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Title: Downstream Processing of Microalgal Biomass for Biofuels

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Ganti S. Murthy

This thesis documents the work carried out investigating the downstream processing of algal biomass for biofuel production. A life cycle assessment was conducted on a hypothetical algal biodiesel process in which it was found drum drying and cell component separation were life cycle intensive processes. A laboratory experiment was conducted which investigated the potential of using a dilute acid pretreatment followed by phospholipase and cellulase enzymes to simultaneously separate cells into their useful components. It was successfully demonstrated that cells can be degraded in this fashion and that the sugars produced were fermentable. Another investigation aimed at exploring the potential use of rapid sand filters as a first filtering and concentration step for dilute algae growth cultures such as those grown in open ponds. Finally, an life cycle inventory was compiled for a process which simultaneously produces biodiesel and bioethanol utilizing algae biomass as a feedstock. It was identified

that the production of commercial enzymes was energy and resource intensive and also released the most emissions.

Downstream Processing of Microalgal Biomass for Biofuels

by

Kyle B. Sander

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APPROVED:

Major Professor, representing Biological and Ecological Engineering

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Head of the Department of Biological and Ecological Engineering

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Kyle B. Sander, Author

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CONTRIBUTION OF AUTHORS

Dr. John Bolte has written the Microsoft Visual Basic^{TM}code which performs a 4th order Runge-Kutta approximation which was used in the modeling portion of the sand filtering experiment. Dr. Ganti Murthy made the biodiesel and bioethanol processing SuperPro simulation model used to compute LCI values in the final combined biodiesel/bioethanol life cycle inventory.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF APPENDIX FIGURES

DEDICATION

My Parents, My Sister and Kelsey

Chapter 1 – Introduction

1.1 History of algae as an energy source

The most rudimentary fuel pathway to extract energy from algae in its useful form is to consume it [Hills and Nakamura, 1978]. Nori and Spirulina are popular mainstay food items in East Asian countries. People have been consuming algae and seaweeds for centuries and the practice continues today.

Anaerobic digestion of algae is perhaps the next most basic technology by which energy can be extracted from algae. Gouleke et al. [1956] realized that the removal of wastewater lagoon algae may be advantageous or necessary and a method for ultimate disposal which "lends itself to conservation" would be the "controlled anaerobic digestion" of the removed biomass. In this process algae biomass (with an added high carbon supplement) is used as a substrate in a digester for anaerobic fermentation. The community of microorganisms present in a digester metabolize components of algae biomass into a biogas which consists of primarily CH_4 and CO_2 with impurities of hydrogen sulfide, oxygen, nitrogen, hydrogen and other gases. This gas can then be combusted to produce heat and electricity. The reason for the added supplement of high carbon material (hay, wood pulp, etc.) is to bring the C/N ratio from that of native algae biomass (10) to the optimum for anaerobic digestion (20) [Sialve et al., 2009].

1.2 Aquatic Species Program

The beginning of what would identify algae and their cell components as prime candidates as feedstocks for *liquid* biofuels was the Aquatic Species Program [Sheehan et al., 1998b], a research effort headed by the U.S. Department of Energy. This 18 year interdisciplinary effort succeeded in isolating 3,000 total strains of algae, 51 "high value" strains, identifying and characterizing the ACCase enzyme (an enzyme essential to the algal lipid synthesis pathway) from a diatom, demonstrating open pond systems designed for the mass culture of algae, performing a cost analysis of a proposed system and identifying some of the technology challenges which needed to be addressed if an algal biofuels industry was to ever exist.

General outcomes from these investigations were that growth rates experienced on a laboratory scale could not be extrapolated to a pond system, due to the complex nature of a pond environment and increased number of variables introduced when moving from laboratory to large scale (diurnal light shifts, exposure to contamination, changing temperature conditions, less controlled gas exchange). Typical biomass productivities achieved were between 15-30 $g/m^2/day$. Deliberate nitrogen limitation and starvation to induce lipid accumulation gave mixed results.

All outdoor mass culture focused on the use of open raceway style ponds because of their low cost. Up to 60% of the CO_2 introduced was utilized by the algae, proving to be an effective technology. One important detail to emerge from the study was that "such a system whereby, algae biomass would be providing several quads of energy, is not resource limited" [Sheehan et al., 1998b] The input

demands for a system of such magnitude were, albeit large, not out of the scope of reasonability. Furthermore, they established through a cost analysis, that the open pond method of cultivation (or something equivalently low tech and inexpensive) is the only option for mass cultivation on such a scale. All other options for growth and harvest were simply too expensive.

Surprisingly enough, the Aquatic Species Program made light that the one area which was not in need of major technological work was the actual growing of the algae. Industrial cultivation of algae has been practiced for many decades in the commercial production of beta-carotene, astaxanthin and neutraceuticals. Furthermore, large scale demonstrations were done in 1982 in Roswell, NM as part of the Aquatic Species Program.

Lastly, harvesting and extraction were two areas where current technology falls short of the needs of an algae energy system. The recalcitrance of algae to degradation and the seemingly species specific cell wall covering make for challenging cell component separation.

1.3 Beyond the Aquatic Species Program

With these needs identified, researchers have set out finding new ways to elucidate the secrets of microalgae and technologies which are better suited to processing them for biofuel production. Efforts were focused on many fronts ranging from a continued effort to understand the biochemistry of microalgae to developing methods for extracting their products to finding new harvest techniques. The guiding

hand under which all of these efforts did and continue to operate is economics [Benemann, 2008]. Transportation fuel product produced from microalgae should not cost more than a few dollars per gallon to produce. Use of extravagant high-tech harvest and extraction technologies can be justified when the product being produced (i.e. a neutraceutical or omega-3 fatty acid) is worth many times more than that of a transportation fuel. However when the final product is a low value commodity, low tech options for downstream processing are what was and still is preferred [Mendes et al., 1995, Harun et al., 2010].

Downstream processing can be defined as every process which occurs after algae biomass is grown up until delivery of fuel to consumers and resulting emissions from combustion. These processes, in the case of an algal biodiesel system, include a method for removing water (about 99.9% w/w) from the growth culture, separating cell components into their usable constituents, converting cell components into their useful products (be they energy related or not), transporting and storing products and product intermediates, and ultimately delivering products to the end use consumer.

Water removal from a solid liquid system (as an algae growth slurry is often viewed), while technologically simple has presented itself as a major obstacle in algae processing. Ultimately, the subsequent extraction and/or cell component separation process will determine how and to what extent dewatering must take place. The difference in dewatering needs between component separation processes is not trivial and can vary substantially. For example, a hexane extraction process requires biomass with no greater than 9% moisture [Sheehan et al., 1998a].

If a large percentage of the moisture must be removed, a sequential process will most likely need be employed as different dewatering technologies have maximal percentage removal levels above which they are ineffective or would require impractical time scales to operate [Kothandaraman and Evans, 1972]. An example of such a series of technologies would be continuous centrifugation (concentrating the slurry to 20 % solids) followed by solar drying (further concentrating the slurry to 40% solids) finished by thermal drum drying (achieving the final required solids concentration of 91%).

Cell component separation can comprise one or more methods of either chemical or physical processes. Physical disruption methods apply physical force to rupture cells and free intracellular components. Chemical cell component separation processes involve the use of a solvent to extract solutes by taking advantage of chemical differences between the target solute and other substances present (e.g. polarity) [Cooney et al., 2009]. In regards to biodiesel derived microalgae, the cell desired component is the non-polar lipid fraction primarily made up of triacylglycerols (TAG).

Because of their prevalent use in cellulosic and starch based ethanol production, enzymes are more and more being considered "low tech" and, more importantly, "low cost." Previous work has been done using lipase enzymes to catalyze biodiesel production and separate algae cell components. The work carried out and documented here aimed to explore the use of common enzymes (phospholipases and cellulases) as cell component separation mediators in an aqueous environment. Another benefit enzyme technology might bring about is the possibility of simul-

taneous production of biodiesel and bioethanol from algae biomass. Cellulase and phospholipase enzymes might be used as a disruption and separation strategy either as stand-alone or in conjunction with other methods.

1.4 Research Needs Moving Forward

Marching toward a reality of algal derived liquid transportation fuels will require maintaining goals similar to that of the ASP and pursuing it with the expanded knowledge, technology and resources of the current day. Not many of the technology challenges which the ASP faced have been convincingly resolved, yet each person facing the issue must realize that no fundamental or general design barrier stands in the way of substituting several quads of fossil based transportation fuel with micro algal derived liquid fuels [Sheehan et al., 1998b]. Cultivation demonstrations and decades of commercial cultivation have given algae agronomy the technology to successfully grow algae even on a large scale and substantial research is needed in developing algae processing technologies. Through the works documented in this thesis and the references to other works, we hope to have made some headway in the human desire for renewable, sustainable algal derived fuels.

This thesis documents four pieces of work which support the goals of overcoming the technical challenges of bioconversion of microalgal biomass into liquid biofuels. First, an LCA was conducted to assess the sustainability of an algal biodiesel process. This LCA led to the discovery that the drying step was by far the most life cycle intensive step. The next section of this thesis describes a project

aimed at investigating a process utilizing rapid sand filters to capture and recover algae biomass. The next section of this thesis describes experiments conducted to investigate the feasibility of cell component separation using enzymes in aqueous environments, which would eliminate the need for drying. Both of these projects were aimed at developing a low cost concentrating technology and aqueous algal processing technology. The final section describes the life cycle inventory of a proposed process using algae biomass to simultaneously produce biodiesel and bioethanol.

Chapter 2 – Life Cycle Analysis of Algae Biodiesel

2.1 Abstract

Background, aim and scope: Algae biomass has great promise as a sustainable alternative to conventional transportation fuels. In this study a well-to-pump life cycle assessment (LCA) is performed to investigate the overall sustainability and net energy balance of an algal biodiesel process. The goal of this LCA is to provide baseline information for the algae biodiesel process.

Materials and methods: The functional unit was 1000 MJ of energy from algal biodiesel using existing technology. Systematic boundary identification was performed using RMEE method using a 5% cutoff value. Data for this study was obtained from US LCI data base and GREET model. The data was organized in a Microsoft Excel spreadsheet with a transparent interface to simulate multiple scenario and sensitivity analysis. Algae dewatering is the most significant energy sink in the process and therefore two alternate technologies were evaluated. Carbohydrates in coproducts from algae biodiesel production were assumed to displace corn as feedstock for ethanol production.

Results and discussion: For every 24 kg of algal biodiesel produced (one functional unit, 1000 MJ algae biodiesel), 34 kg carbohydrates and cellulose co-product are also produced. Total energy input without solar drying is 3,292 and 6,194 MJ for

the process with filter press and centrifuge as the initial filtering step, respectively. The net energy ratio of a filter press process (energy input into the process/energy value of product) was found to be -9.9. Without the inclusion of the coproducts credits, net energy ratio is 3.3 (3291 MJ total process energy/1000 MJ functional unit). Net CO_2 emissions are -110 and 46 kg/functional unit for filter press and centrifuge case respectively. Other criteria pollutants which are created (per functional unit) during the process utilizing a filter press are -0.23 kg of VOC's, -2.3 kg of NO_x, -1.2 kg of CO, -0.34 kg of particulate matter and 1.29 kg of SO_x. In addition to the -105 kg of total air emissions per functional unit, 18.6 kg of waterborne wastes, 0.28 kg of solid waste and 5.54 Bq are created. The largest energy input (89%) is in the natural gas drying of the algal cake. It is interesting to note that although net energy for both filter press and centrifuge processes are -9943 and -7041 MJ/functional unit respectively, CO_2 emissions are positive for centrifuge process while they are negative for filter press process. Additionally, 20.4 m^3 of wastewater per functional unit is lost from the growth ponds during the 4 day growth cycle due to evaporation and needs to be replaced for each growth cycle.

Conclusions and recommendations: This LCA has quantified one major obstacle in algae technology; the need to efficiently process the algae into its usable components. Thermal algal dewatering requires high amounts of fossil fuel derived energy (3,556 kJ/kg) of water removed and consequently presents an opportunity for significant reduction in energy use. Current technology would be relatively inefficient, unreliable (in the case of solar drying) or inadequate on an industrial

scale. The potential of green algae as a fuel source is not a new idea, however this LCA and other sources clearly show a need for new technologies to make algae biofuels a sustainable, commercial reality.

2.2 Introduction

About 28% of the 99.3 quads of total energy which were consumed in 2008 [EIA, 2008] were used in the transportation sector. The world needs alternatives to provide transportation energy needs [Dale, 2008]. Biofuels from renewable bioresources provide alternatives to liquid fossil fuels, that are critical for terrestrials and air transportation sectors. Although other automobile technologies using plug-in electric, natural gas and hydrogen may provide alternatives to terrestrial transport, presently there is no such alternative for air transportation sector.

Due to limitations in production capacity, first generation feedstocks such as corn and soybeans cannot meet all the transportation fuel needs. Additionally, food vs. fuel issues, requirement of intensive agricultural inputs, land use and fresh water use are some of the limitations for large scale production of the first generation of biofuels. Second generation feedstocks, using cellulose in non edible plant biomass address some of the concerns such as food vs. fuel. Though (ligno)cellulosic feedstocks do not use human food resources, they still require arable land, fresh water and some agricultural inputs for their production. Long term impacts of sustained biomass harvest on soil quality, nutrient management are still being studied. Additionally, infrastructure for large scale production,

transportation and processing is in the initial stages of development.

Biofuels from algae feedstock are the "third generation" of biofuel feedstocks as they can potentially address most of the concerns about first and second generations fuels. Algae are autotrophs that utilize CO_2 and sunlight through photosynthesis. Algae can be used to obtain the essentials of life such as oxygen, organic carbon and vital nutrients [Hills and Nakamura, 1978, Borowitzka and Borowitzka, 1988, Lembi and Waaland, 1989, Shelef and Soder, 1980]. Some strains of algae accumulate high lipid/starch content, thus algae can be used as a feedstock for producing liquid biofuels. Algae have shorter growth cycles as compared to other terrestrial plants and hence the biofuel productivity potential from algae is orders of magnitude higher than terrestrial crops such as soybeans. Algae can be grown in waste water unfit for crop irrigation or municipal use. Versatility of algae to grow in diverse climatic conditions, waste waters and heavy metal sequestration capacity has been well documented [Sheehan et al., 1998b, Mehta and Gaur, 2005, Grima et al., 2003, Ceron et al., 2008]. Many processes have been proposed for growing single celled algae and converting it into liquid fuels [Chisti, 2007, Aresta et al., 2005, Sheehan et al., 1998b]. Algae biofuels is a rapidly advancing area with many studies focusing on production, harvesting and processing technologies. However there are relatively few studies on the long term sustainability and life cycle analysis of the algae to biofuels pathways.

As with any potential new technology, the long term sustainability of algae production as well as its impacts on the environment are critical concerns. These questions deserve special consideration because such questions have been raised in

the case of other biofuels. It is prudent to answer these questions before any large scale production of biofuels from algae biomass.

