Algae to Biodiesel Conversion and Scale-Up

CHE 451: Senior Design Project
Department of Chemical and Biomolecular Engineering

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EXECUTIVE SUMMARY

In recent years, global warming, world oil supply and demand, and energy security have all played a part in the push for alternatives to petroleum-based fuels. Biodiesel is a viable alternative to the current use of petroleum-based fuels in the transportation sector because it is a renewable resource and can be used in traditional diesel engines with little or no modifications. Furthermore, it is environmentally friendly in that burning biodiesel does not result in a net carbon dioxide increase.[1] Overall particulate emissions are also less for biodiesel compared to regular diesel.[2] The Algae Biodiesel Group has been investigating the potential for algal oil to be used as an alternative oil source for producing biodiesel. Algae are a promising feedstock for biodiesel because it may have higher yields than oilseed crops currently used.[3]

The goal of this project is to successfully produce one liter of biodiesel from *Scenedesmus* algae, a strain of algae common to North Carolina. In order to accomplish this goal, the project was divided into four distinct steps: growth, harvesting, extraction, and conversion. *Scenedesmus* algae have been growing in the Undergraduate Teaching Laboratory since December 2006 in a photobioreactor (PBR). Algae are periodically separated from water by harvesting algae through dissolved air floatation. Oil will then be extracted from the algal biomass using solvent extractions and an autoclave operated at elevated temperatures. This algal oil will then be converted into biodiesel using a transesterification process. During each step, scale-up possibilities will be studied for a pilot-plant design to determine the feasibility of producing algal biodiesel as a new alternative energy source for commercial sale.

The algal species *Scenedesmus* was chosen for this study for several reasons. This strain is native to North Carolina and was found in high concentrations in ponds operated by the NCSU Fish Barn. *Scenedesmus* also exhibits a high natural oil content ranging from 14 to 40% by weight.[4] Additionally, bristles are present on the corners of each cell to help space the algae for maximum nutrient and light absorption.

A cell count calibration is being developed to determine the life-cycle stage of the algae. A UV-Visual Spectrophotometer and a hemocytometer are used to determine the concentration of algal cells. On-going cell counts are used to create a cell count calibration. Should the amount of cells decrease, nutrients are added to the PBR. If cell counts continue to increase, no additional nutrients are required; however, if cell counts stabilize after adding nutrients, harvesting is required.

In order to separate algae from suspension a dissolved air floatation (DAF) procedure was used. DAF is a separation technique that uses a pressure gradient to raise particle aggregates to the top of a suspension. DAF requires a fraction of the input water to first be pretreated with a flocculant and then pressurized. The remainder of the liquid is in the depressurization column;

[1] McCarl, et al. 2005
[2] Biodiesel Emissions
[3] Sheehan, et al. 1998
[4] Microalgae

this serves as the flocculation zone. DAF was chosen as a separation method because of the low energetic input and theoretical high yield; algal recoveries up to 98% have been obtained.[5] Chitosan and alum were both tested as possible flocculants, with alum providing a better separation of algae from suspension. By using DAF, algae concentration increased from 6,800 cells/mL to 314,000 cells/mL.

Oil will be extracted from the concentrated algae obtained from the DAF technique using three methods: Bligh and Dyer solvent extraction, ethanol solvent extraction, and liquefaction. Ethanol solvent extraction involves combining dried algae mass with ethanol. Oil will separate from the algae cells and dissolve into the ethanol. The ethanol will then be evaporated, leaving oil behind. Bligh and Dyer solvent extraction involves combining wet algae and a chloroform, methanol, and water solution. The oil will separate from the algae cells into the chloroform phase creating an immiscible layer, which can allow the algal oil to be decanted and collected. Liquefaction involves using an autoclave operating at 250°C and 3 MPa in the presence of sodium carbonate as a catalyst. A combination of elevated temperature and pressure break down the cell walls allowing for increased oil yields from the algal cells compared to solvent extraction. All three of these methods will be studied to determine the maximum amount of oil that can be extracted from the algal cells.

Once algal oils have been obtained, conversion to biodiesel will be conducted using a transesterification reaction. Preliminary experiments for the transesterification of soybean oil into biodiesel have been performed. By establishing a method for the conversion of soybean oil, the group will be prepared to repeat the procedure once algal oil is obtained. The acid value of the algal oil will be found through a titration with sodium hydroxide, which will determine the amount of basic catalyst to add. The algal oil will then be converted into biodiesel using methanol as a solvent over a sodium hydroxide catalyst. After the transesterification reaction, glycerin will be separated from biodiesel using a separatory funnel. The biodiesel will then be washed with Magnesol® using a Büchner funnel. In order to determine the physical properties of the produced biodiesel, future GC/MS tests will be performed.

Scale-up feasibilities are being studied using SuperPro software. The overall process has been designed to allow both 99% biodiesel and 99% glycerin products to be separated. A 1,000 gallon biodiesel/week pilot plant will be designed and an economic feasibility analysis of both the biodiesel and glycerin products will determine the plant's profitability.

The Algae Biodiesel group is currently on schedule to achieve the projects goals. In the coming weeks, the group expects to extract oil from both the harvested algae and commercially available algae paste. Once algal oil has been obtained, a transesterification procedure will be conducted similar to the soybean oil procedure. In addition, an economic analysis of the simulation process will be completed to help determine the feasibility of a large-scale algae-to-biodiesel production facility.

[5] Sim, Goh, and Becker 1988

INTRODUCTION

Motivations

In recent years, global warming, world oil supply and demand, and energy security have all played a part in the push for alternatives to petroleum-based fuels. The Intergovernmental Panel on Climate Change (IPCC) affirms that during the 20th century, the Earth's average temperature increased by 0.6°C and will continue to increase anywhere from 1.5°C to 4.5°C by the year 2100.[6] This increase in global temperature is enough to cause flooding in coastal regions and make storms like Hurricane Katrina a more common occurrence.[7,8] The major force in rising global temperatures is anthropogenic carbon dioxide emissions, which accounts for 80% of all greenhouse gases produced.[9]

Furthermore, the supply for petroleum-based fuels is declining. Currently, the Organization of Petroleum Exporting Countries (OPEC) supplies 60% all the world's oil needs and a quarter of US oil needs. These statistics are disturbing on two levels: (1) OPEC oil production is expected to plateau by 2010 and then decline by about 2030 and (2) the Middle East, where most participating nations in OPEC are located, is considered a region of political instability.[10] Oil outside of OPEC is also expected to peak, with predictions for peak oil production ranging from 2018 to 2025. The US Energy Administration projects that in 20 years, US demand for oil will increase by 33%. In addition, the US is facing increased competition for oil from China. In 2002 China's total energy consumption was less than that of many European nations, but by 2010, China is expected to be the second largest importer of oil.[11,12]

In 1980, the Carter Doctrine, stated in reference to oil that "an attempt by any outside force to gain control of the Persian Gulf region will be regarded as an assault on the vital interests of the United States of America, and such an assault will be repelled by any means necessary, including military force."[13] This policy of resource hegemony has fueled opposition in the form of asymmetric warfare against the US What the media now labels "terrorism" is a response to decades of resented US presence in the Middle East. Global security depends on the US to shift its foreign policy away from resource dominance.[14] Furthermore, the Middle East itself is a region of political instability. If any disruption in oil supply were to occur there, a global oil crisis could result.[15]

[6] McCarl, et al. 2005 300-301
[7] Weart 2003 6
[8] Sweet 2006 2
[9] McCarl, et al. 2005 300
[10] Kerr 2005
[11] Salameh 2003
[12] Wright 2006
[13] Carter 1980
[14] Heinberg 2003 229-230
[15] Salameh 2003

With such a large percentage of US oil coming from the Middle East and increased competition and predictions of peak oil, it is vital that the US obtain a secure alternative energy source that reduces the nation's dependence on foreign oil. The threat of global warming also puts pressure to find an energy source that is cleaner than the fossil fuels currently used.

One option for an alternative energy that has received much attention in recent years is hydrogen. The use of hydrogen fuel cells is environmentally friendly for the consumer as the only combustion product is water. However, the energy output of liquid fuels is much higher than that of a fuel cell, even when the hydrogen is stored at 250 atmospheres.[16] Implementing a system that would allow safely transporting the fuel cell creates an extremely difficult engineering scenario, one that cannot be bolted-on to current vehicles. Automobile producers would have to invest in new materials and more costly manufacturing practices. Additionally, the immediate technology still requires the use of fossil fuels as the source of hydrogen.[17]

In contrast to the hydrogen alternative, biodiesel is a more immediate option as a renewable fuel source. Biodiesel consists of alkyl esters of long chain fatty acids that are derived from oils and fats produced by organisms.[18] Considering that 27% of US greenhouse emissions are produced from the transportation sector and approximately two-thirds of the oil used in the US is used for transportation, biodiesel is well-suited to replace petroleum fuel use in automobiles.[19,20] In addition, biodiesel would require very little change to our existing transportation infrastructure. Unlike many other alternative fuels, biodiesel can be used in traditional diesel engines with little or no modifications to the engine or fuel system. Furthermore, over forty federal and state fleets are already using biodiesel blends.[21]

Additionally, biodiesel has many environmental benefits. Sulfur emissions are virtually eliminated with biodiesel, since plants do not contain any sulfur. Sulfuric oxides are the chief components in the production of sulfuric acid, which produces acid rain. In addition, carbon monoxide, various hydrocarbons, ozone, and particulate matter emissions are greatly reduced compared to regular diesel.[22] Also, biodiesel produces no net increase in atmospheric carbon dioxide concentrations. Plants and other photosynthetic organisms remove carbon dioxide from the atmosphere. When biodiesel derived from these photosynthetic organisms is burned, the carbon dioxide is released into the atmosphere again, only to be recycled by other plants. In contrast, burning fossil fuels releases 100% of the carbon stored without recycling it.[23] Biodiesel is also energy efficient in that it can yield up to 320% more energy than is required in its production.[24]

[16] http://www.unh.edu/
[17] Solomon, et al. 2004
[18] National Biodiesel Board 2006
[19] McCarl, et al. 2005
[20] Union of Concerned Scientists
[21] Environmental Protection Agency 2002
[22] Biodiesel Emissions
[23] McCarl, et al. 2005
[24] Sheehan,et al. 1998a

Feasibility of Microalgal Biodiesel

Recently, an economic feasibility assessment was conducted on the production of biodiesel by Zhang et al.[25] They concluded that the break even price of biodiesel for the least expensive process studied would be $644 per ton, or $1.05 per gallon. This price does not include government subsidies, which would also reduce the price of biodiesel. In comparison, the average price per gallon of gasoline and diesel is, respectively, $2.292 and $2.567 (November 27, 2006 average).[26] Additional reductions in the break-even price of biodiesel are determined by the sale price of glycerin. An increase in the price of glycerin would reduce the price of biodiesel, however, glycerin prices are falling daily. Furthermore, they determined the cost of the feedstock oil plays a significant role in the feasibility of producing biodiesel. It is important to note that this price is the cost of manufacturing biodiesel -- not the market price for the fuel.[27]

Around the world, many countries are embracing biodiesel as a new form of commercial energy. The United Nations reports that biodiesel produced by developing countries can provide heat and energy while also helping to alleviate poverty by reducing petroleum costs.[28] Brazil has become a world leader in biodiesel production, with over $500 million invested in two new biodiesel production facilities. This increase in biodiesel production has allowed the Brazilian government to require a 2% addition of biodiesel to petroleum diesel by 2008.[29]

There are a number of animal fats and plants that can be used to produce biodiesel. Animal fats that are usually used include lard, yellow grease, and tallow. Plants that are typically grown for biodiesel include corn, cottonseed, peanut, rapeseed, and soybean.[30] Currently, the supply of biodiesel from animal fats and plant oils is not enough to completely support the world's energy needs. It is also not economically feasible to rely completely on plants and animal fats for biodiesel. Plants grown specifically for biodiesel compete for land with plants that are grown specifically for agriculture. A large amount of land conversion from forestry and food agriculture would be required; furthermore, not all land may be suitable for crops.[31] In addition, an increase in crops would be conducive to increased amounts of pesticide and fertilizer, which would damage the environment.[32]

Microalgae has been investigated as a potential source for biodiesel. The US Department of Energy reports that biodiesel produced from algae could see yields greater than oilseed crops.[33] Microalgal biodiesel still has the environmental benefits of other biodiesel sources. It is estimated that if a 100 MW thermal plant using coal was replaced by algal biodiesel, carbon dioxide could be mitigated by 1.5×10^5 tons/year using just 8.4×10^3 ha of cultivation area, a result

[25] Zhang, et al. 2003
[26] Weekly Retail Gasoline and Diesel Prices
[27] Zhang, et al. 2003
[28] United Nations
[29] Dooley
[30] Ma & Hanna 1999
[31] McCarl, et al. 2005
[32] Hill et al. 2006
[33] Seehan, et al. 1998

that is very promising in light of global warming.[34] In contrast to agricultural sources for biodiesel that require large tracts of land, algal biodiesel requires water and carbon dioxide. Algae, collected from sewage ponds, have created a potential for farmers to become energetically self-sustaining. This can be made possible through an economically friendly method of producing biodiesel from sewage ponds. Pre-existing ponds can be used to grow algae requiring only outside equipment to extract and convert the algae to biodiesel, reducing costs. In addition, algae ponds can be located next to coal-direct plants to capture generated CO_2. Commercial production of biodiesel from algae has already been achieved recently by New Zealand based Aquaflow Bionomic Corp, however this technology is proprietary.[35]

[34] Sawayama, Minowa, Yokoyama 1999
[35] Kiong

TECHNICAL BACKGROUND

Algae convert inorganic material into organic material through photosynthesis. Photosynthesis provides the algae with the energy necessary for growth and reproduction. Carbon is fixed into organic compounds, and oxygen is released as a byproduct. Excess energy is stored within the algal cell in the form of lipids and fatty acids.

Algae Biology

Algae have evolved differently around the world, and it is estimated that there are anywhere from one to ten million different species of algae.[36] Aquatic algal organisms can be microscopic, as seen on the surface of a pond, or macroscopic, as seen in the kelp forests of the ocean. These microalgae are made up of only one cell, while the macroalgae are comprised of branches of cells. Terrestrial algae also exist, and can survive in moist climates, or from a symbiotic relationship with lichens.[37] Algae exist as either prokaryotic or eukaryotic organisms. The only prokaryotic species of algae is *cyanobacteria*, which belongs to the phyla *Cyanophyta*. All species in the *Cyanophyta* phyla are microscopic. All other species of algae are eukaryotic and belong to the following phyla: *Rodophyta*, *Chlorophyta*, *Ochrophyta*, *Haptophyta*, *Dinophyta*, *Cryptophyta*, *Euglenophyta*, and *Glaucophyta*.[38] Macroscopic and microscopic algal species both exist within each phylum. The algae discussed in this paper are focused primarily on microscopic algae rather than their macroscopic counterparts.

