Lamers. Algae oil from small scale low input water remediation site as feedstock for biodiesel conversion. Guelph Engineering Journal, (2), 24-38. ISSN: 1916-1107. ©2009.

Algae oils from small scale low input water remediation site as feedstock for biodiesel conversion

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Algae have been proven as a viable lipid source for conversion into biodiesel, and are also able to remediate contaminated waters, making them a very useful biotechnological microorganism. This feasibility study focuses on the Ridgetown Campus Soybean Biodiesel plant where an algae growth reactor, as demonstrated by this report, can be a useful addition to the plant. The plant is capable of producing 800,000 L of biodiesel annually from locally grown and processed soybean oil. The biodiesel plant aims to operate on a closed loop, recycling and reusing as many coproducts from biodiesel production as possible. An important part of the process will be the handling of the wash water required in the cleaning process of the biodiesel, which could reach 800,000 L of water per year. This report explores the viability of growing algae in photo-bioreactors using wash water from the biodiesel plant supplemented with corn powder hydrolysate and COMBO media as a growth medium. Growing green algae *Chlorella protothecoides* under heterotrophic growth conditions, a lipid content of 55.2% dry weight can be achieved. These lipids are viable for conversion into biodiesel through transesterification and these algae-produced lipids could add 39,704 L of biodiesel to the annual Ridgetown production. Meanwhile by reusing the water this has helped remediate the wash water from the biodiesel plant.

Nomenclature

χ	=	biomass concentration, g/L
S	=	limiting substrate concentration, g/L
μ	=	specific growth constant, 1/h
r_χ	=	rate of biomass growth, h/Lh
$Y_{\chi/S}$	=	yield of biomass from substrate, g/g

I. Introduction

As a renewable, biodegradable, and non-toxic fuel, biodiesel is a viable option for meeting part of the future energy requirements of automobiles, trucks and tractors. Fossil fuel resources are being depleted very quickly and replacements will soon be needed. Biodiesel can be used as a direct substitute for petroleum diesel in most diesel engines, and it can be made from a range of renewable feedstocks. Algae are known to have a high fatty acid concentration when grown in certain conditions and are a viable source for lipids for the biodiesel conversion process.

This study investigates the feasibility of growing algae species in biodiesel plant wash water for two purposes; to remediate the contaminated water, and to grow the algae in conditions conducive to oil production for use as a biodiesel conversion feedstock. It has been proven by Xu et al., 2006 that it is possible to convert microalgal oil into biodiesel meeting ASTM standards. The U.S. Department of Energy's Aquatic Species Program conducted by the National Renewable Energy Laboratories (NREL) also proved microalgal oil not only a viable feedstock for biodiesel conversion but that alga can be used to reduce carbon emissions from industrial plant flue gas [12]. This paper will outline the design criteria in order to use algae as an oil feedstock on a small scale low input biodiesel operation on the Ridgetown campus in south western Ontario.

A. Biodiesel

The list of benefits associated with replacing petroleum diesel with biodiesel is extensive. It is stable in sealed containers so it can be transported and stored for prolonged periods of time. It has a higher cetane value than petroleum diesel resulting in smoother, more complete combustion in engines. Emissions from biodiesel combustion are lower than petroleum diesel, with decreased particulate matter, lower sulphur concentration, and biodiesel can be

created from a wide range of renewable resources. It is currently seen as a viable replacement in the transportation industry and even airlines are giving biodiesel a lot of attention because it can possibly be used in aircraft. The first transcontinental flight fuelled by pure, B100 biodiesel was completed this year by Green Flight International disproving a lot of doubts that people have had in the fuel [2].

Downsides of biodiesel are also present; it tends to gel at higher temperatures than petroleum diesel, it is subject to biodegradation by microorganisms, it produces higher nitrous oxide (NO_x) emissions, has a lower energy content than petroleum biodiesel, and the price to process biodiesel from raw oils is high [9, 10]. Biodiesel gelling is a common concern with biodiesel in cold climates, and occurs when wax crystals form. Gelled fuel is not usable in an engine because it will clog the fuels injectors and fuel pumps. When biodiesel is thawed from the gelled form it is again useable but gelling is a nuisance for cold weather use. The "cloud point" for B100 (pure biodiesel) from wasted vegetable oils is around 14°C which is an indication that wax crystals are about to form [9, 10]. The biodegradation of biodiesel is only a factor if it is not properly stored in sealed sterile containers since it contains carbon sources and water which is a niche for microbiological activity. The lower energy content is minimal compared to petroleum diesel. Kemp (2006) claims that there is only a 8% decrease in the amount of energy in biodiesel compared to petroleum diesel and this will result in only a 5% increase in fuel consumption by volume.

1. Transesterification

Any substance with a considerable amount of fatty acids is a potential feed stock for biodiesel conversion through transesterification. Triglycerides (TAGs), three fatty acids molecules esterified with a molecule of glycerine suitable for biodiesel conversion, come from a variety of sources; animal fats, raw vegetable oils from oil seed plants, used vegetable oils from restaurants, and algae.