One of the methods to assess sustainability (and to some degree overall renewability) is Life Cycle Assessment (LCA). Life Cycle Assessment is a procedure for compiling information about the production and consumption of a good, normalizing and displaying the results in a useful and accurate format. The mass and energy inputs into production, processing, utilization and recycle as well as the environmental impact of production and consumption of the good are considered.

A Well-to-Pump LCA is a general descriptive term used in transportation fuel LCA to indicate an LCA including processes from the extraction of resources from the earth to delivery of fuel at refueling station.

Many of the contrasting conclusions from several LCA's and resulting debates can be traced to less rigorous system boundary definition [Wang, 2005]. Relative Mass, Energy and Economic value (RMEE) is a system boundary selection protocol proposed by Rayolds et al. [2000]. RMEE method is a systematic method, that is objective, repeatable and quantitative to ensure fair comparison between systems with very different configurations. In this method, individual system processes are chosen and information is compiled for these specific unit processes. This information is then used to delineate the system boundary.

Presently, there are no large scale commercial operations to produce and process algae feedstock in biodiesel, so a whole algal biodiesel processes is not readily available to model. The process technology for the algae based biodiesel process is based on current state-of-the-art industrial technology. Such component

technologies are well characterized and used in other industries, or have been used in large scale algae culture for other purposes such as wastewater remediation. Different aspects of algae technology have been studied for decades, yet a detailed LCA of a practical whole algae system has not been done to enable an analysis of energy input and environmental impact. Goal of this LCA is to establish baseline information for the process of making algal biodiesel, to which other transportation fuel LCA's can be compared. Understanding the environmental burdens of the algal biodiesel production will allow insight into inherent sustainability. Information from this LCA will be useful in identifying energy and emission bottlenecks in the process. This information can be used to provide impetus for further technological advancement of algal biodiesel and reduce overall energy use and environmental impact of a future algal biodiesel process.

Therefore, the specific objectives of this paper are to:

1. Perform well to pump LCA for the production of 1000 MJ energy from algal biodiesel.

2. Establish baseline information for algal biodiesel process.

3. Assess sustainability of algae biodiesel by characterizing energy use and emissions.

2.2.1 Scope of This Study

The goal of this LCA is to provide a baseline information for the algae biodiesel process. The system analyzed is typical of what the authors believe an algae system would look like if it were implemented today. This model proposes using currently existing industrial technology (both in algae processing and other industries). This approach was taken for two reasons: If algae is to be grown large scale as a fuel source, early systems will most likely use already existing technology components. Data for such component technologies is readily available and verifiable. The functional unit for the LCA analysis is 1000 MJ of energy from algae biodiesel "well-to-pump."

2.2.2 Data Organization and Display

The data was organized in a Microsoft Excel spreadsheet. Efforts were made to make this data compilation/model as transparent and user friendly as possible. The data which is deemed variable are listed on a separate worksheet and can be changed. Changing these values will result in a spreadsheet recalculation. This feature increases transparency, allows for a multitude of calculation scenarios and also allows the model to easily undergo many sensitivity analyses.

2.2.3 Specificity

Data used in this report, in all cases where a distinction is appropriate (such as electricity prices, efficiency values), is specific to the United States. All attempts were made in using the most up-to-date data available, however some data was chosen over newer data because of its greater specificity and usefulness.

2.3 Life Cycle Inventory

2.3.1 Relative Mass Energy and Economic Value Method

In choosing a complete system boundary, the RMEE method was employed [Raydolds et al., 2000]. The RMEE method uses a predefined "cut off ratio" to the functional unit on the basis of mass, energy and economic value. For a process input, a relative ratio of that input to the functional unit is determined for all three values (mass, energy and economic value). Starting with the unit process closest to the functional unit, the three RMEE ratios are calculated for each input. If any of the three RMEE ratios are larger than the predefined cut off ratio for a given input, the upstream process of that input is included in the system boundary. This is done until all upstream processes are below the cut off threshold. A cut-off ratio of 5% was chosen for this LCA. Therefore all processes which contribute $> 5\%$ mass, energy or economic value with respect to the functional unit (defined as 1000 MJ of energy from algae biodiesel) are included.

2.3.2 System Boundary

Algal biodiesel conversion process (Fig. 2.1) analyzed in this LCA is similar to a process which may be implemented if a plant were to be built today. It must be noted that the analysis will be significantly different if new technologies lead to radically different process layouts.

The process starts with culturing a strain of algae in photobioreactors/indoor ponds to prepare inoculum. The inoculum will be used as a seed culture for the open ponds. Open ponds, based on standard design [Borowitzka and Borowitzka, 1988], consist of a lined, shallow raceway (0.18 m deep and 1,115 m length) in which water containing algae is circulated by paddle wheels. Wastewater after secondary treatment is used as nutrient medium. The wastewater is assumed to have all nutrients necessary for algal growth except carbon. Carbon dioxide may be supplied from external sources such as flue gases from boilers, furnaces or power plants to stimulate the growth of algae in the open ponds, although flue gas sparging is not considered in this LCA. Typical harvest-growth-harvest cycle is assumed to be 4 days [Borowitzka and Borowitzka, 1988].

After growth of algae to their harvest concentration, they are separated from the wastewater by one of two processes; filtered through a chamber filter press or centrifuged in a self cleaning plate separator centrifuge followed by drying in a natural gas fired dryer. The algae are dried to 9% moisture, the required moisture content for the hexane extraction step [Sheehan et al., 1998a]. Alternatively, algae could be partially solar dried after the initial separation step (filter press

or centrifuge) and before the natural gas fired dryer to offset some of the drying costs (Fig. 2.2). However, the nominal results reported in this LCA are calculated without solar drying.

After the harvesting and drying steps, lipids present in the algae are extracted using a solvent (hexane) extraction process. Growth, harvest and oil extraction from algae are assumed to be performed at the same site. Algal oil is transported 150 km to a separate facility for conversion to algae biodiesel. Hexane extraction process and transesterification of resulting algal oil into biodiesel were modeled based on a previous soybean biodiesel LCA [Sheehan et al., 1998a].

The final process included in this LCA is the transportation and distribution of the biodiesel as described by the GREET model [GREET, 2008]. This involves transporting the biodiesel from a conversion plant through a distribution chain to a refueling station. The functional unit is described as 1000 MJ of biodiesel at a refueling station. Since this is a well-to-pump LCA, this LCA does not capture the emissions created resulting from the use of algae biodiesel in the vehicles.

2.3.3 Algae Dewatering

Algae dewatering is the most significant energy sink in the process and therefore a more detailed analysis was performed for this step. Dewatering freshly harvested algae slurry can be accomplished in two alternate pathways (Fig. 2.2). Both scenarios have three distinct steps to take the algae/wastewater mixture from a dilute 5×10^{-2}% w/w (0.5 g algae/L) to 91% algae. Only the first step of the two path-

ways is diferent. In the first process, a self cleaning plate separator centrifuge is used while in the second process chamber filter press is used as a first dewatering step.

Both scenarios allow for user defined amounts of solar drying as the second step so as to reduce the amount of water to be removed (and subsequent energy use) in the drum drying step. It is assumed there are no energy inputs (beyond the pumping requirements) to be accounted for in the solar drying process, no air emissions created, no mass lost and any water captured is sent back into further steps in the conventional wastewater treatment process from which the water was originally taken from. The third step in both scenarios is a natural gas fired dryer.

2.3.4 Data Quality Indicators and Notable Assumptions

2.3.5 Data Sources

Two primary data sources used to compute LCI environmental outputs and energy inputs were the US LCI database (created and maintained by ACLCA) [LCI, 2008] and the GREET model (Greenhouse Gases, Regulated Emissions and Energy use in Transportation) version 1.8 [GREET, 2008].

Data sources for process parameters and energy inputs are shown in Table 2.1. Since transesterification of algal oil is yet to be practiced on a large scale, little data exists. Data for the growth and harvest portions of the LCA were taken from Borowitzka and Borowitzka [1988], Shelef and Soder [1980], Richmond [1986],

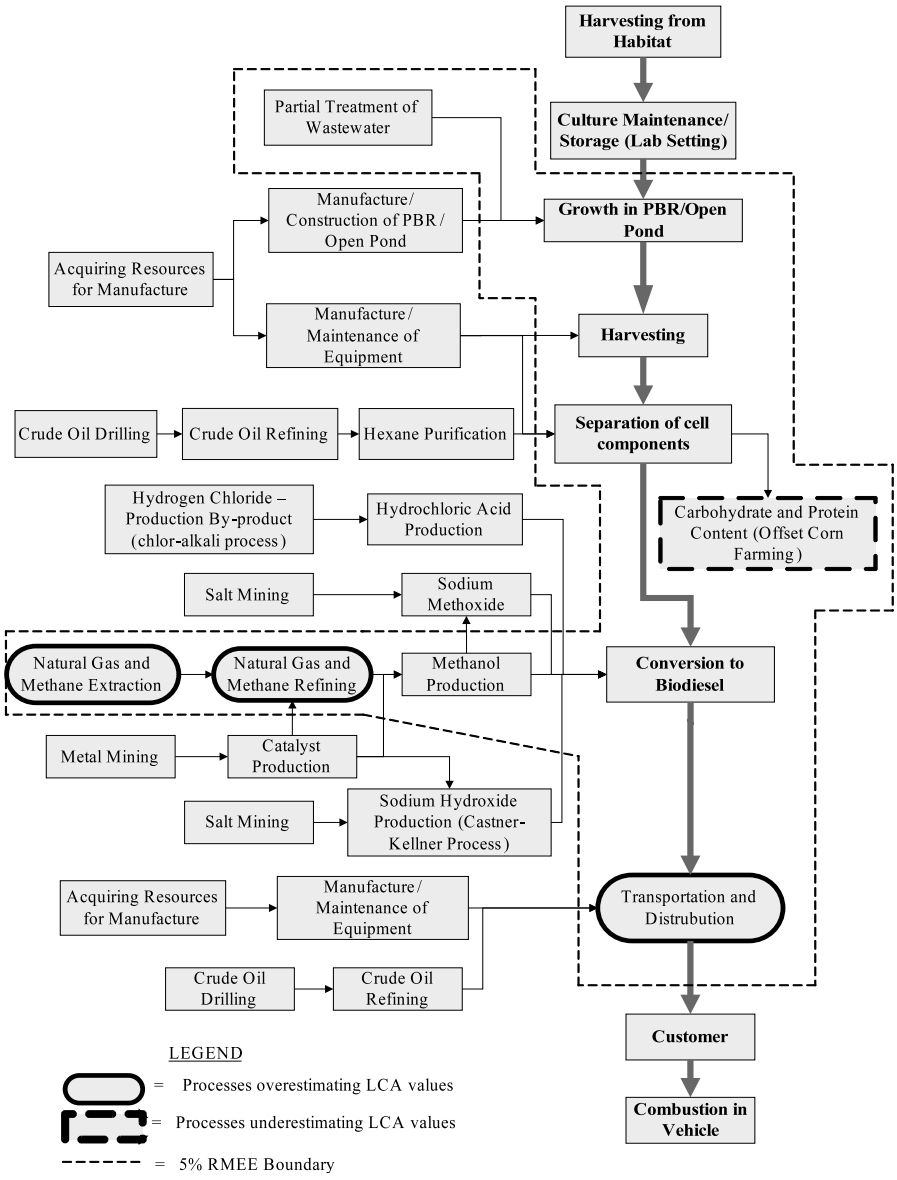

Figure 2.1: Process Flow Diagram

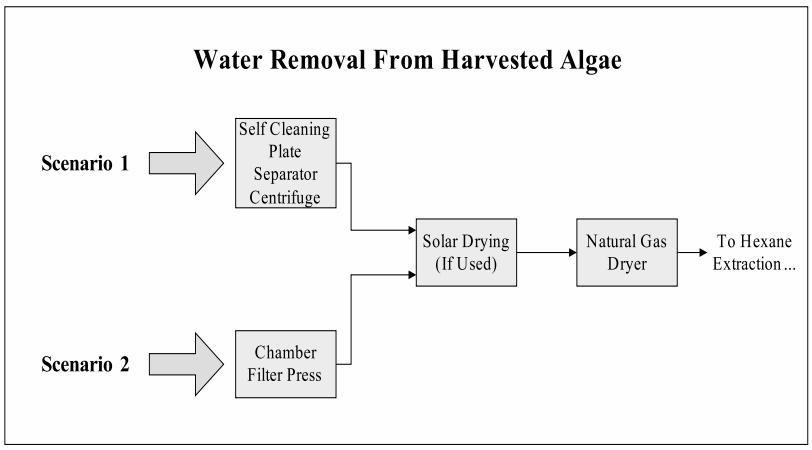

Figure 2.2: Water Removal Process Flow

Lembi and Waaland [1989].

Data for the soybean crushing (oil separation) and biodiesel conversion processes was obtained from Sheehan et al. [1998a]. Some of the steps such as grinding, hull cracking, flaking and other pre-extraction processing steps specific to soybean feedstock were omitted due to the differences between algal biomass and soybeans. Also, data was specified in terms of outputs per mass of whole beans delivered to the processing plant. Therefore, reported values were adjusted to units of outputs per "mass processed" rather than per "mass delivered."

Additional assumptions (Table 2.2) were made in regard to use of the soybean biodiesel model developed by Sheehan et al. [1998a]. Hexane extracted algal oil was assumed to have similar composition as soybean oil. The lipid percentage

Table 2.1: Summary of Data Sources

Unit Process	Data Source	Data Gathered from Source
RMEE Analysis	[GREET, 2008]	Natural Gas Boiler Efficiency Density of Diesel Fuel, Biodiesel;
	[Akers et al., 2006]	Heat of Combustion Diesel Fuel, Biodiesel
Growth	[Borowitzka and Borowitzka, 1988]	Electricity Requirements, Paddle Wheel and Pond Design, 1988
Harvest and Drying	[Shelef and Soder, 1980]	Operating Parameters of Separations Unit Process Equipment, 1980
	[Sheehan et al., 1998a]	Natural Gas Dryer Efficiency,1998 (National Oil Processors' Association)
	[LCI, 2008]	Natural Gas Dryer Emissions
Separation	[Sheehan et al., 1998a]	Hexane Extraction Process Information, 1998
	[Borowitzka and Borowitzka, 1988]	Dry Algal Cell Components
	[LCI, 2008]	Natural Gas Boiler Inputs and Emissions
Algal Oil Transportation	[LCI, 2008]	Diesel Truck Transportation Information
Biodiesel Conversion	[Sheehan et al., 1998a]	Base Catalyzed Transesterification
MeOH Prod. and Trans.	[LCI, 2008]	MeOH Production and Transportation Missing CO_2 Emission Information
Biodiesel Trans. and Dist.	[GREET, 2008]	Transportation and Distribution of Biodiesel Inputs and Emissions
Natural Gas Extraction and Processing	[LCI, 2008]	Natural Gas Extraction and Processing Inputs and Emissions
Coproduct Offset	[GREET, 2008]	Inputs and Emissions
	[Nielsen and Wenzel, 2005]	Density of raw corn

of the algal feedstock and working volume in the separation process were adjusted for typical algal values [Borowitzka and Borowitzka, 1988].