It is often difficult to provide a well-defined set of characteristics for algae because there is so much diversity even within the various phyla of algae. Cell wall, storage products, and photosynthetic pigments vary in each phylum. Table 1 shows various distinguishing features of the major algae groups. All of the species in these phyla contain the photosynthetic pigment *a*. *Chlorophyll* pigment *a* absorbs light energy, which is ultimately converted to energy for the cell in the form of adenosine triphosphate (ATP). Accessory pigments, which vary according to phyla, help transfer light energy to pigment *a*. Excess energy from the photosynthesis process is stored in the form of polysaccharides.[39] As seen in Table 1, the storage products can also vary according to phylum. The form of storage depends upon how the glucose units link together. A link between the α-1,4 linked glucans produces a starch storage product. A link between the β-1,3-linked glucans produces a chrysolaminarin, laminarin, or a paramylon storage product.[40]

[36] Barsanti and Gualtieri 2006
[37] Graham and Wilcox 2000 6
[38] Sze 2006 1-3
[39] Sze 2006 2
[40] Sze 2006 7

Table 1: Characteristics of Algae Phylum. [41]

Algae Phylum	Photosynthetic Pigments	Storage Products	Cell Covering
Cyanobacteria	chlorophyll *a* phycocyanin allphycocyanin phycoerythrin beta-carotene xanthophylls	cyanophycin granules, cyanophytan starch (glycogen)	Peptidoglycan
Glaucophyta	chlorophyll *a* phycocyanin allophycocyanin phycoerythrin beta-carotene xanthophylls	Starch	some cellulosic
Euglenophyta	chlorophyll *a*, b betap-carotene, other carotenes and xanthophylls	paramylon	proteinaceous pellicle beneath plasma membrane
Cryptophyta	chlorophyll *a*, c phycocyanin phycoerythrin allophycocyanin alpha, beta-carotene xanthophylls	Starch	proteinaceous pellicle beneath plasma membrane
Haptophyta	chlorophyll *a*, c beta-carotene xanthophylls	chrysolaminaran	$CaCO_3$ scales common
Dinophyta	chrlophyll *a*, c beta-carotene xanthophylls	Starch	cellulosic plates in vesicles beneath plasma membrane
Ochrophyta	chlorophyll *a*, c beta-carotene xanthophylls	chrysolaminaran, lipids	some naked; some with silica/organic scales; cellulose, alginates in some
Rhodophyta	chlorophyll *a* phycocyanin phycoerythrin allophycocyanin alpha, beta-carotene xanthophylls	floridean starch	cellulose, sulfated polysaccharides; some calcified
Chlorophyta	chlorophyll *a*, b beta-carotene, other carotenes and xanthophylls	Starch	wall of cellulose/other polymers; scales on some; some naked; some calcified

Lipids are hydrocarbons used for storage that are essentially insoluble in water. Algae contain lipids as both membrane components and storage products. Lipids help control the flow of material into and out of the cell wall. They are responsible for the fluid nature of algal cells, which allow materials to be transferred through the cell.[42] The mechanism for lipid production in algal cells is not fully understood. The lipid content of algae can vary with environmental conditions. The Aquatic Species Program found that by decreasing various nutrients, such as sulfur and nitrogen, algal oil production could be increased. The Aquatic Species Program also

[41] Barsanti and Gualtieri 2006
[42] Horton, et al. 2006 10-11

studied the enzyme acetyl-CoA carboxylase or ACCase. This enzyme is believed to catalyze an early reaction in lipid synthesis.[43] If this enzyme could somehow be cloned or magnified, the lipid content of an algal cell could be controlled. Table 2 below shows lipid contents for various algae species. The Aquatic Species Program determined that diatoms and green algae organisms showed the most promise in extracting algal oil.

Table 2: Lipid Contents of Various Algal Species. [44]

Species	Phyla	% Lipid
Porphyridium	Rodophyta	9-14
Prymnesium	Haptophyta	22-30
Euglena	Euglenophyta	14-20
Dunaliella	Crysophycean	6-8
Spirogyra	Chlorophyta	11-21
Scenedesmus	Chlorophyta	12 – 40
Chlamydomonas	Chlorophyta	21
Synechoccus	Cyanobacteria	11
Chlorella	Chlorophyta	14-22

Life Cycle of Algae

The life cycle of algae cultivated in limited volume such as in tanks and aquariums is characterized by five phases: the lag or induction phase, the exponential growth phase, declining relative growth phase, stationary cell numbers phase, and finally death.

Induction Phase

During the induction phase the cells are preparing themselves for division by activating enzymes and increasing their metabolism. They are not yet strong enough for cell division and continue to grow in size and strengthen while absorbing nutrients and light. Microalgae taken from a culture exhibiting exponential growth and placed in a new medium will still show a lag upon transfer. Using a re-enriched medium from a previous cultivation that exhibited fast growth will cause a decrease in lag time in a new microalgae culture, as shown in Figure 2.[45]

[43] Sheehan, et al. 1998b
[44] Microalgae
[45] Fogg, G. E., 1965. 12.

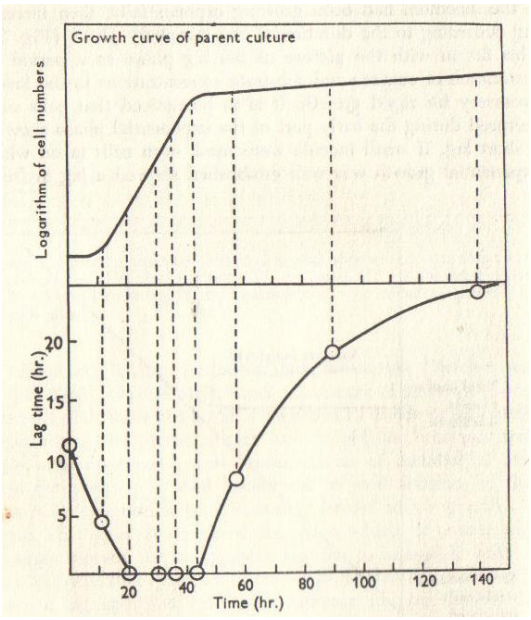

Figure 1: Comparison of lag time and number of cells versus time. [46]

Exponential Phase

The exponential phase of growth occurs after the microalgae have adapted to the medium and have increased their metabolism to the point that cell division can occur. The growth of large populations of non-synchronously dividing cells is characterized by the equation:

$$W = W_o e^{(kt)} \quad (1)$$

where k is the relative growth constant. The growth constant is a function of temperature, light intensity, number of algae cells, the volume of algal material, concentrations of nutrients such as N_2 in the cells, as well as other environmental factors. The value of k increases up to a maximum temperature of around 35°C.[47] Above this temperature, the algae move into the declining relative growth phase and equation (1) is no longer valid.

Reagents	Per Liter
$NaNO_3$	0.075 g
$NaH_2PO_4 \bullet H_2O$	0.005 g
Microelement Stock Solution	1 mL
Vitamin Solution	1 mL
Microelement Stock Solution	
$FeCl_3 \bullet 6\ H_2O$	3.150 g
Na_2 EDTA	4.160 g
$MnCl_2 \bullet 4\ H_2O$	0.180 g
$CoCl_2 \bullet 6\ H_2O$	0.010 g
$CuSO_4 \bullet 5\ H_2O$	0.010 g
$ZnSO_4 \bullet H_2O$	0.022 g
$Na_2MoO_4 \bullet 2\ H_2O$	0.006 g
Vitamin Solution	
Biotin (Vitamin H)	0.5 mg
Thiamine HCl (Vitamin B_1)	100 mg
Cyanocobalamin (Vitamin B_{12})	0.5 mg
pH = adjusted to 8.0 with 1 M NaOH or HCl	

Table 3: Composition of the medium supplemented by f/2 formula.[48]**f/2**

Exponential Phase: Growth Conditions

Certain growing conditions such as temperature, pH, lighting, and CO_2 must be controlled to grow any freshwater algae culture in limited volume. Although freshwater algae can withstand temperatures from 10-35°C, optimal growth temperatures of the culture range from 18-22°C.[49] Temperatures less than 16°C and higher than 35°C will hinder cell division; however, studies show that adding high levels of some nutrients, such as B vitamins at 300 times the necessary concentration, allows microalgae to grow in extreme temperature conditions.[50] Optimal levels of nutrients to cultivate algae are shown in Figure 3. Transitions of temperatures should not exceed 2°C in a short period of time to avoid shocking the algae. Microalgae can

[46] Fogg 1965 14
[47] Fogg 1965 17
[48] Gaultieri, et al. 2006 231
[49] Gaultieri, et al. 2006 213
[50] Fogg 1965 21

withstand a pH range of 7-9; however, most algae grow optimally with a pH of 8.2-8.7.[51] Many factors affect the pH of the medium including temperature and CO_2. Failure to maintain the pH could result in a "complete cultural collapse"[52] as many cellular processes will fail. Lighting is a major factor in the success of algae cultivation as it is a necessary element for photosynthesis. Microalgae react best to light in the blue spectrum, as this spectrum contains the most energy. Filters can be used to obtain the right intensity and wavelength of light. Many algae species grow faster initially in low light, but reach a population saturation point more quickly than algae that grow under intense light, as shown in Figure 4. This means that more light does not mean faster growth, but higher microalgae concentration. Most algae cultures do not grow well under constant illumination. Lighting should be cycled throughout the day using a light/dark (LD) ratio of 14:10 or 12:12 hours.[53]

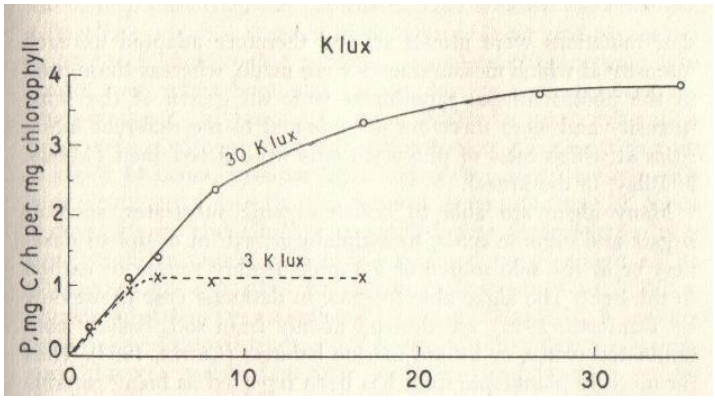

Figure 2: Relationship between chlorophyll production and light intensity .[54]

Concentrations of both O_2 and CO_2 should be maintained in limited volume cultivation. CO_2 should make up 1% of the aeration feed to the medium. An excessive amount of CO_2 will cause the pH to rise, potentially causing damage to the cultivation; however, some extra CO_2 will act as a buffer to the CO_2/HCO_3^- imbalance.[55]

Declining Relative Growth Phase

The algae continues to grow exponentially until one or more of the following five conditions occur: the nutrients become exhausted, the supply of CO_2 becomes exhausted, the pH of the medium is altered by metabolic activity of the culture, the intensity of light is reduced by self-shadowing, and/or autoinhibition. Onset of these conditions is characteristic of the transition into the declining relative growth phase. Exhaustion of nutrients and CO_2 is caused by inadequate amounts of CO_2 and nutrients, such as nitrogen and iron, being added to the system. This causes photosynthesis in the cell to slow, impeding the culture's ability to strengthen for division; this in turn slows the overall growth rate. During photosynthesis, cultures release organic acids as byproducts into solution. Over time, unless proper water changes are performed, increasing concentrations of these organic acids alter its pH causing the water to become more acidic.[56] Freshwater algae thrive in a pH range of 8.2 to 8.7.[57] If pH changes are too drastic the metabolisms of the cells will slow, causing a declining rate of growth. Over time as concentrations of algae in the medium increase, the amount of self-shading also increases, causing the overall light intensity received by the cells to decrease. The maximum algal layer

[51] Gaultieri, et al. 2006 213
[52] Gaultieri, et al. 2006 213
[53] Gaultieri, et al. 2006 213
[54] Fogg 1965 22
[55] Gaultieri, P., et. al. 2006. 214.
[56] Fogg, G. E., 1965. 28.
[57] Gaultieri, P., et. al. 2006. 213.

thickness before self-shading begins to take its toll on growth is 6mm.[58] Self-shading is an exponential function of density, as seen in Figure 5.

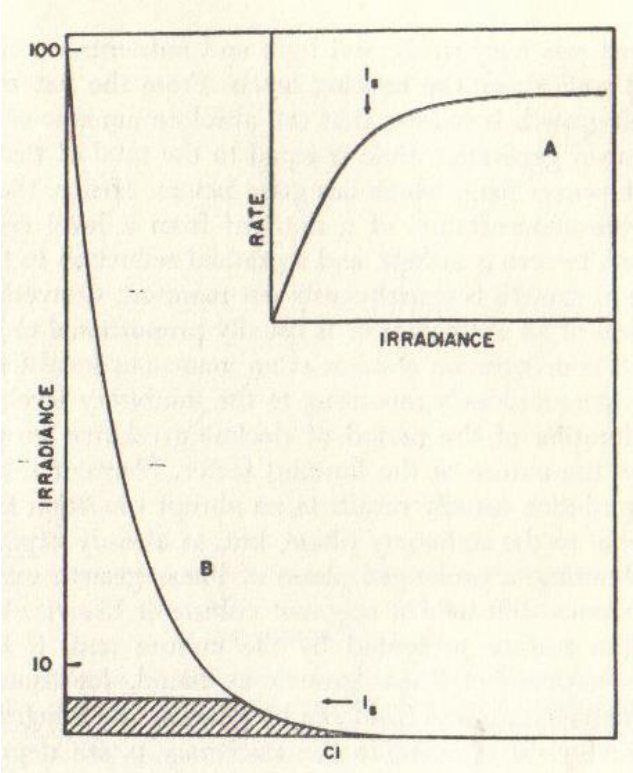

Figure 3: Relationship between irradiance and algae concentration.[59]

Autoinhibition is the last condition that can promote the declining relative growth phase. During metabolism, algae produce substances that are toxic to them. These toxins begin to accumulate in the medium shortly after the exponential growth phase begins. High concentrations of the substance bring algae growth to a standstill.[60]

Stationary Cell Numbers and Death Phase

Shortly after one or more of the limiting growth conditions takes effect, the fourth phase, characterized by a stationary number of cells, begins. During this phase, there is no accumulation of algae cells in the media. Following this is the last phase, death. The stationary phase may last for weeks before the death phase begins, or transition may occur directly from the exponential growth phase to death.[61]

Algae Selection Criteria

The microalgae selected for this study must meet several criteria. Most importantly, it must be native to North Carolina. It should be a major constituent of the lagoon ponds found in this area. This is to maintain the possibility that it could be extracted locally and refined to biodiesel in the future. The algae should have a high growth rate so that the population can quickly replenish itself after harvesting. The selected culture should also have a high oil concentration. Oil concentrations in microalgae vary from 10-60% by weight.[62] The more oil produced by the cell, the more will be able to be extracted for production of biodiesel. The last major criteria for the selected algae strain is it must be able to thrive in conditions easily reproduced in a limited volume cultivation facility. It should have a relatively high threshold for pH, temperature, hardness, and other water conditions. The operators of the tank should be able to keep the algae alive and thriving without being overly attentive to medium conditions.

Scenedesmus is in the *Chlorophyta* family and is native to North Carolina. It was found to be the algae of highest concentration in samples taken from a local lagoon. *Scenedesmus* exhibits

[58] Fogg, G. E., 1965. 31.
[59] Fogg 1965 28
[60] Fogg 1965 28
[61] Fogg 1965 32
[62] Graham, et al. 2000

optimal growing conditions of 20-25°C, as well as good response to the f/2 fertilizer as a means for nutrient addition in limited volume cultivation.[63] It also is unique in that adding glucose to the medium will increase yield in times where self-shadowing has lowered the intensity of light received by the cells.[64]

Concentrations of both O_2 and CO_2 should be maintained in limited volume cultivation. CO_2 should make up 1% of the aeration feed to the medium. An excessive amount of CO_2 will cause the pH to rise, potentially causing damage to the cultivation; however, some extra CO_2 will act as a buffer to the CO_2/HCO_3^- imbalance.[65]

Reactor Design

The type of microalgae selected will primarily affect reactor design as growth conditions vary with each strain of microalgae. Pre-existing ponds are the most ideal sites for producing algae due to their low capital costs, while algae grown in a laboratory offer an advantage in converting microalgal oils to biodiesel in a controlled environment. Reactor design will focus on four topics: algae growth, harvesting, oil extraction, and converting oil extracted from microalgae to biodiesel.

