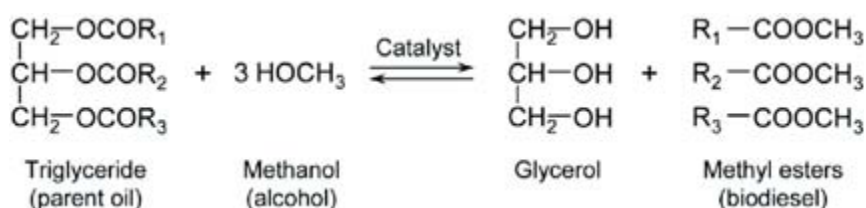


Figure 1 - Transesterification of oil to biodiesel
(Christi 2007)

An oil, or triglyceride, is combined with an alcohol, generally methanol, and goes through transesterification to become methyl esters (biodiesel). The by-product, glycerol, is also of commercial value in the pharmaceutical industry.

Biodiesel is a cleaner burning fuel than conventional diesel, and produces fewer greenhouse gas emissions. Biodiesel blends of any concentration can be used in any diesel engine. The only disadvantage is that in cold temperatures, biodiesel tends to lose viscosity, particularly in blends with a high concentration of biodiesel. This has already begun to be addressed, however, with the use of fuel additives and engine block or fuel filter heaters and storing vehicles indoors (OEE). Furthermore, algal biodiesel has a lower melting point than other biodiesels, and thus possesses better cold climate properties. Technology producing and using biodiesel has been available for over 50 years (Chisti 2007). Therefore, biodiesel is potentially an ideal alternative to fossil fuel use.

The obstacle then becomes producing sufficient quantities of the oils needed to make biodiesel. In the United States, biodiesel is produced primarily from soybean oil. It is also produced, to a lesser

extent, from canola oil, palm oil, corn oil, waste cooking oil, animal fat, and jatropha oil.

Unfortunately, biodiesel production from any of the conventional feedstocks mentioned here cannot realistically meet the tremendous demand for transport fuels. It would require 0.53 billion m³ of biodiesel to replace all the transport fuels consumed in the United States. Producing even half of this would require an unsustainably large land base (Chisti 2007). Therefore, a new feedstock is required. Microalgae, like all plants, uses sunlight to produce oils, but it does so more efficiently than crop plants. The table below summarizes the land area required for various feedstocks to meet 50% of the transport fuel needs of the United States.

Table 1 - Land area required to meet 50% of U.S. transport fuel needs

Comparison of some sources of biodiesel			
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.

(Chisti 2007)

Microalgae is an ideal feedstock for the following reasons. Many species of microalgae are extremely rich in oil (Rodolfi et al.). An oil content of 20-50% by weight on a dry mass basis is easily attainable. Microalgae also reproduce very quickly, and commonly have doubling times of 24 hours or less. They are largely a non-food resource. Microalgae production can also take place on non-productive land, such as desert land, that is of too poor quality for conventional crops (NREL).

They can utilize brackish or saline water as well as sequester waste CO₂ from other processes (NREL). Ideally, microalgal biodiesel would be carbon neutral. All the power needed to produce the algae would come from biodiesel or from methane produced by the anaerobic digestion of biomass residue left after the oils had been extracted from the algae.

Microalgae is thus an ideal feedstock for the production of biodiesel. The remaining problem is that it is not currently available to the general public. This is because it is not yet cost-effective from an industry perspective.

The potential for the use of algae as a source of renewable fuel is therefore quite considerable. However serious research on this application of algae is fairly recent. Among the new prospects, there exists the possibility of improving lipid productivity through nitrogen deprivation as will be explained subsequently on this report. It would therefore be interesting to investigate the design of an algae cultivation system with this idea in mind.

1. Objectives and Scope

The objective of this project is to design a small-scale algae cultivation system to produce biodiesel. This project will therefore adopt a systems approach to optimize both biomass production and oil content of the algae cells. Not only must the system be productive, it must also try to be cost and energy efficient. This project will attempt to achieve this end by the use of a photobioreactor system which takes into account the full cycle of producing the biodiesel, all the way from choosing an algal strain suited to this end to designing a photobioreactor system that would allow this algae to grow in optimal conditions and finally the harvesting of the algae lipids that would be converted into biodiesel. The system will also incorporate the property of some algal species to increase their lipid content through nitrogen deprivation. Indeed some sources point to the possibility of N depriving algal cells in a two step process: Phase I would be dedicated to increasing biomass productivity in a nitrogen rich medium, while phase II would increase lipid content in a nitrogen deficient medium.

Another objective of this project is to determine the efficiency of this system by conducting an energy balance. The initial cost and the added cost of production (fixed and variable cost) will be compared to the present cost of diesel in order to compare the cost of this method of production compared to the current price of diesel.

Finally, the greatest sign of success from this project would be to build it and prove that the system is functional. This could perhaps also serve as the basis for a larger scale photobioreactor system in the future. A brief analysis will therefore also be conducted to see if the design could be scaled-up to an industrial level from the initial small scale.

2. Location and Context

Algae growth is possible in a wide variety of climates such as tropical or even arid climate if the proper nutrients are provided. However, the small scale photobioreactor facility will be designed to operate outside in a temperate climate with an average yearly temperature above 20°C with no more than 15°C of fluctuation between seasons as well as a solar intensity of (). A Mediterranean climate with the following temperature distribution would be ideal:

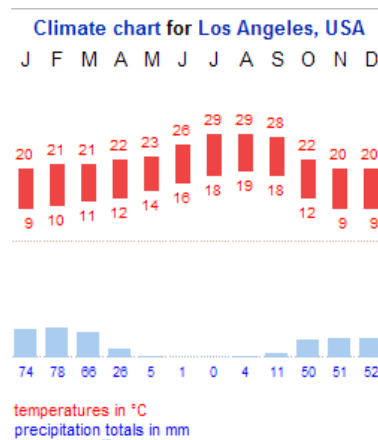


Figure 2 - Climate chart of Los Angeles

Designing for a plant in a colder or a less sunny climate would have had an aversive effect on the growing conditions for the algae and would severely complicate the design and the economics of the project. This experimental design is to pave the way for further development with the aim of achieving larger scale photo bioreactor plants that would obviously designed in the most favorable conditions to optimize costs and reduce risks. This photobioreactor project is too small to really make land use an issue, but up- scaling a plant would entail taking this into account. This implies that the plant would have to use land that is unsuitable for agriculture. The following map shows world location where such large facilities could be built without compromising food production. The green areas are used for agriculture. The rectangle represents the area with sufficient lighting for algae to be cultivated .

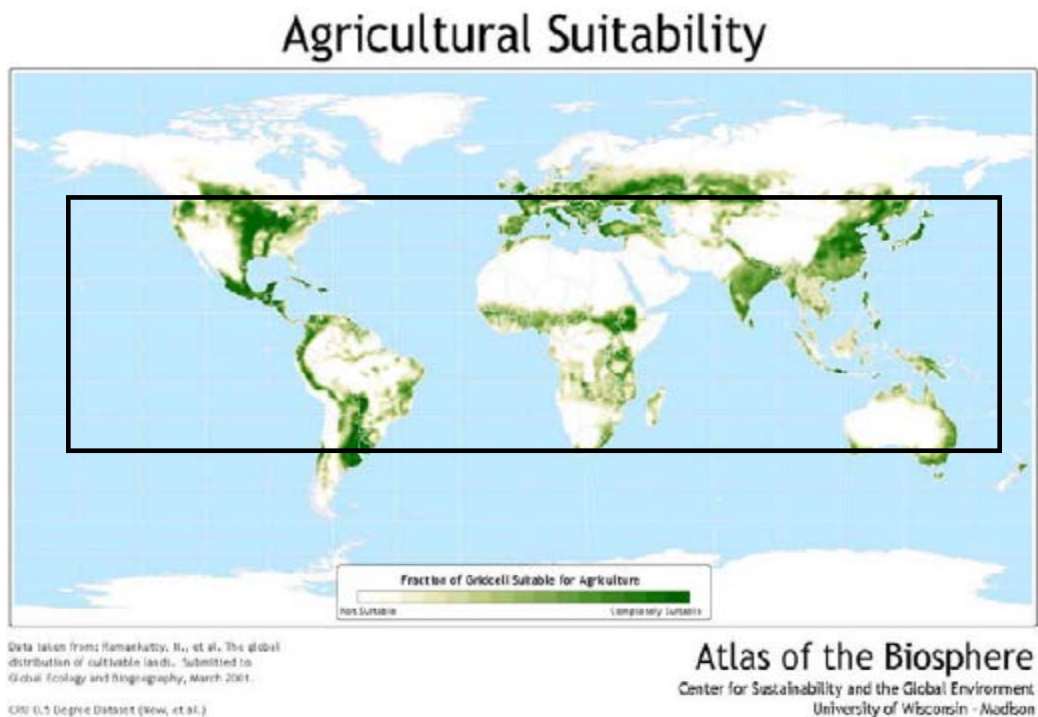


Figure 3 - Land suitable for agriculture and algae cultivation

Source: http://www.sage.wisc.edu/atlas/maps/suit/atl_suit.jpg

A large plant also requires water and depending on the design, also some CO₂ from the exhaust of a power plant. In the case a marine algae strain was selected than a coastal area with free access to seawater. In our case we would simply require free water and a small CO₂ source from a heating system for instance.

3. Literature Review

4.1 Biology concepts relating to algae

The algae strain grown in the photobioreactor system is the first major system element to be chosen. Indeed the photo bioreactor is then tailored to meet the specific need of this algal strain in order to maximize its growth and its lipid productivity. This section will therefore clarify important concepts about algae biology and the important parameters to look for when selecting an algal strain for the purpose of this project.