Triglycerides + 3 Methanol ➔ Glycerol + 3 Methyl Esters

Figure 1. Transesterification process from oil to biodiesel.

Transesterification of 1 mole of triglyceride requires 3 moles of methanol for the complete equilibrium reaction. The reaction is an equilibrium process, meaning it can be made to proceed towards reactants or products by providing an excess of the appropriate compounds. Industrial processes will often react with a molar ratio of methanol to oil a lot higher than 3:1 to ensure that the above reaction will proceed in the forward direction towards products [1, 7, 13]. A catalyst is also required to ensure the reaction proceeds at a reasonable rate. Depending on the type of transesterification, either alkali or acidic, the corresponding catalyst is chosen. Alkali transesterification is more commonly used instead of acid catalyzed transesterification because of its increased efficiency and reaction speed [7]. Transesterification is usually carried out at room temperature but reaction rate can be increased with higher temperatures. Proper mixing is also a key concern. Haas, et al., (2004) conducted experiments to increase the efficiency of the transesterification reaction. It was found that reactions at 60°C with constant agitation and with a molar ratio for methanol/TAG/NaOH of 226:1:1.6 with a reaction time of 8h were the most efficient. Methanol again is at a concentration well above the stoichiometric amount stated in Fig. 1 to ensure the process goes through to completion. Constant agitation ensures that proper mixing occurs and that methanol comes into contact with the oils as much as possible. Temperature aids in the reaction because an increased temperature will decrease the activation energy required for the catalytic reaction to occur and thus speed up the process.

2. Downstream Processing

When the methyl esters are produced they are referred to as fatty-acid-methyl-esters, or FAME. FAME can also be referred to as raw biodiesel. The glycerol co-product can be easily separated through sedimentation and will generally settle to the bottom of the reactor within 12 hours, a visible separation line can be noticed. The reactor used should therefore have a drain located at the bottom for the removal of the glycerol once the settling is complete. A distinct change will be seen once FAME begins to drain out because the viscosity dramatically increases [9]. The other major component, unreacted methanol, will need to be removed from the raw FAME as well. This can be done through distillation and will be more energy efficient if done when the products are still warm. Distillation should be done at a temperature of 80°C. The evaporated methanol can be recovered through condensation and thereafter it can be reused [9]. The efficiencies of the clarification steps are limited by how well the glycerol settles and the accuracy of the distillation, generally 90% efficient in removal [9]. Typically, a small amount of these materials will

still be present in the raw FAME so an additional washing step is required. The hydrophobic FAME will not mix with water. Therefore it can be cleaned by either bubbling water mixed in with air through an air sparger, or sprinkling water lightly over top and letting it settle to the bottom [9]. Contaminants will be picked up by the water and it is recommended by Kemp (2006) to wash at a 3:1 ratio of raw FAME to water and to complete three washings per batch. This removes 99% of the contaminants. If this process has been done correctly, the FAME should be suitable for blending or pure combustion in a diesel engine, or for use in a furnace as a substitute for heating oil.

3. Biodiesel on a farm

When biodiesel is made on small scales on a farm it can easily be used for either the equipment on the farm or heaters of barns or houses. Diesel tractor engines are generally built tough to withstand farm wear and tear which tends to be more severe than regular diesel engine use. A farmer will not need to be concerned with exact levels of blending if they were to mix the biodiesel themselves. If the tractor was previously fuelled with petroleum diesel, fuel filters will need replacement within weeks of switching to biodiesel because biodiesel is known to clean diesel engines of built-up carbon when first used [10]. Another point to mention about using biodiesel in traditional diesel engines is that if used for a prolonged period of time plastic hoses and washers that come in contact with the fuel should be replaced because biodiesel is known to be corrosive to plastics. The other option of using biodiesel on the farm is using it in heaters and furnaces. Barns and farm homes usually have a fuel oil furnace that can safely utilize biodiesel. Using biodiesel in a furnace would require the farmer to store the biodiesel inside during cold winter months which will help prevent biodiesel gelling.

B. Algae

Algae are can be thought of as small energy conversion and storage units that filter air and water while creating useable biomass for human consumption or use. They are able to convert solar energy and carbon dioxide into biomass and oxygen at high efficiency rates. As a result algae are highly beneficial to a society in need of energy resources. They are very versatile and hardy, do not require much maintenance when growing and grow in very dense cultures. Another benefit of algae is their lipid vacuoles, which can compose up to 80% of their dry weight, and may serve as a viable feedstock for conversion into biodiesel [1, 4, 8, 12, 13].

1. Metabolism

It is estimated that there are 30,000 to 50,000 different species of algae in the world ranging from single cell (one micrometer diameter) organisms to giant seaweeds (over 50 meters long) [3]. Their versatility allows them to grow all over the world in many different climates ranging from hot water springs, to dark ocean depths. Algae mainly grow in aquatic environments. They can be heterotrophic, feeding off of organic carbon as a carbon source, or autotrophic, fixing carbon dioxide as a carbon source. Most are photosynthetic, requiring natural light as a source of energy, while some species are lithotrophic, feeding off of inorganic matter as a source of energy. Some species are able to switch their metabolism depending on their environment adding to their versatility and hardiness. Algae are categorized as being eukaryotic and thus have complex eukaryotic cells. This complex cell development is what leads algae to having lipid vacuoles that are important if algae were to be considered as an oil source for biodiesel. The lipid vacuoles are primarily used as a floatation device during aquatic growth or as protection from cold environments [3].