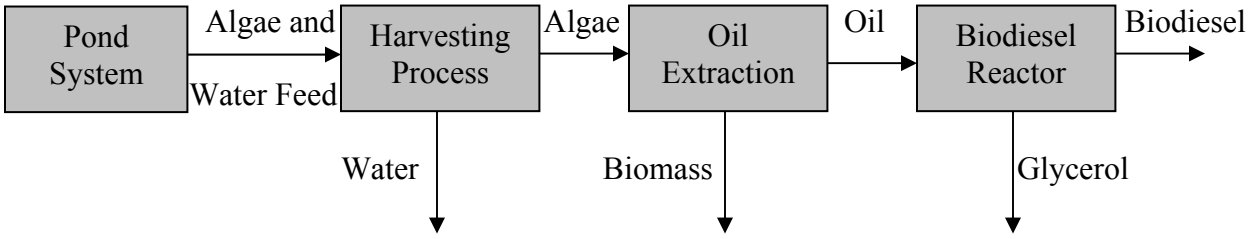

Figure 4: BFD for production of biodiesel.

A feed consisting of algae and water from the pond source will enter into a separator. Once the algae are separated from the water, the lipids can be extracted. Waste biomass will be discarded as the oil enters into a catalyzed reactor to be converted into biodiesel. The biodiesel product will be collected from the reactor. Algae harvesting, oil separation and conversion to biodiesel will be described in detail in the following sections.

Two types of microalgae reactor systems will be discussed: open and closed systems. Open pond systems require a lower capital cost, as they are pre-existing. Factors affecting growth such as contamination and temperature cannot be controlled when exposed to the environment. Closed pond systems can be constructed in a laboratory allowing for control over these factors, especially bacteria. However, closed pond systems have been found to be too expensive for microalgae fuel production.[66]

[63] Fogg 1965 20
[64] Fogg 1965 24
[65] Gaultieri, et al. 2006 214
[66] Pedroni, et al. 2001

Open Pond System

Samples from open pond systems such as North Carolina State University operated Fish Barn contain *Scenedesmus* and diatom algae as well as bacteria and other multicellular species. As stated earlier, this report will focus on the study of *Scenedesmus*, a form a green algae and a member of the *Chlorophyta* family. This study of one particular type of algae will be difficult with an open pond system. Contamination of ponds open to the environment will play a permanent risk in the open pond design.

The following table taken from Pulz shows the different types of open pond systems and their required parameters.

Table 4: Types of Open Systems and technical variables required.[67]

Type of System	Variables
Cuvette, container	Material (glass, plastic)
Natural Water	Turbulence development (pumping, stirring)
Raceway Pond	Flow Direction (horizontal/vertical)
Incline surface device	Surface to Volume-Ratio

Natural water and raceway ponds are the most heavily researched form of open pond systems.[68,69] These designs require constant mixing and flow for optimum algae growth. Continual mixing allows for nutrients, CO_2, and solar irradiance to reach all areas of the pond to improve algae production.

Commercial open pond systems employ a raceway type design where water is constantly mixed by use of motorized paddle wheels. The paddle-wheel mixers eliminate concentration gradients in nutrients and biomass.[70] Furthermore, mixing allows algae to constantly be brought back to the surface of the pond.[71] Carbon dioxide is bubbled into the pond to assist in algae growth. These ponds are found to be shallow (depth of 0.1 m) as sunlight can only penetrate up to 0.2 m into water; thus pond area is more important than volume.[72] As the depth of the pond increases, the cell density of algae cultivated lowers.[73] In addition, lining over the bottom of the pond is generally required either by using a non-membrane (asphalt, concrete) or membrane lining (sheet plastic, rubber).[74]

Some possible attempts to control contamination in the open pond systems included covering the ponds with plastic sheet covers or constructing a green house over the pond. These methods allowed for a brief extension of cultivation periods for the ponds and helped maintain CO_2 levels and temperatures. Increases in biomass productivities have also been found. However capital costs involved with covering the ponds proved these methods to not be

[67] Pulz 2001
[68] Singh and Rekha 2001
[69] Chaumont 1993
[70] Sheehan, et al. 1998b
[71] Sheehan, et al. 1998b
[72] Sheehan, et al. 1998b
[73] Sheehan, et al. 1998b
[74] Chaumont 1993

feasible.[75]

Open Pond System: Advantages and Disadvantages

The Aquatic Species Program reports that open pond systems have the distinct advantage of being placed next to power plants. CO_2 expulsion gas can be pumped directly into open ponds, which allows for waste gas to be used directly by the ponds to assist in algae growth.[76]

Whereas open pond systems are found to be less expensive than closed systems, several disadvantages have been found. Low temperatures hinder the production of algae; in order to produce algae year-round, the pond will require an enclosure in order to control the pond temperature. Open ponds can experience significant evaporation loss, diffusion of CO_2 to the atmosphere, permanent contamination risk, and require a long period of time for net production (6-8 weeks).[77] However, production of algae from an open pond system was found to require only 0.1 to 0.2 g/L of algal biomass in order to initiate growth of algae. This is relatively small compared to the 2 to 8 g/L required for closed pond systems.[78]

Closed Pond System

Closed systems, unlike open systems, offer better control over of growth conditions, including nutrient addition and temperature control. By controlling these parameters, it is possible to maximize the amount of algae produced from a system. A photobioreactor (PBR) is a system that provides an artificial environment for the growth of phototrophic microorganisms, such as microalgae, under the presence of light. Growth of specific strains of algae is directly dependent on the conditions of the system. Temperature, pH, nutrient and CO_2 additions will determine which strain of algae will dominate growth.

Japanese Research and Development programs using closed PBRs have been able to isolate over 10,000 strains of microalgae for tolerance to high CO_2, temperature, salinity, and O_2 evolution rates.[79] Green algae studied averaged a growth of 17 g/(m^2·day). In some cases, cultures supplied with CO_2 and O_2 can reach algae concentrations of 40 mg/L.[80]

The following table taken from Pulz shows the different types of closed pond systems and their required parameters.

[75] Chaumont 1993
[76] Pedroni, et al. 2001
[77] Pulz 2001
[78] Pulz 2001
[79] Pedroni, et al. 2001
[80] Pulz 2001

Table 5: Types of Closed Systems and technical variables required.[81]

Type of System	Variables
Plastic sleeves	O_2 removal > CO_2 input
Fermenter-like Tank	Type and duration of illumination
Tubular PBR	Temperature Control
Laminar PBR	Sterilization

Research in fermenters,[82] tubular PBRs,[83] and laminar PBRs[84] show promise as methods of producing high concentrations of microalgae. When choosing a photobioreactor, three main factors must be considered: a light source, CO_2, and nutrients. These factors are dependent on various other factors such as pH, temperature, structural design, and mixing mechanisms.[85] Examples of PBR designs are shown in Figures 5 and 6.

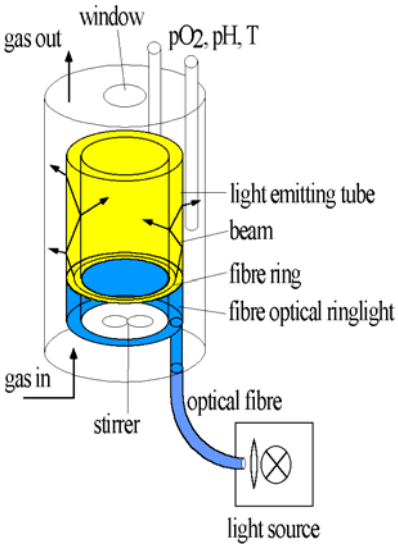

Figure 5: Diagram of a Vertical Photobioreactor.[86]

Tubular PBRs allow for operator control over the temperature of the system. To control temperature, the algae culture is heated at the beginning and end of the light phase while at thorough turbulent flow. The system is connected to an open-channel raceway to allow circulation of the culture as well as diffusion of heat to the entire culture. This eliminates any areas of over-heating in the system.[87]

Light penetration into the system is a parameter that must be controlled. Some systems are not completely transparent, which allows little light into the system. Instead they are heated from within by their respective light sources. Other systems employ transparent columns allowing for light penetration. These systems are generally made of glass or plastic acting as a solar receptor, depending if natural or artificial light is used. Chaumont, et al. reports that systems imitating solar collectors have been found to produce yields ranging from 20 to 25 g/(m^2·day). Pumps are used to circulate the culture through the system while also allowing air, CO_2, and nutrients to be added to the system and for O_2 removal from the system. In addition, replacing the pump with an airlift has been found to increase productivity by 75% in microalgae cultures.[88]

Masojídek, et al. studied growth of *Chlorella* and *Scenedesmus* algae. Each species were grown in both a fermenter and a tubular PBR, with the fermenters located indoors and the tubular PBRs located outdoors. The PBRs (outdoor apparatuses) consisted of ten parallel glass tubes

[81] Pulz 2001
[82] Masojídek, et al. 1999
[83] Masojídek, et al. 1999
[84] Lee, et al. 1996
[85] Kommareddy and Anderson 2005
[86] Cso-Gör
[87] Chaumont 1993
[88] Chaumont 1993

(length of 2 m, I.D. of 48.4mm) connected by PVC U-bends, similar to the apparatus shown in figure 9.

Figure 6: Diagram of a Horizontal PBR.[89]

The PBRs allowed for a total volume of 50 liters each. These reactors were placed in an isothermal water basin to maintain growth temperatures at night. Chlorophyll concentrations of each reactor were adjusted with fresh medium each morning to allow for a constant concentration of 7 μg/mL. Addition of CO_2 was used to maintain a pH of 6.8 for continual growth of algae. Dissolved oxygen concentrations were kept to 20 mg/L via nitrogen gas. The speed for each reactor was set to 0.46 m/s, corresponding to a Reynolds number of about 11,000 (fully turbulent flow). *Chlorella* and *Scenedesmus* algae provided a biomass yield of 21.5 and 27.5 g/(m^2·day), respectively.[90]

The cultures in the indoor fermenters were grown in plate-parallel cuvettes under tungsten bulbs operating at 100-μmol photons/(m^2·s). The systems were maintained at 30°C and 35°C for *Chlorella* algae and *Scenedesmus* algae, respectively. Fresh medium, Chlorophyll, was also provided to the systems to maintain a concentration of 10 μg/mL. For 120 minutes, the bulbs provided 1000-μmol photons/(m^2·s) of energy to the system. During this period of high intensity light exposure, the cultures were bubbled with air at a 2% excess of CO_2 to promote strong algae growth. Light and dark cycles of 12 hours each were used. No data on yields for either algae strain grown indoors were provided. Compared to the *Chlorella* algae strain, *Scenedesmus* was found to have a higher capacity to adapt to extreme irradiance, due to an effective quenching mechanism and high photosynthetic capacity.[91]

Lee, at al. studied enclosed outdoor PBRs used to grow *Chlorella*, a member of the *Chlorophyta* family. The photobioreactor was placed horizontally, to allow no light to diffuse from the underside of the reactor. Glucose was used as the main carbon source to the reactor, providing both CO_2 and energy to the system in addition to light. In theory, this reactor would allow complete conversion of the glucose provided to the system. During the day, sunlight and carbon is converted in algal cells, while at night, only carbon contributes to algae growth. This allows for constant growth and constant conversion of glucose throughout the entire day.[92]

Water temperature was maintained at 32 to 35°C by cycling water from water batch maintained 32°C. The pH of the algae and water was kept at 6.6. Before use of the PBR, the reactor was sterilized with a 2-6% vol. hydrogen peroxide solution and exposed to sunlight for one day to remove any containment from the system. As a problem with all outdoor systems, the

[89] SpirulinaSource
[90] Masojídek, et al. 1999
[91] Masojídek, et al. 1999
[92] Lee, et al. 1996

system experienced an evaporation rate of 100 mL/day. Extra water was added to the system to maintain water levels to counteract this. Algal biomass production for the system was found to be as high as 127 g/(m^2·day) during the day and 79 g/(m^2·day) at night.[93]

Closed Pond System: Advantages and Disadvantages

Photobioreactors provided several advantages over open pond systems. These enclosed systems allow for control of temperature, extending cultivation periods. Through these reactors, higher algal cell densities can also be achieved in reduced harvesting volumes while also reducing processing costs. By enclosing the system, contamination can be prevented, allowing for algal product use in markets ranging from health food to pharmaceuticals.[94] In addition, closed systems will prevent evaporation of water, which is important in dry conditions.

For the purpose of laboratory experiments, the use of closed pond reactors is ideal. Growing and harvesting microalgae indoors will allow for the control of growth conditions, in turn maximizing the amount of microalgae produced.

Algae Harvesting

Because algae can be present in such amounts in water, a key component in the production of algal biomass is harvesting algae in a way that is both efficient and economical. In the past, algae harvesting has been studied extensively for the purpose of water treatment where it has traditionally been accomplished by flocculation followed by a step to separate algae from water (Figure 9).[95] The degree to which flocculation occurs depends largely on the pH of the water, the salinity of the water, and the chemical flocculant used.[96,97,98,99] Algae is normally in a dispersed state and is very small in size (between 5 and 50 μm), which makes harvesting difficult.[100] The addition of a chemical flocculant creates a matrix involving the algae and the flocculant, generating larger particle sizes and allowing for greater efficiency of algae removal with subsequent harvesting steps.[101]

Flocculation

The regulation of pH in an algal suspension is vital to ensure effective flocculation of algae. *Chaetoceros sp.*, a genus of diatoms, was found to flocculate without chemical addition at a pH of 10 and above. However, at pH 10, other organic ions and nutrients precipitate out of the water.[102] Aluminum sulfate, commonly known as alum, is often used for flocculation, though the optimal pH for flocculation depends on the species of algae. For *Botryococcus braunii*, a species of green algae with a relatively high lipid content, the optimal condition for flocculation required

[93] Lee, et al. 1996
[94] Chaumont 1993
[95] Bilanovic, Shelef, and Sukenik 1988
[96] Bilanovic, Shelef, and Sukenik 1988
[97] Divakaran and Pillai 2002
[98] Millamena, Aajero, and Borlongan 1990
[99] Tenney, et al. 1969
[100] Tenney, et al. 1969
[101] Millamena, Aajero, and Borlongan 1990
[102] Millamena, Aajero, and Borlongan 1990

200 mg/mL alum at pH 11. These conditions resulted in an algae recovery of 90%.[103] In *Chaetoceros*, *Tetraseimis*, and *Skeletonema*, the optimal pH for alum flocculation occurred between 5.5 and 6.5, although the algal cells began to deteriorate below pH 6.5. Once deteriorated, the cells were unable to flocculate. It is important to note for purposes of growing algae cultures that cell viability is not affected by alum.[104] However, alum flocculation is not always an effective method. In a study done of a water storage res ervoir, alum flocculation barely resulted in the removal of 50% of the organic matter present, which consisted largely of algal species.[105]

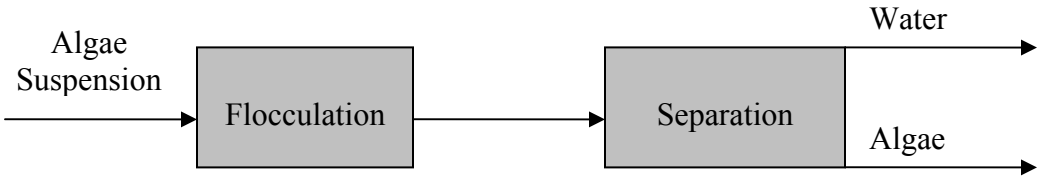

Figure 7: BFD for Separation of Water and Algae.