A) Algae in the environment

Algae form a vast group of eukaryotic, hototrophs which do photosynthesis in both marine and freshwater ecosystems. Microalgae in particular are the most primitive plant species to have appeared on Earth. They make up the most of the mass of organisms called phytoplankton that are found near the surface of water and they are the base of the food chain in aquatic ecosystems. Furthermore they are the primary fixer of CO₂ in the atmosphere by carrying out 80% of the world's photosynthesis. (John Sheehan 1998)

The growth cycle of algae in an outdoor environment as it is encountered in the natural environment follows a cycle in which the bacteria and the algae interact together: The algae being the primary producers oxygenate the water, allowing growth of aquatic aerobic bacteria, and serve as a food source for the zooplankton. On the other hand, bacteria brake down wastes that are inputs to the ecosystem into simple compounds readily usable by the algae. This interdependence is described by the following chart:

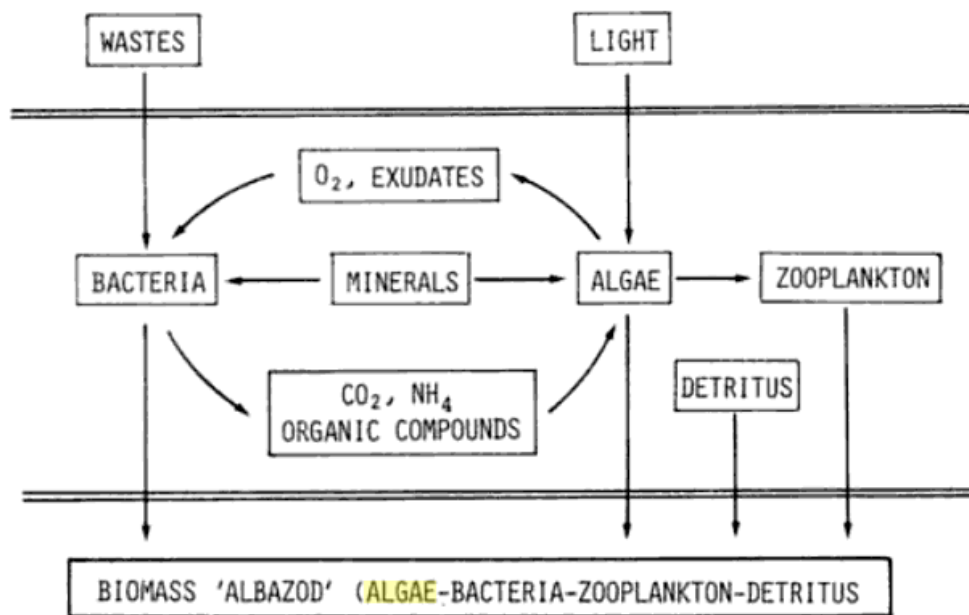


Figure 4 - Interaction of main factors influencing algae growth
(Becker 1994)

A key feature of microalgae is while their mechanism of photosynthesis is similar to that of higher plants; they are generally more efficient converters of solar energy because of their simple cellular structure. Indeed all microalgae cells do photosynthesis unlike in plants where most cells serve other purposes. In addition, because the cells grow in aqueous suspension, they have more efficient access to water, CO₂, and other nutrients. For these reasons, microalgae are capable of producing 30 times the amount oil per unit area of land, compared to terrestrial oilseed crops.

B) *Classification of Algae*

Algae are classified into six groups according to the form of their thalli (whether they are unicellular, coenocytic, filamentous or plantlike), the structure of their wall and the pigments they produce. Micro algae can pertain to four of these groups.

Table 2 - Classification of algae

Phylum	Thallus Structure	Wall Composition	Pigments	
			Chlorophylls	Others
Euglenophyta: euglenoids	Unicellular	No wall	a,b	
Pyrophyta: dinoflagellates	Unicellular	Cellulose	a,c	Carotenoids
Chrysophyta: diatoms	Unicellular, coenocytic, filamentous	Silica	a,c	Carotenoids
Chrysophyta: green algae	Unicellular, coenocytic, filamentous or plantlike	Cellulose	a,b	
Phaeophyta: brown algae	Plantlike	Cellulose and algin	a,c	
Rhodophyta: red algae	Plantlike	Cellulose	a	Phycobilins

(John L. Ingraham 2000)

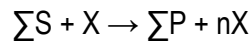
The pigments of the chlorophyll (a,b or c) are especially important since they dictate the habitat for the strain, since it will develop best in an environment rich in the wavelengths of light its pigments absorbs. The main pigments present in the algae are the base of the common names of three groups: green algae, brown algae and red algae in the table above. The other three groups are classified according to morphological differences: euglenoids are single motile cells with two flagella of unequal length. Dinoflagellates are singles cell which usually have two flagella, one

wrapped in a groove around the middle of the cell while the other extends from the groove. Diatoms are single cell organisms enclosed in a rigid silica shell. The Size of these micro algae is measured in μm with typical sizes being under 10 μm .

C) Growth Kinetic of Algae

Reproduction of bacteria is an autocatalytic reaction meaning that the speed of growth is directly related to cell concentration:

substrates + cells \rightarrow extracellular products + more cells



Where:

S: substrate concentration (g/L); X: cell mass concentration (g/L);

P: product concentration (g/L); n: increased number of biomass.

This leads to the expression of the net specific growth rate (1/time):

$$\mu_{\text{net}} \equiv \frac{1}{X} \frac{dX}{dt} = \mu_g - k_d$$

Where μ_g Gross specific growth rate (1/time)

k_d The rate of loss of cell mass due to cell death or *endogenous metabolism*: during the stationary phase, the cell catabolizes cellular reserves for new building blocks and for energy-producing monomers.

Integrating the specific growth rate equation gives the number of cells as a function of time:

$$X = X_0 e^{\mu_{\text{net}} t}$$

Algae growth has the same characteristics as microbial growth. Because individual cells grow larger only to divide into new individuals, microbial growth is defined not in terms of cell size but as the increase in the number of cells, which occurs by cell division. When algae is in a closed medium as it is the case in a photo bioreactor it follows the same typical phases of the batch microorganism growth: lag phase, exponential phase, stationary phase, and depletion phase :

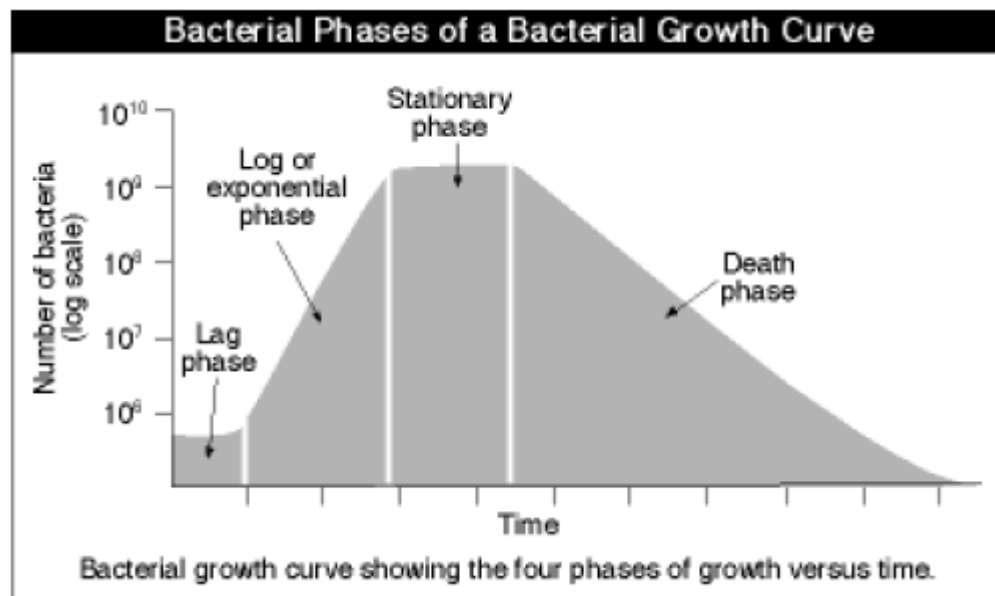


Figure 5 - Phases of a bacterial growth curve
(Abedon 2003)

Lag phase: This phase is preceded by a lag phase in which the algae in which enzymes necessary for growth are synthesized.

Exponential growth: In this phase, the cells have adjusted to their new environment and multiply rapidly (exponentially). The growth is balanced, all components grow at the same rate, and growth rate is independent of nutrient concentration as nutrients are in excess. The rate of growth is maximal during the exponential growth phase. In which the doubling time or the time required to double the microbial mass is given by:

$$\tau_d = \frac{\ln X / X_0}{\mu_{\text{net}}} = \frac{\ln 2}{\mu_{\text{net}}} = \frac{0.693}{\mu_{\text{net}}}$$

Stationary phase:

Growth inhibition (GI) occurs when cell concentration increases beyond a certain threshold beyond which the productivity starts to decrease due to insufficient nutrient concentration as well as, in certain cases, excretion of metabolites by the algae themselves to avoid overpopulation. Biomass productivity therefore decreases compared to the previous phase. In this phase cell lysis may

occur and viable cell mass may drop due to growth inhibition. This in turn can lead to a second growth phase known as cryptic growth using decay products of cells. Endogenous metabolism occurs by catabolizing cellular reserves for new building blocks and energy-producing monomer (maintenance energy) thereby leaving less energy for growth.

The rate describing the conversion of cell mass into maintenance energy or the loss of cell mass due to cell lysis is expressed by:

$$\frac{dX}{dt} = -k_d X$$

Where k_d is the rate constant for endogenous metabolism.

Death Phase:

The living organism population decreases with time, due to a lack of nutrients and toxic metabolic by-products. The rate of death can be described as follows:

$$\frac{dN}{dt} = -k_d' N$$

Where k_d' is the first order decay constant.

Instantaneous growth rate:

This parameter is used to determine the growth of algae over a certain period of time. It is therefore useful in comparing the efficiency of various growing mediums:

$$K = \frac{\ln N_t - \ln N_0}{t}$$

Where N_t = Final cell count, N_0 = initial cell count, and t = time (d)

The time to grow algae before it can be harvested therefore depends on the growth rate of the algae. For the algal strains considered for biodiesel production (Nannochloropsis, Isochrysis or Dunaliella for example), the growing period for a full batch of algae is usually between 20 and 30

days before it is harvested. However, this is highly variable according to growing conditions. The entire point of designing photo bioreactors is to make a batch of algae in the shortest time possible. The doubling time can be measured experimentally either by measuring the concentration of chlorophyll a in a sample taken from the reactor.

The total change in substrate for a batch system is given by:

$$\Delta S = \Delta S_{\substack{\text{assimilation} \\ \text{into biomass}}} + \Delta S_{\substack{\text{assimilation} \\ \text{into an} \\ \text{extracellular} \\ \text{product}}} + \Delta S_{\text{growth energy}} + \Delta S_{\substack{\text{maintenance} \\ \text{energy}}}$$

The equation above is important in assessing how effectively the nutrients are used. High Maintenance energy means that a lot of the energy is not devoted to algal biomass. However it is quite hard to evaluate the true importance of each of these parameters on practical terms.