2. Microalgal Lipids

Depending on growth conditions, lipid content of algae can vary greatly. It has been shown that *Chlorella protothecoides,* a species of green algae, grown under heterotrophic conditions has four times more lipid content than when grown in autotrophic conditions [13]. Other factors like lighting and temperature also greatly affect algae composition. Some species of algae which grow in cold aqueous environments generally have higher lipid content. Algae can adapt to their surroundings and create lipid vacuoles that are used as a protection from the cold [1, 12, 13]. Growth with intermittent lighting, known as light-dark cycling, has been shown to increase the growth rates and lipid content of certain algae species. Chisti, (2007), found that light-dark cycling improved growth rates of algae when mixing increased to allow for 10ms cycling times. This cycling is thought to allow the photosynthetic elements in the algae to rejuvenate during the cycling and improves photosynthetic cell efficiencies.

Another technique proven to increase the lipid content in algae is nutrient deficiency during growth. Although seemingly counter-intuitive by limiting certain substrates such as nitrogen algae increase their lipid content, it does prove beneficial. Some algae species stop creating nucleic acids and proteins when nutrients are limited and therefore stop reproducing but continue to produce lipids. This technique is known as a lipid trigger [1, 13]. So far, this has only been shown to work under laboratory controls. It improves the relative lipid content making for a better

harvest when it is time to remove the algae from the growth media. A lipid trigger may be done right before harvesting to increase the overall lipid content 130-320% depending on growth conditions [13]. Generally lipid content on a dry weight basis can range from 10-80%. Chisti, (2006) outlines several species and their potential lipid contents in table 1 below.

Table 1: Oil content from microalgal species, Chisti (2006)

Microalga	Oil Content (% dry wt.)
Botyococcus braunii	**25-75**
Chlorella sp.	**28-32**
Crypthecodinium cohnii	**20**
Cylindrotheca sp.	**16-37**
Dunaliella primolecta	**23**
Isochrysis sp.	**25-33**
Monallanthus salina	**>20**
Nannochloris sp.	**20-35**
Nannochloropsis sp.	**31-68**
Neochloris oleoabundans	**35-54**
Nitzchia sp.	**45-47**
Phaeodactylum triornutum	**20-30**
Schizochytrium sp.	**50-77**
Tetraselmis sueica	**15-23**

It has been proven that the lipids produced by the algae are viable for biodiesel conversion by several studies [1, 12, 13]. These have all shown or developed algae based biodiesel as a viable fuel according to ASTM standards. The lipids found in the different algae species do vary, so consideration must be put to which oils the species produce and if that oil is suitable for use as a biodiesel. The amount of saturation and length of the hydrocarbon chain, along with where the double bonds exist, greatly affect the quality of the diesel produced [1, 12, 13].

3. Bioreactors and Metabolite Processing

The main types of bioreactors used to grow algae are raceway ponds or photobioreactors. Both use sunlight as the main source of lighting and have specific limitations. Photobioreactors are used for growing dense populations and are able to grow only one strain of species. Raceway ponds are exposed to the local environment and can easily be contaminated and as a result of layout and have difficulties developing dense populations of algae [4].

Grima et al. (2003), outline various processes for recovering microalgal biomass and metabolites such as lipids from these bioreactors. They found that harvesting and extraction of the metabolite of the algae accounts for 60% of the overall costs of production, and the growth of algae is 40% of the overall cost [4]. Common techniques used for the harvesting are centrifugation, filtration or gravity sedimentation and are commonly preceded by a flocculation step. Recovery is a major hurdle to overcome in the processing of algae since microalgae usually range between 3-30μm in diameter. Some species only grow into very dilute populations, especially considering growth in outdoor raceway ponds, which make it even harder to harvest and isolate the small algae cells. The key to efficient and productive harvesting is being able to process a large amount of volume in a relatively small amount of time. For low value products where purity is not of outmost concern, gravity sedimentation works well and can be enhanced by the use of flocculation. Centrifugal harvesting works well for high valued products but risks damaging the product. Filtration works if filtering equipment is available that is fine enough to capture the algae and if a large throughput can be achieved. Filters tend to clog with large amounts of filtrate so complexity and costs quickly increase to account for having to clean the filters. After the algae are separated from the growth media they will still have a significant amount of water which may not be suitable depending on the final product.

In the case of biodiesel from algae, water content should be reduced to a minimum before the transesterification step. Common drying methods are spray drying, drum drying, freeze drying or sun drying. Freeze drying becomes very expensive in large scale production but does produce the most pure results. Removal of oils can be done through solvent extraction using, for example, hexane. Hexane-based solvent extraction is the standard method for lipid extraction from microalgae [12, 13].