Polymers are also commonly used chemical flocculants. Generally, cationic polymers are able to induce flocculation due to the negatively charged surface of the algal cells. Nonionic and anionic polymers do not cause flocculation, although they are able to attach to the algal cells. While the interaction between cationic polymers and the algal cells is electrostatic, anionic and nonionic polymers only interact with the algal cells through hydrogen bonding. The optimal pH for flocculation using cationic polymers depends on the polymer used. The optimal pH for a cationic polyamine organic polymer (molecular weight 5×10^6) was between 2 and 4 while for a polyacrylamide (molecular weight 1×10^7) was 7.[106,107]

Chitosan is a cationic polyelectrolyte synthesized from the deacetylation of chitin, which comes from shellfish.[108] As a chemical flocculant, maximum reduction in turbidity was achieved at pH 7 and a concentration of 5 mg/L.[109] Furthermore algal cells are still viable and able to reproduce after flocculation with chitosan.[110] As with other chemical flocculants, the flocculation of algae using chitosan is dependant on pH. A study done on water samples containing eleven species from six different classes of algae showed that adjusting the pH to 6.5 before the addition of chitosan and then re-adjusting the pH to 8 after the addition resulted in the most effective removal of algae.[111] Chitosan is an extremely desirable option as a chemical flocculant because it is environmentally safe and effective compared to other chemical flocculants. It is widely used in the food and medicine industry and is available for less than $2 per kilogram in the US[112,113]

[103] Lee, et al. 1998
[104] Millamena, Aajero, and Borlongan 1990
[105] Hejzlar et al. 1998
[106] Tenney, et al. 1969
[107] Bilanovic, Shelef, and Sukenik 1988
[108] Lubian 1989
[109] Bilanovic, Shelef, and Sukenik 1988
[110] Divakaran and Pillai 2002
[111] Lubian 1989
[112] Lubian 1989
[113] Divakaran and Pillai 2002

However, chitosan, as well as alum and other cationic polymers, is not effective when the salinity of the water is greater than 0.1 M.[114]

Algae Separation from Water

After flocculation, algae can be separated from water by a variety of methods, including sedimentation, flotation, or filtration.[115] Centrifugation can also be used to separate algae from water, but it is an energy intensive process and is not as economically desirable as other alternatives.[116] Filtration is a relatively low energy separation technique for algae harvesting. However, when using filtration, weak flocs will break apart and go through the filter. Furthermore, large flocs can block the pores of the filter, especially if the flocculant dosage is too high. If external pressure is applied to blocked pores, cells are then able to pass through the filter, an undesirable result.[117,118] Flotation is usually preferred over sedimentation for large scale processes because it can be carried out at relatively high overflow rates and still have high efficiencies. The amount of algae removed by flotation compared to sedimentation is about the same; however, flotation results in slurries that are almost twice as dense as those by sedimentation and is also considerably faster.[119] Using flotation, air bubbles pass through an algal solution while biomass attaches to the bubbles, increasing their buoyancy.[120]

Dispersed air flotation, also known as froth flotation, bubbles air through an algal suspension so that a froth containing algae is created and can be taken off the top of the solution.[121] However, in order to collect large amounts of algae using this flotation method, and frothing agent, such as sodium dodecylsulfate (SDS) or *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide (CTAB) must be used. When chitosan is used as a flocculant in addition to SDS, algae recovery increased from 20% cell removal to over 90%. In contrast, chitosan used with CTAB decreased from 86% to 36% cell removal.[122] Regardless of the effectiveness of algae removal by froth flotation using SDS or CTAB, both frothing agents are toxic to human health. As such, it is not desirable to use them on a large scale.[123,124]

Dissolved air flotation

Dissolved air flotation (DAF) is similar in theory to dispersed air flotation but does not require any frothing agent. It has been proven to thicken sludge from water treatment plants and has also been effective in algae removal.[125,126] The apparatus required for DAF can be relatively simple to construct, consisting of a vertical cylinder that has a pump and an inlet for dissolved air coming in through the base.[127,128] Because oversized bubbles often break up flocs, a saturation

[114] Bilanovic, Shelef, and Sukenik 1988
[115] Bilanovic, Shelef, and Sukenik 1988
[116] Sim, Goh, and Becker 1988
[117] Rawlings, et al. 2006
[118] Sim, Goh, and Becker 1988
[119] Lee, Koopman, and Lincoln 1992
[120] Sim, Goh, and Becker 1988
[121] Levin, et al. 1962
[122] Liu, Chen, and Ju 1999
[123] SDS MSDS
[124] CTAB MSDS
[125] Doccko, et al. 2006
[126] Sim, Goh, and Becker 1988
[127] Lee, Koopman, and Lincoln 1992

tank is needed to create a supersaturated solution of air in water. As the flocs float up, a compaction zone containing concentrated amounts of algae is created.[129] Both alum and chitosan are extremely effective in algae removal when used with DAF. However, the dose point of alum greatly affects the rising rate of the flocs formed whereas the dose point of chitosan does not. This indicates that the flocculant contact time is not a significant variable for chitosan.[130] Flocculation with 50-mg/L chitosan followed by DAF can result in 98% of algae recovery. The use of other polymers as flocculants prior to DAF is not advantageous because they are not as effective at low concentrations and are potentially more toxic than chitosan. Furthermore, chitosan is effective at neutral pH, which eliminates risks due to high acidity or alkalinity.[131]

Oil Extraction

Solvent extraction of bio-oils historically has the highest yield of any extraction process, thus it sets the standard for comparison.[132] Extraction techniques involving ethanol-supercritical carbon dioxide ($scCO_2$) co-solvents as well as acetone and ethanol extraction processes were studied in comparison with hexane as a solvent for lipid extraction from *Spirulina*. Among these solvents, ethanol provided the highest yield of bio-oils, with hexane providing the poorest yield for standard solvents (73% and 33% of total lipids, respectively). With the high fatty acid content in algal bio-oil, further processing can be reduced when using an alcohol solvent.[133] The general solvent oil extraction process is shown in Figure 8.

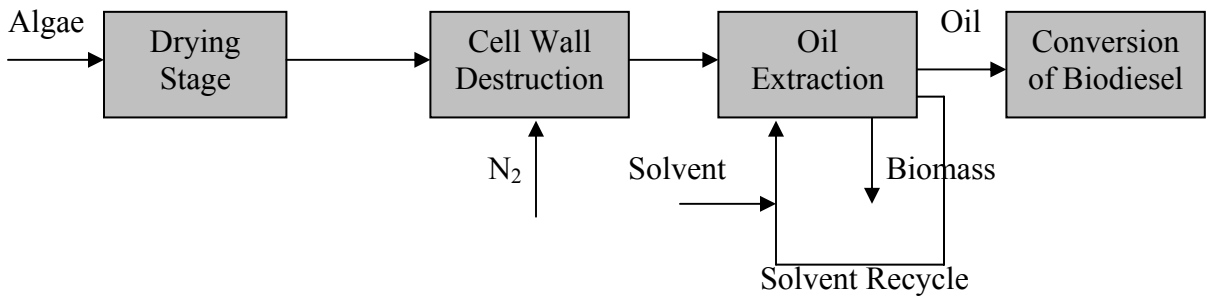

Figure 8: BFD for solvent extraction using hexane.

Bligh and Dyer developed a method for total lipid removal based on the phase behavior of a ternary mixture of methanol, chloroform, and water. This mixture will be monophasic at the proper concentrations of solvents, and will become biphasic if the solvent balance is perturbed.[134] Upon extraction, the lipids will be found in the chloroform phase. The solvent is then evaporated and the lipids are collected.[135] This method has generally set the standard for lipid extraction due to its ease and efficacy. A downside to the use of this solvent mixture is the

[128] Doccko, et al. 2006
[129] Sim, Goh, and Becker 1988
[130] Lee, Koopman, and Lincoln 1992
[131] Sim, Goh, and Becker 1988
[132] Mendes, et al. 2006
[133] Mendes, et al. 2006
[134] Bligh and Dyer, 1959
[135] Bligh and Dyer, 1959

toxicity of chloroform. The Occupational Safety and Health Agency (OSHA) mandates the prolonged exposure limit (PEL) of chloroform to 50 ppm; thus the use of this method would also require extensive ventilation. [136] This method relies heavily on the water content of the sample[137], which would be a variable in algal lipid extraction. Due to water being a variable in the extraction technique, automation of this process is difficult, as the solvent ratios must be kept in proper balance.

Supercritical CO_2 Extraction

Much interest has been placed on the use of $scCO_2$ as the solvent for biomass extraction. Current solvent extraction methods for the removal of oils from soybeans are centered on hexane extraction. Energy balances were conducted comparing the current technology with $scCO_2$ extraction.[138] The energy balance was found to be favorable to hexane extraction. There are two main drawbacks to $scCO_2$: soybean oil does not have a high solubility in the fluid (57g oil/1000 g solvent) and compression of gaseous CO_2 to solvating $scCO_2$ requires a pressure of 592 atm at ambient temperature. However, a proper co-solvent can be chosen to increase the solubility of the oil in the fluid, which makes the energy balance more favorable to $scCO_2$ extraction.[139] Additionally, if membrane separation processes are utilized and are found to separate nearly 100 percent of the carbon dioxide, additional energy savings can be exploited due to reduced solvent losses and a consequent reduction in compression requirements.[140] Both of these technologies also require preprocessing, which, from an energetic standpoint, mainly consists of drying the biomass. Figure 9 shows a block flow diagram for lipid extraction using $scCO_2$.

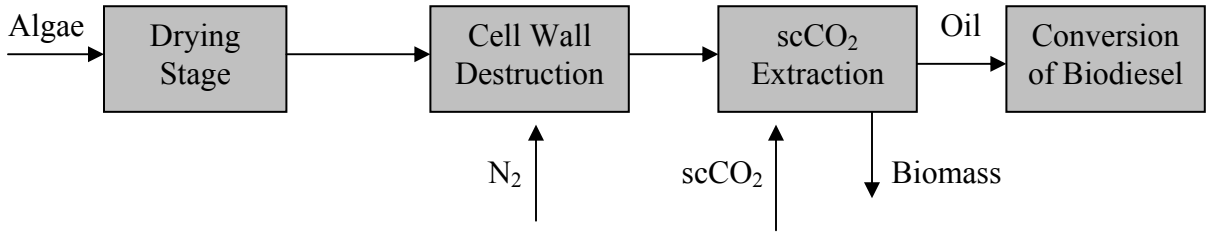

Figure 9: BFD for $scCO_2$ lipid extraction.

Research has been conducted into the use of $scCO_2$ as an extraction method for algal oil as well.[141] These lab-scale experiments were conducted using methanol as a co-solvent in the extraction process. Again, drying the biomass is a major factor in the process. The procedure requires the algae to be dried away from sunlight at ambient temperature for 5-8 days. Although this saves energy for the lab scale experiment, scaling up the process will require a drying step. Additional energy requirements come in the form of grinding up the cell wall in an inert atmosphere before $scCO_2$ extraction.

[136] OSHA
[137] Bligh and Dyer, 1959
[138] Li, et al. 2006
[139] Li, et al. 2006
[140] Li, et al. 2006
[141] Aresta, et al. 2005

Reducing the outside energy requirements of these processes can be implemented by the use of solar cells. Again, even with methanol as a co-solvent, the solubility of algal oil is low in $scCO_2$ – 45 g oil/1000 g solvent.[142] Although using methanol as a co-solvent provides very little effect on the solubility of the oil in the solvent, further treatment of the crude oil is not required. Since high yields of free-fatty acids can be directly extracted from the algal oil, extraction in $scCO_2$-co-methanol solvent produces biodiesel rather than crude bio-oils. Additionally, increasing the temperature from ambient temperature to the critical temperature of carbon dioxide (31.1°C) allows the pressure required for the process to be reduced to 72.8 atm, which would reduce the energetic input.

Solvent extraction

Comparing $scCO_2$ oil extraction to extraction using liquefaction was conducted by Aresta et al.[143] Liquefaction is a process in which biomass enters an autoclave and is heated to moderate temperatures and pressures for a given amount of time (Figure 10). It was found that liquefaction consumes less energy than an extraction process, which involves $scCO_2$, most importantly in the preparation step. Liquefaction does not require drying of the organic matter; consequently wet biomass can be processed, and the preparatory drying step can be eliminated.[144] The process can also be used to produce alternative liquid fuels in addition to lipids, due to fiber and protein degradation in algal cells.[145]

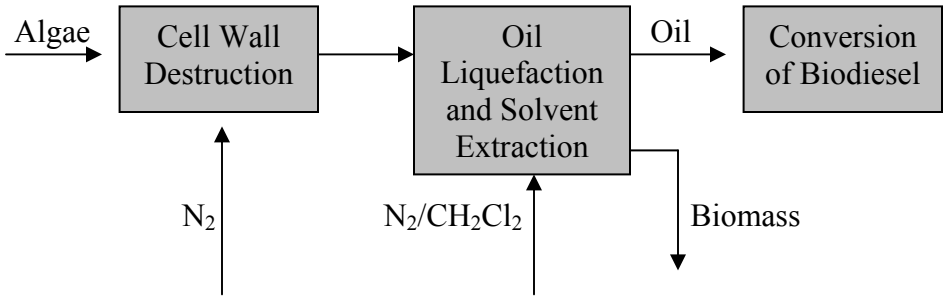

Figure 10: BFD for liquefaction process.

Liquefaction provided higher yields at 300°C than solvent extraction using hexane. Due to the lower processing temperatures for liquefaction (200-400°C), it has been deemed more energetically favorable than pyrolysis (500°C) and gasification (2000°C). Catalysts such as sodium carbonate have long been used in the liquefaction process, and its effect on the yield of algal oil is significant.[146] A catalyst dosage of 5-wt% sodium carbonate increases the yield by roughly 5 percent as compared to the non-catalyzed process. Maximum yield is obtained at 340°C for 30 minutes with 5-wt % catalyst. Degradation of the oils may occur at the longer holding time, which reduces the yield. Net energy production has been reported, with the maximum (39.5%) occurring with the maximum yield of oils.

[142] Aresta, et al. 2005
[143] Aresta, et al. 2005
[144] Dote, et al. 1993
[145] Dote, et al. 1993
[146] Yang, et al. 2004

The required lipid purification step in liquefaction is a downside to using the process. Methylene chloride is used in some experiments to fully extract the oil.[147,148] This chemical can be replaced by acetone, although acetone is not as effective a solvent. Other liquefaction processes utilize chloroform[149], and contain the same hazards as the Bligh and Dyer solvent extraction technique. It is imperative that the methylene chloride is recycled in the process, as it is regulated in the United States and known to deplete the ozone layer.[150] Additionally, residual nitrogen is present in the waste liquid, half of which is present as ammonia.[151] Phosphates are also present in the wastewater. Both the phosphates and nitrogen-containing compounds can be reduced by secondary wastewater treatment by *Botryococcus braunii*, which in turn also can be used as a biomass source.[152] The wastewater composition from the liquefaction step can be altered depending on the type of algae used. *B. braunii* is preferred due to the low nitrogen and phosphorous requirements for growth as compared to *Dunaliella tertiolecta*, which has a higher fertilizer requirement and lower oil content.[153] The species of algae used will determine the net energy production. The algae with the higher oil content will inevitably produce more energy per process.