2.3.6 Coproduct Allocation

One of the largest input and output values (depending on parameter settings) in this LCA is the coproduct offset. In the United States, 97% of ethanol produced is made using corn as a feedstock [Shapouri et al., 2006]. Typical algae biomass has a substantial fraction of carbohydrate, particularly after the lipid has been removed. The carbohydrate fraction can be further converted to simple sugars and fermented into ethanol using a process similar to a dry grind corn ethanol conversion process. Thus, this LCA supposes that algae carbohydrate byproduct will become a feedstock for an ethanol conversion process, offsetting the currently used corn feedstock. Algae meal was chosen to have the same ethanol yield as wheat straw, since residual algae meal and wheat straw have similar glucan content [Kim and Dale, 2003]. Since algae do not contain lignin, it is anticipated that ethanol conversion process involving algae meal will not require a harsh lignocellulosic pretreatment. Based on preliminary studies [Sander and Murthy, 2009], algae "pretreatment" would consist of a process similar to corn dry grind ethanol liquefaction process.

The allocation was done using the system expansion method of allocation (Fig. 2.3). A theoretical yield was calculated for the algae meal, based on the assumption that 30% of the carbohydrates are cellulose [Ververis et al., 2007].

Table 2.2: Summary of Assumptions

Process	Assumptions
Growth	Wastewater substrate has ample nutrients for algal and bacteria growth. Raceway pond operated to maximize algal growth. Wastewater taken after secondary treatment. Algae are 30% lipids, 37.5% Protein, 31% Carbohydrates and 1.5% Nucleic Acids.
Harvest	Dryer requires 3,556 kJ/kg (850 kcal/kg) water removed [Sheehan et al., 1998a]. Dry algae has a density of 1 g/mL. Filter Press capture is 90%. No mass loss in dryer or during solar drying. Filter cloth replacement not included LCA due to lack of reliable data.
Separation	No energy input or emissions from solar drying. Algae delivered to hexane extraction at 9% moisture. Overall extraction process is 92.5% efficient. Hexane extraction is 96% efficient. Algae residuals leaving hexane extraction are at 12% moisture [Sheehan et al., 1998a].
Biodiesel Conversion	Distance traveled from extraction to conversion site = 150 km. Overall mass yield = 96.4%. MeOH to algal oil molar ratio 6:1 Transesterification reaction yield 99%. Density of algal oil = 0.93 g/L. No losses in glycerin settling tanks. Counter current biodiesel washing utilizes water equal to 20% w/w of biodiesel feed.
R.M.E.E.	Default GREET model assumptions. Density of algal biodiesel = 0.87 g/mL.

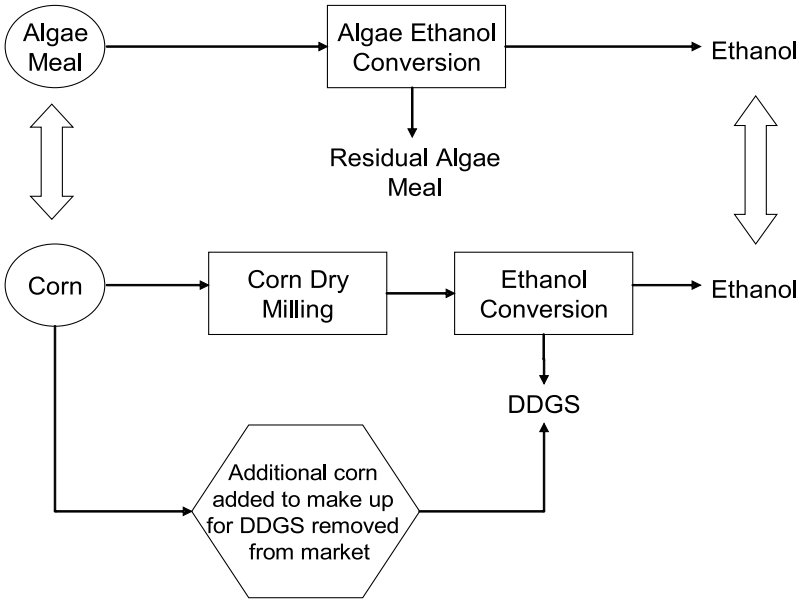

Figure 2.3: Coproduct Allocation Strategy

It is assumed hemicelluloses are not fermented in such a process. A cellulose to ethanol yield of 85% was assumed, resulting in 1.57 gallons (4.68 kg) of ethanol per functional unit. Assuming a corn dry grind ethanol yield of 0.387 L/kg corn (2.6 gallons/bushel), the algae meal coproduct from one functional unit would replace 13 kg of corn input.

Protein matter made up the bulk of the remaining algae meal. If algae were to replace the corn input into the ethanol process, 5.22 kg of DDGS would not be produced. Kim and Dale [2002] state a displacement ratio of 1.077 units of corn for 1 unit of DDGS. This would necessitate adding 5.62 kg of corn back into the system and bringing the net amount of corn displaced to 11.8 kg.

Residual algae meal, consisting mostly of protein and minerals, has not been evaluated as a replacement for other products. It is not definitively known at

present whether residual algae meal can replace DDGS or other protein rich products. For this reason, the residual algae meal was not assigned a displacement value nor were any of the inputs and emissions from algal biodiesel allotted to it. However, on a protein w/w basis, one functional unit of algae residual meal could replace 93.7 kg of DDGS (with a 30% w/w protein content) or 4.8 kg of urea fertilizer (assuming 50% bioavailability of the nitrogen in algae meal).

2.4 Results and Discussion

2.4.1 Overall Energetics and Sensitivity Analysis

The base case for all results reported in this LCA are calculated for the algae composition of 30% lipids, 31% carbohydrates, 37.5% proteins and 1.5% nucleic acids. The model allows for the algae compositions to be varied, resulting in different scenarios. Overall energetics can be described in terms of total energy and net energy input to the process as defined below.

$$\begin{aligned}
\text{Total Energy Input} &= \sum \text{Sub Process Energy Inputs} \\
\text{Net Energy Input} &= \text{Total Energy Input} - \text{Coproduct Allocation} \\
\text{Net Energy Balance} &= \text{Net Energy Input} - \text{Energy in Functional Unit, 1000MJ} \\
\text{Net Energy Ratio} &= \text{Net Energy Input/Energy in Functional Unit, 1000MJ}
\end{aligned}$$

Table 2.3: Total Energy Sensitivity

Algal Lipid Content (% w/w)	Total Energy Input (J/MJ Algae Biodiesel)	Net Energy Input (J/MJ Algae Biodiesel)
40	2,500	-6,103
30	3,292	-9,943
20	4,878	-17,620
15	6,470	-25,293
10	9,665	-40,628
5	19,347	-86,531

Sensitivity of total and net energy input to algae lipid composition at constant carbohydrate to protein ratio is shown in Table 2.3. This model is particularly sensitive to the lipid content of the algae being grown. This is due to the fact that the functional unit of the LCA was set as 1000 MJ of algal biodiesel. In most algae species, there is typically a larger percentage of carbohydrates than lipids in an algae cell. With such a large percentage of the algae cell being carbohydrates, algae's potential as an ethanol feedstock cannot be ignored. According to the model used, for every 24 kg of algal biodiesel produced (one functional unit, 1000 MJ algae biodiesel), 28.1 kg carbohydrates and cellulose co-product are also produced. With less than 2% lignin [Ververis et al., 2007], algae also circumvents the issue of processing a lignin-laden material. As the lipid content of algae decreases, a larger amount of residual algae mass is processed into ethanol resulting in larger coproduct credits. Therefore total energy increases due to increased processing energy, as algae lipid content decreases. However net energy also increases due to higher coproduct credits as algae lipid content decreases (Table 2.3).

Total energy input with no solar drying is 3,292 and 6,194 MJ for the process

with filter press and centrifuge as the initial filtering step, respectively. Overall net energy input for the process with no solar drying was -9,943 MJ and -7,041 MJ when a filter press and centrifuge were used as the initial filtering step, respectively (Table 2.3). To achieve zero net energy balance without coproduct allocation for a filter press process, the algal slurry would have to be solar dried to 19% w/w moisture. Solar drying process is well documented and has been used for many years in Asia for drying food quality algae and in agriculture [Kadam, 2001, Hills and Nakamura, 1978]. Although not infeasible, this process relies on the sun as its driving force and would be too slow for large scale commercial applications.

The largest energy input is in the natural gas drying of the algal cake. This process comprises 69% of the entire energy input into the process. Algae carbohydrates displaces corn, which uses petroleum intensive corn farming during its production. If the carbohydrate coproduct is not considered, a significant amount of solar drying would be needed to achieve negative net energy ratios. Huo et al. [March] report a net energy ratio 0.15 for soybean biodiesel using the displacement method for allocating co-product offsets. For an algal biodiesel process using a filter press, this ratio is -9.9 (-9,942 MJ total process energy/1000 MJ functional unit).

Overall results of this well-to-pump LCA for a functional unit produced using two types of harvesting processes are presented in Fig. 2.4, Fig. 2.5 and Table 2.4. Solid waste in both processes is waste oil and grease skimmed during the biodiesel conversion process. These are the only solid waste streams generated in the entire process. The solid wastes from wastewater treatment are not included

Table 2.4: Well-to-Pump Algae Biodiesel Process Wastes

Dewatering Process ⇒ / Wastes ⇓	Centrifuge	Filter Press
Air (kg)	55	-104
Waterborne (kg)	18.60	18.60
Solid (kg)	0.28	0.28
Radioactive Species (Bq)	5.54	5.54

Table 2.5: Well-to-Pump Algae Biodiesel Net Air Emissions

Dewatering Process ⇒ / Emissions (kg) ⇓	Centrifuge	Filter Press
VOC	-0.18	-0.23
CO	-1.22	-1.24
NO_x	-2.13	-2.26
Particulate matter (PM 10 μm)	-0.19	-0.20
Particulate matter (PM 2.5 μm)	-0.14	-0.14
SO_x	2.59	1.29
CH_4	0.72	-0.03
CO_2	46.17	-110.44
Other	8.44	8.44

in this LCA. Radioactive species is a byproduct of steam generation in a natural gas boiler [LCI, 2008].

A well-to-pump GHG emission (measured as CO_2 equivalents of CO_2, CH_4 and N_20) of -0.074 kg CO_2 equivalent /1000 MJ of soybean biodiesel was reported by Huo et al. [March]. Well-to-pump GHG emissions for conventional gasoline is 119.0 kg of GHG per 1000 MJ of gasoline in GREET model [GREET, 2008]. Equivalent GHG emissions for a filter press algae biodiesel process with coproduct allocation would be -112.6 kg CO_2 equivalent/1000 MJ of algal biodiesel. Net CO_2 emissions are -110 and 46 kg/functional unit for filter press and centrifuge

Table 2.6: Fertilizer and Pesticide use in GREET Model

Chemical	Amount Applied g/kg(g/bushel)
Nitrogen	16.52(420.00)
P_2O_5	5.86(149.00)
K_2O	6.84(174.00)
$CaCO_3$	47.28(1,202.00)
Herbicide	0.32(8.10)
Insecticide	0.03(0.68)

Table 2.7: Corn Farming Energy Mix in GREET Model

Fuel Source	Amount of Energy kWhr/kg$\times 10^{-3}$ (BTU/bushel)
Diesel Fuel	65.86 (5,715.00)
Gasoline	26.48 (2,298.00)
Natural Gas	21.15 (1,835.00)
Liquefied Petroleum Gas	24.42 (2,119.00)
Electricity	7.69 (667.00)
Total	145.61 (12,635.00)

case respectively. The natural gas drying process alone accounts for 45% and 39% of the CO_2 emissions (excluding the coproduct credits) from the filter press and centrifuge process, respectively. It is interesting to note that although net energy for both filter press and centrifuge processes are -9943 and -7041 MJ/functional unit respectively, CO_2 emissions are positive for centrifuge process while they are negative for filter press process. This reinforces the need for a comprehensive analysis of all impact categories when assessing sustainability of biofuels using LCA. Other criteria pollutants which are created (per functional unit) during the process utilizing a filter press are -0.23 kg of VOC's, -2.3 kg of NO_x, -1.2 kg of CO, -0.34 kg of particulate matter and 1.29 kg of SO_x resulting in a net -105 kg of total air emissions per functional unit (Table 2.5), 18.6 kg of waterborne wastes, 0.28 kg of solid waste and 5.54 Bq are created (Table 2.4).

Evaporative water loss during algae growth is the largest quantity of water consumption. Evaporative loss of 13.716 cm/month was estimated based on evaporation tables from Bakersfield, CA [CDWR, 2007]. Evaporative water loss accounts for 10% from total volume of 201.42 m^3 algae culture lost during four days of growth. This water was not included in the LCA totals for fresh water use because wastewater was assumed to be used as the water source. This volume of waste water can be produced in four days by 34 people with an estimated waste water output of 0.375 m^3/person-day [Kenny et al., 2009]. If algae are grown in open pond systems with thousands of acres of surface area and utilize wastewater, the evaporation make-up demands must be considered in design and sourced appropriately.