II. Problem Definition

In Ridgetown campus the planned biodiesel facility will face a problem dealing with the large volume of used wash water. With over 800,000 L of biodiesel being produced annually, a lot of water will have to be sent to a treatment plant or sewer systems after used for washing. Additionally, a disposal approach goes against the Ridgetown projects goal of being a closed loop biodiesel operation. This feasibility study will examine the following problem: Ridgetown biodiesel operation will not fulfill its closed loop goal because it plans to use fresh water to clean its biodiesel afterwards expelling this used water into water treatment facilities. There are other limitations which add to the problem statement and they will be addressed in the following pages.

A. Ridgetown Campus Biodiesel Project

Along with the Southwestern Ontario Bioproducts Innovation Network (SOBIN) and the University of Guelph, Ridgetown Campus plans to operate an oilseed processing and biodiesel production facility starting in summer 2009. The project aims to be small scale and operate on a closed loop in partnership with local farmers. The primary oil feedstock will be soybeans from local farmers. Oil will be extracted from the beans and the residual defatted soy will be fed back to the farmer's livestock. Meanwhile, the oil will be processed into biodiesel at Ridgetown. To close as many loops as possible, Ridgetown campus will look for ways to add value to every aspect of the biodiesel operation. Utilizing byproducts (or "coproducts") of the operation in an optimal manner will be a key element of the Ridgetown project. Ridgetown will require a large amount of methanol and potassium hydroxide catalyst for the biodiesel conversion process along with a very large quantity of water for washing the raw biodiesel of any residual contaminants. The plant aims to produce upwards of 800,000 L of biodiesel annually which will require approximately 800, 000 L of fresh water, 154, 288 kg of methanol, and 9, 000 kg of catalyst (see Appendix 1 for calculation details). The chemical reactants are not available on site and will be bought, however water could potentially be reused if there was a system to remediate and clean the used wash water. The small scale, low input characteristics of the Ridgetown plant will be considered as a constraint to this design analysis and attention will be given to minimize the resources needed for the process.

The climate has cold winter months, December through until March, and warm summer months, April through until November. It is common for biodiesel facilities to shut down, or at least minimize operations during colder weather to avoid the risk of gelling so it is assumed, by looking at local weather data, that the biodiesel facility will only be operational during the warmer 8 months of the year. The campus has lecture and greenhouse buildings on site and the biodiesel facility is housed in separate structure. The following assumptions will be made about the campus and biodiesel operation for this study;

- There is space available for an algae bioreactor to be installed on site near the biodiesel facility,
- Wash water is easily collected and there is space available for water storage tanks,
- Indoor temperature of barns or buildings is sufficient for efficient transesterification and biodiesel storage without clouding, and
- There is a capable person on site to control algae growth operations and maintenance.

Since it is a small scale operation, consideration will be given to the size and complexity of the bioreactor and operations chosen for the algae growth. The reactor size will be kept as small as possible to keep the process simple.

B. Biodiesel Production

Ridgetown is capable of producing 800, 000 L of biodiesel annually and this will be considered as the basis for this analysis. The following assumptions will be made concerning the biodiesel production;

- 8 month operation period per year,
- biweekly batches are produced, therefore there will be sixteen 50 000 L biodiesel batches per year,
- The biodiesel facility is operating on a steady state basis throughout the year,
- It will be assumed that the facility reliably converts oil to biodiesel and that there will be no major shutdowns during the year of this analysis,
- Two weeks is enough time to complete one batch of biodiesel including clean up, downtime due to malfunctions or possible errors, raw FAME contaminant extraction, washing and preparation for the following batch, and
- After the two weeks the wash water will be available as a growth media.

1. Washing

Using fresh water for each wash will require 16 666 L per wash. Three washes per batch will equal 50 000 L of fresh water per batch.

2. Contaminants

The methanol, catalyst and glycerol are assumed to be extracted to minimal but not negligible concentrations. Through distillation 90% of the methanol can be recovered and through sedimentation 90% of the glycerol can be assumed extracted. From the washing process it is stated by Kemp (2006) that 99% of the contaminants are washed into the water. Therefore, after the washing of the biodiesel the water will contain the following components.

- Using a 6:1 molar ratio of methanol to oil 9, 643 kg of methanol will be used in the each batch. Half will be used for the conversion into FAME and half will be unreacted. From the distillation 90% of this methanol is recovered for reuse and the other 10% will need to be washed out. From that, washing will remove 99%, leaving 477 kg of methanol in the wash water.
- During transesterification, 4,620 kg of glycerol will be produced. It is assumed that 90% can be removed by settling and 99% of what was left will again be washed out into the water leaving 457 kg of glycerol in the wash water.
- Generally catalyst is added to equal 1% of the total reactant weight. This is 565 kg per batch. Again it will be assumed that 90% of that will be used, and 99% washed out into the water leaving 51 kg in the wash water.