D) Conditions influencing growth

There are many important factors that affect the generation time of the organism: pH, temperature, salinity as well as nutrients. Microalgae do not react linearly to these changing environmental conditions; when outside the optimum range, the growth rate declines sharply. Here is a chart explaining the impact of temperature variation on the growth rate of a microorganism:

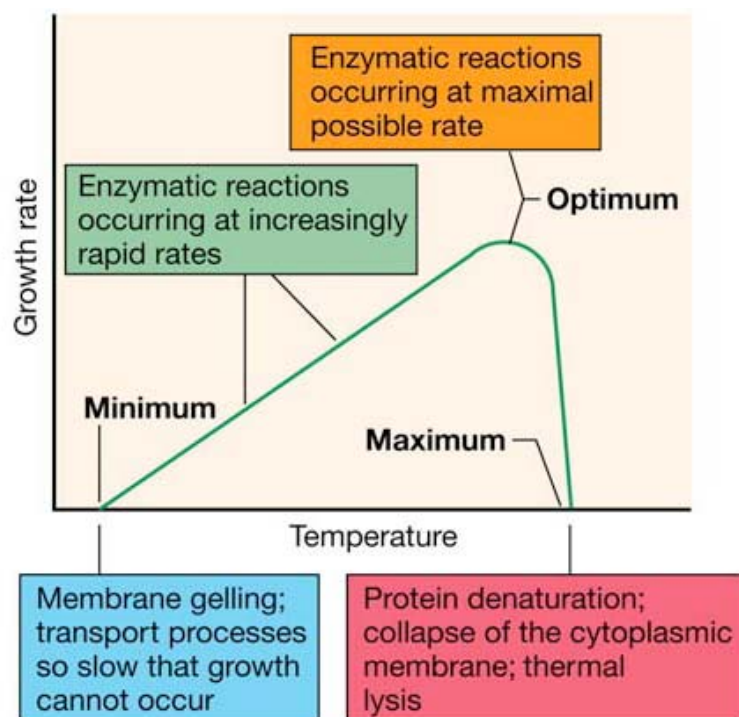


Figure 6 - Growth rate as a function of temperature
(John L. Ingraham 2000)

Each algal strain has optimum growing conditions for each of these parameters. However, in the real environment algae are subjected to environmental changes and can develop even outside of optimum conditions. (Tawfiq Institute for Scientific Research 1999) Another requirement is therefore the resistance of the algae to fluctuating environmental conditions. Even though a PBR is a controlled medium it is still subject to temperature changes and keeping all parameters of the growing medium within a narrow range can prove very complicated and expensive thereby greatly increasing costs. Therefore an algae strain must be relatively tolerant to fluctuations of the composition of its growing medium (nutrients and pH) as well as temperature and irradiance without a dramatic fall in its productivity.

Finally another important factor influencing growth is resistance to contamination. Indeed it is far easier to grow algae without having to sterilize the growing medium continuously. Contamination leads to a decrease in algae biomass productivity as the growing medium serves as a substrate to competing organisms as well as a higher death rate for the algae. Resistance to contamination is essential if most important when the algae is grown in open ponds, but is also an issue when

considering a large scale PBR plant since it would be hard to ensure total protection from contamination at a large scale.

E) Biomass productivity and lipid productivity

Algal biomass is always made up of these three main components:

- ☐☐ Carbohydrates
- ☐☐ Protein
- ☐☐ Natural Oils

The most important component for biodiesel production is the natural oils that can be converted to biodiesel. The percentage lipid composition varies of these fatty acids varies according to the algae strain within a range of 10 to 40% under natural conditions. The lipids present are mainly made up of polyunsaturated lipids. (John Sheehan 1998)

The Strain must have a high lipid productivity which is dependent on two factors, biomass productivity and lipid content. Biomass productivity is the rate at which the algae reproduce, whereas lipid content is the percentage of lipids in the total mass of the algae. Generally, biomass productivity and lipid content are inversely related. Indeed, this can be explained by the high metabolic cost of synthesizing lipids. Some studies imply that highly enriched iron medium may lead to an increased productivity but this is not well established. (Zhi-Yuan Liu 2008) This implies that there is a tradeoff between having a high biomass yield and high lipid content. Here is the list of algal strains from which a few will be investigated as being suitable for biodiesel production:

Table 3: Biomass productivity, lipid content and lipid productivity of 30 algal strains

Microalgae	Biomass productivity (g/L/day)	Lipid content (% biomass)	Lipid productivity (mg/L/day)
Marine strains			
<i>Porphyridium cruentum</i>	0.37	9.5	34.8
<i>Tetraselmis suecica</i> F&M-M33	0.32	8.5	27.0
<i>Tetraselmis</i> sp. F&M-M34	0.30	14.7	43.4
<i>Tetraselmis suecica</i> F&M-M35	0.28	12.9	36.4
<i>Phaeodactylum tricornutum</i> F&M-M40	0.24	18.7	44.8
<i>Nannochloropsis</i> sp. F&M-M26	0.21	29.6	61.0
<i>Nannochloropsis</i> sp. F&M-M27	0.20	24.4	48.2
<i>Nannochloropsis</i> sp. F&M-M24	0.18	30.9	54.8
<i>Nannochloropsis</i> sp. F&M-M29	0.17	21.6	37.6
<i>Ellipsoidion</i> sp. F&M-M31	0.17	27.4	47.3
<i>Nannochloropsis</i> sp. F&M-M28	0.17	35.7	60.9
<i>Nannochloropsis</i> CS 246	0.17	29.2	49.7
<i>Isochrysis</i> sp. (T-ISO) CS 177	0.17	22.4	37.7
<i>Pavlova salina</i> CS 49	0.16	30.9	49.4
<i>Pavlova lutheri</i> CS 182	0.14	35.5	50.2
<i>Isochrysis</i> sp. F&M-M37	0.14	27.4	37.8
<i>Skeletonema</i> sp. CS 252	0.09	31.8	27.3
<i>Thalassiosira pseudonana</i> CS 173	0.08	20.6	17.4
<i>Skeletonema costatum</i> CS 181	0.08	21.1	17.4
<i>Chaetoceros muelleri</i> F&M-M43	0.07	33.6	21.8
<i>Chaetoceros calcitrans</i> CS 178	0.04	39.8	17.6
Freshwater strains			
<i>Chlorococcum</i> sp. UMACC 112	0.28	19.3	53.7
<i>Scenedesmus</i> sp. DM	0.26	21.1	53.9
<i>Chlorella sorokiniana</i> IAM-212	0.23	19.3	44.7
<i>Chlorella</i> sp. F&M-M48	0.23	18.7	42.1
<i>Scenedesmus</i> sp. F&M-M19	0.21	19.6	40.8
<i>Chlorella vulgaris</i> F&M-M49	0.20	18.4	36.9
<i>Scenedesmus quadricauda</i>	0.19	18.4	35.1
<i>Monodus subterraneus</i> UTEX 151	0.19	16.1	30.4
<i>Chlorella vulgaris</i> CCAP 211/11b	0.17	19.2	32.6

The flasks were incubated at 25°C under continuous illumination in an orbital shaker flushed with CO₂ enriched air.

(Rodolfi L and N 2008)

Furthermore, the lipid type prevalent in the algae is also important as this will simplify the refining process. The fats produced must be preferably in triacylglycerol (TAG) form, in which there are three long carbon chains known as fatty acids connected to a glycerol backbone. The fat content varies for each algae strain, but it can go as high as 60% of lipid content. (Assaf Sukenik 1993)

F) Nutrient requirements

Like other microorganisms, micro-algae require a certain set of energy inputs (heat and light) and nutrients in order to grow. Some algae can grow in extreme conditions of cold and it is therefore

necessary to consider the optimal temperature range for each specific range. The inorganic nutrients needed for algae growth are carbon oxygen, nitrogen, phosphorous and sulfur. It is the CO_2 dissolved in the water that is used for photosynthesis. Trace elements such as metals as well as growth factors (vitamins), that are essential to metabolism but cannot be synthesized by the algae itself, are also necessary for growth. It is important to note that nitrogen is a constituent of amino acids necessary for protein synthesis but is present in lipids which only contain glycerol and three fatty acids all built from carbon. (John L. Ingraham 2000)

The most important way off assessing if a growing medium is suitable for algae growth is its C:N: P ratio. The C:N:P ratio used to grow phytoplankton is the Redfield ratio with C: N: P= 106 : 16 : 1. It is always better to find a more precise C: N: P ratio for the particular strain chosen since the Redfield ratio is an average of what is usually encountered. None the less variability in the C: N: P ratio is quite small between algal species (<10%) and this ratio can therefore be used if more precise is unavailable. However if the growing medium has a C: N: P ratio which deviates greatly from the Redfield ratio, then either nitrogen or phosphorous inhibition occurs as shown in the plot below. (Oceanography 1999)

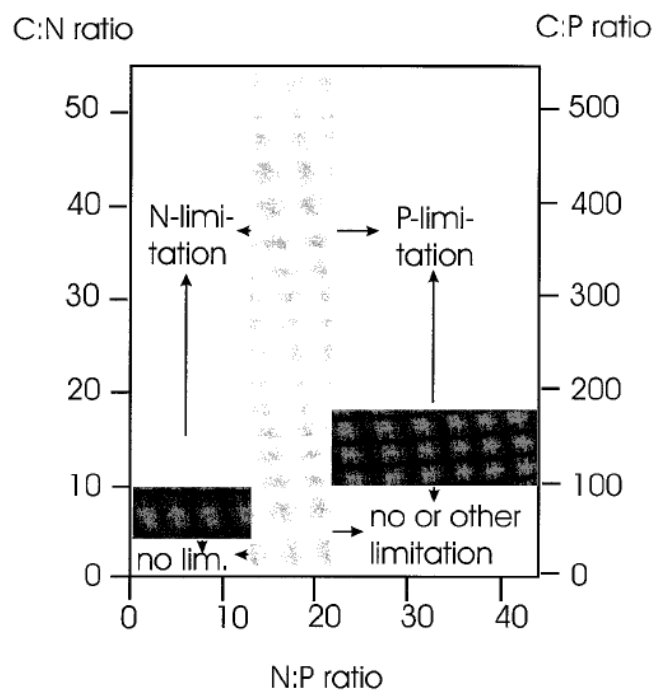


Figure 7 - Schematic diagram on the use of nutrient ratios as indicator of nitrogen or phosphorus limitation.
(Oceanography 1999)

The nutrients are readily available in sea or fresh water in small quantities which explains why algal growth remains limited unless high amounts of nitrates and phosphorous are added to the aqueous medium thereby leading to eutrophication. It is important to note that in a natural ecosystem the nitrogen and phosphorous as well as other essential elements are to a portion of the nutrients are provided by the breakdown of organic waste by other bacteria. In the case of a photo bioreactor, nutrients can be provided directly in usable form into the growth medium.