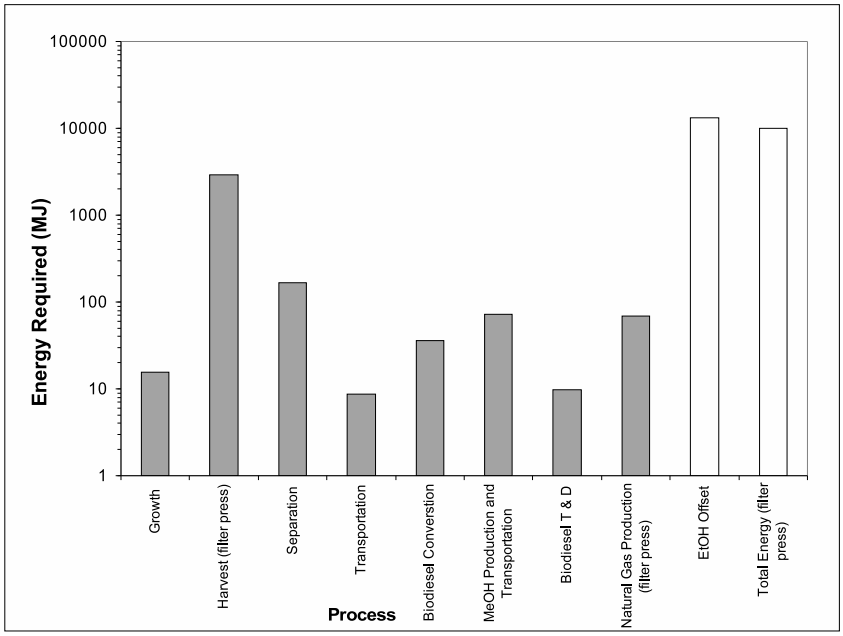

Figure 2.4: Total and Net Energy Utilizing a Filter Press for Algae Dewatering

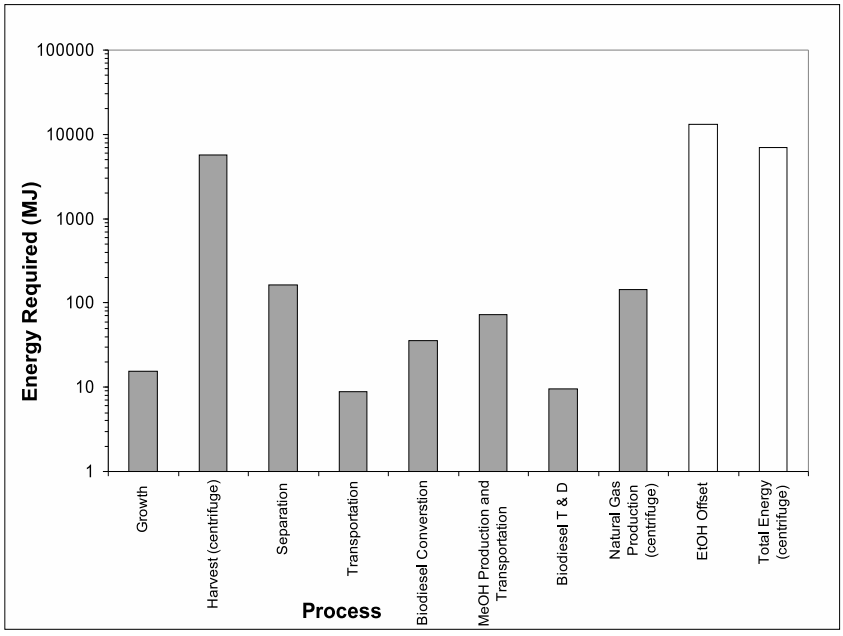

Figure 2.5: Total and Net Energy Utilizing a Centrifuge for Algae Dewatering

One process improvement which might be made to reduce overall energy use and GHG emissions would be to degrade the algal biomass enzymatically in aqueous solutions [Sander and Murthy, 2009]. This would obviate the need to remove water and the energy input required for such water removal. In addition, CO_2 emissions would be reduced by 45% as well. Another processing method may be the anaerobic digestion of algae biomass. The algae drying step will not be necessary if it is to be anaerobically digested, however the pre-filter step will still be necessary.

2.4.2 RMEE Method Inconsistencies

Due to difficulties in obtaining LCA data, RMEE boundary selection method could not be followed completely. In some instances, the inconsistencies were overestimated to the $> 5\%$ limit for RMEE boundary selection process. In other cases data for the processes that accounted for $> 5\%$ could not be obtained. All the inconsistencies in the RMEE boundary selection process are shown in Fig. 2.1 and are discussed below.

The US LCI data was used for the transportation of algal oil from the production facility to the biodiesel conversion facility. US LCI data couples diesel fuel emissions with the "secondary" energy of transporting and distributing that diesel fuel from the refinery. Therefore diesel emissions could not be uncoupled from the secondary emission effects as required by RMEE method.

In some instances, electricity input to a process was within the system

boundary (as defined by the generic system unit), yet the emissions associated with the generation of that electricity fell outside of the RMEE boundary. One example of this is in the growth process of this LCA. No emissions are attributed to the growing of algae, although electricity is used to operate paddle wheels and pump wastewater/algal slurry. The electricity is $< 5\%$ RMEE boundary and thus the upstream electricity generation process in not included in the LCA. While it does not cause an inconsistency with the system boundary, it is a point meriting discussion.

Other violations of the RMEE boundary are present in the apparent exclusion of combustion emissions from U.S. LCI data during methanol production. This may be from a lack of data because CO_2 is not currently an industrially regulated emission. No resolution to this issue is yet available; hopefully future versions of this model will show differently.

The coproduct offset from corn farming used from the GREET model was considered as one data (i.e. energy use and emissions for the entire process of corn farming using GREET assumptions as one data set). This violates the fundamental RMEE selection process because it does not start with the process closest to the functional unit and move outward. Since the boundary was not selected in accordance to RMEE, the coproduct allocation offset is a violation of the RMEE boundary. A past LCA utilizing the GREET model [Wang et al., 2007] as well as the GREET model itself provide some insight into the processes included in the corn farming. Corn farming processes in GREET includes the processes of farming and collection of corn. The process includes the application of fertilizers

and pesticides as shown in Table 2.6. The production of farming equipment is not included.

A portion of the CO_2 emissions (195 g/bushel corn) are calculated based on potential land use changes. Total energy input into corn farming and collection is 145.61 $\times 10^{-3}$ kJ/kg (12,635 BTU per bushel) of corn. This is an estimate in the GREET model for corn farming practices in the year 2010. A breakdown of the fuel mix for corn farming is shown in Table 2.7. Despite the efforts to quantify the individual processes, the extent of deviation is difficult to quantify and therefore this may cause the results reported to be $< 5\%$ RMEE cut-off limit.

2.5 Conclusions

This LCA has quantified one major obstacle in algae technology, the need to efficiently process the algae into its usable components. Thermal algal dewatering requires high amounts of fossil fuel derived energy (3,556 kJ/kg (850 kcal/kg)) of water removed and consequently presents an opportunity for process improvements to reduce energy use. Current technology would be relatively inefficient, unreliable (in the case of solar drying) or inadequate on an industrial scale. The removal of water is an intermediate step in the final separation of algae components, and ultimately may not be necessary. If there is a separations process capable of separating the algae into its components without having to undergo the energy intensive process of water removal, this would fulfill the needs of sustainable algae bioprocessing. Enzymes may provide a pathway to developing such a process. The

potential of green algae as a fuel source is not a new idea [Sheehan et al., 1998b], however this LCA and other sources clearly show a need for new technologies to make algae biofuels a sustainable, commercial reality.

Chapter 3 – Filtration of Raceway Grown Algae Through Rapid Sand Filters

3.1 Introduction

3.1.1 Algae Separation and Dewatering

Cultured algae in engineered systems possess potential to one day replace liquid petroleum fuels. Development of effective, economical downstream processing of algae biomass continues to be a major bottleneck to progress [Sheehan et al., 1998b]. One of the most energy intensive processes in algae-to-fuel technologies is the removal of culture broth to increase biomass concentration [Sander and Murthy, 2010]. Many proven technologies have been investigated for their potential use in harvesting algae such as sedimentation, flocculation, centrifugation and filtration.

The two most economical process options from this list are sedimentation and filtration. Sedimentation is most effective when suspended solids to be removed are greater than 100 μm in diameter and 50 mg/L in concentration [Sincero and Sincero, 2003]. Popular green algae open-pond culture species being proposed as biofuel precursors are usually less than 100 μm in diameter but greater than 50 mg/L. However, this rule of thumb is of course ignoring any surface chemistry effects which may play an impact in either of these processes.

Sedimentation of algae species is governed by stokes law and depend on the size of the algae and the efficiency of floc formation. Floc formation is also sensitive to environmental conditions (in the case of outdoor growth) and operating parameters [Al-Shayji et al., 1994]. Algae settling times are between 0.1 and 2.6 gm/hr depending on size and tendency to floculate [Choi et al., 2006]. The density of the algae culture is independent of settling time [Choi et al., 2006]. As any industrially grown algae for biofuel purposes will most likely be grown outdoors, variability introduced by the weather and changing algae species will need to be understood to effectively use flocculation and settling as harvest tools.

Granular filtration technology has been in use for over 100 years as a wastewater remediation tool. The objective of granular filtration is to remove suspended solids from a water source thereby reducing the amount of turbidity. Most modern water treatment protocols call for outlet streams containing less than one NTU (nephelolometric turbidity units) [EPA, 2010]. It is understood and taken as fact that the turbidity of water is an indication of the presence of disease causing organisms [EPA, 2010].

By far the most popular method of removing suspended solids in drinking water is through granular filtration [EPA, 2010]. The most common form of granular filters in use today are rapid filters, operating at flowrates between 4 and 50 m/hr. Granular filters utilize deep beds of small diameter particles to remove solid material from water as it is flowed through the bed. Typical substances used as bed material are sand, diatomaceous earth (DE), anthracite coal and garnet, activated carbon and synthetic resins [Crittenden et al., 2005, Montgonmery, 1985]. Proper-

ties desired in filter bed media are a uniform size and sphericity and a recalcitrant surface.

3.1.2 Gravity Filter Operating Parameters and Factors

Granular filters can be operated under gravity, under pressure or under vacuum [Crittenden et al., 2005], the former requiring the least direct energy input while the two latter allow for more pressure to overcome head losses. One principle operation parameter which drastically affects the performance of a granular filter is the water flux through the filter. Generally, faster flowrates (i.e. higher flux) equate to less capture by the filter and consequently more volume may pass through the filter before backflushing is required. Also, a general rule in granular filters is that a smaller effective particle size (d_e) leads to higher suspended solids removal but shorter run times and more frequent backwashing. This can be overcome by increasing the depth of the sand bed, potentially allowing more void volume to capture solids. However, if the void spaces are too small relative to the retained particles, or the flowrate is too slow, the upper portions will become clogged without effectively making use of the increased bed height. An empirical correlation between sand filter bed height and effective particle size was documented by [Montgonmery, 1985] for different bed porosities. This correlation is only valid for beds utilizing sand as the filter media and not other particles which may have different filtering properties.

A number of different mechanisms act to trap and retain solids in the filter

bed such as impaction, straining and attachment. The size of the suspended solids present relative to the size of filter bed particles and the Reynolds number of the fluid flowing in the interstitial areas of the bed will result in different mechanisms being dominant [Crittenden et al., 2005, Montgonmery, 1985]. Furthermore, beds can be constructed of uniform sized media, or various layers of differing sized media. A metric used to describe granular filter beds is the "uniformity coefficient." The uniformity coefficient is defined as:

$$U_c = \frac{d_{60}}{d_{10}} \tag{3.1}$$

The value d_{10} refers to the sieve size which will retain all but the finest 10% of particles of the granular bed. Similarly, the value d_{60} refers to the sieve side which will retain all but the finest 60% of particles of the granular bed. The value d_{10} is also referred to as the "effective size" of th filter material. The uniformity coefficient for a rapid sand filter should not exceed 1.5 [Sincero and Sincero, 2003].

As the feed water flows through the filter bed, solids from the incoming water stream become entrained in the bed while allowing the water to pass through. At the beginning of a filter run, a sand filter bed has a characteristic head loss which must be overcome to push water through the bed. As more and more solids are deposited in the filter bed, this head loss increases due to ever shrinking void spaces between the particles. This continues until the filter cannot accept anymore solids (so called filter "breakthrough"), the head loss cannot be mechanically overcome or until the filter cannot produce water meeting turbidity specifications [Sincero

and Sincero, 2003, Degremont, 1973, Crittenden et al., 2005, Montgonmery, 1985].

At this point, the filter must be cleaned by backflushing. Backflushing is done by introducing wash water in the reverse direction of normal filter flow at a rate such that the sand bed achieves an "expanded" bed volume 15-25% larger than the normal bed volume. Fluidization of the bed media serves to free the trapped particles through strong convective flows and the particles impacting each other. Backflushing is done until the washwater runs clear from the filter.

Sand filtration is typically a unit process done in combination with other solids removal processing, most notably sedimentation and flocculation (either chemical flocculation or autoflocculation). Sedimentation is typically performed first to remove the largest particles with shortest settling times. Flocculation is either done in tandem with sedimentation or directly following sedimentation. Flocculation of suspended solids allows the subsequent filtering step to be more effective [Degremont, 1973].

3.1.3 Past Studies

Past studies [Konno and Sato, 1986, Grima et al., 2003, Harun et al., 2010, Kothandaraman and Evans, 1972, Borchart and O'melia, 1961] have indicated that sand filtration of algal cultures is effective only in capturing algae of relatively larger dimensions such as *Spirulina platensis*. These studies suggest typical sand filters are ineffective at capturing algae of smaller dimensions such as *Chlorella* or *Dunaliella*, which measure only a few microns at their narrowest. Borchart and

O'melia [1961] report obtaining 33, 22 and 10 percent removal efficiencies through a 107 cm deep sand bed of sand sizes of 0.316, 0.397 and 0.524 mm respectively. Furthermore, no difference was found across flowrates ranging from 0.2 and 2.0 gpm/sq.ft. Converse to these studies, Naghavi and Malone [1986] utilized sand beds with much smaller effective particle size (0.064 - 0.2 mm) and smaller operational flowrates (80-160 LPM/m^2) but much smaller bed depths (3-12 mm) and achieved more than 97% removal of *Scenedesmus quadricauda* in all cases. Furthermore, it is concluded that if a media effective particle size fine enough to capture algae was used, it would clog too fast and backflushing would be too frequent for practical purposes.

Given the mixed results of past studies, current opinion still seems to hold that filtering algae through granular media is not an effective process technology for concentrating biomass [Grima et al., 2003, Harun et al., 2010, Merkele, 2007]. Also, the problem of head loss in the filter was addressed by Kothandaraman and Evans [1972]. This review states gravity filters are not a promising technology due to their limited practical head pressure availability, yet this can be overcome, if economically feasible, by operating a granular filter under pressure or under vacuum.

3.1.4 Objectives of This Study

We propose an overall process similar to one suggested by Kothandaraman and Evans [1972] whereby algae in culture is separated in a series of three sequential

dewatering procedures; a culture at 0.02-0.04% w/w biomass is first taken to 1-4 % w/w biomass through "concentration" (either sedimentation or filtration) and then to 8-20% through "dewatering" (using a centrifuge or filter press) and then, if needed, dried to 85-92% w/w biomass. As Kothandaraman and Evans [1972] make light of, preconcentration of algal culture using a low tech solution (filtering or sedimenting) will reduce the power demand significantly in subsequent dewatering processes. The reason centrifuges have been shunned from use as culture concentrators is their high power requirement [Shelef and Soder, 1980]. Furthermore, if any pretreatment of the cell mass is required for downstream component separation and processing, this can be accomplished in the concentration recovery step. An example would be backflushing a sand filter with hot dilute acid. This might, in effect, clean the sand filter, collect the concentrated algae biomass and pretreat the biomass all in one step.

The specific objectives of this study are:

1. Determine which filter configuration (of three effective particle sizes) best collects algae from a culture solution

2. Determine which filter configuration allows for the best recovery (through backflushing) from culture solution

3. Determine if algae biomass can be concentrated by at least a factor of 10 using rapid sand filtration

3.2 Materials and Methods

3.2.1 Raceway Pond Design

Algae was grown in a 1211 L single-loop open raceway pond. The pond body was made of galvanized steel sheet metal, and the inside surface of the pond was coated with a commercial pick-up truck bed liner to prevent corrosion.