Table 2. Wash water contaminants

Contaminant	Concentration, kg/L
Methanol	0.029
Glycerine	0.028
Catalyst	3.06×10^{-3}

C. Algae Biodiesel

For this process to be feasible the biodiesel produced from the algae lipids must meet the ASTM D975-08a standard for on and off road vehicle use, or must meet ASTM D396-08b for home heating use. To be used as a blend it must meet ASTM D7467-08. Xu et al. (2006) successfully created biodiesel that met these ASTM standards by using the species *Chlorella protothecoides.* The same species will be used for this analysis to ensure that similar quality can be achieved.

D. Criteria

The following criteria were used to guide the final system design for this project.

- The cost of the overall operation should be kept low so that design is achievable,
- Ease of use and operation is considered so that the process is possible,
- The required inputs should be minimal to coincide with Ridgetowns projects scope,
- The biodiesel produced from the algae should be the highest quality and quantity possible, and
- The design should not interfere with regular biodiesel operation.

III. Design and Analysis

Based on the above criteria and design constraints the following system was designed. Algal biodiesel is a viable option for fuel and pilot plants are currently being installed around North America, Europe, and Australia to produce biodiesel from algae oils. Xu et al. (2006) have proven that high quality biodiesel can be produced with photobioreactors and therefore their experimental procedures will form the basis for the design to grow *Chlorella protothecoides.* A definitive harvesting and downstream processing system has not been developed by algae biodiesel producers yet, as far as this author has found, and so the harvesting systems will be a combination of existing technologies.

A. Growth Reactor Design

Photobioreactors are the proven best option over raceway ponds for several reasons. Photobioreactors can produce much denser cell cultures than raceway ponds. On a cost basis they are advantageous as well because they take up considerably less space. The control system is more complex in photobioreactors because climate is controlled more strictly than is possible with raceways ponds, therefore adding to initial capital costs. An advantage to photobioreactors is their sterility and ability to reliably grow one specific species of algae. One concern that Sheehan et al. (1998) noted about raceway ponds is that indigenous algae species tended to contaminate the ponds. As a result contaminated ponds generally have lower lipid content than the pure species ponds. Photobioreactors are closed to the ambient environment and do not have this problem. Controls would have to be in place to keep the system clean from foreign debris and contamination. This can be done through air filters and through standard operating procedures which enforce sterile working procedures.

A common photobioreactor has the photo tubes separate from the airlift and degassing section. This allows the airlift and degassing zone to be kept indoors. At the degassing zone the media parameters of temperature, pH, oxygen concentration, substrate concentration and flow rate will be controlled.

1. Operating Temperature

This is very dependant on the species being used. It is recommended that the media temperature be kept near 28°C. *C. protothecoides* prefer this temperature and it should not impose an excessive energy requirement to maintain this temperature during growth. Natural respiration of the algae may be able to keep the temperature near this level. During warm summer months it may become too hot in the photo tubes and a cooling system may be required. During the nights some heating may be required. These can be controlled by installing heating coils somewhere in the reactors volume by sterile means or with heating wraps around the outside of the reactor. Coils are recommended because of increased energy efficiency but do have with higher initial capital costs.

2. Media

The algae will need to be supplemented with a glucose source to help promote dense population growth. Corn powder hydrolysate (CPH) was used by Xu et al., 2006 with excellent results. It is used as a glucose substitute because it is cheaper and it will be available near the Ridgetown campus since it is a common farm product. Xu et al. used an average concentration of CPH of 10 g/L over a period of 250 hours. An average cell concentration of 15.5g/L was found and was reached within 184 h. This is suitable for this design because it can be achieved with the two week period. Trace elements will also be required to supplement the algae for optimal growth. A common media is chosen easily purchased from chemical industry suppliers, like Fisher. The media chosen is COMBO media which is known to promote algae growth.

3. pH and Oxygen

Air will need to be pumped through the phototubes and it would be beneficial for algae growth if CO_2 was added into the air flow. Algae thrive off higher CO_2 conditions and will grow to denser populations [1, 12]. But unused CO_2 may cause a problem because it would decrease pH making the environment unfavourable for algae. Therefore a pH controller will be needed in the degassing zone so that fresh air may be added to lower the CO_2 concentration. The degassing zone will also be used to remove any oxygen that the algae have converted. If the oxygen level rises too high, will become toxic to the cells and cause algae death. Therefore fresh air again will be required to lower the total dissolved oxygen concentration.

4. Air flow rate

Air flow rate can also be correlated to the media flow rate because both will control the turbulence inside the phototubes. Algae will settle on the bottom of still media and will compress and kill any algae that may be on the bottom of the settlement. Therefore continuous agitation is required to ensure proper mixing and suspension of the algae cells. Proper mixing is also very important to increase the CO_2 and substrate suspension. When turbulence increases so does the probability that an algae cell can come into contact with substrate or CO_2 and thus increasing the nutrient uptake rate. This will result in better growth. Another benefit in keeping the turbulence as high as possible without damaging the cells is that the light-dark cycling would be increased with higher turbulence. This would give the cells better efficiency in photosynthesis and thus have better growth rates. A value of air flow 16 666 L for every 4 hrs is recommended because fresh air will have passed through all parts of the reactor. This is stated enough air flow by Xu et al. 2006.