G) Effect of nitrogen deprivation

A significant body of research shows that algae grown in nitrogen-depleted medium develop a higher lipid content than algae grown in nutrient-sufficient conditions (Weldy and Huesemann, Dempster et al.). Under nitrogen deprivation algae are unable to synthesize proteins which are made up of amino acids containing nitrogen. This implies that the energy from photosynthesis cannot serve to increase the biomass (number of cells) and is therefore channeled towards lipid production since fatty acids do not require any nitrogen. This allows the cell to store energy in order to restart growth as soon as nitrogen becomes available once again. Reaction to nitrogen deprivation is highly variable according to strain and it is therefore crucial to select a strain that shows increased lipid productivity under such nitrogen deprivation conditions. However, it is important to note that increased lipid productivity due to nitrogen deprivation is done at the expense of biomass productivity. This is where the idea of a two step process comes into mind as it enables to first maximize biomass productivity in a nitrogen rich medium and then place the algae in another nitrogen poor medium where lipid content can be maximized under nitrogen deprivation.

Algae are grown in a photobioreactor in the first step because it offers better protection from contamination and higher productivity due to an optimized growing medium. Secondly, algae are transferred to a pond where it will undergo nitrogen deprivation and therefore have higher lipid productivity. (Rodolfi L and N 2008)

H) *Light/Dark cycle*

Algae undergo respiration during the night and consume part of their lipid reserves built up during day as a source of energy. The percentage decrease in fat over night is typically in the range of 15% to 20% of lipids (5) for algae grown in natural conditions. (G. Chini Zittelli 1999) Most algae undergo similar losses in fat at night, but it is none the less important to look for, if possible, algal strains which can minimize this loss.

It is important to note that algae have two light/ dark cycles. The first is the light hours/ night hours cycle which can be interpreted as an activity/rest cycle in which the algae culture is subjected to light for a certain number of hours and then left in the dark for the rest of the day. Each strain has an optimal light hours/ dark hours cycle in which it will achieve maximal productivity. Indeed, the algae undergoes photosynthesis during light hours and recuperates during dark hours. In fact having too many light hours damages the algae and decreases their productivity. The optimal light dark cycle for a certain strain is obtained from suppliers or the literature.

The second light dark cycle occurs very fast within the growing medium as algae move rapidly from areas of sunlight to areas of shade. Indeed, in a photobioreactor, algae at the center are in the shade while the ones on the sides are in light. This light/ shade cycle must be kept as short as possible to attain maximum productivity.

1) Photoinhibition

Another important type of inhibition to consider is photoinhibition in which the algae are subjected to too much light energy, beyond their maximum photosynthetic capacity. In fact, beyond a certain level of irradiance, the algae are permanently damaged. The damaged algae therefore devote their biosynthesis to repairing the damage instead of multiplying or producing lipids, hence decreasing productivity. Photoinhibition typically occurs for light intensity much lower than the maximum light provided on a summer day. Irradiances between 1800 and 2200 $\mu\text{E m}^{-2} \text{s}^{-1}$ are typical in summer in the Mediterranean but are inhibition occurs as low as 200 $\mu\text{E m}^{-2} \text{s}^{-1}$ for some algae strains. Therefore choosing a strain with photoinhibition occurring at a higher light regime would be preferable. (Amos Richmond 2003)

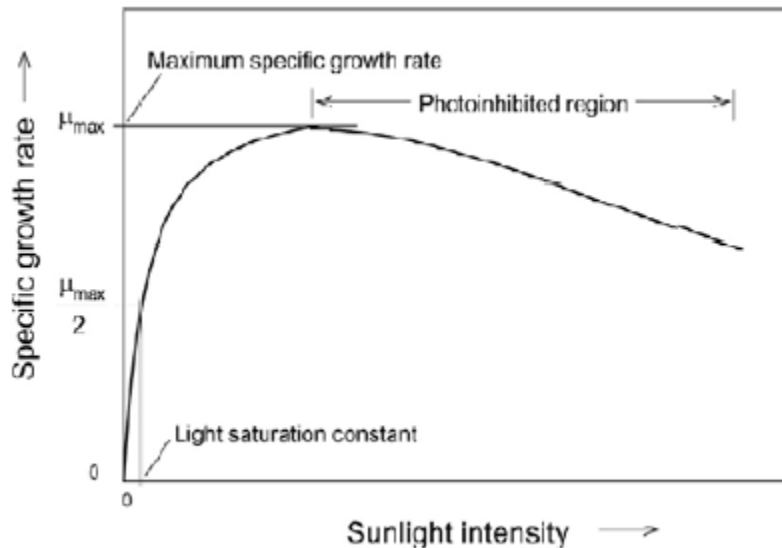


Figure 8 - Effect of light intensity on the specific growth rate of microalgae
(Chisti 2007)

J) Likely algae candidates

With these key parameters in mind, the selection of the algal strain can proceed. In the table below are listed both marine and fresh water strains from which the algae strain will be selected. The algae below are the ones on which the most documentation and the most knowledge was available:

Table 4 - Algal group and culture collection of origin of 30 strains

Algal group	Strain	Culture collection	Freshwater/marine
Diatoms	<i>Chaetoceros calcitrans</i> CS 178	CSIRO Collection of Living Microalgae, Australia	Marine
	<i>Chaetoceros muelleri</i> F&M-M43	F&M Culture Collection, Italy	Marine
	<i>Phaeodactylum tricornutum</i> F&M-M40	F&M Culture Collection, Italy	Marine
	<i>Skeletonema costatum</i> CS 181	CSIRO Collection of Living Microalgae, Australia	Marine
	<i>Skeletonema</i> sp. CS 252	CSIRO Collection of Living Microalgae, Australia	Marine
	<i>Thalassiosira pseudonana</i> CS 173	CSIRO Collection of Living Microalgae, Australia	Marine
Green algae	<i>Chlorella</i> sp. F&M-M48	F&M Culture Collection, Italy	Freshwater
	<i>Chlorella sorokiniana</i> IAM-212	IAM Culture Collection, University of Tokyo, Japan	Freshwater
	<i>Chlorella vulgaris</i> CCAP 211/11b	Culture Collection of Algae and Protozoa, UK	Freshwater
	<i>Chlorella vulgaris</i> F&M-M49	F&M Culture Collection, Italy	Freshwater
	<i>Chlorococcum</i> sp. UMACC 112	University of Malaya Algae Culture Collection, Malaysia	Freshwater
	<i>Scenedesmus quadricauda</i>	ISE—CNR Culture Collection, Italy	Freshwater
	<i>Scenedesmus</i> sp. F&M-M19	F&M Culture Collection, Italy	Freshwater
	<i>Scenedesmus</i> sp. DM	Istituto di Biofisica—CNR, Italy	Freshwater
	<i>Tetraselmis suecica</i> F&M-M33	F&M Culture Collection, Italy	Marine
	<i>Tetraselmis</i> sp. F&M-M34	F&M Culture Collection, Italy	Marine
	<i>Tetraselmis suecica</i> F&M-M35	F&M Culture Collection, Italy	Marine
	<i>Ellipsoidion</i> sp. F&M-M31	F&M Culture Collection, Italy	Marine
Eustigmatophytes	<i>Monodus subterraneus</i> UTEX 151	UTEX, Culture Collection of Algae at University of Texas, USA	Freshwater
	<i>Nannochloropsis</i> sp. CS 246	CSIRO Collection of Living Microalgae, Australia	Marine
	<i>Nannochloropsis</i> sp. F&M-M24	F&M Culture Collection, Italy	Marine
	<i>Nannochloropsis</i> sp. F&M-M26	F&M Culture Collection, Italy	Marine
	<i>Nannochloropsis</i> sp. F&M-M27	F&M Culture Collection, Italy	Marine
	<i>Nannochloropsis</i> sp. F&M-M28	F&M Culture Collection, Italy	Marine
	<i>Nannochloropsis</i> sp. F&M-M29	F&M Culture Collection, Italy	Marine
Prymnesiophytes	<i>Isochrysis</i> sp. (T-ISO) CS 177	CSIRO Collection of Living Microalgae, Australia	Marine
	<i>Isochrysis</i> sp. F&M-M37	F&M Culture Collection, Italy	Marine
	<i>Pavlova lutheri</i> CS 182	CSIRO Collection of Living Microalgae, Australia	Marine
	<i>Pavlova salina</i> CS 49	CSIRO Collection of Living Microalgae, Australia	Marine
Red algae	<i>Porphyridium cruentum</i>	Istituto di Biofisica—CNR, Italy	Marine

(Rodolfi L and N 2008)

The strains that will be investigated more in detail are the ones with the highest lipid productivity. For the purpose of this project, the fresh water strain *Chlorococcum* as well as the marine strains *Nannochloropsis* and *Pavlova Salina* will be investigated more in detail next semester.

4.2 Methods for algae cultivation

A) Description of the Algal Cultivation System

A cultivation system for algae can therefore be seen as a process in which the process converts inputs to outputs. An algae cultivation system can be described as a process in which inputs are on the left and outputs on the right as shown by the following chart:

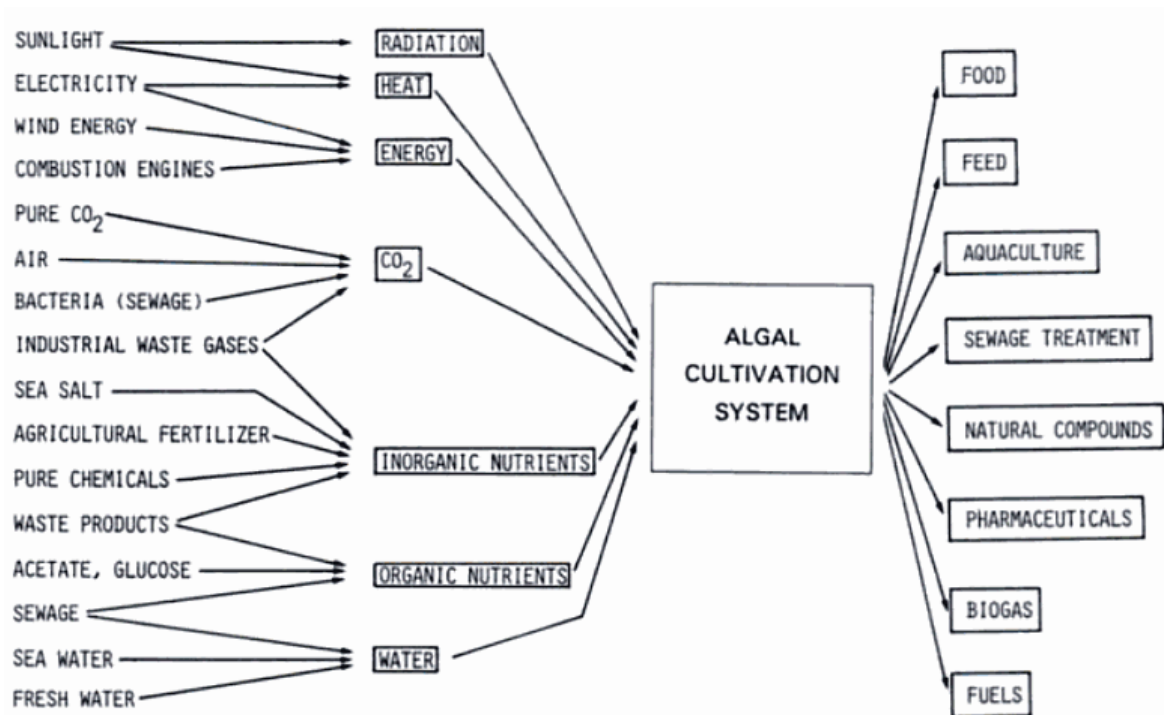


Figure 9 - Potentials of algal biomass: raw material, energy sources, products and applications