Conversion to Biodiesel

Once the oil is extracted, it must be processed further to convert it into biodiesel. Raw fats and oils (glycerides) cannot be used directly in diesel engines without modifications to the fuel systems. The glycerides can contaminate the lubricating oil, induce the formation of carbon deposits in the engine, and affect engine durability, among other issues. The oils must be converted into derivatives to become compatible with diesel engines. Although other methods exist (pyrolysis, microemulsion, and blending of oils) current emphasis is on the process known as transesterification (alcoholysis).

Figure 11 shows the process of converting oil into biodiesel. First the catalyst and alcohol are mixed together. This mixture is then fed to a reactor, where it is then combined with raw oils and continuously stirred. Next, the mixture of glycerin, biodiesel, and unreacted methanol is fed to a separator. Biodiesel and methanol are separated from the glycerin byproduct by use of either density loops or gravity settling. Finally, biodiesel and methanol are purified through evaporation to allow for collection of pure biodiesel.[154]

[147] Dote, et al. 1993
[148] Aresta, et al. 2005
[149] Yang, et al. 2004
[150] EIA/DOE 1999
[151] Sawayama, et al. 2005
[152] Sawayama, et al. 2005
[153] Sawayama, et al. 2005
[154] Russel, et al. 2005

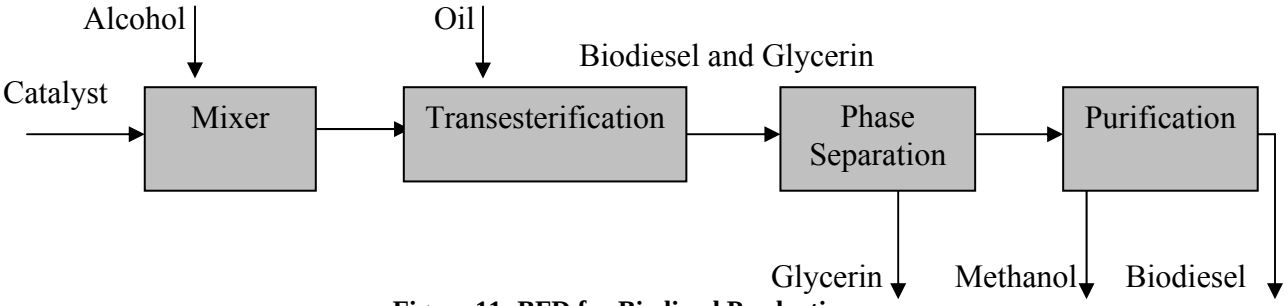

Figure 11: BFD for Biodiesel Production.

Transesterification

In transesterification, fats or oils are reacted with an alcohol to produce esters and glycerol in a one step reaction that is illustrated in Figure 12. The catalyst and alcohol are mixed together prior to the reaction with glycerides.[155] The ester products can be used directly in diesel engines without blending it with petroleum diesel fuel.[156]

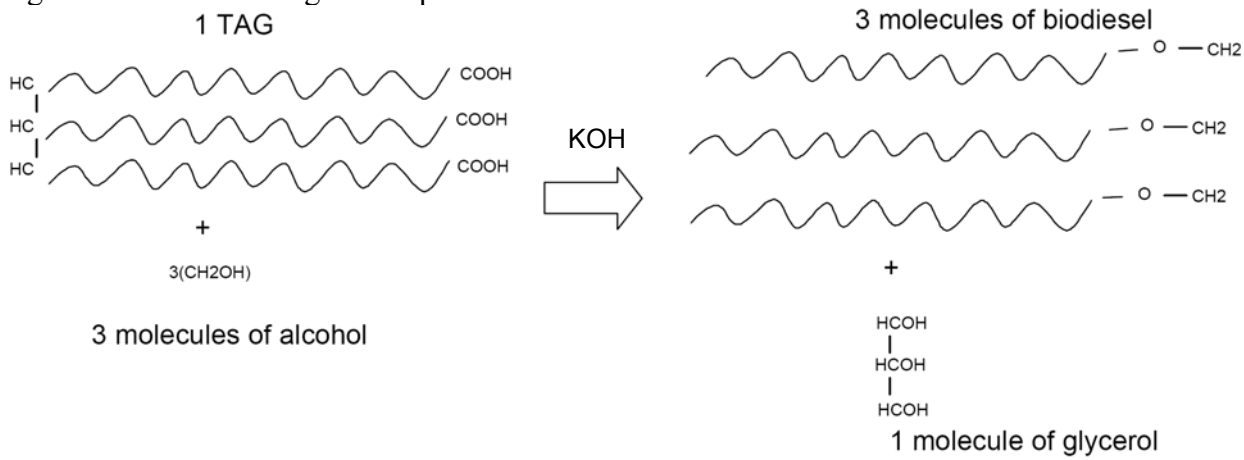

Figure 12: Transesterification of triglycerides with alcohol.[157]

Alcohol Selection

A variety of alcohols can react with oil, including methanol, ethanol, propanol, butanol, and amyl alcohol. Methanol is used most frequently not only because it is inexpensive, but also because it can react quickly with glycerides due to its polar nature and status as the shortest chain alcohol.[158] It is also worth noting that methanol allows the simultaneous separation of glycerol. In order to obtain glycerol separation with ethanol, both the oil and ethanol must be largely water-free. However, ethanol is preferred over methanol as it is derived from agricultural products and is renewable.[159]

The type of biodiesel is determined by the alcohol used in the transesterification process. Methanol produces methyl esters and ethanol produces ethyl esters.[160] The performance and heat

[155] Teall, 2005
[156] Ma and Hanna 1999
[157] Sheehan, et al. 1998b
[158] Zhang, et al. 1997
[159] Demirba 2003
[160] Otera 1993

contents of these esters are comparable. However, methyl esters produced slightly higher power and torque than ethyl esters, as demonstrated by engine tests. Some desirable attributes of the ethyl esters over methyl esters are significantly lower smoke opacity, lower exhaust temperatures and lower oil viscosity.[161]

It is important to note, although the final molar ratio of alcohol to glyceride is 3:1, a commonly accepted molar ratio of alcohol to glyceride is anywhere from 6:1 to 30:1 to ensure complete conversion.[162]

Catalyst Selection

Transesterification can either be alkali-catalyzed or acid-catalyzed. Acid catalysts include sulfuric acid, hydrochloric acid, and phosphoric acid. Alkali catalysts include sodium hydroxide, potassium hydroxide, carbonates (such as K_2CO_3), and various alkoxides (such as $NaOCH_3$).[163]

Acid-catalyzed processes have limitations. Most notably is the fact that transesterification occurs 4000 times slower with an acid catalyst than with an alkali catalyst at equimolar concentrations. Consequently, much more acid catalyst needs to be used and reaction times will be longer to produce similar yields. Acid-catalyzed reactions are typically used in situations in which either the glyceride or alcohol has a high water content. [164]

Alkali-catalyzed processes are characterized by having sensitivity to reactant purity, particularly with regard to the presence of water and free fatty acid content. Water causes the unfavorable side reaction of hydrolysis of the produced ester, resulting in saponification under alkaline conditions.[165] This side reaction consumes the catalyst and reacts with the esters, decreasing the yield. The free fatty acids can also react with NaOH or KOH, causing saponification. This soap that is formed also interferes with separation of the esters from the glycerol.[166]

NaOH and KOH are most commonly used in industry, due to their low cost and their fast reaction rate. However, a recent study by Miao et. al. indicated a lack of success in performing an alkali-catalyzed reaction for oil from microalgae. Miao points out that this may be due to the high acid value of the algae studied, which is 8.97 mg KOH/g (8.97 mg of KOH are required to neutralize 1 g of microalgae oil). Rather, sulfuric acid was used for an acid-catalyzed process. Sulfuric acid was used rather than other mineral acids due to its high acidity and wide availability.[167]

Reactor specifications

The maximum yield for algal biodiesel was found to be 75% at 50°C with a catalyst concentration of 60%.[168] The reaction was conducted isothermally as a batch process at

[161] Encinar, et al. 2002
[162] Demirba 2003
[163] Fukuda, et al. 2001
[164] Fukuda, et al. 2001
[165] Zhang, et al. 2003
[166] Basu 1996
[167] Miao, et al. 2006
[168] Miao, et al. 2006

atmospheric pressure. However, large-scale production may benefit in the development of a continuous reactor. Advantages of a continuous reactor include more fuel to be produced per unit of labor, the low cost and time for producing a large amount of fuel, and the potential for consistently producing a higher quality fuel through optimizations and design improvements.[169]

An option for a continuous reactor is an acid- catalyzed process. However, due to the presence of sulfuric acid for this process, it has been suggested that the reactor and piping be composed primarily of stainless steel to prevent corrosion.[170] Despite the high costs related to the use of stainless steel piping, acid catalyzed processes have been shown to have lower break-even prices than their alkali-catalyzed counterparts. The main factors in the after-tax rate of return were determined to be the cost of the feed oils, the plant capacity, and the sale price of biodiesel; the bare module costs associated with the plant are not as relevant.[171]

Although the sizing of the reactor is largely dependent on the volume of biodiesel desired, one should build a reactor large enough to accommodate the extra amount of catalyst needed as compared to an alkali-catalyzed process. It was suggested that the reactor be 2.1 meters in diameter and 6.3 meters long to handle approximately 1050 kg/hr of oil.[172] A plant design by Teall and Sickels showed production of biodiesel operating at a 98% conversion rate using methanol and KOH at 150°F and 20psi.[173] It is important to note; however, that algal oil has not been tested using this process. Due to a high fatty acid content, excess catalyst will be required to neutralize the algal oil for the reaction to proceed.[174]

Separation of Biodiesel and Glycerin Byproduct

In small-scale laboratory processes, separation by density and solubility of glycerin and biodiesel is conducted in a separating funnel. The less dense biodiesel is then collected and washed with petroleum ether and then followed by washing with water at 50°C. The ether is then allowed to evaporate, leaving behind the purified biodiesel.[175]

For larger scale processes, a more sophisticated technique can be employed as proposed by Petersen et al. This process, known as specific gravity separation, allows for the simultaneous separation of the esters and glycerin. After the reaction has taken place, the products are pumped to a settling tank. The contents are allowed to settle for 2 ½ - 3 hours and to cool to room temperature. Because the glycerin has a higher density than the biodiesel, it settles to the bottom, while the biodiesel is allowed to settle to the top. The glycerin is then drained out the bottom and allowed to pass through a density loop. The density loop creates a pressure head that is able to pump the biodiesel out of the top of the tank without the use of a pump as it is displaced by the density loop. The glycerin and biodiesel are collected separately. Since a pump is not used to remove the biodiesel from the settling tank, both energy and money are saved.[176]

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[169] Peterson, et al. 1999
[170] Zhang, et al. 2003.
[171] Zhang, et al. 2003.
[172] Zhang, et al. 2003.
[173] Teall 2005
[174] Miao, et al. 2006
[175] Miao, et al. 2006
[176] Petersen, et al. 2006

Biodiesel Production

The results presented in Table 5 present physical properties of microalgae biodiesel obtained from experiments performed by Miao et al. These properties are compared to those of petroleum diesel and biodiesel standards determined by the American Society for Testing and Manufacturing (ASTM).

Table 6: Comparison of diesel fuels.[177,178]

Properties	Microalgae Biodiesel	Petroleum Diesel	ASTM Biodiesel Standard
Density (kg/L)	0.864	0.838	0.86-0.9
Kinematic Viscosity (mm^2/s at 40°C)	5.2	1.9-4.1	1.9-6.0
Flash Point (°C)	115	75	Min 130
Solidifying Point (°C)	-12	-50 to 10	--
Heating Value (MJ/kg)	41	40-45	--
Acid Value (mg KOH/g)	0.374	Max 0.5	Max 0.5
Cold Filter Plugging Point (°C)	-11	-3.0 (Max -6.7)	Summer max 0; winter max < -15
Hydrogen/Carbon Ratio	1.81	1.81	--

Several similarities can be found when comparing the properties of microalgae biodiesel to petroleum diesel fuel. The density, solidifying point, heating value, and hydrogen/carbon ratio of both fuels are close to identical or within the ranges recorded, varying by less than 5%. Microalgae biodiesel has the distinct advantage of having a higher flash point temperature, proving it to be a safer fuel to handle. The cold filter plugging point is less than that of petroleum diesel, which is favorable to proper vehicle maintenance. The viscosity of microalgae biodiesel is greater than that of petroleum diesel, which may cause incomplete combustion, carbon deposition in the injectors, and fuel build-up in lubricating oils.[179] Blending the fuels together can minimize these problems. For example, a 25/75 biodiesel/petroleum diesel blend lowers the kinematic viscosity to 4.88 mm^2/s.[180] With the exception of flash point temperature, all of the physical properties of microalgae biodiesel conform to the ASTM standards for fuel-grade biodiesel. Considering all of these factors, biodiesel presents a feasible alternative to petroleum diesel.

[177] Miao, et al. 2006
[178] National Biodiesel Board
[179] Encinar, et al. 2002
[180] Ma and Hanna 1999

Biodiesel Purification

After the transesterification process and glycerin removal, the remaining biodiesel still contains contaminants. Adsorbent media have been investigated as purification methods for used vegetable oil, with the hopes that the media will return the vegetable oil to its initial viscosity, color and fatty acid content. Of the factors influencing the optimal adsorbent media, the most relevant to biodiesel production is the free fatty acid (FFA) content. It was determined that magnesium silicate was most effective in reducing the FFA content of used vegetable oil, with 3% magnesium silicate, provided the greatest reduction in FFA.[181] Similar studies revealed comparable reductions in FFA when compared to untreated fryer oils. A mixture of three adsorbents (Hubersorb® 600, 3 wt.%; Magnesol®, 3 wt.%; Frypowder®, 2 wt.%) reduced the FFA content by 61.9% after 32 consecutive hours of cooking. When the oil was filtered daily during the study, the FFA content was reduced by 64.2% for the same mixture of adsorbents.[182] Of these adsorbents, Magnesol® is currently being marketed as a water-free wash for biodiesel.

Magnesol® has been used as an adsorbent for the purification of vegetable oil, with notable success in reducing the FFA content.[183] This trademarked product from The Dallas Group would eliminate the water-washing step that is generally involved with biodiesel manufacturing.[184] It was found that biodiesel purified with Magnesol® met all ASTM standards, while biodiesel washed with water from the same batch did not meet the standards. Furthermore, the fuel washed with Magnesol® had a lower soap and sodium content than the fuel washed with water.[185]

[181] Maskan, Bagci. 2003
[182] Lin, Akoh, Reynolds. 2000
[183] Maskan, Bagci. 2003
[184] Bryan, 2005
[185] Bryan, 2005

PROJECT GOALS

Piedmont Biofuels, a biofuels cooperative, has developed a biodiesel production facility that uses alternative forms of biologically derived oil. The ultimate goal of this project is to design a production facility that can produce up to 1000 gallons/week of biodiesel derived from algae. In order to achieve this goal, a series of experiments on a laboratory scale will be completed to determine a method to effectively harvest algae, extract oil, and convert the algal oil to biodiesel. Ideally, the method chosen should be relatively simple to scale up to a larger scale so that future production of algal biodiesel is economically and energetically feasible.