5. Lighting

The phototubes are placed outside and should be oriented for maximum solar exposure. It would be beneficial to angle them up to receive the most possible direct solar irradiance during the operation months. If reflective surfaces were placed behind the tubes reflected light could also be harnessed to light the backside of the tubes to increase illumination. The tubes may also be kept under a thermostable cover to keep the temperature of the media more consistent. This would require a transparent layer surrounding the tubes, possibly glass, thus keeping the air around the tubes at a more consistent temperature. This would alleviate some heating requirements and would also keep the temperature within more constant range.

6. Size

If biweekly batches of biodiesel are made it would make sense to design the bioreactor to operate on a biweekly basis. Algae cell densities suitable for harvest can easily be created within the two week period and there would be enough time for clean up and prepare for another algae batch. A continuous state system may also be beneficial and would maintain a constant stream of algae output. A continuous system would require more resources for storage of the wash water and processing of the algae biomass on a continuous basis, but would greatly increase efficiency of algae culture. With a well run continuous reactor the cells can be kept in an exponential growth period for a prolonged period under the right conditions. The size requirement for a continuous reactor would require an 80% working volume reactor that allows for 20% volume increase to maintain control of flocculation or addition of media. This means that if 16, 666 L of wash waters are added every two weeks, a 20 000 L, bioreactor volume would allow an 83% working volume and 17% volume increase tolerance, which is sufficient. This volume is the total of both the degassing zone and phototubes combined.

7. Maintenance

During the two week period regular maintenance would involve ensuring that enough media is available and that all process controls are in working order. It is estimated that during the two weeks only one week is necessary for the algae to grow into a dense enough population suitable for harvesting. That leaves one week for regular maintenance before beginning of the next algae batch. Under continuous reactor control there would be no down time between the batches. A steady state of algae cells would be maintained within the reactor and under proper control exponential growth is maintained. Continuous reactors have enough controls in place to limit the growth rate and ensure that during the two week period enough wash water can be used so that the wash water stock does not back up. This type of control can only be established based on individual reactors. Any controller of a bioreactor will tell you that every one has its own personality and should be controlled accordingly. This two week rate can be controlled by the maintenance operator after its personality is characterized.

B. Harvesting

During a continuous reactor operation, the algae would be continually harvested at the end of the tubes. For a process of this size a filtration system would be suitable. Grima, et al. (2003) notes that for a process that processes less than 2 m^3/d of media and algae, filtration is possible. The Ridgetown process would only have to processes 1.2 $m^{3/}$daily of media and algae. If the scale of the project were to increase above this level, centrifugation is seen as the preferred option. Separated algae biomass can be dried and treated with solvent to extract oils. The drying step is important to allow for the extraction of intracellular products like lipids and sun drying is seen as the best approach for an operation of this scale. Sun drying is a more energy efficient technology and more applicable to the Ridgetown process. A simple covered and sterile platform and conveyor system could be used to effectively dry the algae after harvest. This also alleviates complicated technology associated with freeze drying or other technologies.

Winter conditions are not of concern because of the 8 months processing schedule. Summer irradiance is assumed enough to dry the algae effectively. After drying, solvent extraction is done and centrifugation is able to separate the solvent and oil sufficiently. Hexane is commonly used for lipid extraction from algae [1, 4, 13].

Because this project is based on farm scale, efficiency of the extraction is estimated to be lower than industrial or laboratory processes. The farm settings make for the need of a simple to employ harvesting system due to minimal technology and expertise available on site. The solvent extraction will be a one step esterification step that requires hexane, ethanol and acetyl chloride. This will have to be performed under strict watch by experienced personnel. For the purpose of this study an extraction efficiency of 70% was estimated. Studies show that the extraction of oil from algae through the hexane extraction method can be estimated to be 70% efficient [4, 13].

The remaining algae biomass which has been de-oiled contains large amounts of nutritional products. There is a large concentration of proteins, carbohydrates, and minerals which may be suitable for feed for livestock. Using the de-oiled algae would require an additional processing step to ensure the algae are viable for feed to livestock and the stockpiling of the algae. The cost may be too high for small amount of biomass but if a large enough amounts of algae are available the cost may proof worthwhile. It may be a procedure Ridgetown should investigate further to fulfill the closed loop objective.

C. Biodiesel Production

Since the equipment is already present there are no extra requirements to use algae oil as the feedstock of the transesterification process. It will likely be possible to add the oils from the algae in with the soybean oils. No studies could be found that examined mixing algal oil with other biodiesel feedstocks.

IV. Results

It will require a complex processing procedure to install an algae growth and extraction process into the existing Ridgetown biodiesel facility, but adding this capability will be beneficial. Nothing has been found that suggests that the wash waters are toxic or an unacceptable media for the algae. Research has been conducted where the flue gas from a natural gas energy power generation plant has been pumped through an algae photobioreactor after having gone through some scrubbers. The algae were able to not only survive but also thrive with the excess CO_2 [1, 8, 12]. It is assumed that the contaminants in the flue gas are a great deal worse than the contaminants in the wash waters of this project so it can be safely stated that the algae can grow on it. For the analysis it was taking into consideration that the lag time of the algae will be increased because of the contaminants so conservative estimates were made.