(Becker 1994)

The cultivation of algae takes many factors into consideration. The cultivation system is designed around the algae strain to be grown. Indeed the algae strain is the starting point of the design and is the system is then tailored to its needs to achieve maximum productivity. The aim of this project is to use a two step process taking into account nitrogen deprivation: during Phase I, the objective will be vigorous algae growth while in Phase II of the algae growth will take place in nitrogen-depleted conditions, with the objective of raising the lipid content of the algae. The above diagram can therefore be decomposed further to identify the various components of the process:

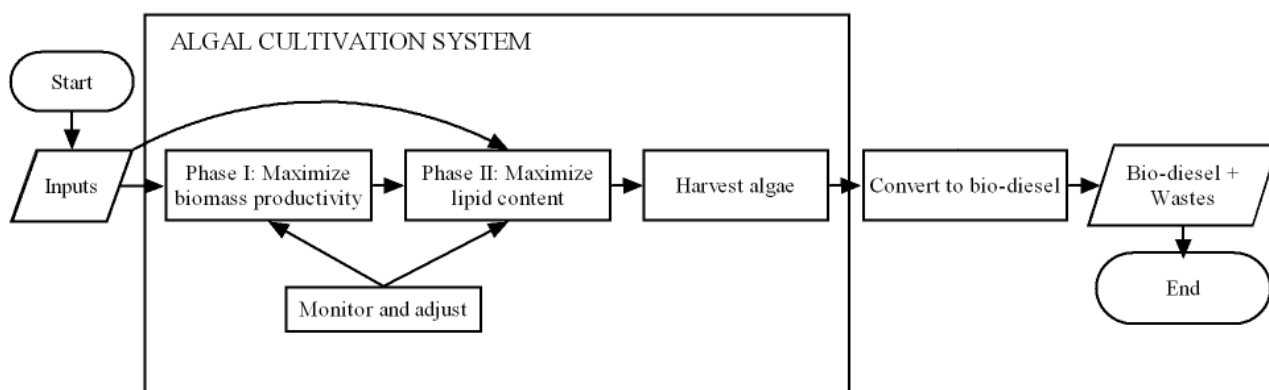


Figure 10 - Algal cultivation system using a two phase process.

An essential step for the design of the cultivation system is determining the method through which it will be done: open pond or photobioreactors, or both. The following sub-sections present the various techniques used to cultivate algae. Harvesting will be examined in the following section of this report. The table below presents the strengths and limitations of several types of cultivation systems that are analysed in the following subsections of this report.

Table 5 - Prospects and limitations of various culture systems for algae

Culture systems	Prospects	Limitations
Open ponds	Relatively economical, easy to clean up after cultivation, good for mass cultivation of algae	Little control of culture conditions, difficulty in growing algal cultures for long periods, poor productivity, occupy large land mass, limited to few strains of algae, cultures are easily contaminated
Vertical-column photobioreactors	High mass transfer, good mixing with low shear stress, low energy consumption, high potentials for scalability, easy to sterilize, readily tempered, good for immobilization of algae, reduced photoinhibition and photo-oxidation	Small illumination surface area, their construction require sophisticated materials, shear stress to algal cultures, decrease of illumination surface area upon scale-up
Flat-plate photobioreactors	Large illumination surface area, suitable for outdoor cultures, good for immobilization of algae, good light path, good biomass productivities, relatively cheap, easy to clean up, readily tempered, low oxygen build-up	Scale-up require many compartments and support materials, difficulty in controlling culture temperature, some degree of wall growth, possibility of hydrodynamic stress to some algal strains
Tubular photobioreactors	Large illumination surface area, suitable for outdoor cultures, fairly good biomass productivities, relatively cheap	Gradients of pH, dissolved oxygen and CO ₂ along the tubes, fouling, some degree of wall growth, requires large land space

(Ugwu 2008)

B) Open air systems

Open ponds were the first and the most studied method for the mass-cultivation of microalgae. They usually consist of natural waters (lagoon, pond) or of artificial waters (containers, man-made ponds). The following types of open air systems exist: “shallow big ponds, tanks, circular ponds and raceway ponds” (Ugwa et al., 2007). The most popular type is the paddle-wheel raceway pond, shown below, because it is the most productive open air system (Chisti 2007).

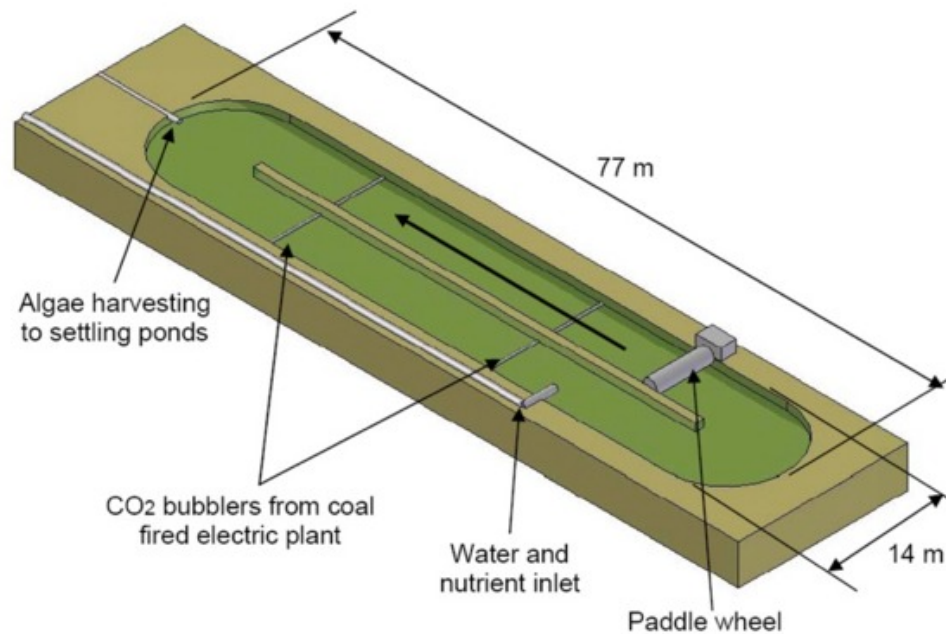


Figure 11 - Raceway pond.
(BioLink)



Figure 12 - Large scale raceway pond algae cultivation
Source: <http://www.ieagreen.org.uk/newsletter/dec80/images/biofixation.JPG>

The greatest advantage of this type of culture system is that it is the most economical system. These systems have a very low cost (compared to other systems) and are fairly simple to construct.

Due to the nature of open air systems, however, certain limitations exist. Temperature and light intensity cannot be regulated. A deeper pond has the advantage of reducing the diurnal variation in temperature, but would reduce the available light to the culture. Another limitation of this system is the low concentration of algae in the pond which tends to make harvesting less cost effective. They also demand a lot of land, which was one of the major problems with ethanol. Open ponds cannot be built everywhere because if the place is too hot there is a lot of evaporation and diffusion of CO₂. If it is constructed in a place that is too cold, ice can develop on top and the temperature will be too low for the algae to reproduce (depending on the type of algae), which drastically decreases the efficiency of the system.

It is important to note, however, that since a two-phase process is being examined, biomass maximization is only the primary objective during one part of the growing process. During the second phase of growth, the objective is rather to refine the composition of the existing biomass. Therefore, the strictly controlled environment of the photobioreactor is not necessary. Phase II could thus take place in an open air system, which is less costly. Nutrient and physical requirements of the algae are to be met during Phase I as much as possible. In phase II, only nitrogen input will be purposely depleted. There will be inputs of other nutrients and possibly CO₂.

The culture could be transferred, as a batch, from the photobioreactor to the open air system. This would free up the photobioreactor to begin production of a new batch sooner than if Phase II took place in the photobioreactor. Using batch culture has the advantage of periodically emptying out the open air system. Since the open air system is vulnerable to contamination by other, hardier strains of algae because it is open to the environment, regularly flushing it out prevents those other strains of algae from taking root and choking out the desired strain (Borowitzka 2005).

Several parameters are taken into account when designing an open air system. Firstly, the depth of the pond must be determined. The deeper the pond is, the less light filters through the culture, and thus less light is available to the algae. However, as the pond becomes shallower, more land surface is required to contain the same volume of culture, and one of the aims of the project is to maximize algal output per unit of land base. Furthermore, increased pond area leads to increased evaporation losses over the pond surface. (Borowitzka 2005). The following equation, determined empirically (Oswald 1988), demonstrates the relationship between the depth of the pond and the concentration of algae.

$$D_p = 6000 / C_c$$

D_p = depth of pond (cm)

C_c = concentration of algae (mg/L)

Secondly, the walls may be elevated concrete or excavated earthen walls. The earthen pond can lead to insect contamination, and the sloping of the walls makes constant flow within the pond more difficult to maintain. Concrete walls eliminate these problems, but are expensive and the rough surface of concrete leads to increased friction, which reduces water flow. Concrete walls also tend to deteriorate when the culture is grown in saline water. Whether saline or fresh water is used is determined by the strain of algae chosen. Additionally, at a further cost, a liner may be added to reduce seepage (Borowitzka 2005).

Thirdly, the pond may be mixed or unmixed. Mixing generally takes place using a paddlewheel, because paddlewheels have been shown to be the most efficient and easy to maintain for the purpose of mixing algal cultures. In unmixed ponds, the only mixing that takes place is caused by the wind flowing over the surface of the pond. Mixing is an added cost, but it minimizes the settling of algae cells, and prevents temperature and oxygen stratification within the medium. Mixed ponds demonstrate yield rates of up to 10 times those of unmixed ponds. This higher density of algae makes harvesting less costly (Borowitzka 2005).