The pond was operated at a culture depth of 51 cm, which is almost twice as deep as conventional raceway ponds [Shelef and Soder, 1980]. The inner race of the pond was constructed of acrylic sheeting to which red and blue LED light strips were fastened to the bottom 15.2 cm. This sheet was then put into a sleeve, also constructed of sheet acrylic, to keep the lights and electrical connections from being submerged in water. One strip of blue lights and two strips of red lights were fastened along the length of the wall sheeting. The motivation for this design was to reduce the footprint of open ponds while maintaining the algae harvest concentrations and light availability of conventional open ponds. Another issue which arises upon increasing the depth of open ponds is an increase in abundance of unwanted organisms. This especially becomes a problem in the lower, relatively unmixed regions where light does not often penetrate to. In these regions, bacteria and rotifiers can take hold and decrease the algal biomass potential of an open raceway pond. Both of these problems might be abated by the light provided by the LED lights placed in the bottom of the pond.

The pond was mixed using a paddlewheel constructed of four 30 cm square blades made of acrylic affixed to a central acrylic shaft 35 cm in length. This

shaft was then placed on the outside of and affixed to a mild steel shaft 1 meter in length. This rod was turned by a 1/3 horsepower (1725 rpm) motor which was gear-reduced twice to turn at 60 revolutions per minute. The paddlewheel shaft, as well as the secondary gear reducing shaft, were both supported by bearings mounted in plastic and clamped to the inner race sleeve on one end and the outer wall on the other. Custom length v-belts and v-belt pulleys transfered turning power from the motor to the two shafts. A schematic of the pond and paddlewheel configuration can be seen in figure 3.1. A picure of the pond during operation can be seen in 3.2

3.2.2 Raceway Pond Operation

The pond was operated inside of a greenhouse and the temperature was kept constant at $21^oC \pm 1^oC$. The LED light strips along the bottom of the inner race were continuously illuminated as well as two 1200 Watt Sodium Grow Lights 1.5 meters above the pond surface. The greenhouse was enclosed by a translucent roof and the pond was subject to natural diurnal variations. The pond rested on a 0.5 m bigh bench. Nutrients were supplied by adding an amount of MiracleGro All Purpose Plant Food Fertilizer^{TM} consistent with 2.94 mM available Nitrogen.

The temperature of the pond was measured in the top 10 centimeters of liquid as well as in the bottom 10 centimeters of the pond. The ambient temperature inside of the greenhouse was also measured and logged. The pH of the pond was kept at 6.5 ± 0.2 by injecting CO_2 into the culture medium through an aquarium sparging

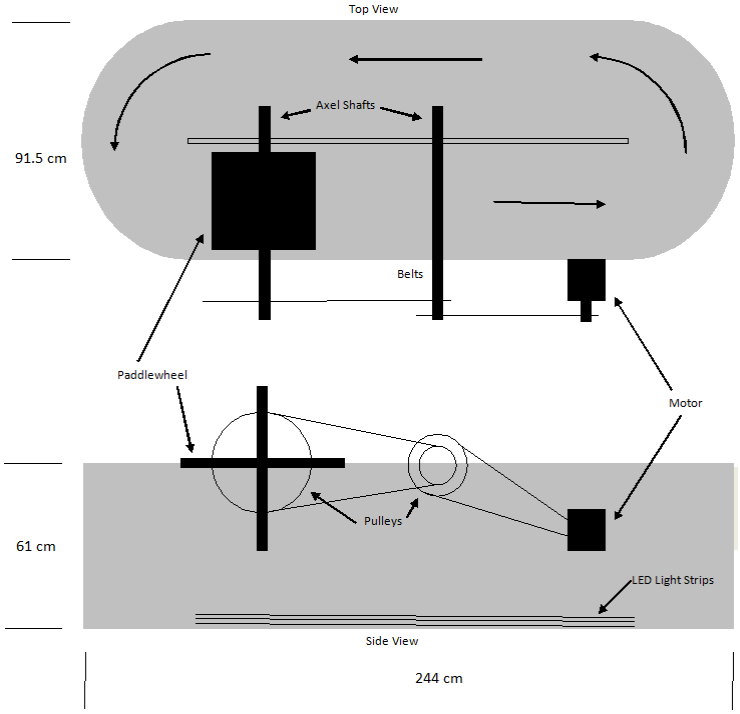

Figure 3.1: Open Raceway Pond Diagram and Dimensions

Figure 3.2: Open Raceway Pond

stone. The pH was controlled using a pH controller (Milwaukee Testers SMS122 pH meter, Milwaukee Instruments, Inc., Rocky Mount, NC) CO_2 solenoid regulator (Milwaukee Testers MA957 CO_2 regulator, Milwaukee Instruments, Inc., Rocky Mount, NC). Ambient light intensity was logged using a Li-Cor PAR spherical light sensor connected to a LI-COR variable resistance signal adapter (both LI-COR Biosciences, Lincoln, NE) which converted the μS signal of the meter to millivolts. This converted signal was then amplified 100 times through a dual instrumentation operational amplifier (Model EI-1040, Electronic Innovations Corp., Lakewood, CO) and sent to the LabJack U12 data acquisition module (LabJack Corporation, Lakewood, CO).

3.2.3 Inoculation and Semi-Batch Operation

A 2 L inoculum of *Scenedesmus dimorphus* was grown under aseptic laboratory conditions in Proteose medium (UTEX recipe with Bristol medium and Proteose Peptone) in a 4L flask. After the culture had reached exponential growth, it was transfered to a 110 L conical bottom cylindrical tank which was continuously circulated using two small aquarium pumps. The pH was controlled using the same pH controller mentioned above. The culture was allowed to grow for 8 days and this inoculum was transfered to the raceway pond while the culture was still in the exponential growth phase.

The open raceway pond was operated in semi-batch fashion. Daily biomass samples were taken to monitor biomass concentration in the pond. When the

biomass concentration reached the desired maximum value, 32% of the pond volume (385 L) was removed and replaced with an equivalent volume of UV sterilized water which had been allowed to equilibrate to ambient temperature and a corresponding amount of MiracleGro All Purpose Plant Food^{TM}was also added. The water was sterilized by continuously pumping it through a 50 W SMART High Output UV Sterilizer (Emperor Aquatics, Pottstown, PA) for at least 3 days.

During the first two cycles, a full logistic growth curve was allowed to develop to determine the maximum growth concentration potential for the pond. For the remaining cycles, the biomass concentration was cycled when the biomass concentration achieved 90% of the maximum biomass concentration potential. The pond was cycled in this fashion about every four days or as dictated by algae growth. Three 50 mL samples were taken daily at approximately the same time (about 8:30 am each morning) and measured in a spectrophotometer at 680 nm to monitor growth.

The evaporation rate of water from the pond was also measured. This was done by marking a "fill line" on the inside wall of the tank and each day filling the pond back to the fill mark, noting how much water was used to do so. Municipal hose water was the source used to refill the pond. The evaporation rate was found to be 18.5 liters/day.

3.2.4 Modeling of Raceway Pond Growth

In order to better understand the growth behavior of this open pond, a three state, two input biomass growth model was developed to predict growth in the pond. The model system (visualized in Fig. 3.3) incorporates a state function for biomass, CO_2 and O_2. Inputs into the model were irradiation (from natural sunlight and the lights along the bottom of the tank) as well as CO_2 (injected to control pH).

All equation parameters and constants were first sourced from literature (see Table 3.1). The parameters which impacted results the most were the specific maximum growth rate (r_{max}), the specific respiration constant (r_a) and the death and maintenance constant (k_d). These were then fit using a least sum of squares method. The model was solved numerically in a Microsoft Excel^{TM} spreadsheet using a 4^{th} order Runge-Kutta approximation with a timestep of 0.05 hr over a simulation time of 24 hrs.

The model was created using the assumption that illumination and CO_2 were the only growth limiting factors. Relating light intensity to specific growth rate was done using the Steele Equation [Lapidus and Amundson, 1977]. This equation accounts for light saturation and light limitation in algae using an exponential equation. Light distribution in ponds varies with depth and location. Local irradiance values measured at 99 equally spaced points were measured. These values were used to calculate the average irradiance based on the velocity of water at each point. This weighted average pond irradiance (I_{ave}) was then correlated to the ambient irradiance (I_{amb}) by using a calculated multiplier (k_i). A linear correlation

was developed, with an R^2 value of 0.945, using the following equation:

$$I_{ave} = \frac{\sum v_i I_i}{\sum v_i} = k_i I_{amb} \tag{3.2}$$

Furthermore, biomass is assumed to decrease at a biomass and oxygen dependent rate due to respiration. Since CO_2 concentration was injected to regulate pH, CO_2 saturation was not limiting at the concentrations seen in the pond. It is known that respiration losses are more impactful at night when algae cells are not photosynthesizing at daytime rates [Grobbelaar, 1985]. This term of the equation is much smaller than the daytime photosynthesis biomass accumulation rates and is only apparent at times of low irradiance. A maintenance and death term was also incorporated into the biomass equation.

The remaining terms in the state equations for CO_2 and O_2 were due either to the uptake or release of gaseous species by the algae or diffusion. The diffusion coefficients were taken from literature and equilibrium constants were calculated using Henry's law. CO_2 injections occured for 3 min every hour. This was verified during previous pond operation. CO_2 injection simultaneously led to O_2 removal. Diffusion during forced bubbling diffusion had a negligible effect on the gaseous make up of the culture. The dominant diffusion term was across the surface of the pond. Due to constant paddlewheel mixing, the pond was assumed to achieve equilibrium with the ambient air (assumed to be 0.3 % CO_2, 21% O_2 and the balance N_2).

Another constraint of the model are that the system is not temperature depen-

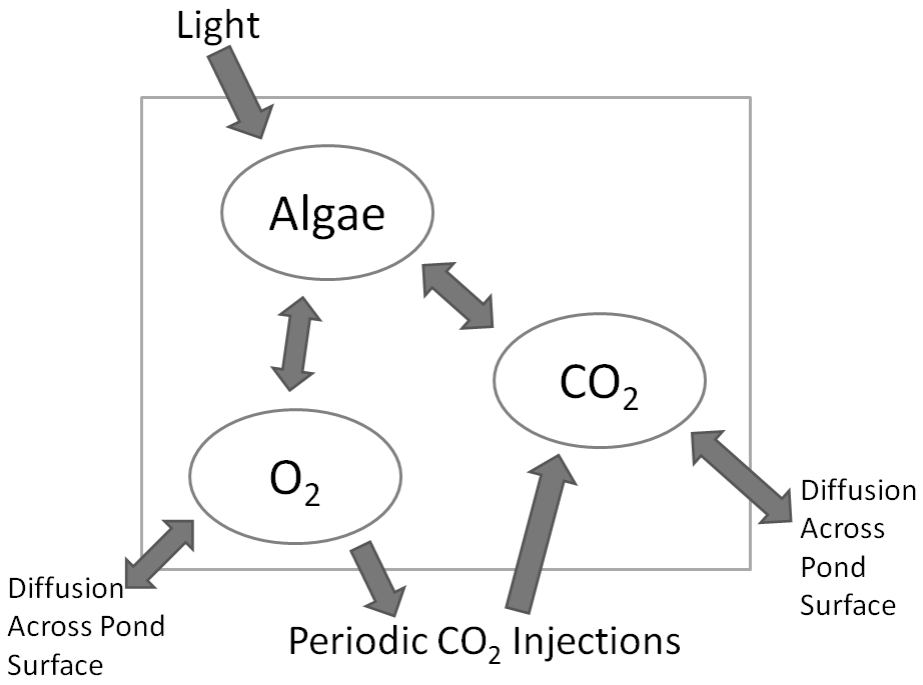

Figure 3.3: Model Block Diagram

dent. As this pond was operated in a greenhouse, the temperature (both in the pond and inside the greenhouse) was held at $20^oC \pm 1^o$C. The effect of temperature on algae growth was not modeled. The light input was also approximated as the positive portion of a sine wave with a base constant value substituted for the negative portions of the sine wave 3.4. As this method does not represent the actual light curve well, later iterations of this model might incorporate actual irradiance data as an input. The CO_2 injection frequency and duration were also assumed to be constant, though this assumption was verified, physiologically this frequency would change within the system throughout the different stages of algal growth, as the uptake of HCO_3 increases as growth rates approach their maximum.

Another critical assumption of this model is that the system is not macronutrient limited. Each successive semi-batch growth cycle was begun with the addition of the equivalent of 2.94 mM and 1.03 mM of nitrogen and phosphorous, respectively. Similar concentrations of these elements is found in popular laboratory medias (Bristol's, P49 and TAP media). Nitrogen and Phosphorous species were assumed to be available in sufficient concentration at all stages of growth and were not included in the growth model. This assumption should be validated however to ensure neither phosphorous or nitrogen are limiting growth in this system.

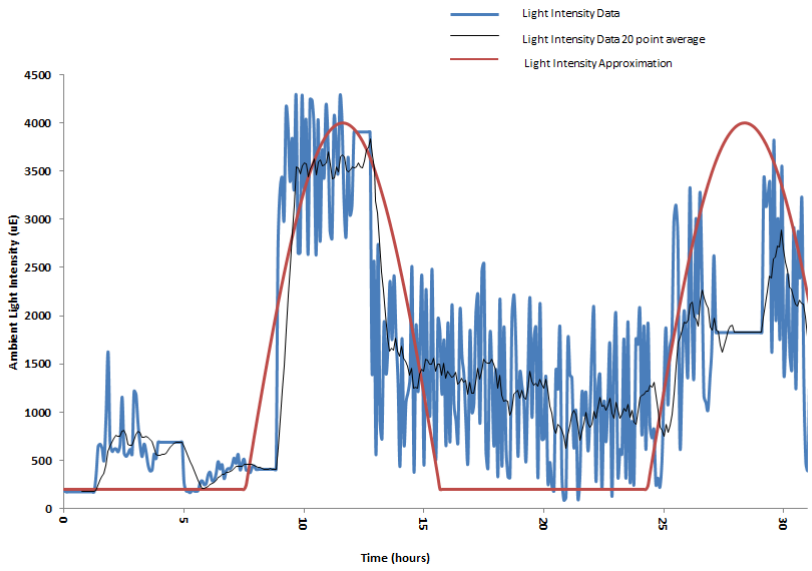

Figure 3.4: Light Input Approximation

The model consists of the following equations:

$$\frac{dA}{dt} = Ar_{max}\frac{I_{ave}}{I_{max}}e^{1-\frac{I_{ave}}{I_{max}}}\frac{CO_2}{Km_{CO_2}+CO_2} - Ar_a\frac{O_2}{Km_{O_2}+O_2} - Ak_d \tag{3.3}$$

$$\frac{dCO_2}{dt} = 4\pi r(D_{CO_2})(k_{H,CO_2}P_{Bubble,CO_2}MW_{CO_2} - CO_2)\frac{F}{\frac{4}{3}\pi r^3}t_{bubble} - 1.566Ar_{max}\frac{I_{ave}}{I_{max}}e^{1-\frac{I_{ave}}{I_{max}}}\frac{CO_2}{Km_{CO_2}+CO_2} + 1.566Ar_a\frac{O_2}{Km_{O_2}+O_2} - D_{CO_2}\frac{CO_2 - k_{h,CO_2CO_{2,air}}}{L}(SA_{pond}) \tag{3.4}$$

$$\frac{dO_2}{dt} = 4\pi r(D_{O_2})(k_{H,O_2}P_{Bubble,O_2}MW_{O_2} - O_2)\frac{F}{\frac{4}{3}\pi r^3}t_{bubble} + 1.139Ar_{max}\frac{I_{ave}}{I_{max}}e^{1-\frac{I_{ave}}{I_{max}}}\frac{O_2}{Km_{O_2}+O_2} - 1.139Ar_a\frac{O_2}{Km_{O_2}+O_2} - D_{CO_2}\frac{CO_2 - k_{h,CO_2CO_{2,air}}}{L}(SA_{pond}) \tag{3.5}$$