Commercially available algal samples native to North Carolina will be used as a starter culture for a small-scale indoor algae "raceway." The raceway design, proposed by the US Department of Energy, is an efficient way for algae cultures to have access to light and carbon dioxide (Figure 1).[186] By using native algae species, a realistic model can be developed to determine how much biodiesel can be produced using algae from agricultural waste ponds in North Carolina.

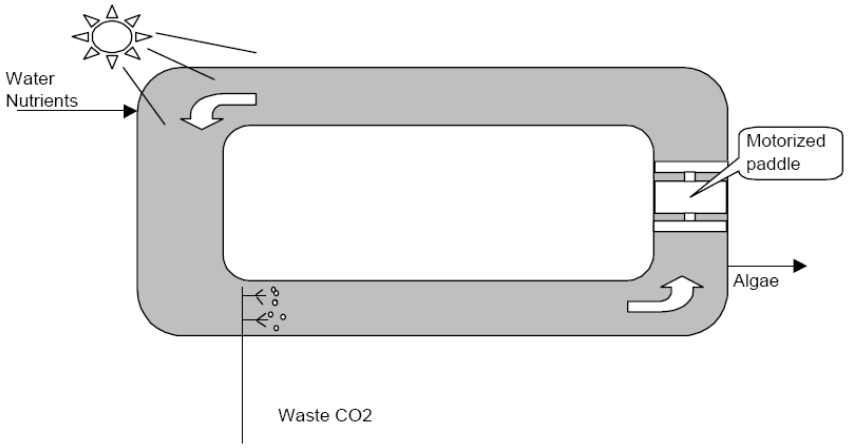


Figure 13: Design of an algae pond raceway.[187]

The algae will be grown continually under optimal growth conditions and harvested at regular intervals so that the indoor culture will be healthy and viable. Temperature, pH, light intensity, nutrient availability, and carbon dioxide access will all be controlled to maintain exponential growth of algae. By periodically harvesting algae, overpopulation, which may lead declining growth rates, in the indoor raceway can be avoided.[188] Potential harvesting techniques include flocculation, filtration, sedimentation, and flotation.[189] The design of the indoor raceway will be modified so that the water pumped out to harvest the algae can be returned to the raceway.

[186] Seehan, et al. 1998
[187] Seehan, et al. 1998
[188] Gaultieri, et al. 2006 213
[189] Bilanovic, Shelef, and Sukenik 1988

While algae is being grown and harvested, methods of extracting algal oil will also be investigated. Oil is commonly extracted from biological sources using chemical solvents, liquefaction, or supercritical carbon dioxide.[190,191,192] One step in this process will be to determine which method of extraction is preferred based on economic feasibility, environmental impact, and resource availability. Oil will be extracted from commercially available algae paste, and then if successful, from algae grown and harvested from the indoor raceway.

The primary goal for the year is to produce one liter of algal biodiesel. This will be achieved by developing a process, which extracts oil from algae and converts it into biodiesel.

[190] Miao and Wu 2006
[191] Yang, et al. 2003
[192] Mendes, et al. 1995

PLAN OF ACTION

A water sample obtained from the pond at the North Carolina State Aquaculture center contained numerous algal species. Of these species, *Scenedesmus*, from the phylum Chlorophyta, was present in large concentrations. This species exhibits long filaments on the outside of the cell that help space the algae allowing maximum light and nutrient absorbency. This makes it an ideal candidate for cultivation. The water sample from the pond also contained algae consuming organisms, so a pure culture of *Scenedesmus* will be used to grow the algae to maximize yield.

The *Scenedesmus* strain was obtained from Carolina Biological Supplies. The strain was placed in two flasks containing 5ml/L F/2 medium and allowed to grow. Once the concentration of algae has reached 200,000 cells/ml, the algal species will be placed in the photobioreactor containing the f/2 medium (Figure 15). A metal halide fixture on a 12:12 hour light to dark ratio will provide necessary light for photosynthesis while a power-head pump will circulate the water to minimize algae self-shadowing. Carbon dioxide will be bubbled into the photobioreactor. The medium composition will be tested at least twice a week and kept constant by adding necessary amounts of f/2 formula. The temperature will be monitored over the period of this experiment. The *Scenedesmus* strain of algae should be kept from 18°C to 25°C. If necessary, a heater will be added to the tank to keep the water temperature constant. The pH of the medium will be tested and kept at a range of 6.8-7.6 by adding necessary amounts of CO_2. The amount of light emitted by the metal halide will be tested using a light meter. The use of a hemocytometer will allow the algal cells in a given sample to be counted. A photospectrometer will measure the absorbance of a given sample. A calibration curve relating cell count to absorbance will be created. The algae will be allowed to grow until one of a number of limiting conditions is reached that cause the number of cells to remain stationary. These limiting factors include self-shadowing, carbon fixation, and medium depletion.

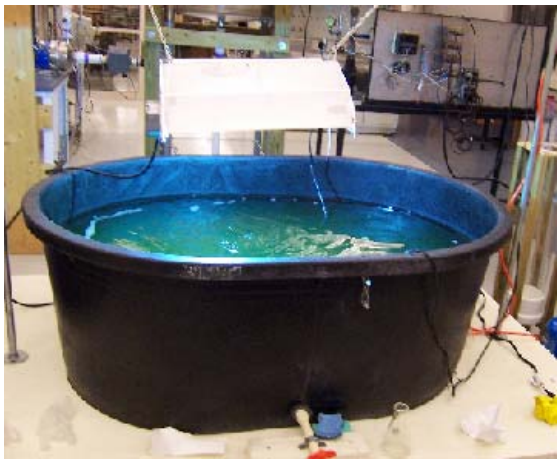


Figure 14: Algae growing in the photobioreactor found in the undergraduate teaching lab.

In order to harvest algae, approximately 33 gallons (one-third the total volume) from the algae tank will need to be removed in order to separate the algae from the liquid. Algae will be harvested when the algae culture reaches stationary cell numbers phase. Approximately 50 mg/L of chitosan, used as a flocculant, will be added to the algal suspension removed from the tank. A dissolved air floatation apparatus (Figure 15) will be built by connecting a pressure vessel to an acrylic or PVC tube, which will serve as a flotation column. The vessel requires two inlets and one outlet. Pressurized air and liquid from the algae tank will flow into the pressure vessel so that the algal suspension is saturated with air at 40 psig. From there, the saturated algal suspension will travel to a flotation column, where the pressure drop will allow the algae to concentrate in the upper layer of the

column, forming a compaction zone. Oil can then be extracted from the algae removed from this compaction zone. It is anticipated that harvesting will be required once every one to two weeks.

Figure 15: DAF system for separation of algae from suspension.

Due to the ease with which solvents are used as extraction media for biologically derived-oils, implementing a process that relies on this technology would be sufficiently simple. As mentioned earlier, one drawback to solvent extraction is the health concerns of the solvent mixture. A blend of chloroform, methanol and water would provide the highest yield, whereas a reduction in health concerns with the use of hexane causes a decrease in oil production.

Liquefaction solves the issue of health concerns, while maintaining high oil solubility. An alternative to solvent extraction would be to obtain an autoclave that is capable of maintaining temperatures and pressures required for this process, generally above 300°C and 2 MPa. The high oil production that is achieved with liquefaction counterbalances the stringent conditions. Conversely, due to the high cost of the process vessel, purchasing an autoclave is prohibitively expensive.

The plan of action for the oil extraction step is to obtain an autoclave on campus and use that for liquefaction. If an autoclave is not found by the beginning of January, the oil will be extracted using solvents. Liquefaction in an autoclave would be easily scalable, and reduces the health hazards as compared to solvent extraction. Additionally, the yields are comparable to solvent extraction using the Bligh and Dyer method (a ternary mixture of methanol, chloroform, and water in a 2:1:0.8 volume ratio) and would not require the drying step that hexane extraction requires. However, if an autoclave is not available, hexane extraction will be used, and very small experiments will be run using the Bligh and Dyer method for comparison purposes. Additionally, extraction will be conducted on algae paste in case the algae cannot be grown or harvested.

To produce approximately one liter of useable biodiesel from oil, an acid/base transesterfication process will be used. Following a transesterfication processes, methanol and NaOH will be required to react with algal oil in order to produce biodiesel where sodium hydroxide will be used as the catalyst for the reaction.

The procedure for producing biodiesel is as followed:[193]

One liter of algal oil will be heated to 60°C for 15 minutes in order to separate any water from the oil. The oil will then be placed into a settling tank over night. The following day, the oil

[193] Kac

will be heated to 35°C in order to dissolve any solid fats present. Next, 80 mL of 99%+ pure methanol will be added to the heated oil. Add a stir bar rotating at 500 rpm to the oil and mix the contents together for five minutes. One milliliter of 95% H_2SO_4 will then be added to this mixture. The mixture will then continue to be heated at 35°C and mixed for one hour. The mixture will then be unheated while being mixed for another hour. Allow the mixture to settle overnight.

While the mixture is settling overnight, a sodium methoxide mixture will be prepared. 120 mL of methanol and 3.1 g NaOH will be mixed together until the sodium hydroxide is completely dissolved. Allow this new mixture to settle overnight as well.

The next day, half of the prepared sodium methoxide mixture will be added to the unheated oil mixture and mixed for five minutes. This new mixture will then be heated to 55°C. The rest of the sodium methoxide mixture will then be added and mixed together at 500 rpms. After 25 minutes, start draining the glycerine by-product from the bottom of the mixture. Allow the top biodiesel product to sit for one hour. A transesterfication reactor used in past biodiesel experiments is located on campus. It can be used for this process.

Determination of Acid Value of Oil

One possible problem that might be encountered during the biodiesel conversion of oil is a high acid value of fatty acids in *Scenedesmus* algae. In oils with high acid content, free fatty acids can react with the catalyst, causing saponification and hindering biodiesel conversion. These acids can consume the catalyst before it has a chance to participate in producing the biodiesel product. Because the free fatty acid content in *Scenedesmus* algae is unknown, an additional amount of alkaline catalyst may have to be added to neutralize the algal oil to enable it to be suitable for conversion. In order to determine the free fatty acid content of the algae, a titration procedure will be utilized.

A pH indicator will be obtained for the titration. Phenolphthalein, an indicator that detects pH's 7 and higher, can be used to determine when the solution becomes neutral. A small amount of algal oil will be measured and placed in a beaker, along with a pH indicator. NaOH will be slowly titrated into the beaker until the indicator indicates a pH of 7. The process will be repeated to ensure the amount of NaOH added is the same. The ratio of the amount of oil to the amount of NaOH needed to neutralize the solution will be added to the catalyst that will be needed in the conversion process.

Washing of Biodiesel

An adsorbent, such as Magnesol® or other activated silicate, will be used to wash the biodiesel. This will reduce the presence of any non-water soluble substances and greatly decreases the chance of transesterification.

Testing of Algal Oil and Biodiesel

Algal oil and the biodiesel produced from the transesterification process will be tested in order to determine if the conversion was successful or not. The specific gravity data and molecular weight of oil are based upon experiments performed by Miao and Wu. These assumptions are shown in the table below:

Table 7: Comparison of Algal Oil and Biodiesel.[194]

	Oil extracted From algae	**Biodiesel produced through Transesterification**
Density (g/L)	912	864
Molecular weight (g/mol)	933	296

For the molecular weight for biodiesel, the molecular weight of methyl oleate is assumed, a methyl ester that is a main component of biodiesel. Data is not available for the exact composition of biodiesel produced from algal oil, so this value may differ from the actual molecular weight. A simple mass scale will be used to determine the density of the reactant and product, while Mass Spectroscopy will be used to determine the molecular weight of the product.

[194] Miao and Wu, 2006

PROCESS DESCRIPTION

To effectively cultivate *Scenedesmus* algae indoors, the PBR used by last year's senior design team was outfitted with a 250 watt metal halide lamp and two 300 gph pumps. The new lamp provides the wavelength and light intensity necessary for optimal algae growth, while the new pumps prevent algae self-shadowing that occurs when cell concentrations increase. After the algae were cultured in 250 mL flasks, they were transferred to the PBR where higher yields could be obtained. Water level and conditions such as pH, nitrite, nitrate, and ammonia were monitored throughout the cultivation process to ensure the algae were experiencing optimal growth conditions.

Concentration Determination

A UV-Visual Spectrophotometer is used to determine the absorbance of algae at a specific wavelength. A relationship between the absorbance of algae and the algal cell count is also being developed through the use of a hemocytometer and microscope. Drops of algal solution from the bottom of the photobioreactor are placed on square grids located on the hemocytometer. Between seven and ten squares are analyzed and recorded. The average amount of cells per square is found and divided by the volume of the square to give a concentration of algal cells per unit volume in the PBR.

Algae Harvesting

Flocculation coupled with dissolved air flotation is used to separate algae from the algal suspension. Either aluminum sulfate or chitosan can be used as flocculants. Once the flocculant is added to the algal suspension, 15% of the suspension is saturated with air at 40 psig and the rest is poured into the flotation column open to the atmosphere. When the pressurized suspension is released to the column the pressure drop causes the air to come out of solution, carrying the algal cells to the top of the suspension. This results in a layer of concentrated algae cells at the top of the suspension, which can be collected. Oil is then extracted from the concentrated algae using either solvent extraction or liquefaction.

Oil Extraction

There are three methods of extraction used to obtain oil from algae – solvent extraction using ethanol, solvent extraction using the Bligh and Dyer mixture, and liquefaction followed by solvent extraction. The final process design will depend on the experimental data, as the technique that provides the best balance between oil yields and chemical hazards will be chosen as the extraction method.

With ethanol solvent extraction, the water from the concentrated algae slurry is fully evaporated off leaving only the algae. After this, the dried algae are transported into a vessel where it is agitated in the presence of ethanol. The oils are extracted into the ethanol solvent and a filtration step is introduced to remove the solid biomass from the liquid oil phase; the ethanol is

then boiled off and recycled. The remaining algal oil then continues to the biodiesel conversion step.

In the Bligh and Dyer method for oil extraction, the algal slurry does not require a drying step if the water content is below eighty percent. The slurry is moved into a process vessel where methanol and chloroform are added under high agitation. The slurry-solvent mixture is agitated for five minutes, then the biomass is filtered, and the remaining liquid allowed to separate. The methanol is decanted off and recycled which is followed by the purification of the lipids through flash distillation of chloroform. The chloroform is recycled and the remaining algal oils continue to the biodiesel conversion step.

The third method for oil extraction is liquefaction, which requires the use of an autoclave. In liquefaction, the biomass is moved to a pressure chamber where nitrogen and sodium carbonate are introduced and the pressure is raised to 3MPa. The temperature is raised isobarically to 250°C and held for thirty minutes. The pressure and temperature are reduced and the biomass is transported to a vessel where methylene chloride is added under agitation. The lipids are extracted and the biomass is filtered and removed. The solvent is removed via flash distillation and the algal oils are processed further in the biodiesel conversion step. Alternative solvents to methylene chloride can be used for the extraction.