Fourthly, CO₂ may be added to the medium. This will result in increased growth, but may not be cost-effective. The simplest way of increasing CO₂ in the medium is through passive transfer, by

increasing the contact area between the atmosphere and the surface of the pond (Becker 1994). As previously discussed, the cost-effectiveness of increasing pond area depends on the cost of land and water. Addition of CO₂ may also occur by sparging gas bubbles into the medium or by lining the pond bottom with plastic and pumping CO₂ under the plastic. The plastic prolongs the contact between CO₂ and the medium (Vasquez and Huessler 1985).

Fifthly, a cover may be added to the pond to decrease the risk of contamination from other strains of algae, and to eliminate grazing pressure on the algae.

C) Photobioreactors: description

Due to the limitations of open ponds most of the research in algal cultivation is conducted using photobioreactors. A photobioreactor is a closed system that contains a biologically active environment which is sustained with light, energy, heat and nutrients. They have the main advantage of being a closed system, which eliminates the risk of contamination but more importantly allows for a better control of the conditions surrounding the cultivation of microalgae. Different types of algae need different conditions in order to reproduce at their most effective rate, which is easily facilitated by the photobioreactor, which allows parameters to be controlled more tightly. Although photobioreactors are quite efficient in increasing productivity, the design can be further optimized by the use of a two phase system. The first phase is the photobioreactor in which the algae is cultivated and the second phase is a pond in which the algae is harvested in order to get a greater biomass.

When designing a photobioreactor the first step is to determine if it shall be constructed indoors or outdoors. Building it indoors is advantageous as the user has total control over temperature and the lighting, which is provided artificially. All types of algae have certain temperature constraints. In an indoor system it would be easy to permanently maintain the required temperature. On the other hand having it indoors would more than triple the cost of keeping it outside. In the cost one must include the building, the heating and all the artificial lighting plus the cost of construction of the photobioreactor and the costs of operations. Having the system outdoors would be much cheaper as it drastically brings down the cost. It provides free lighting and for the algae types that demand

lower temperatures they can be attained through the natural conditions depending on the location. Of course, one has to take into account the fact that conditions are not always perfect. In most cases the photobioreactor is designed for optimal conditions, but the fluctuations in temperature may diminish the productivity, which has to be taken into account in the design. Although algae strains need periods of darkness in order to obtain a greater efficiency (Janssen et al. 2001); productivity will drop if night periods are too long. Also since it is outside, the required carbon dioxide inputs can be taken from the atmosphere or from other sources that emit carbon dioxide (for example, the wood pellet system). The oxygen outputs of our system will also be thrown back into the atmosphere.

D) Airlift photobioreactor

The air-lift is the first major component of the photobioreactor and is used in all the different models. It is a “device that serves to circulate the culture through the solar receiver (tubes)” (Fernandez et al., 2001). This is the main place where all the gas exchanges occur. Carbon dioxide enters the system while oxygen is removed. It is of the greatest importance to evacuate oxygen from the system because it accumulates within the tubes and prevents photosynthesis. As the fluid circulates through the system, a gas-liquid separator found at the top of the air-lift prevents the oxygen from going back into the system (Fernandez et al., 2001). So far, the air-lift has been the mechanism the best suited to perform this function (Richmond et al., 1993). Carbon dioxide is necessary to the cultivation of micro-algae and the air-lift pump also helps to control the concentrations of CO₂ that will be sent into the system. Another advantage to the air-lift is that it causes mixing within the system, allowing the algae to get more sunlight. This component is usually very small compared to the rest of the system as it is one of the darkest parts. If the fluid stays in it too long productivity will be diminished due to a lack of light. Researchers also prefer this system because the flow patterns and circulation times within are easy to follow and understand (Eriksen, 2008).

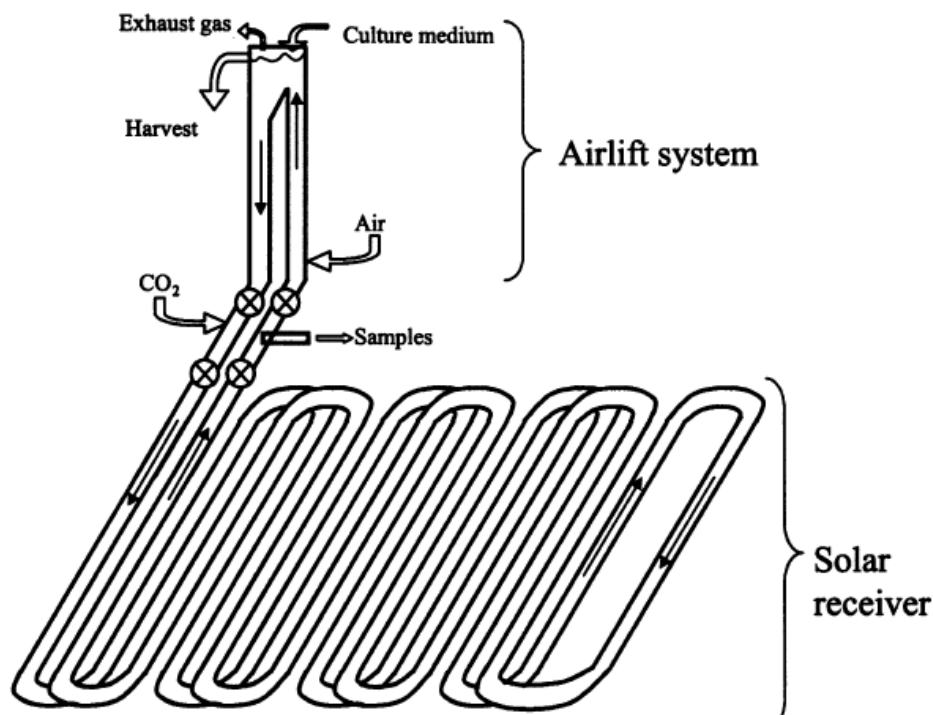


Figure 13 - Airlift photobioreactor system
(Molina et al., 2001)

E) Tubular photobioreactor

A tubular photobioreactor consists of an air-lift system and a solar receiver. The solar receiver is essentially long lines of tubes that intertwine over a large surface. The tubes are usually made of transparent glass or plastic. The diameters vary between 3 to 6 cm and the lengths between 10 and 100 m (Janssen et al., 2001). They can be placed in many different ways of which the most common is laid horizontally or set up vertically. Its main advantage is that it has a large surface area. Since it is spread on a long and large distance and because it has tubes of a small diameter, it allows more of the medium to be in contact with sunlight, allowing for good biomass productivities (Ugwu et al., 2007). It is also one of the cheapest systems as the pipes do not have to be of high quality. In his research, Miyamoto used commercially available glass tubes that were at a very low cost. Also, instead of cleaning and sterilizing the tubes they could just be thrown away and replaced by new ones. On a small scale this works, but on a larger scale it may get more complicated. In terms of productivity, tubular photobioreactors have a higher biomass density, but vertical-column photobioreactors have a higher biomass yield.

As great as this system may seem it still has some major flaws. The first one is that it has poor mass transfer, problem which increases as the system is scaled up. Oxygen tends to build-up within the tubes if there is poor mixing and circulation. There are also problems with the circulation of carbon dioxide. This becomes a problem because re-carbonation is needed when the pH gradient within the tubes gets too high (Ugwu et al., 2001). The final problem is land-space. This systems demand a lot of space as the tubes can extend for long distances. Fortunately, since it is a closed system, any type of flat non-arable land can be used to hold the system.

F) Vertical-Column photobioreactor

Vertical-column photobioreactors can also use the airlift system and they can have one or many vertical tubes that are lined up. Unlike the tubular photobioreactor, the medium does not pass through all the tubes and the columns have a bigger diameter. The tubes have diameters in the ranges of 15 to 20 cm and they can go up to 5m high. Since the tubes are much larger, a good mixing system has to be put in place. For vertical columns this system is referred to as the bubble column (Janssen et al., 2001). They are aerated from below which causes air bubbles to rise and cause mixing within the medium. This allows the algae that is not exposed to light to get more and the light/dark cycle can be profitable to the algae. Small fans can also be installed within the column to mix the fluid. Yet, the high velocities and turbulence flow caused by the fans create shear stresses which have been shown to damage the microalgae (Eriksen, 2008). This type of mixing exerts a low shear stress on the walls of the column and demands little energy (Ugwu et al., 2008). In some cases, columns with much larger diameters have used internal light sources. Although this is efficient it is not cost effective. Another advantage is that they are quite easy to clean and to sterilize since they are rather large and can be opened from the top and the bottom allowing the waste water to flow out of the tubes.

Its disadvantages are that the materials for the column are much more expensive as the column must be made sturdier (Ugwu et al., 2008). There is also a smaller surface area of irradiance.. As was said before internal illumination systems have been tried, yet have turned out to be way too expensive for the little more biomass it would produce.



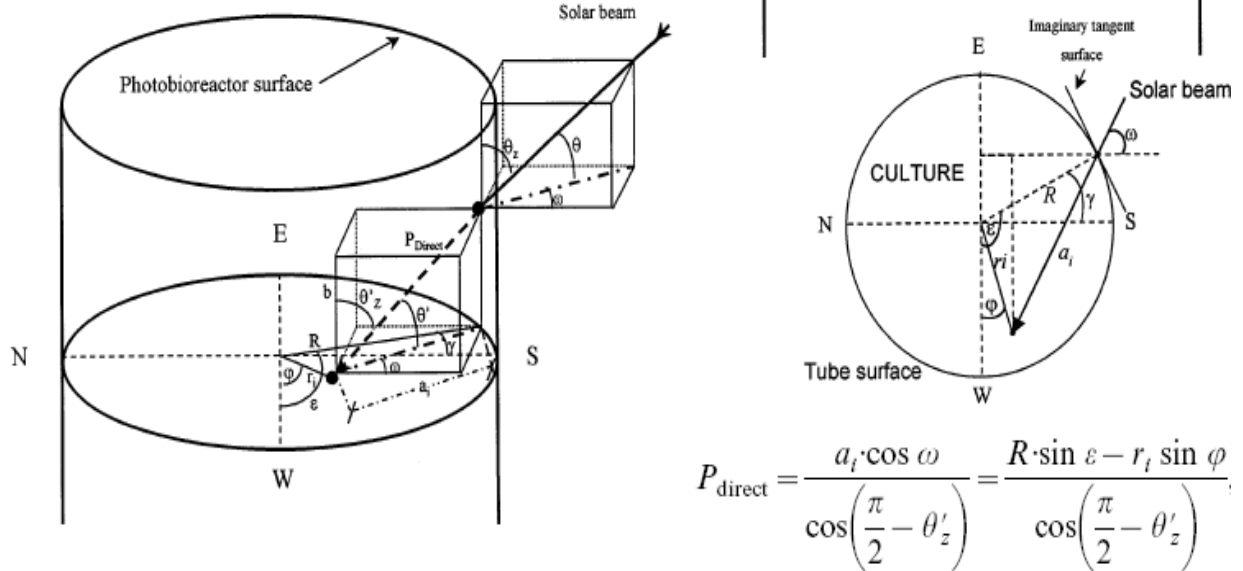
Figure 14 - Vertical column photobioreactors