Two data sets were collected over two consecutive 24 hr periods. One data set was used to calibrate the model (using a least squares fit approach) while the other data set was used for comparison. Ambient irradiance, CO_2 injection time, pH,

Symbol	Definition	Value (units)	Source
A	Algae State	(g/m^3)	
CO_2	CO_2 State	(g/m^3)	
O_2	O_2 State	(g/m^3)	
r_{max}	Algae Growth Rate Constant	0.052 (hr^{-1})	[Grima et al., 1996]
r_a	Algae Respiration Constant	0.005952 (hr^{-1})	[Erikson, 1999]
k_d	Algae Death and Maintenance Constant	0.00385 (hr^{-1})	[Grima et al., 1996]
I_{max}	Maximum Light Intensity	1020.3 ($\frac{\mu mol}{m^2 \bullet sec}$)	[Grima et al., 1996]
I_{ave}	Average Light Intensity in Pond	Calculated ($\frac{\mu mol}{m^2 \bullet sec}$)	
I_{amb}	Ambient Light Intensity	Measured ($\frac{\mu mol}{m^2 - sec}$)	
Km_{O_2}	Saturation Constant - O_2	0.5 (g/m^3)	[Rubio et al., 2003]
Km_{CO_2}	Saturation Constant - CO_2	2.64 (g/m^3)	[Rubio et al., 1998]

Table 3.1: Model Parameters and States

Symbol	Definition	Value (units)	Source
D_{O_2}	Diffusion Coefficient of O_2 in Water	7.09 $\times 10^{-6}(\frac{m^2}{hr})$	
D_{CO_2}	Diffusion Coefficient of CO_2 in Water	6.98 $\times 10^{-6}(\frac{m^2}{hr})$	
k_{H,CO_2}	Henry's Law Constant	34 $(\frac{mol}{m^3\ atm})$	
k_{h,CO_2}	Henry's Law Constant	0.8317 (unitless)	
k_{H,O_2}	Henry's Law Constant	1.3 $(\frac{mol}{m^3\ atm})$	
k_{h,O_2}	Henry's Law Constant	0.0318 (unitless)	
r	Radius of CO_2 Injection Bubbles	0.003 (m)	Estimated
t_{bubble}	Time of Rise for CO_2 Injection Bubbles	3 (seconds)	Estimated
P_{Bubble,O_2}	Partial Pressure of O_2 in Bubbles	0 (atm)	Assumed (pure CO_2 injected)
P_{Bubble,CO_2}	Partial Pressure of CO_2 in Bubbles	1 (atm)	Assumed (pure CO_2 injected)
MW_{CO_2}	Molecular Weight of CO_2	44.01 (g/mol)	
MW_{O_2}	Molecular Weight of O_2	16 (g/mol)	
F	CO_2 Gas Flowrate	4.368 $\times 10^{-5}$ $(\frac{m^3}{sec})$	Measured
L	Pond Depth	0.51 (m)	Measured
SA_{pond}	Pond Surface Area	1.82 (m^2)	Measured
$CO_{2,air}$	Carbon Dioxide Concentration in Air	0.761 $(\frac{g}{m^3})$	
$O_{2,air}$	Oxygen Concentration in Air	300 $(\frac{g}{m^3})$	

Table 3.2: Model Parameters and States (continued)

pond temperature and ambient temperature were also logged during this time. The first data set was used to calibrate three parameters of the model, the maximum growth constant (r_{max}), respiration constant (r_a) and the death and maintenance constant (k_d). This was done be setting up a Microsoft Excel spreadsheet in which the difference could be found between predicted and actual biomass concentrations at all points measured (approximately every 4 hr during growth). The square of the difference was taken at each point and summed. This value was termed the "least square" for this investigation. Each of the three above mentioned parameters was individually allowed to change (using the "solver" function) while the other two parameters were kept as the literature values so as to minimize the "least squares" value. Results from this are shown in table 3.3.

3.2.5 Uniform Media Rapid Sand Filter Design

The algal sand filters were designed using the principles of rapid sand filtration. Rapid sand filtration is most commonly used in wastewater and stormwater treatment to remove suspended particles and reduce turbidity. The three predominant and interrelated parameters in uniform media rapid sand filter design are particle size, bed height and pressure drop across the sand bed. Typically, large scale uniform grade rapid sand filters are designed to operate at 2 bar and 1 meter in depth [Degremont, 1973]. An increase in bed particle effective size (d_e) will require a larger bed depth to achieve the same solids removal of a bed with smaller effective particle size. Generally, a larger bed depth will be characterized by a larger

pressure drop across the bed, necessitating higher inlet pressure.

Montgonmery [1985] published a summary of many high rate sand filters reported in literature correlating effective bed media size to bed depth for different bed porosities. In designing the sand filters for this investigation, a bed porosity of 0.45 was assumed and the following correlation was used to determine bed heights of the three different effective particle sizes investigated. Further, the bed heights were cut in half due to available pumping capacity.

ASTM grade C-144 grade paver's sand was sieved into three grades and used as sand bed material. The three sieve sizes used were 150-300 μm, 300-600 μm and 600-840 μm and, assuming uniform particle size distributions, correspond to effective particle sizes (d_e) of 165 μm, 330 μm and 624 μm. According to the relation given by [Montgonmery, 1985], the corresponding bed heights for these three effective particle sizes are 20 cm, 36 cm and 67 cm, respectively. The three bed heights used in this investigation were half the size suggested by Montgonmery [1985].

The sand filters for this experiment were housed in acrylic cylindrical tubes 9.52 cm in diameter and 1 meter long. The ends were capped with PVC pipe ends into which 0.77 cm barbed fittings were attached. Diffusers were made from 10 cm acrylic disks with many small (1 mm diameter) holes. The filter tubes were housed in a frame and operated 50 cm off of the ground. A schematic of the experimental apparatus can be seen in figure 3.5.

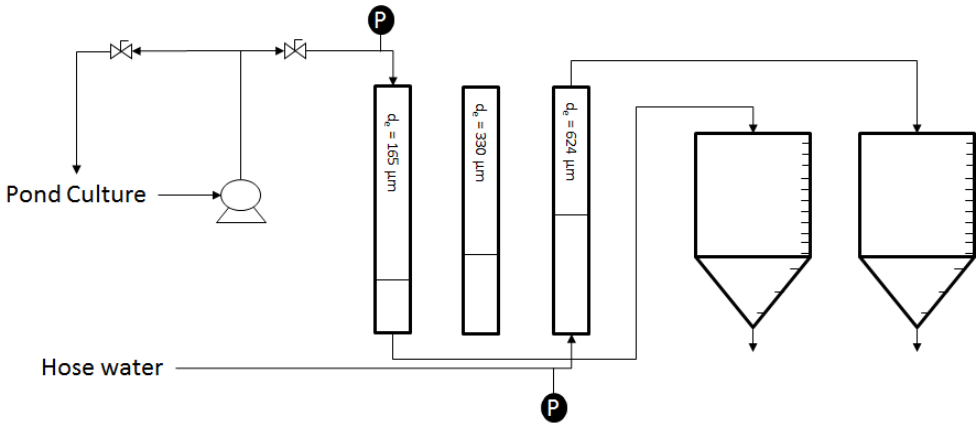

Figure 3.5: Sand Filter Experiment Setup

3.2.6 Uniform Media Rapid Sand Filter Operation

Algal culture was pumped the top of the filters using a centrifugal pump operating at 0.7 bar head. Each filter run consisted of pumping 100 liters of algal culture through a sand filter. The culture to be filtered was stored in a 400 liter conical bottom tank. The pump was then connected to the filters and the recycle loop was then placed into the culture storage tank. The pump was primed with the culture being recycled back into the culture storage tank. The filter outlet was then placed into the outlet metering tank. This tank was a 110 liter conical bottom tank which was marked with volume graduations every 5 liters up to 100 liters.

To begin a filter run, the recycle loop valve was closed and the valve controlling flow into the filters was opened. The filter quickly filled the bed with liquid volume and began steady flowthru in less than 10 liters of flow through the filter. Once the volume in the outlet metering tank reached 100 liters, the valve between the pump and the filters was closed and the recirculation valve was again opened. After all samples and measurements had been taken for the filtering run, the outlet metering tank was drained and rinsed free of algae.

Upon completion of a filter run, the pressure inside the filter was relieved by slowly removing the inlet tube from the top of the filter. The diffuser plate was removed before backflushing to prevent interference with the fluidized sand bed during backflush. A hose configured with an in line pressure gauge was attached to the bottom of the filter (where the filter outlet had previously been attached). The hose was then turned on slowly until the bed was fluidized. The backflush

water proceeded up through the filter bed, and into the outlet metering tank, where all samples and measurements were taken. Backflush ran until the water leaving the filter was visually clear. The hose was then removed and the water left in the filter allowed to drain out of the filter. The diffuser plate was cleaned and replaced 2 cm above the filter bed.

This procedure was repeated in the two remaining filters for each semi-batch cycle output. Throughout the experiment, each unit filtered 100 liters of culture three separate times. The time between filterings was dependent on the growth in the open pond and was about 4 days.

3.2.7 Design of Experiment and Analysis

The open raceway pond was allowed eight cycles to reach a biological community steady state. In that time, the culture of *Scenedesmus dimorphus* was outcompeted by primarily two other species: *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. This culture ranged in concentration from 72 mg dry biomass/liter (mg/L) to 95 mg/L.

100 L of this culture was then filtered through each of the three filters. Each sand filter was ultimately filtered and backflushed three times to obtain average data. Each filter run consisted of pumping 100 liters of algae culture through the filter. Samples of the filter outlet were taken every 10 liters. A composite sample was taken of the inlet stream at the culmination of each 100 L filtering run. The

flowrate was measured every 10 liters as well by filling a 1L vessel and recording how long it took to do so. The inlet pressure remained constant throughout the filter run at 0.7 bar, yet both the superficial velocity and liquid height above the sand filter both changed throughout the run.

The objective of the experiment was to obtain operating data for each filter all operated at the same conditions to assess performance. Each semi-batch culture removal (385 liters) was used to put 100 liters through each filter once (300 liters total). During normal and backflush operation, pressures and flowrates were measured after every 10L pumped. Samples were also collected every 10L.

3.2.8 Analysis

Absorbance at 680 nm was used to assess the biomass concentration using a UV-Vis Spectrophotometer (Shimadzu PharmaSpec UV-1700, Shimadzu Corporation, Columbia, MD). A biomass correlation curve was created for each run by filtering three dilutions of backflush, encompassing the range of all samples taken, through preweighed and dried glass fiber filter papers (#934-AH 42.5mm, Whatman Ltd.). 100 mL of each dilution was filtered in triplicate and averaged to give a three point biomass-absorbance correlation.

Parameter	Literature Value (hr^{-1})	Least Squared Fit Value (hr^{-1})
r_{max}	0.052	0.050
r_a	0.00595	0.00704
k_d	0.00385	0.00488

Table 3.3: Least Squares Parameter Fit

3.3 Results and Discussion

3.3.1 Growth Modeling Results

Results from this exercise were successful in some regard and lacking in others. The overall line of the graph was quite close with an acceptable least squared fit. Over the 24 hr investigation period, while using fitted parameters, the model predicted finishing biomass concentration well. The overall shape of a logistic growth curve was observed in the model. The final biomass concentration predicted was slightly higher than the observed biomass. Furthermore, the predicted model does follow the observed increased growth rate in times of high ambient irradiance. Reasons for this may have been the inaccurate approximation of light input data, the exclusion of macronutrient states from the model or inaccurate saturation constants for the gaseous species.

A logistic growth curve was observed in the growth trial. The slope of the lag phase predicted by the model, even with fitted parameters, was much steeper than what was observed, which was basically a flat lag phase with no growth observed. The final biomass prediction did improve after parameter fitting, however the overall biomass curve shape prediction did not improve upon parameter fit-

ting. Conversely, the increase in growth rate during high irrandiance time was under-predicted by the model. Further investigation into improving the shape of the predicted growth curve to better resemble the observed logistic growth curve needs to be done.

In addition to improving the model's predictive ability, this model and data can be used to develop a control strategy for this system. The value of this tool is such that the model can be observed and controlled in a number of different ways based on the objective of the system, whether it be carbon sequestration, biofuels feedstock production, the production of other products, wastewater treatment, ecosystem maintenance or other objectives.

3.3.2 Filtration Experiments

While sand filters proved to be useful in increasing concentration of algae biomass, the results varied greatly and were largely unpredictable. There were no perceivable trends in the percent of algae captured. A probable reason for this is the reduction of all sand bed depths to half of their design height. This had the effect of reducing the amount of void space in the filter available for capture. It should be mentioned that each trial run was performed on a different day. For all filters, the time between the first and second trial was 5 days. The time between the second and third trial was 11 days. The time between filter runs was needed to allow algae to grow.

The highest achieved recovery (filter and backflush recovery) was 73% of the

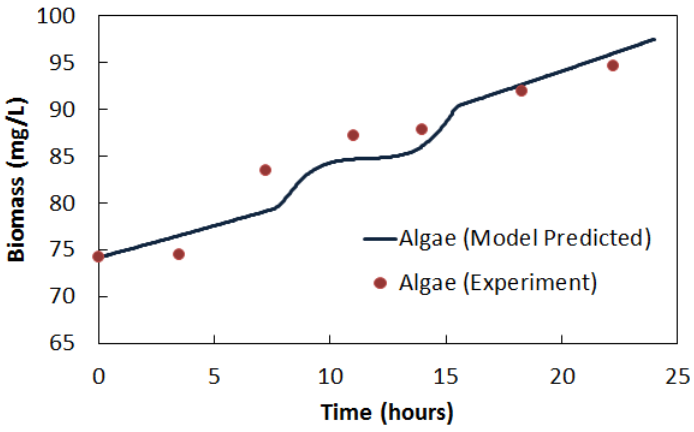

Figure 3.6: Fitted Parameter Model and Observed Growth Values

total biomass inputted into the filter. This was achieved on the first trial using the filter with effective particle size of 0.165 mm. The lowest biomass recovery was observed to be 17.6%. This was observed in the filter with effective particle size of 0.624 mm in the third trial. No other discernable patterns were readily apparent given this data.