A. Algae Biodiesel Production

The following estimates of algae growth, lipid production and biodiesel output were generated using the values outlined in Section III, and the algae growth model seen in the appendix.

Using the growth parameters set out by Xu, et al. (2006), noted in section III A above, and through calculations shown in the appendix, it was found that 6, 016 kg of algal biomass can be created every two weeks. Accounting for the limitations of harvesting and the extraction process, this amounts to 2, 324 kg of oil every two weeks. Using this oil in the transesterification process would results in 2, 481 L of biodiesel. This equals an annual production of 39,704 L of algae biodiesel.

B. Water Remediated

If after the algae have been harvested the water it is suitable for reuse, essentially 800, 000 L of water has been saved. Ridgetown should look into a low end water treatment facility to ensure that the water is safe to be reused in the biodiesel procedure since contaminants from the algae growth will be present. Treatment with chlorine after filtration may be all that is required since the only major contaminants would be algae biomass and unused substrates. Algae have an excellent capability to remediate water and it can safely be assumed that any glycerol, methanol, or catalyst is mostly converted into biomass or other organics and remain in minimal concentrations. The primary concern in the remnant water will be unharvested biomass and unused substrates. To remove these contaminants the proposed small scale water treatment plant can be used. A diatomaceous earth filtration type system will suffice. The algae species and substrate are of large enough size that a 5 micro meter filter can be used to effectively remove 99% of the algae and substrates. Afterwards the water will need to be treated according to local water treatment guidelines to make it able for re-use.

V. Conclusions

A. Recommendations

This has only been a feasibility study and it should not be treated as a viable solution until further investigation has been made. The following recommendations should be noted before proceeding.

- Laboratory and pilot scale testing of algae production operations are needed to ensure that the aforementioned operations are viable and will perform as predicted. It is unclear how well the algae will grow in the wash water so conservative estimates were made, but it is possible growth rates and oil contents may differ from those observed in the referenced papers. Laboratory studies will give a better understanding and analysis on the potentials of the wash water as a media.
- Different algae species should be investigated for growth potential using photobioreactors.
- A chemical analysis should be carried out on samples of the wash water from the biodiesel plant to obtain accurate of the mean concentrations and typical ranges of the contaminants.
- A cost feasibility study should be conducted to see if a process of this type would be economically feasible. It is believed that the cost of the algae biodiesel would be very high compared to any other lipid-sourced biodiesel and especially high compared against petroleum diesel.
- Solar irradiance at the Ridgetown campus should be investigated to ensure that enough illumination is available for algae to grow.
- The feasibility and process of extracting oil from algae should be investigated in more detail. The goal of on-farm implementation puts limits on the technological complexity of the oil extraction process. It may be that drying, pressing and solvent extraction is viable, but the feasibility and costs (capital and energy) of this process need to be investigated.

B. Recommended Course of Action

From this feasibility analysis it is recommended that laboratory studies should be conducted in light of the recommendations mentioned above to test the viability of this process. Ridgetown stands to benefit from this process if it will alleviate a lot of cost associated with the costs of water use and disposal. Since the biodiesel project is set out as a learning and pilot scale operation, with further research other beneficial uses of the algae may be found. The dried and pressed algae may prove to be an excellent feed for the livestock in the area. Algae have been known to be excellent sources of proteins and minerals.

Overall more research and investigation is recommended on this topic. Algae biodiesel may prove to be a viable resource for future fuel needs. Ridgetown may develop an excellent water remediation process and renewable energy supply by growing algae on the wash water. This procedure may become a viable source for future energy supplies meanwhile supplementing waste water treatment plants.

VI. References

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VII. Appendices

A. Growth Modeling

For the purpose of the feasibility analysis growth of algae must be modeled. Generally, growth occurs exponentially if reactor conditions are sufficient. After inoculation of the algae there is a lag phase which precedes the exponential growth phase. The lag phase represents the species need to acclimatize to their surroundings. A stationary phase will occur over time when substrate becomes limiting or the population becomes too dense and non-optimal growing conditions result. The exponential growth can be modeled as

$$\chi = \chi_o \exp^{\mu maxt} \quad (1)$$

Where χ is the biomass concentration in g/L, χ_o is the initial biomass concentration in g/L, μ_{max} is the maximum growth rate in units of 1/h, and t is the time measurement in h. In practice, the maximum growth rate is generally not fulfilled in a reactor since it is dependant on the amount of substrate present. A Monod parameter, K_s, units of g/L, relates the sensitivity of the specific growth to the amount of substrate and accounts for nonoptimal growth conditions. Usually a specific growth rate term, μ_s, is calculated and used in equation 1. S, here, is equal to the limiting substrate concentration, g/L.

$$\mu_s = \mu_{max} S / K_s + S \quad (2)$$

The algae doubling time t_d is

$$t_d = \ln 2 / \mu_{max} \quad (3)$$

Doubling time can also be found with the specific growth rate calculated in equation 2 if the substrate concentration is known.

$$Y_{\chi/S} = \Delta\chi / \Delta S \quad (4)$$

Equation 4 can be used to calculate the yield, $Y_{\chi/S}$, of biomass, $\Delta\chi$, per substrate use, ΔS. If the yield is given it can be used to calculate end or initial biomass and substrate concentrations. The rate of biomass production, r_χ, in g/Lh is

$$r_\chi = \mu\chi \quad (5)$$

Using the above equations the growth of any organism, whose growth parameters are known, can be modeled. This is a simplified set of equations when considering living organisms. However all of the parameters that may affect organism growth cannot be fully modeled, i.e. toxic metabolite development, substrate use for product development not associated with biomass growth or population density affects. These equations can be modified to model the effect of some of these negative parameters, but the model described by equations 1-5 will be sufficient for this feasibility study.