Source: <http://www.sunxenergy.com/research.htm>

G) Lighting in Photobioreactors

The productivity of the photobioreactor will highly depend on the irradiance that will hit the culture surface and how efficiently the energy of the sun rays will be converted into chemical energy by the algae (Tredici et al., 1997). If the sunlight continuously hits one single point of the surface area, it will become saturated with light, causing photoinhibition (reduction of the capacity of photosynthesis within the algae) (Grima et al., 1999). A simple solution to this problem is the “spatial dilution of light”, in other words it is to try and spread the light rays on a greater surface area. Tubular systems manage quite well to accomplish this task since the curved surface of the tubes allows the rays to be directed towards the middle of the tube, touching some of the algae in the darker regions. Of course, the diameter and curvature of the tubes surface are of the greatest importance to achieve the greatest pathway for the light so that it touches everything. It has been suggested that short periods of light followed by periods of darkness can allow the algae to use the light more efficiently (Tredici et al., 1997). Below is a figure showing the penetration and dispersion of light within the tubes.

Figure 15 - Penetration and dispersion of light in a photobioreactor tube
(Molina Grima et al., 1999)



$$P_{\text{direct}} = \frac{a_i \cdot \cos \omega}{\cos\left(\frac{\pi}{2} - \theta'_z\right)} = \frac{R \cdot \sin \varepsilon - r_i \sin \varphi}{\cos\left(\frac{\pi}{2} - \theta'_z\right)},$$

where the parameter a_i is

$$a_i \frac{r_i \cos \varphi - R \cdot \cos \varepsilon}{\sin \omega} = \frac{R \cdot \sin \varepsilon - r_i \sin \varphi}{\cos \omega}.$$

$$I_{Bt}(r_i, \varphi) = I_{Bt} \exp(-K_a C_b P_{\text{direct}}),$$

$$P_{\text{disperse}}$$

$$= \sqrt{(r_i \sin \varphi - R \cdot \sin \varepsilon)^2 + (r_i \cos \varphi - R \cdot \cos \varepsilon)^2}.$$

Nomenclature :

a_i path length parameter defined

C_b biomass concentration (kg m³)

I_B hourly PA direct irradiance on a horizontal surface

$I_{Bt}(r_i, \varphi)$ direct local hourly PA irradiance inside vertical column (μE m⁻² s⁻¹)

I_{Dt} hourly PA diffuse irradiance on inclined surface (μE m⁻² s⁻¹)

$I_{Dt}(r_i, \varphi)$ local hourly disperse PA irradiance inside vertical column (μE m⁻² s⁻¹)

K_a absorption coefficient (m² g⁻¹)

P_{direct} distance traveled by a direct incident ray from the tube's surface to any internal point

(r_i, φ) (m)

P_{disperse} distance traveled by disperse radiation from the tube's surface to any internal point (r_i , φ) (m)

R radius or hydraulic radius (m)

r_i distance in polar coordinates (m)

ε angle shown in Fig. 3

θ_z zenith angle of the Sun

θ'_z zenith angle of the Sun modified by refraction in the culture

φ angular position in polar coordinates

ω angle corresponding to the solar hour

H) Instrumentation and Control

Whichever strain of algae is selected will have specific requirements in terms of nutrients, light conditions, temperature, carbon dioxide, water flow rate, salinity and pH. Design of both Phase I and Phase II structures will have to include ways of monitoring some of these parameters either constantly or at regular intervals, so that adjustments can be made to avoid system failure. It is likely that only temperature and pH could be monitored at low cost. Other parameters such as nutrient input and aeration rates would have to be controlled by the proper design of the flow rate of the water through the reactor. The pumps would have to be set to allow for a precise flow rate in the photobioreactor. Aeration would also have to be controlled tightly.

4.3 Harvesting techniques

Harvesting has been, and continues to be, one of the largest obstacles in the commercial production of microalgae. These difficulties arise primarily from 3 factors. Firstly, microalgae are very small in size, generally measuring less than 25 micrometers in diameter. Many species of algae are so small that sieves and filters cannot reliably strain them. Secondly, unlike many crops which are harvested once or twice a year, microalgae must be harvested every 2 to 3 days. Rather than one harvest yielding a significant crop, harvesting microalgae is a nearly continuous process with low yields per harvest. Thirdly, as microalgae are aquatic and therefore harvesting involves

concentrating a large volume of water containing microalgae relatively little microalgae. For example, processing over 100 000 gallons of this solution would yield less than 0.1 tons of biomass (dry weight basis). These 3 factors make harvesting of microalgae either extremely costly, unreliable, or both.

The chief existing processes for microalgae harvesting are centrifugation, sand filtration, microstraining, chemical flocculation, and bioflocculation:

- Centrifugation delivers a pure, uncontaminated product, but is the most expensive process, and is therefore preferred when microalgae is being used in the health food industry. However, for use in the biofuels industry, the cost is prohibitively high.
- Sand filtration is effective only for low concentrations of microalgae, and is therefore not appropriate as a primary removal process.
- Microstraining is effective either if the mesh size is very small or microalgae very large. However, in the case of small mesh size, the cost is much higher.
- Chemical flocculation is a harvesting method that causes algal cells to aggregate. When a critical size is achieved, the algal masses, or flocs, either rises to the top of the pond or sink, depending on their density. This flocculation is caused by the addition of chemicals. It is therefore expensive, and results in polluted effluent water, requiring treatment.
- Bioflocculation is the method that offers the most promises. During this process, the algal cells spontaneously aggregate into flocs. When the flocs become large enough, they either rise to the top of the pond or sink to the bottom, depending on their density. The sinking, or rising, velocity, is calculated using Stokes Law:

$$V = \frac{2}{9} * g * r^2 * (\rho' - \rho) / \mu$$

V = velocity

g = acceleration of gravity

r = radius of particle

ρ' = density of particle

ρ = density of medium

μ = absolute viscosity

The following observations have been made in scientific studies. First, senescent cultures usually sink faster than actively growing cultures. This is because growing algae cells hold a negatively charged surface layer and thus repel each other. Second, nutrient limited algae usually sink faster than nutrient sufficient algae. Management and acceleration of this process could provide a low cost and efficient method of microalgae harvesting. (Augenstein 1982)

The table below summarizes the reliability and cost of these 5 harvesting processes.

Table 6 - Comparison between harvesting processes

Process	Removal reliability	Estimated cost (c/lb)
Centrifugation	High	50
Sand filtration	Fair	30
Microstraining	Poor	5
Chemical flocculation	High	40-45
Bioflocculation	Not established	1-2

(Augenstein 1982)

It should be noted that often these techniques are often not sufficient alone. They are a primary harvesting process. Sun-drying is often used to complete the concentration process.

5. Revised Objectives and Scope

After an extensive literature review of research conducted in the field of algae cultivation and photobioreactor design, it was found that a great number of projects have already been devoted to optimizing algal cultivation for biodiesel production. Given this, it was realized that the initial goal set for this project was unreasonable. The initial objective was essentially to generally optimize the productivity of an algae cultivation process. When starting this project, it was thought that it would be innovative. It was later recognized that the initial goal would merely have resulted in a repetition of the results of other researchers who have previously designed photobioreactors.

The revised objective of this project is to design a system in which microalgae will be cultivated and turned into a biofuel. It will take all the steps into account, from the cultivation of the algae to when the biodiesel is put in the car. This will be done through a two phase process of which the first phase is the cultivation of the microalgae with a photobioreactor in order to have a high biomass yield. The second phase is the harvesting of the microalgae through a pond in order to gain a higher oil and lipid content. The system will be on a small scale and any individual should be able to construct it in their basement or backyard. The materials will be cheap and easy to obtain. The goal of this project is not necessarily to make it efficient or cost-effective, but to show that it can be done on an individual scale. The scale-up of biodiesel production from algae may not necessarily occur through huge industries, but through the multiplication of small units in people's backyards.

Since there are no biofuels made from algae on the market, it can only be produced on an individual level. The cost will be of the materials and installation. There will also be operational costs such as lighting and heating, depending on the climate of the area and whether the system is indoors or outdoors. For the moment it is impossible to know whether the system is cost-effective. If the cost of this system is more than the consumer would pay for traditional fossil fuels, the values of the citizen come into play. Are they willing to put a monetary value on the conservation and protection of the environment? Although the cost of production of biofuels through algae may be higher than that of fossil fuel, it will be up to the consumer to decide what value the environment has to them, and then to choose which one they will use accordingly.

6. Methodology

6.1 Design Approach

The first step taken was to define the problem statement. The next was to formulate an objective. This objective was to optimize algal productivity within a small system. Research in literature on algae biology and previous experimental designs was conducted in order to obtain background knowledge involved in the design of algae cultivation systems.

To be precise, this involved reading articles in biotechnology journals as well as reading important sections of biology books relevant to this topic. The design team also met several times with Dr. Vijaya Raghavan, Dr. Darwin Lyew as well as with Anil Kumar Patel, a student undertaking research on algae strains for the purpose of biodiesel production. The information gathered gave the design team preliminary ideas about potential design paths. At this point, the system components (Phase 1, Phase 2, Harvesting and Conversion to biodiesel) were established. The following step of the design process was then problem refinement, in which the preliminary ideas were examined in more technical detail.

After a considerable literature review and problem refinement, it was found that the initial objective was vague and unrealistic. Due to the extensive research that has already been conducted in this field, it was recognized that it was unlikely that this project's results could exceed those already achieved in the literature.

Therefore, the problem of bringing biodiesel produced by algae to the general public was addressed from a new angle. The revised objective is to design a small scale system that could be built and run by an individual, in his residence, to produce algae and convert it to biodiesel. However it was decided that the two phase process would be retained. This objective was deemed more appropriate to the time frame, budget, and level of expertise available. Furthermore, its scale, potential building materials and construction techniques are much better defined.