3.3.3 Mass Balance Discrepancy

To check for consistency, a mass balance was calculated for the algae going through the system. It was assumed the only algae coming into the filters was that pumped in and no algae was growing in the filters. The only method for algae to leave the filters was by passing through the filter or being backflushed out of the filter. When this analysis was conducted, it became apparent in some cases large, non-trivial amounts of the algae which was entering the filters were not accounted for when leaving the filters (see table 3.4). Between each filter run, after backflushing had been completed, the filters were let be. It may be the case that in these runs algae was accumulating in the filter and being held until the next run. This may have been caused by inadequate backflushing technique or insufficient length of backflush. This may also have been caused by algae-sand particle interactions which are too strong to be undone by the backflushing technique used.

To correct for this, a second mass balance was calculated whereby it was assumed the unaccounted for mass from a previous run was assumed to have stayed in the filter and been carried over into the next trial. Performing an analysis in

Filter d_e (mm)	Mass Not Accounted For (% of mass input)		
	Run 1	Run2	Run3
0.165	8.5	33.4	-28.7
0.33	30.3	37	43.6
0.624	13.6	12.3	0.5

Table 3.4: Sand Filtration Mass Balance Discrepancy

this way only leads to larger discrepancies in the mass balance.

In order to check how much algae was being retained in the sand beds, ash testing was performed on samples taken from each sand bed. This was done according to the NREL "Determination of Ash in Biomass" protocol. It was thought that if any residual algae remained in the sand bed after the third experimental trial, the ashing of sand bed particles would show a gravimetric change due to the combustion and volatilization of retained algae. This test was performed using 30 gram samples from each sand bed and was performed 10 days after the final filtration experimental run. None of the samples ashed had an appreciable amount of solids retained on them. It is unclear at this time why the mass balance from the original filtration experiment was unable to close or why the ashing test did not show the presence of any residual solids. One possible explanation is that algae respired during this time and biomass decreased.

3.3.4 Changes in Flowrate and Filter Capture

Expected flowrate trends across the filters was observed. Expected trend in which the filter with smaller effective particle size becomes clogged after only 30 L of

culture had been passed through the filter. The filter with effective particle size of 0.33 mm started at an average flowrate of 111 LPM/m^2 and slowed to 73 LPM/m^2.

Capture by the filter alone seems to show what was expected from past studies. The filter with the smallest effective particle size (0.165 mm) had the highest capture (62 % average) while the filter with the largest effective particle size (0.624) captured the least algae. The first trial run through the 0.165 mm effective particle size filter recorded an experiment-wide high capture of 81% of the biomass passed through it, this substantially less capture than was recorded by [Naghavi and Malone, 1986]. This may also have been a consequence of their use of a lesser flowrate through the filter bed.

3.3.5 Issues With Experiment Design

Because of the failure to close the mass balance, this experiment was unable to provide many of the conclusions sought, such as which filter would capture the most algae and which filter would most be easiest to recover algae biomass from through backwashing. Some reasons for this might be the culture put through the filter was a mixed culture which included both algal and bacterial species. The dimensions of these organisms varied greatly and ultimately were not appropriate for an investigation of this kind. Also, important parameters of filter operation were not controlled and/or kept constant throughout the filter run. These uncontrolled parameters were the pressure above the filter bed, the superficial flowrate through the sand bed, the consistency of the feedstock fed to the filter beds or the back-

washing regime. One parameter held constant in this experiment was the pressure of the inlet feed pump feeding the filters. As resistance in the filters increased throughout each run and the flowrate through the filters slowed, the feed rate into the filter was not adjusted accordingly so as to keep the liquid level constant above the filters. This is not consistent with how industrial sand filters are operated nor does it allow for operation under constant operating regimes for investigation purposes.

Design parameters and rules were not adhered to faithfully in the design and construction of these sand filters. The most prominent deviation from literature stated design was halving the original height of each sand bed. This was done to accomodate operation of the sand filters under the limited head pressure available.

Also, no performance or consistency check was done on the sand beds after each filtering and backflushing cycle to ensure the filters were to perform as they had before. All of these factors likely had a role in causing the less than desired result of this experiment. Because of this, a new experimental design has been developed which will address the shortcomings of this design. The algae will be grown in aseptic conditions to a high density which will then be diluted prior to filtering. This will ensure uniformity in the culture being filtered. Furthermore, considerations will be taken to keep both filter inlet pressure and superficial velocity through the bed constant throughout the filtering period. The backwash regime will be constant and controlled (using prescribed and measured inlet pressures and flowrates). An air scour procedure will also be added to the backwash regime, as is typically done with industrial sand filters. The filters will be significantly reduced

in size and the volume of each filter run will only 3 L. Preliminary checks will be done to ensure the most constricting filter will be able to maintain constant flowrate under the allowed pressure and to determine whether different stages of filtering (ripening, effective filtering and breakthrough) can be found given the filtering volume provided. Details of this investigation and suggested procedural improvements and including design calculation details, can be found in appendix A.

3.4 Conclusion

The proposed study failed to find a promising sand filtering technology for the purpose of reliably capturing more than 90% of the biomass introduced and concentrating it by a factor of 10. A mixed culture of algae biomass was grown under semi-batch conditions in an open pond to a concentration between 72 and 94 mg/L. This culture was then used to investigate the collection and concentrating potential of three different sand filters. Each filter had 100 L of culture pumped through and was immediately backflushed. The highest overall biomass recovery obtained was 73% using a sand filter with bed height of 10 cm and effective particle size of 0.165 mm. The highest filter capture was 81% also using a sand filter with bed height of 10 cm and effective particle size of 0.165 mm.

Due to some of the shortcomings of this experiment design, a second experiment has been designed to take into consideration those factors left out of this experimental design and better address the questions originally posed.

Chapter 4 – Enzymatic Degradation of Microalgal Cell Walls

4.1 Introduction

Extraction of algal lipids in a cost effective and environmentally safe way is one of the key challenges for the commercial success of algae biofuels. Lipid extraction technology that does not involve an energy-intensive dewatering step prior to lipid extraction may be key for the development of such cost effective process. Dewatering harvested algae is energy intensive due to the low concentrations present [Shelef and Soder, 1980]. A biodiesel conversion process which does not require anhydrous conditions and is robust to the presence of relatively high amounts of fatty acids is desirable for algal biodiesel production [Chisti, 2007]. Aqueous phase extraction using enzymes is one such technology alternative for extraction of algal lipids. A process catalyzed by lipase enzymes might be such a process [Meher et al., 2004]. Kaieda et al. [1999] suggests a process using lipases from Rhizopus oryzae. However key challenges remain in development of aqueous extraction technologies as algal cells vary greatly in compositions between the species. Multiple fuel products can be made from the different components which make up the algal cell. A system which is flexible to accommodate the processing needs of algal feedstocks of differing compositions may also be desirable. This strategy will allow for the processing of the algae to change as the composition of the algae feedstock

changes.

Algal cell walls are known to consist of multiple layers [Dawes, 1966]. Some cell walls are rich in neutral sugars while some contain only trace amounts of sugar [Imam et al., 1985]. Such neutral sugar rich algae species will be more suitable for ethanol production. The algal cell wall might be treated as any other cellulosic ethanol feedstock as the algae cellulose is often present in a matrix which may be inaccessible to enzyme activity. Pretreatment technologies may increase the susceptibility of algal cellulose to enzymatic hydrolysis.

Industrial scale cellular disruption of single celled microorganisms has traditionally been accomplished using mechanical or thermal methods [Chisti and Moo-Young, 1986, Mendes-Pinto et al., 2001, Doucha and Livansky, 2008, Ceron et al., 2008, Grima et al., 2003]. Cell disruption is performed to release intracellular products into the culture broth making them available for further separation processes, most notably chromatography or solvent extraction. Traditionally, industrial algal processing is used to recover low concentrations of high value products ($>$ \$1000 per ton) [Grima et al., 2003]. Examples of high value products are astaxanthin from *Haematococcus pulvialis* [Mendes-Pinto et al., 2001] and long chain organic acids and proteins from a variety of species [Eriksen, 2008, Belarbi et al., 2000, Ceron et al., 2008, Grima et al., 2003]. In the case of *Haematococcus pulvialis*, cell disruption enhances the ability of the astaxanthin to become biologically assimilated by fish [Mendes-Pinto et al., 2001].

Mechanical cellular disruption methods used industrially include bead milling, high pressure extrusion and to a lesser degree ultrasonication [Borowitzka and

Borowitzka, 1988, Grima et al., 2003, Richmond, 1986]. Thermal treatment serves the dual purpose of lysing cells while removing residual water and is commonly performed in natural gas fired drum dryer [Richmond, 1986]. Thermal methods of cell disruption, as well as the shear created by mechanical disruption, can serve to further degrade or denature the desired products [Richmond, 1986, Fleurence, 1999, Grima et al., 2003]. Thermal degradation of triglycerides yields several compounds, many of which cannot be converted to biodiesel. Algal lipids naturally have a higher concentration of free fatty acids than other lipid sources (up to 2%). Creating more fatty acids will further complicate the downstream biodiesel conversion process. Properly using enzymes to lyse algal cells will leave these triglycerides intact and available for biodiesel conversion. Furthermore, the residual biomass has an energy value and can be converted into various energy forms [Rittman, 2008]. Anaerobic digestion of the residual biomass can form methane gas [Afi et al., 1996, Borowitzka and Borowitzka, 1988] or the algae "hulls" can also be used in a microbial fuel cell to create electricity or hydrogen [Logan, 2004].

Enzymatic cell wall degradation is not widely practiced in industry at the present time because cell lysing enzymes have traditionally been cost prohibitive. High cost of enzymes stems from their production and from the fact that they usually cannot be recovered and recycled after they are used [Chisti and Moo-Young, 1986]. However, even with their cost, enzymes do retain some significant advantages over other methods of algal cell disruption, such as their degradation selectivity. Selectivity of enzymes is important in extraction of delicate and marginally stable chemicals. Algal cell walls are more recalcitrant than the cell walls of other

microorganisms. Most microalgal cell walls contain cellulose and some species have an additional tri-laminar sheath (TLS) containing algaenan, a substance known for its resistance to degradation [Allard and Templier, 2000, Versteegh and Blokker, 2004]. Degrading these biopolymers using mechanical methods will require excess energy usage and multiple passes through disruption equipment. Therefore, the use of enzymes to lyse algal cell walls may be advantageous as compared to other methods.

We propose and test the feasibility of an enzymatic process to degrade cell walls of unicellular algae. Intracellular components such as lipids may be recovered for further processing into algae biodiesel. In this paper, two experiments are carried out. In the first experiment, enzymatic lysing of whole microalgal cells was investigated (further called the "lysing experiment"). In the second experiment simultaneous saccharification and fermentation of lysed algal cells (further called the "fermentation experiment") was performed. A third SSF experiment was performed utlilzing a higher solids content.

Specific objectives of these experiments are the following:

1. Demonstrate degradation of microalgal cell walls using cellulase and lipase enzymes.

2. Visualization of enzymatically degraded algae cells.

3. Determine the fermentation potential of pre-lysed acid pretreated microalgal biomass.

4.2 Materials and Methods

The algae biomass for the fermentation experiment was donated by Kent Sea Tech Corporation. The algae were grown in open ponds and separated using a centrifuge. Algae biomass was frozen until further use (called "fresh algae" henceforth in this chapter). Algae for the whole cell lysing experiment was *Chlorella vulgaris* grown non-axenically in a 30 L bioreactor. Upon inspection under 1000X magnification, it was realized the algae was predominantly cell fractions and remnants of already disrupted cells. This biomass was predominantly comprised of outer cell walls which had been ruptured and cell components. Cell components resembling diatom frustules were also present.

4.2.1 Moisture Content Determination

Moisture content of the fresh algae was determined by drying pre-weighed fresh algae on a pre-dried No. 42 Whatman Filter Paper and drying overnight at 95C. The dried slurry and paper were weighed until a stable weight was reached. Fresh algae had 9.1% w/w dry algae biomass.

4.2.2 Whole Cell Evaluation with Cellulases and Phospholipases - The "Lysing Experiment"

Chlorella vulgaris grown non-axenically in a 30 L photobioreactor was used for the lysing experiment. Preliminary staining was done prior to the lysing experiment to

determine cell viability using methylene blue. Methylene blue (reagent grade) was purchased from Mallinckrodt Chemical Company (Hazelwood, MO). 1 mL of the algae sample was incubated with 10 μL of the diluted methylene blue (0.01g/mL in DI water) for 5 minutes. The stained sample was wet mounted and imaged at 1000X.

50 mL whole-cell fresh algae samples at a concentration of 0.25% w/w and a pH of 5.0 was placed in 125 mL Erlenmeyer flasks. The enzymes used were Accellerase 1000 (an optimized mixture of cellulase and hemicellulase), GZYME G699 (A1 Phospholipase) and LysoMax (A2 Phospholipase) were donated by Genencor (Genencor, Palo Alto, CA). All enzymes were added at 5% (w/w of whole-cell fresh algae) level, in accordance to the manufacturers suggested dosing. Enzymes were added to flasks according to design of experiment (Table 4.1). The flasks were kept at 40^{o}C in constant temperature shaker baths throughout the duration of the experiment.

Culture broth samples of 1 mL each were collected at 0, 6, 12, 18 and 24 hours. A small aliquot ($< 10\mu$L) was taken from each sample and imaged at 1000X using a light microscope (Motic BA300, Motic, Richmond, British Colombia). While this strategy of imaging did not allow for the continuous observation of the same cells, an attempt was made to image cells most representative of the sample at that given time as seen in (Fig. 4.1).