B. Calculations

800, 000 L Biodiesel / y	8 months of the year
50, 000 L Biodiesel / batch	16 batches / y, assumed

1. *Washing*

   W_i / 50 000 L_{BD} = 1 L_{H2O} / 3 L_{BD} (ratio given by Kemp, 2006)

   W_i = 16, 666 L_{H2O}

2. *Water Contaminants*

Table B1: Stoichiometric calculation for 50, 000 L FAME production batch, made from soybean oil at the Ridgetown facility. Calculation used to calculate methanol required, and glycerol created.

	Oil	Methanol	⇔	FAME	Glycerol
Stoichiometry	1	3	equilibrium	3	1
Chemical Formula	Unknown	CH_3OH		Unknown	$C_3H_5(OH)_3$
Molar Mass	933 g/mol (Xu et al. 2006)	32.04 g/mol		292.2 g/mol (Xu et al. 2006)	92 g/mol
Volume				50, 000 L	
Density				0.88 kg/L	
Mass				44, 000 kg	
n (moles): (MM=mass/n)	50, 228 mol	150, 684 mol		150, 684 mol	50, 228 mol
Mass Required	46, 862 kg	In Excess (Use with a ratio well above calculated value here to ensure forward reaction) 4, 821.9 kg Recommend in a 6:1 ratio, so use 9, 643.8 kg /batch			
Mass Created					4, 620 kg

Table B3: Wash water contamination calculation

Contaminant		Methanol	Glycerol	Catalyst
Volume of Wash Water per wash	16, 666 L			
Mass / batch		4, 821 kg (unreacted methanol)	4, 620 kg	Generally 1.0% weight / weight of reactor reactants =0.01×(46,862+9643) =565 kg
Mass Remaining after Distillation or Sedimentation, 90% efficient)		482.1 kg	462.0 kg	56.5 kg
Mass removed in washing (99% efficient)		477.28 kg	457.4 kg	55.9 kg
Concentration		0.029 kg/L	0.028 kg/L	0.003 kg/L

3. Algae Growth

Parameters given by Xu et al., 2006

χ_{max} = 15.5 g/L — max growth reached after 184hr of growth in reactor

k_s = 5 g/L

t_d = 2h

S = 10 g/L — Corn Powder Hydrolysate concentration, average

Calculations (Based on growth modeling section Appendix A)

$t_d = \ln 2 / \mu_{max}$

$\mu_{max} = \ln 2 / 2h$

$\mu_{max} = 0.346h^{-1}$

$\mu_s = \mu_{max} S / K_s + S$

$\mu_s = ((0.346h^{-1})(10g/L)) / ((5g/L) + (10g/L))$

$\mu_s = 0.0576\ h^{-1}$

$r_\chi = \mu\chi$

$r_\chi = (0.0576h^{-1})(15.5g/L)$

$r_\chi = 0.895$ gL/h

Assuming that this rate of growth can be maintained continuously, on average, over the 2 week batch time span then;

0.895 g/Lh × 24h/d × 14d/batch

300g/L

Then with a 20, 000 L bioreactor volume, as designed in section III.A.5.

6, 016, 517 g

6, 016 kg — Algae biomass harvested in bioreactor over the two week period.

4. Extraction

6, 016kg of biomass

Using *C. protothecoides,* lipid concentration of 55.2% lipids dry w.t.

6, 016kg × 0.552 kg oil/kg biomass

=3320.83 kg oil in biomass

Assuming an extraction efficiency of 70%

2, 324 kg oil extracted every two weeks

5. Algae Oil Transesterification

Table B1: Stoichiometric transesterification calculation for two week batch of algae oil

	Oil	**Methanol**	⇔	**FAME**	**Glycerol**
Stoichiometry	1	3		3	1
Chemical Formula	Unknown	CH_3OH		Unknown	$C_3H_5(OH)_3$
Molar Mass	933 g/mol (Xu et al. 2006)	32.04 g/mol		292.2 g/mol (Xu et al. 2006)	92 g/mol
Mass	2, 324 kg				
n (moles): (MM=mass/n)	2, 490 mol				
Mass Required		In Excess (Use with a ratio well above calculated here to ensure full reaction) 239 kg Recommend in a 6:1 ratio, so use 478 kg /batch			
Mass Created				2, 182 kg	
Density				0.88 g/mL	
Volume				2, 481 L	