Biodiesel Conversion

The alkali-catalyzed method using NaOH and methanol is used for the transesterification of algal oil to create biodiesel. Biodiesel conversion is performed as a batch process in a beaker. The algal oil is added to the beaker and placed on a hot plate/stirrer and heated to approximately 50°C. The methanol and NaOH would be added to the beaker, and stirred until dissolved. The mixture would then phase separate as the glycerin has a higher density and is insoluble in biodiesel. The biodiesel phase would be extracted and washed with Magnesol® to remove any remaining impurities in the biodiesel phase.

EQUIPMENT LIST

Photobioreactor

Item	Size	Material	Quantity
Basin	140 gallon	ABS Plastic	1
Ball Valve	½" ID	PVC	1
Metal Halide Lamp	250 W		1
Submersible Power Head	300 gal/hr		2
Piping	½" ID	Steel	
180° joint	½" ID	Steel	1
90° elbow	½" ID	Steel	1
Timer			1
CO_2 Tank	50 lbs		1

Dissolved Air Flotation Apparatus

Item	Size	Material	Quantity
Piping	¾" ID	PVC	
90° elbow	¾" ID	PVC	2
Ball Valve	¾" ID	PVC	2
T-adapter	¾" ID	PVC	1
Piping	½" ID	PVC	
Ball Valve	½" ID	PVC	1
Piping	¾" ID	Steel	
Ball Valve	¼" NPT	Steel	1
Adapted End Cap	6" ID	PVC	1
Screw End Cap	6" ID	PVC	1
Tubing	6" OD	Acrylic	1
Hydro-pneumatic pump tank	2 gallon		1
Piston Pressure/vacuum pump	1 hp		1

EXPERIMENTAL DESIGN

Algae Growth

Algae are cultivated by transferring the test-tube *Scenedesmus* culture obtained from Carolina Biological Supply into four prepared 250 mL flasks. Each flask contains 150 mL tap water conditioned with dechlorinator and 2 drops each of f/2 fertilizer solutions A and B. The flasks are placed in a well-lit window until the medium turns green signaling adequate algae growth. They are then transferred to the PBR where water circulation and a larger volume of medium will allow for greater yields.

The PBR is an indoor cultivation facility consisting of a 140-gallon tank with illumination provided by a 250-watt metal halide lamp. The water is circulated by two 300-gph pumps; one is mounted on the wall of the tank and the other is mounted on the bottom of the tank. In order to create a suitable medium for the algae to grow, adequate amounts of dechlorinator and f/2 fertilizer formula are added to the tap water in the PBR. The lamp is cycled 12 hours on and 12 hours off to simulate the day/night cycle. The pumps circulate the water to minimize self-shadowing that occurs when cell concentrations increase, ensuring that all cells get an adequate amount of light.

Concentration Experiments

Algae concentration experiments started during the third week of algae growth. Samples are taken from five different locations in the photobioreactor. For the UV-Visual experiments, two quartz cuvettes are used to hold the reference and sample solutions. To create a baseline, approximately 1 mL of the f/2 nutrient solution is placed in both the reference and sample cuvettes. The baseline is then measured for the f/2 solution. After a baseline is established, the sample cuvette is emptied. Approximately 1 mL of algae water is placed in the empty cuvette, and the absorbance for that sample is measured. The absorbance of these samples is recorded at both 223 nm and 680 nm.

The concentration of algae can be calculated through the use of the Beer-Lambert Equation, which states:

$$A = \varepsilon l c \quad (2)$$

In equation 2, A is the absorbance, ε is the molar absorptivity coefficient, l is the path length in centimeters, and c is the concentration in molarity. The absorbance at a specific wavelength is measured using the spectrophotometer. The absorbance is related to the algae concentration using a hemocytometer. Cell counts conducted with the hemocytometer allow concentrations of the algal suspension to be measured in units of algal cells per unit volume. Absorbance can also be related to algae wt/v% using a weight analysis. To do a weight analysis, approximately 5 mL of algae solution is placed in the container. This container is placed on a heating plate, and the algae solution is boiled until all the water has evaporated from the algae solution. The container is then weighed again to determine the mass of algae that was in the solution. This mass of algae

divided by the volume of algae solution is the concentration of algae in that sample in units of grams per unit volume.

Algae Harvesting

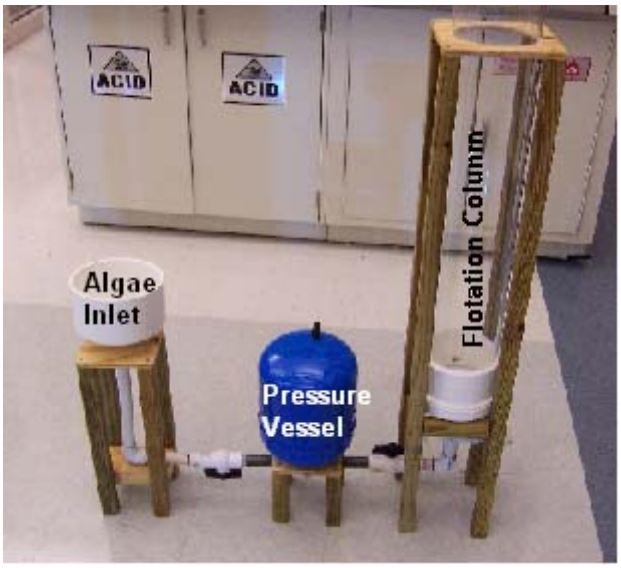


Figure 16: DAF system for separation of algae from suspension.

The procedure for use of the DAF (Figure 16) requires the addition of a flocculant to 14 L of algal suspension. The solution containing flocculant and partially coagulated algae is then added to the DAF. Fifteen percent of the solution is pressurized to 40 psi, while the remaining volume is added to the flotation column. After holding the pressure in the vessel for five minutes, the pressure is released by opening the valve between the column and the pressure vessel. The pressurized water is released over the next ten minutes. Fifteen minutes are allowed for separation following the release of the pressurized water. Following the fifteen minutes, a portion of the quiescent liquid is recycled from the bottom of the column and re-pressurized. Again, fifteen percent of the liquid is pressurized with the remainder in the column.

RESULTS AND DISCUSSION

Algae Growth

During the second week of cultivation in the PBR, the algae entered the exponential growth phase. There was a noticeable cloudy, green tint to the medium on day 9. The water conditions also showed evidence of metabolic activity. This is seen by the gradual increase in pH, decrease in nitrite and the presence of ammonia.

By the 13th day, the medium was opaque and the bottom of the PBR was not visible. The cloudy green tint from four days before became dark green and algae were beginning to grow on the sides of the tank. The water conditions also indicated an accelerated growth rate.

On the 16th day, the average concentration of algae based on five different samples taken from the PBR was determined to be 174 mg/L using the weight analysis method.

Table 8: Nutrient and pH levels of algae growing in the PBR for the past month.

Date	pH	Nitrate (mg/L)	Ammonia (mg/L)	Nitrite (mg/L)
Day 1 (1/20/2007)	7.00	18.00	0.05	2.00
Day 7	7.30	5.00	0.50	0.25
Day 9	7.40	7.50	0.25	1.00
Day 13	7.55	7.50	0.10	0.90
Day 17	7.70	8.50	0.10	0.50
Day 19	8.80	7.50	0.10	1.00
Day 23	8.30	7.50	0.10	0.60
Day 27 (2/15/2007)	7.10	7.50	0.10	0.50

Determining Concentration

A cell count was performed using the hemocytometer on a sample taken from the PBR on the 20th day. The results showed the PBR had an average concentration of 6,800 algal cells/mL. Using this same sample, UV-Visual spectrophotometer was used to determine the absorbance of the samples (Tables 9 and 10). The sample absorbed in the light in the linear range around 219-224 nm for the undiluted sample and for subsequent dilutions in f/2. However, the literature reports that algae absorb well at 680 nm.[195,196] At this wavelength, the undiluted sample had an absorbance of 0.110. Such a low absorbance is more sensitive to noise and so is not considered to be as reliable as higher absorbances in the linear region. It is likely that the algae concentration was not great enough at that time to have a higher absorbance.

[195] Ma et al. (2002)
[196] Raven et al. (2001)

Table 9: UV-Visual Results of undiluted and diluted algae studied at the 222 nm peak.

Samples		Wavelength	Absorbance
Undiluted algae		224 nm	0.561
800 μL algae	200 μL f/2	222 nm	0.426
600 μL algae	400 μL f/2	222 nm	0.221
400 μL algae	600 μL f/2	222 nm	0.152
200 μL algae	800 μL f/2	219 nm	0.086

Table 10: UV-Visual Results of undiluted and diluted algae studied at the 680 nm peak.

Samples		Wavelength	Absorbance
Undiluted algae		680 nm	0.110
800 μL algae	200 μL f/2	680 nm	0.077
600 μL algae	400 μL f/2	680 nm	0.056
400 μL algae	600 μL f/2	680 nm	0.040
200 μL algae	800 μL f/2	680 nm	0.025

DAF

Another cell count was performed from the algae harvested on February 11th. The harvested algae had a concentration of approximately 314,000 cells/mL - two orders of magnitude greater than the unharvested algae previously counted. The absorbance of this sample was measured at 250 nm and 680 nm. The wavelength 250 nm was chosen because it is believed that proteins on the cell wall of algae are absorbing here. At both wavelengths, the absorbance was not in the linear range. A 3:5 dilution in f/2 also resulted in absorbances outside the linear range. Figure 17 shows the range of absorbances for the 3:5 dilutions. A clear peak is seen at 680 nm, confirming previous results in the literature. Because the peak at 250 nm is not as well defined as the one at 680 nm, 680 nm is a more reliable wavelength to measure algae concentration if the cell concentration is high enough. Further cell counts and spectrophotometry measurements will have to be taken in order to correlate cell concentration to absorbance at both 250 nm and 680 nm.

The procedure for use of the DAF requires the addition of a flocculant to 14 L of algae medium. Initial runs of the process were attempted using chitosan as the flocculant. One run was conducted at a concentration of 50 mg chitosan per liter, with poor flocculation. Increasing the concentration of chitosan to 200 mg per liter also provided poor flocculation. Chitosan was found to be insoluble in the medium; aluminum sulfate (alum) was used in the later run. Alum was added at the concentration of 150 mg/L with minor flocculation being noted immediately upon dissolution.

The solution containing alum and partially flocculated algae was then added to the DAF. Fifteen percent of the solution was pressurized to 40 psi, while the remaining volume was added to the depressurization column. After holding the pressure in the vessel for five minutes, the pressure was released by opening the valve between the column and the pressure vessel. The pressurized water was released over the next ten minutes. The tank was presumed to be empty when large bubbles of air entered the column. Upon noticing the large bubbles, the valve

between the column and the pressure vessel was closed. The large bubbles disturbed the concentrated algae phase at the top of the column that had accumulated during depressurization.

Separation increased dramatically over the fifteen-minute flocculation time, as there were no eddy currents that inhibited the floatation of the algae. After the second pressurization and floatation, the algae were at a concentration of 8.1 mg/mL in the floated phase. Further settling of the floated phase indicates that additional separation is possible through centrifugation.

Table 11: UV-Visual results of concentrated algae at both the 250 nm and 680 nm peaks.

Samples		Absorbance at 250 nm	Absorbance at 680 nm
Undiluted algae		4.547	3.999
600 μL algae	400 μL f/2	3.340	3.287

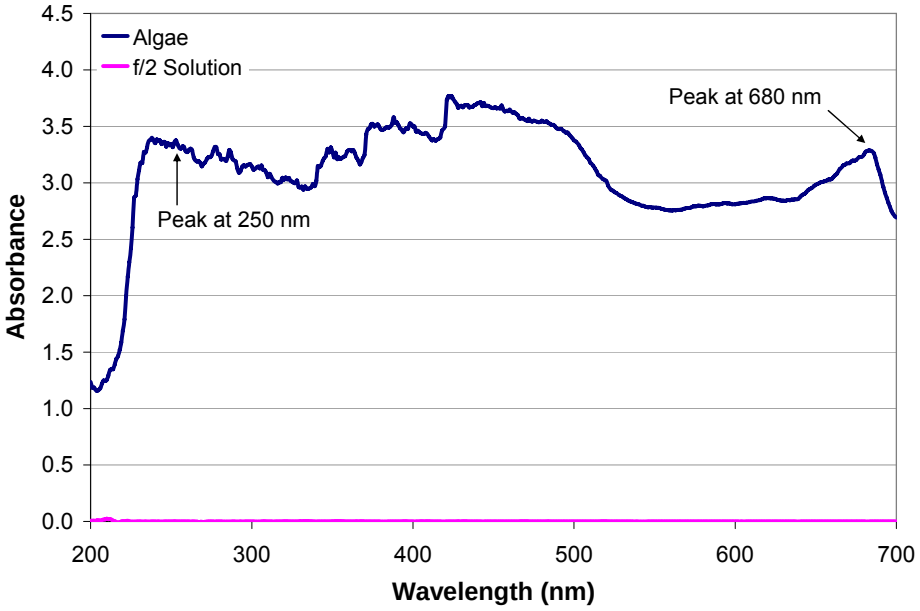


Figure 17: UV-Visual results of concentrated algae from 200 to 700 nm.

SITE LOCATION

The algae biodiesel system can be used in conjunction with a coal burning plant. Carbon dioxide emissions from the coal plant would be sent to the algae PBR. The algae for photosynthesis and cell growth use these CO_2 emissions. Through the use of an algae biodiesel system, coal burning power plants could lower carbon dioxide emissions to the environment. The coal power plant can then use the biodiesel that was converted from algal oil to power utilities in the plant.

An advantageous partnership could be established with Progress Energy, a company that provides energy to customers in the southeastern United States. The company's Asheville plant, which consists of two coal-fired units and two combustion turbine units, would be an ideal location to implement the algae to biodiesel system. As a result of the 2002 North Carolina Clean Smokestacks Act, Progress Energy has decided to invest $190 million to reduce air emissions from the Asheville power plant. This money could aid in the development of an on-site algae to biodiesel process.

Progress Energy also has another large plant in Roxboro, North Carolina. With one combustion turbine, and four coal-fired steam plants, the Roxboro plant is one of the largest power plants in the United States. The application of an algae biodiesel system at the Roxboro plant would help reduce the large amount of carbon dioxide emissions coming from the plant.

The algae to biodiesel at the Asheville or Roxboro site could take on any number of forms. The simplest design would be to develop an open-pond system, in which carbon dioxide emissions from the coal plant are piped into the algal suspension. A disadvantage to this design is that it is more sensitive to outside influences, such as climate change or contamination. A solution to this problem would be to build a greenhouse around the pond. This would allow algae to be grown year-round and would eliminate the threat of species contamination.

ENVIRONMENTAL CONSIDERATIONS

Environmental Impacts of Chemicals Used

Because sustainability is a compelling argument for the use of biodiesel, focus has been placed on creating a process that is as environmentally friendly as possible. Life-cycle analysis should be conducted on all of the chemicals involved in the overall process.

During the oil extraction phase, possible harmful environmental effects must be taken into account when selecting a solvent. Among the standard solvents used in the extraction of bio-oils, a Bligh and Dyer mixture containing methanol, chloroform, and water has been shown to have the highest yields of oil.[197] However, chloroform evaporates easily into the atmosphere, and, although it does break down, it is a slow process. Chloroform dissolves easily in water, where it persists for a significant amount of time.[198] Any leaks from the process should be assumed to enter into the groundwater.