Next semester after continuing literature research, based on analysis of the gathered information, a choice will be made about what types of system components to use, in terms of algal strain, photobioreactor, harvesting system, oil extraction, and conversion to biodiesel process.

Based on the analysis, decisions will then be taken regarding the final design. The decision process will likely involve making compromises about system efficiency and cost. Calculations about component dimensions, flow rate, growth rate of the algae, nutrient requirements and estimated outputs will have to be conducted. Various solutions to provide feedback from the system will also have to be analysed. The type of harvesting technique will have to be examined according to feasibility and cost. Cost will also be taken into account when determining which solution is best in terms of types of materials used and nutrient and energy requirements. A small analysis of the potential scale-up of the system will also be conducted. An energy and cost analysis will be performed on the system as a whole to determine whether the energy output is greater than the energy inputs, as well as whether the system can be built at a cost that members of the public would be willing to pay for.

The final decisions being made, the system will be ready for implementation. This will take the form of design drawing and specifications done with the aid of CAD. Operating conditions for the system as well as output predictions would also be provided. A physical model could also be constructed, although time and budget constraints would make this difficult. However the design team is looking forward to such an undertaking if it is feasible to do so.

6.2 Expected Results

The expected result of this project is a design for an energy and cost efficient photobioreactor system that will produce algae to be used for transformation into biodiesel. The design should be able to operate either indoor in controlled conditions or outdoors in a Mediterranean type of climate. The system should be simple and cheap enough for anyone to build and operate. It will use cheap and easy to find materials and should have an energy ratio output:input greater than 1. Energy output will be calculated according to literature values for biomass yield, oil content, and conversion to biodiesel. Since the needs of people are different from one another and the system will be designed in order to produce a certain quantity of biofuel, tips on how to down size and scale-up will be given.

It is expected that this innovative project will interest environmentally conscious people, who will all be quite willing to invest in this system in order to conserve and protect the environment even though it may cost them slightly more than gas.

The system will also be modeled either physically or by computer in order to show the dimensions of the physical structure, but also to show the interactions within and between the different components.

6.3 Cost Analysis

The cost of the design will be divided into fixed, capital costs and variable, operating costs. The cost of designing the system will be neglected, as this is a project conducted by students, with the primary objectives being education and interest.

The fixed costs will be composed of the costs of the building materials. The cost of all necessary tools will be neglected, as it will be assumed that the builder possesses them. This will have been taken into account when making design decisions, so that it can be considered a safe assumption. The cost of the land will also be neglected, as it is assumed that the builder is constructing the system on land he already owns.

Electrical costs, nutrient inputs, water inputs, and gaseous inputs will make up the variable costs. An analysis of typical lifespans of the materials used in the system will determine whether or not maintenance and repair costs must also be included in the variable cost.

The cost of the labour involved in both the building and the operation of the system will be neglected, as it is assumed that the owner of the system is undertaking the labour, and is donating his time out of goodwill.

The cost analysis conducted will thus not be a direct comparison with the cost of purchasing fossil fuels. This would be an inaccurate analysis, as it would not take into account the value people place on environmental conservation. This value is highly individual, though an attempt at assessing it may be made in order to determine what percentage of the population might be willing to undertake such a project. Simply put, it is not expected that the system designed will be economical. The hope is that it will be beneficial from an environmental perspective, and that a certain subset of the population would be willing to pay for that benefit.

7. Work Schedule

Table 7 - Work Schedule

Month	Activity	Time allocation (hrs.)
Jan-09	Continue literature review	30
	Discussion with professors/experts	6
	Decide what strain of algae to use	2
	Decide what the components of the system will be, based on research	10
Feb-09	Design Phase I	20
	Design Phase II	20
	Design harvesting process	10
	Design Conversion to biodiesel	10
Mar-09	Model system	40
	Conduct energy balance analysis	10
	Conduct cost analysis	10
	Explore potential for scale-up	5
Apr-09	Compose final report	40
	Develop final presentation	9
	Deliver final presentation	1
Total		223

8. Conclusion

To date, an outline of a system is proposed. The aim of this project is to design a small scale system for algal cultivation and conversion to biodiesel. The system should be simple and cheap enough for anyone to build and operate.

The solutions considered so far consists in a two phase process in which algal biomass would first be maximized before undergoing nitrogen deprivation to increase lipid content. The aim is to first model the system using CAD and perhaps making a physical model.

Further research and development of ideas may lead to modification of this preliminary design. The design will continue to be developed and tested according to the method and schedule outlined in sections 6 and 7.

Acknowledgments

We would like to express our gratitude towards the following people:

Dr. G.S.V. Raghavan, Department of Bioresource Engineering

Dr Darwin Lyew, Department of Bioresource Engineering

Anil Kumar Patel, Department of Bioresource Engineering

Bibliography

Abedon, S. T. (2003). "Important words and concepts from Chapter 6, Black, 1999 ", from <http://www.mansfield.ohio-state.edu/~sabedon/black06.htm>.

Acién Fernández, F .G., J .M. Fernández Sevilla, J.A. Sánchez Pérez, E. Molina Grima and Y. Chisti. 2001. Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. *Chemical Engineering Science*. 56: 2721-2732.

AlgaeLink. "Algae Growing Systems."The Netherlands. 20 Nov. 2008
<http://www.algaefuels.org/algaepond.htm>.

Amos Richmond, Z. C.-W., Yair Zarmi (2003). "Efficient use of strong light for high photosynthetic productivity: interrelationship between the optical path, the optimal population density and cell-growth inhibition." *Biomolecular Engineering* **20**: 229-236.

Augenstein, D.C., J.R. Benemann, R.P. Goebel and J.C. Weissman. 1982. "Microalgae as a source of liquid fuels." U.S. Department of Energy, Office of Energy Research.

Augero, E. J., I. G. Borlongan and O. M. Millamena. "Techniques on Algae Harvesting and Preservation for Use in Culture and as Larval Food." *Aquacultural Engineering*. 9 (1990): 295-304.

Assaf Sukenik, O. Z., Yael Carmeli (1993). "Biochemical quality of marine unicellular algae with special emphasis on lipid composition. II. *Nannochloropsis* sp " *Aquaculture* **117**: 313-326.

Becker, E. W. (1994). Microalgae: Biotechnology and Microbiology, Cambridge University Press.

Borowitzka, Michael. "Culturing Microalgae in Outdoor Ponds." Algal Culturing Techniques. Burlington, Mass. : Elsevier/Academic Press, 2005.

Chisti, Y. (2007). "Biodiesel from microalgae." *Biotechnology Advances* **25**: 294-306.

Dempster, T. A., K. M. McGinnis and M. R. Sommerfeld. "Characterization of the growth and lipid content of the diatom *Chaetoceros muelleri*." *Journal of Applied Phycology* 9.1 (1997): 19-24.

Erikson, N.T. 2008. The technology of microalgae culturing. *Biotechnol Lett*. 30: 1525-1536.

G. Chini Zittelli, F. L., A. Bastianini, L. Rodolfi, M Vincenzini, M.R. Tredici (1999). "Production of eicosapentaenoic acid by *Nannochloropsis* sp. cultures in outdoor tubular photobioreactors " Journal of Biotechnology **70**: 299-312.

Huesemann, Michael and Chad Weldy. "Lipid Production by *Dunaliella Salina* in Batch Culture: Effects of Nitrogen limitation and light intensity." U.S. Department of Energy Journal of Undergraduate Research. U.S. Department of Energy. 20 Nov. 2008
http://www.scied.science.doe.gov/SciEd/JUR_v7/pdfs/Lipid%20Production%20by%20Dunaliella.pdf.

Incropera, F. P. and J. F. Thomas. "A model for solar radiation conversion to algae in a shallow pond." Solar Energy. 20 (1978): 157-165.

Janssen, M., J. Tramper, L.R. Mur and R.H. Wijffels. 2003. Enclosed Outdoor Photobioreactors: Light Regime, Photosynthetic Efficiency, Scale-Up, and Future Prospects. Biotechnology and Bioengineering. 81: 193-208.

John L. Ingraham, C. A. I. (2000). Introduction to Microbiology Pacific Grove, CA 93950 USA.

John Sheehan, T. D., John Benemann, Paul Roessler (1998). A Look Back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from Algae. U. S. D. o. E. s. O. o. F. Development, National Renewable Energy Laboratory.

Laliberte, G., J. de la Noue and D. Proulx. "Algae and waste water." Journal of Applied Phycology. 4 (1992): 247-254.

Miyamoto, K., O. Wable and J.R. Benemann. 1988. Vertical Tubular Reactor For Microalgae Cultivation. Biotechnology Letters. 10: 703-708.

Molina Grima, E., A. Fernández, F. García Camacho and Y. Chisti. 1999. Photobioreactors: light regime, mass transfer, and scaleup. Journal of Biotechnology. 70: 231-247.

Oceanography, T. A. S. o. L. a. (1999). "The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal." Limnology and Oceanography **44 (2)**: 440-446.

Oswald, W. J. "Large-scale algal culture systems (engineering aspects)." Micro-Algal Biotechnology. Cambridge University Press. (1988) 357-394.

Pienkos, Philip. "The Potential for Biofuels from Algae." Algae Biomass Summit. 15 Nov. 2007. National Renewable Energy Laboratory.

Richmond, A., S. Boussiba, A. Vonshak and R. Kopel. 1993. A new tubular reactor for mass production of microalgae outdoors. *Journal of Applied Phycology*. 5: 327-332.

Rodolfi L, B. N., Parente I, Padovani G, Bonini G, Chini Zittelli G, Biondi and T. N (2008). "Lipid production from marine microalgae: Strain selection, induction of lipid synthesis and outdoor cultivation in a Low-Cost photobioreactors." Biotechnology and Bioengineering.

Tawfiq Institute for Scientific Research , M., Jaber Al-Shimmari & Peter Dias (1999). "Optimum production conditions for different high-quality marine algae " Hydrobiologia **403**: 97-107.

Tredici, M.R. and G.C. Zittelli. 1998. Efficiency of Sunlight Utilization: Tubular Versus Flat Photobioreactors. *Biotechnology and Bioengineering*. 57: 187-197.

Ugwu, C.U., H. Aoyagi and H. Uchiyama. 2008. Photobioreactors for mass cultivation of algae. *Bioresource Technology*. 99: 4021-4028.

Vasquez, V. and P. Heussler. "Carbon dioxide balance in open air mass culture of algae." Arch. Hydrobiol. Ergeb. Limnol. 20 (1985): 95-113.

Zhi-Yuan Liu, G.-C. W., Bai-Cheng Zhou (2008). "Effect of iron on growth and lipid accumulation in *Chlorella vulgaris* " Bioresource Technology **99**: 4117-4722.