A better candidate for solvent extraction, from an environmental perspective, is ethanol. In literature, ethanol was found to have the highest yields among standard solvents, and is a sustainable chemical that can be derived from feed crops.[199] Unlike many solvents, ethanol does not contaminate ground water supplies.[200] At low concentrations, ethanol is rapidly metabolized by most living organisms without apparent harm. At high concentrations, such as one that could result from a leak or spill, ethanol can have substantial acute effects on a wide range of biota, while it can cause death to many microbes.[201] Ethanol evaporates easily, which results in higher emissions of smog-forming compounds. Ethanol can also act as precursor leading to the formation of photochemical smog.[202]

EPA Regulations

There are currently no laws specifically regulating the release of biodiesel to the environment. However, de facto practice under current EPA rules is to control biodiesel in the same way that animal fats, vegetable oils and petroleum oils are controlled under oil spill laws and regulations. These statutes for oil regulation fall under the Federal Water Pollution Control Act, which states that no oil can be released that affects the public welfare or the environment, including: wildlife, fish, private property, or shorelines.[203]

[197] Mendes, et al. 2006
[198] ATSDR, 1997
[199] Mendes, et al. 2006
[200] Powers et al. 2001
[201] Arena, 1999
[202] Oanh, 2004
[203] FWPCA, 1996

The Oil Pollution Act of 1990 also regulates oil that is released to the environment.[204] The Oil Pollution Act dictates that oil storage facilities and vessels must submit to the federal government plans detailing how they will respond to large spills. Again, biodiesel facilities and tanker vessels are also controlled the same way petroleum facilities and petroleum oil tankers are treated.

The EPA does not regulate the emission of ethanol. However, the EPA has proposed to apply similar air permit regulations for producing ethanol as currently exist for other fuels.[205]

[204] OPA, 1990
[205] EPA Press Release, 2006

RELEVANT SAFETY CONCERNS AND DESIGN ISSUES

The use of systems operating at high pressures and temperatures as well as chemical solvents poses several safety concerns. Knowledge and understanding of potential risks involved can help to minimize accidents. Each step in the overall process involves either equipment or chemicals that pose a serious risk to the health of anyone performing each step.

Chemicals

Chemicals including chitosan, chloroform, and methanol are used in the overall process. Chitosan used for algae separation is a skin, eye, and lung irritant.[206] Special care is used when handling this chemical to avoid contract with the body as much as possible. A fume hood is used to when handling this chemical. Safety glasses, gloves, and mask are worn at all times when handling this chemical.

Chloroform used for oil extraction is extremely harmful and listed as a human carcinogen. Inhalation and ingestion of the chemical can be harmful and may also be fatal. Exposure to chloroform can irritate the skin and cause reproductive damage.[207] This chemical is handled in a fume hood to minimize exposure time. Safety glasses, glove, and mask will be worn at all times when using chloroform.

Methanol used for algal oil conversion is a skin, eye, and respiratory tract irritant. In addition, reproductive and fetal effects have also been documented. Methanol is a flammable liquid. If swallowed, methanol may be fatal or cause blindness. If inhaled, methanol may cause nausea, vomiting, or diarrhea. Methanol can also cause kidney, heart, or liver damage.[208] Safety glasses and gloves are worn at all times when handling this chemical.

As for all chemicals, safety glasses and gloves are worn at all times. Material Safety Data Sheets (MSDS) for each chemical can be found in the appendix.

Equipment

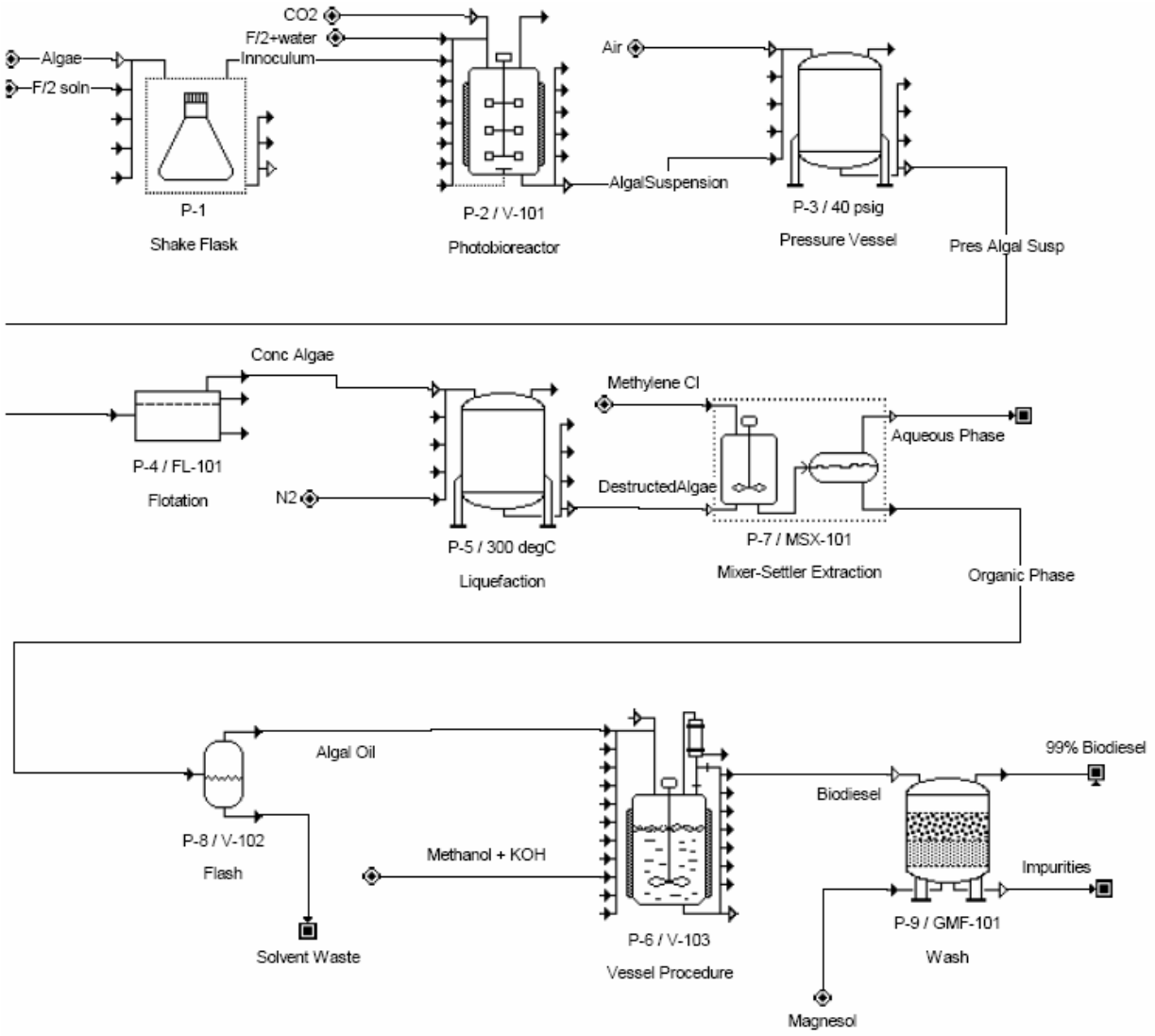
The DAF system used to separate algae from water is operated at 40 psig. Leaks may develop in the system and cause damage to the system or the person operating the device. In order to minimize any damage caused by the device when pressurized, all pressurized areas of the system are constructed out of stainless steel tubing capable of handling elevated pressures. The algal water solution entering into the system is pressured in a water pump able to withstand the pressurized liquid. Areas of the system that are non-pressurized are constructed out of PVC tubing. Special care will be required when operating this device including: always wearing safety glasses and gloves. In addition the device should never be operated alone.

[206] MSDS Chitosan
[207] MSDS Chloroform
[208] MSDS Methanol

An autoclave system used for extracting oil from algae is operated at temperatures exceeding 1200°C. Due to this high operating temperature, steam and high pressures may develop in the system. Heat resistant gloves and safety glasses are worn at all times especially when placing or removing the sample. Solvents including chloroform cannot be autoclaved as these chemicals are flammable and may harm the operator. In addition to the DAF system, this device should never be operated alone.

For algae growth, a CO_2 tank is used to provide the PBR with the necessary amount of CO_2. Small amounts (how much, 1 bubble per 4 sec.?) of CO_2 will flow into the PBR. Because the reactor is located in the undergraduate teaching laboratory, special care will be required to reduce the amount of CO_2 build up in the room. A CO_2 detection device will monitor any build up that may happen in the room. When moving the CO_2 tank, more than one person will be needed to move the tank to its desired location.

PROCESS FLOW DIAGRAM



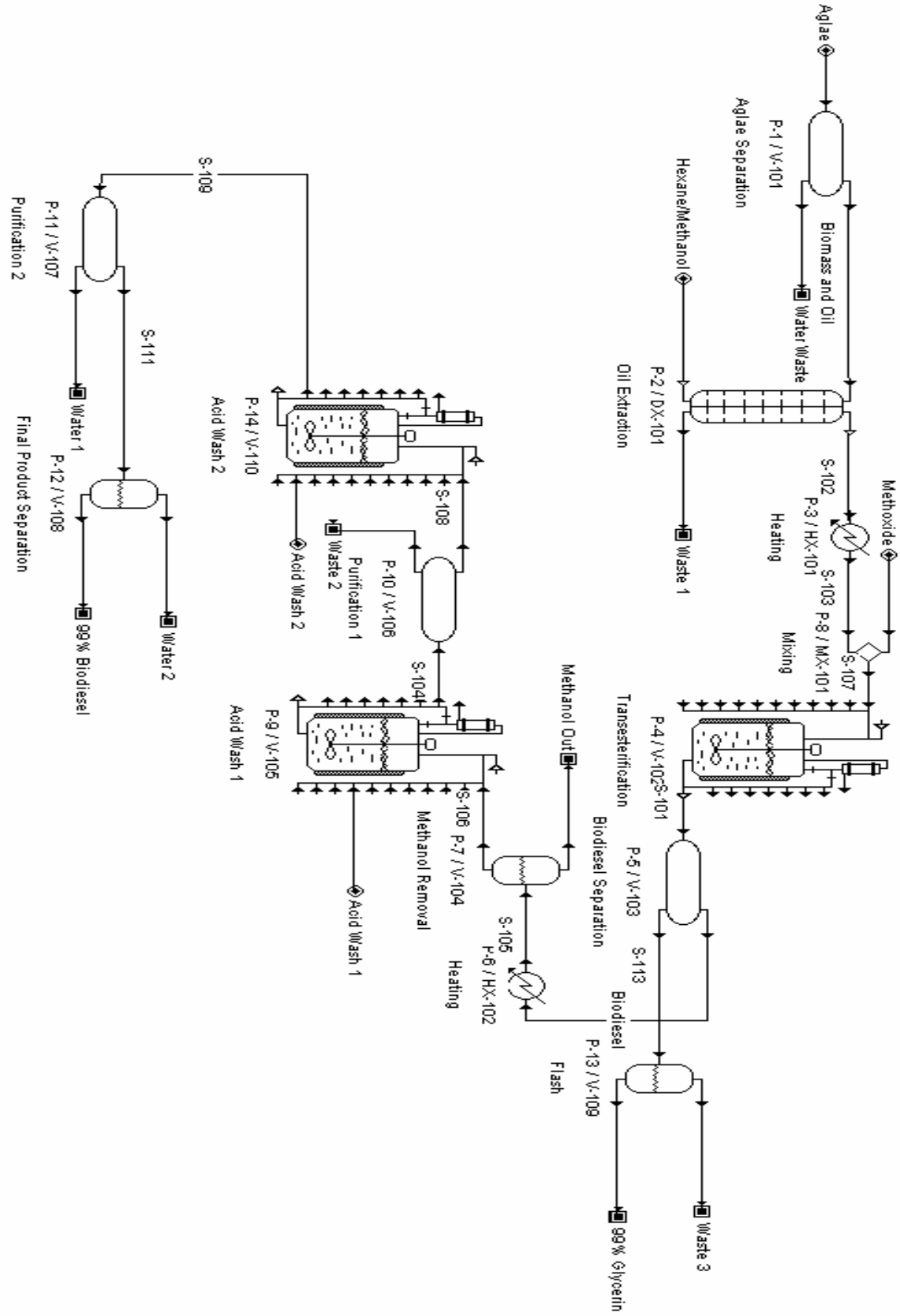
SIMULATION RESULTS

Table 12: Economic analysis of equipment used in overall process.

Name	Type	Units	Size (Capacity)	Material of Construction	Purchase Cost ($/Unit)
V-101	Decanter Tank	1	66.37 L	SS-316	26,000
DX-101	Differential Extractor	1	27.25 L	SS-316	99,000
HX-101	Heat Exchanger	1	0.00 m^2	CS	1,000
V-102	Stirred Reactor	1	45.75 L	SS-316	328,000
V-103	Decanter Tank	1	1.68 L	SS-316	26,000
HX-102	Heat Exchanger	1	0.00 m^2	CS	1,000
V-104	Flash Drum	1	2.25 L	CS	0
MX-101	Mixer	1	35.69 kg/h	CS	0
V-105	Stirred Reactor	1	29.29 L	SS-316	328,000
V-106	Decanter Tank	1	0.13 L	SS-316	26,000
V-110	Stirred Reactor	1	28.38 L	SS-316	328,000
V-107	Decanter Tank	1	0.33 L	SS-316	26,000
V-108	Flash Drum	1	2.21 L	CS	0
V-109	Flash Drum	1	0.93 L	CS	0
				Total Cost	**1,189,000**

The following figure illustrates the SuperPro simulation for this project. Both 99% biodiesel and 99% glycerin products can be obtained. Correct flow rate values are not inputted allowing for a 1,000-galloon/week plant. Recycle streams currently are not connected.

Figure 18: SuperPro simulation of algae to biodiesel process.



BUDGET

Algae Growth

Product	Vendor	Purchase Price
250w Metal Halide Light	eBay	$120.00
Freshwater Water Testing Kit	Petsmart	$17.00
300 gph Powerhead x 2	Petsmart	$80.00
Aquasafe Dechlorinator	Petsmart	$7.00
Scenedesmus Culture x 2	Carolina Biological Supply	$40.00
250W Water Heater	Petsmart	$21.00
Steel for light shroud	Lowes	$25.00
Hemocytometer	eBay	$36.00

Algae Harvesting

Product	Vendor	Purchase Price
Acrylic Tube 4 $^{1}/_{2}$" OD, 5 ft	Aquatic Ecosystems	$70.00
PVC 1 $^{1}/_{2}$" OD, 2 ft	Aquatic Ecosystems	$20.00
PVC to Acrylic joint	Aquatic Ecosystems	$15.00
PVC cleaner, sealer	Lowes	$20.00
Chitosan	Sigma-Aldrich	$30.00
Wood for harvesting stand	Lowes	$15.00
5 gallon pressure tank	Lowes	$65.00
1 hp pump	VWR	$364.00

Oil Extraction

Product	Vendor	Purchase Price
500 mL separatory funnel	VWR	146.24
1L chloroform	Sigma-Aldrich	$32.00
4 L absolute ethanol	Sigma-Aldrich	$59.00
1 L methylene chloride	Sigma-Aldrich	$36.00
1 L Algae paste	Innovativeaqua.com	$100.00

Biodiesel Conversion

Product	Vendor	Purchase Price
3 L methanol	Sigma-Aldrich	$66.00
Vacuum filter/flask	VWR	$205.00

Totals

Total	$1,589.24
20% of Total	$317.85
Total, with 20% cushion:	**$1,863.89**
20% added for shipping, hazard fees, etc	

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