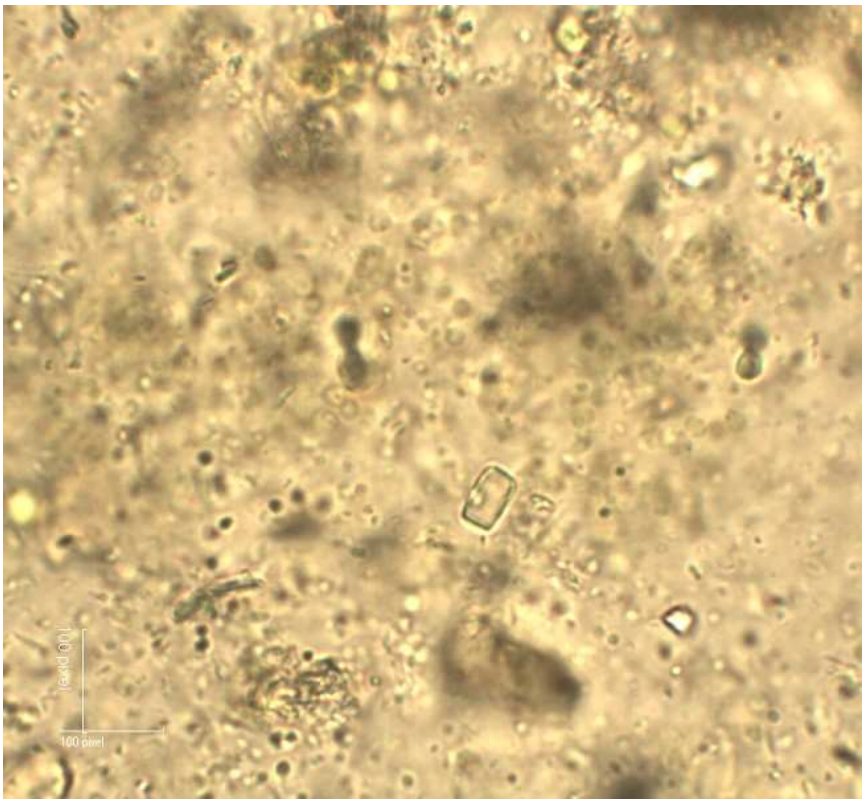

Figure 4.1: Fresh Algae

Table 4.1: Lysing Experiment Design

Experimental Variable	Experiment Trial			
	1	2	3	4
Cellulase		X		X
Lipase			X	X

4.2.3 Cellulases and Lipase Evaluation with Acid Pretreatment - The "Fermentation Experiment"

In the fermentation experiment, fresh algae was diluted to a final solid content of 0.8% w/w using 1.5% w/w sulfuric acid and imaged (Fig. 4.2). This slurry was autoclaved at 121^{o}C, 15 psig for 60 minutes. Slurry pH was adjusted to 5.0 and 80 mL aliquots were placed into 125 mL Erlenmeyer flasks. Cellulases, phospholipases and yeast (Red Star Active Dry Yeast, Lesaffre Yeast Corporation, Milwaukee, WI) were added to the appropriate flasks according to the design of experiment (Table 4.2). Enzymes used were Acccellerase^{TM}1000, GZYME^{TM}G699 (A1 Phospholipase) and LysoMax^{TM}(A2 Phospholipase) which were donated by Genencor (Genencor, Palo Alto, CA). All enzymes were added at 5% (w/w of fresh algae) level to pretreated slurry, in accordance with manufacturers suggested dosing. The yeast was added at a concentration of 0.3% (w/v of pretreated slurry) along with 1 mg/mL of urea as a nitrogen source. The flasks were kept in constant temperature shaker baths at 33^{o}C for the duration of the experiment. Culture broth samples of 1 mL each were collected at 0, 6, 12, 24, 48 and 72 hours. Samples were centrifuged at 10,000 rpm for 10 minutes in a centrifuge (HERMLE Z233 M-2 Microliter Centrifuge, Labnet International, Woodbridge, NJ). The supernatant was removed and filtered through a 0.2μm syringe filter in preparation for HPLC analysis.

Table 4.2: Fermentation Experiment Design

Experimental Variable	Experiment Trial			
	1	2	3	4
Cellulase	X		X	X
Lipase		X	X	X
Yeast				X

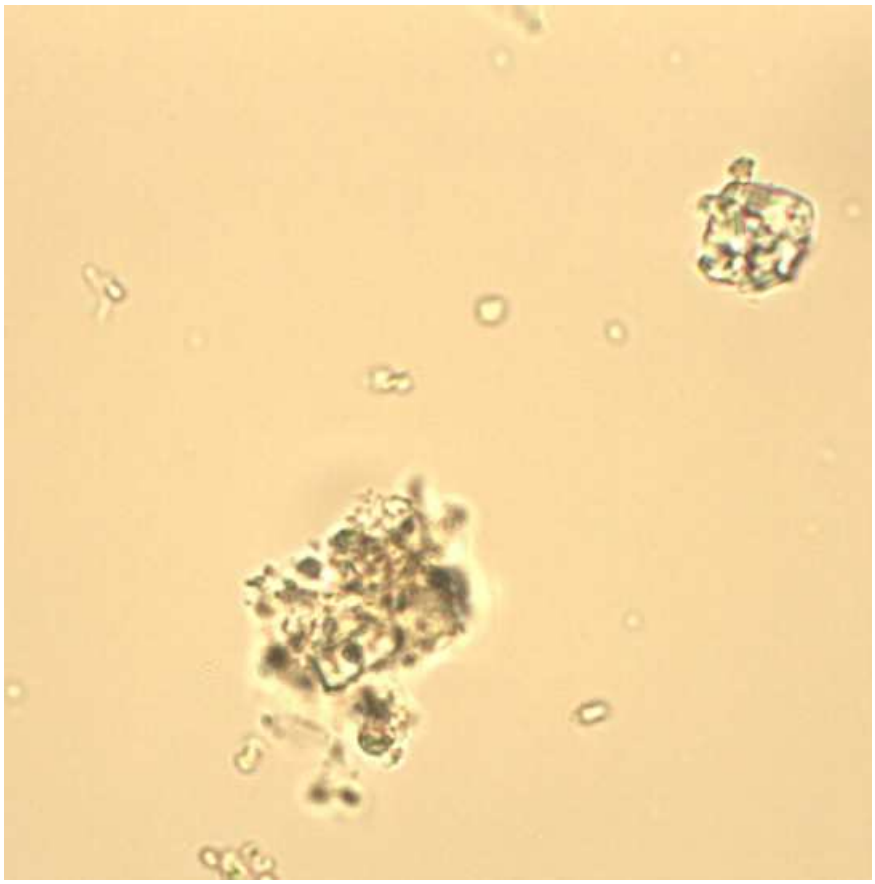

Figure 4.2: Algae Diluted in 1.5% w/w Sulfuric Acid and Autoclaved for 60 Minutes

Table 4.3: High Biomass Experiment Design

Experimental Variable	Experiment Trial			
	1	2	3	4
Cellulase	X		X	X
Lipase		X	X	X
Yeast				X

4.2.4 High Biomass Enzymatic Hydrolysis - The "High Biomass" Experiment

As a confirmation to the previous fermentation expariment, a similar experiment was carried out utilizing a higher solids concentration than the fermentation experiment (Table 4.3). The enzyme dosing, and yeast and urea dosing were all the same as the fermentation experiment. The biomass used for this experiment was algae grown at an experimental pilot facility in New Mexico (Fig. 4.3). It is a relatively high lipid species containing 19.3% w/w total lipids. Lipid analysis was done using an Ankom XT15 Extractor (Ankom Technology, Macedon, NY) utilizing petroleum ether as the solvent. The culture was a single species mix which came frozen and was kept frozen until used. The algae is presumed to be a marine species due to the visible presence of salt when dried. Elemental analysis was done using a Costech ECS 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA). An elemental analysis of the solids was found to be 60% elemental carbon and 4% elemental nitrogen.

The working algae solids content for this experiment was 4.4%. The final acid concentration was 0.7 % w/w algae solids. This mixture was autoclaved for 1 hour

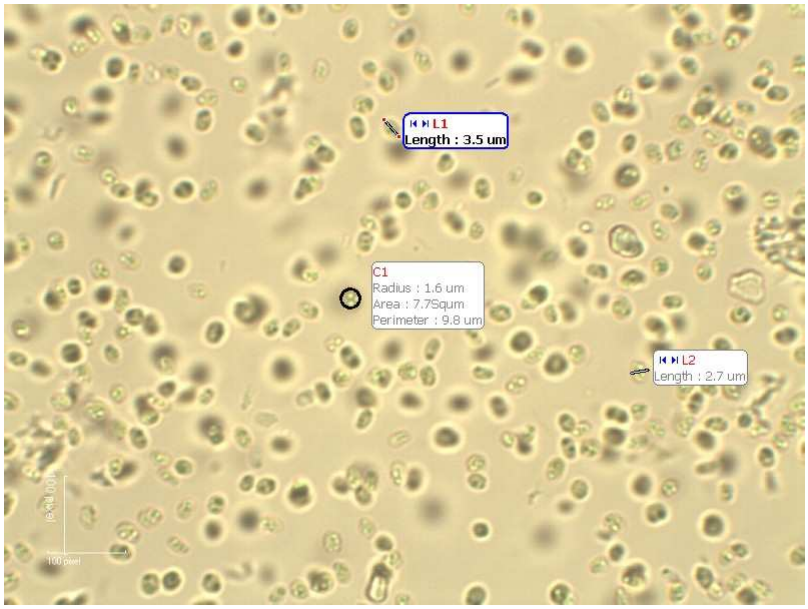

Figure 4.3: Substrate for High Biomass Experiment; Fresh (Not Pretreated)

to simulate a dilute acid pretreatment, after which the pH was adjusted to 5.0.

Samples of 100 mL were placed in 250mL erlenmeyer flasks to facilitate better mixing of the high solids mixutre. Enzymes, yeast and urea were dosed as in the previous fermentation experiment. Protocols laid out in the NREL SSF Experimental Protocols for Lignocellulosic Biomass Hydrolysis and Fermentation [Dowe and McMillan, 2008] were used as a guide and followed where applicable. Samples were held at 30^{o}C through the duration of hydrolysis and fermentation in a constant temperature oscillating water bath.

Time course samples were taken at 0, 3, 6, 24, 48, 72 and 168 hours. As HPLC sample preparation and freezing took upwards of one hour, all time points are offseted as such. Samples were prepared for HPLC analysis in the same fashion as in the fermentation experiment. All products were analyzed for using HPLC as mentioned below.

4.2.5 HPLC Analysis

Supernatant from both experiments were analyzed using an Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA) and a Hamilton PRP-X300 ion exclusion column (250mm x 4.1mm, part #79465, Hamilton Company, Reno, NV) and guard column (part #79460, Hamilton Company, Reno, NV). The HPLC system included the following Agilent 1200 series components: Solvent Degasser, Quaternary Pump, Autosampler/Injector, Refractive Index Detector and Thermostatted Column Heater. The samples (10 μL injected volume) were ana-

lyzed at ambient temperature using 5 mM H_2SO_4 as mobile phase at a 2.0 mL/min flowrate. Glycerol was quantified using the mentioned refractive index detector.

4.2.6 Assay for Glucose

Glucose concentration was determined using a variation of an enzyme assay method [McComb and Yushok, 1958, Washko and Rice, 1961] using glucose oxidase and horseradish peroxidase. The method has been adapted for use in a 96-well microplate and Perkin Elmer Precise 1420 multilabel counter Victor3 V plate reader. Absorbance readings were taken at 531 nm. All chemicals were of reagent grade. D-glucose was purchased from Mallinckrodt Chemical Company (Hazelwood, MO). Horseradish peroxidase type VI-A, crude glucose oxidase, and o-dianisidine were purchased from Sigma-Aldrich Company (St. Louis, MO).

4.3 Results and Discussion

4.3.1 Fermentation Experiment

The species of algae used in this experiment are unknown, although it is most likely a mixed culture. The algae was grown in an outdoor fresh water tank on an organic substrate. It is not known if the centrifuge collection is responsible for the cell lysing of this algae, and because the substrate is so poorly characterized an accurate mass balance cannot be constructed. A chart of theoretical maximum glucose amounts for different biomass carbohydrate weight fractions is given in

Table 4.4: Theoretical Maximum Glucose Conversion

Percent (w/w) Carbohydrate of Algal Cell	Maximum Glucose Concentration Possible (mg/mL)
70	5.6
50	4
30	2.4
15	1.2
1	0.8

table 4.4. These values were calculated using the dry biomass solids previously determined for the acid pretreated algal slurry prior to any enzyme treatments. Abo-Shady et al. [1993] report similar carbohydrate compositions in the cell walls of some green algae species.

Glucose was formed from the activity of the cellulase enzymes on the cellulose fraction of the algal cell. More glucose was formed when phospholipases were used in addition to cellulases than with cellulases alone. It cannot be known whether the low amount of glucose produced was due to low yield from the cellulase enzyme, or from the algae not possessing a cell wall rich in cellulose. Depending on the species, the inner cell wall layer is often the structurally supportive cellulose layer [Wurdack, 1923] which may be encased in other layers of mucilage, pectin, proteins, lipids and sulfonated polysaccharides among other substances. Not only is cellulose itself resistant to enzymatic hydrolysis, these other cell wall substances can make cell wall cellulose less accessible to enzymatic hydrolysis.

Sample preparation took more than one hour and additional time to bring to freezing temperature. Consequently, the first data point collected was not able to capture the kinetics of the cellulases conversion of cellulose to glucose or the

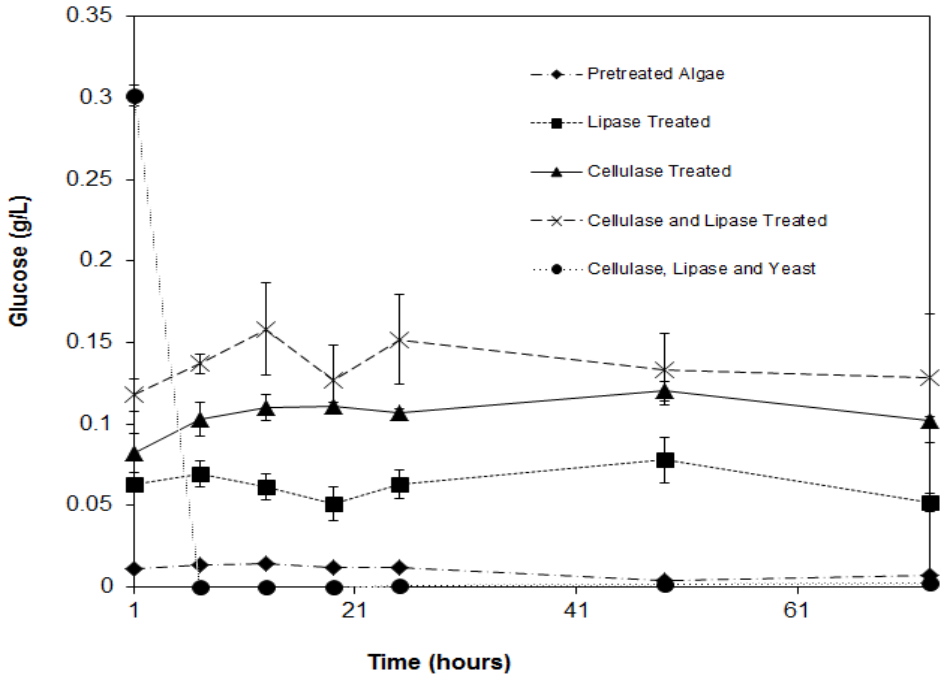

Figure 4.4: Time Course of Glucose Production for the Fermentation Experiment

phospholipases conversion of phospholipids to fatty acids and carbohydrates. Also, conversion of glucose to ethanol by yeast was under the detection limit of the HPLC analysis. Further work needs to be done with algal biomass at a higher concentration to observe this phenomenon. Although ethanol concentrations could not be accurately measured, a reduction in glucose to 0 g/L indicating complete consumption of glucose by yeast was observed in the flasks containing yeast while the other flasks maintained their glucose concentration (Fig. 4.4).

Phospholipids are present in both the main cell wall of algae as well as the

monolayer of the lipid spherosome [Yatsu and Jacks, 1972]. Glycerol phosphate is formed by the enzymatic degradation, with the use of phospholipase A1 (PLA1) and Phospholipase A2 (PLA2) enzymes, of phospholipids. Glycerol phosphate may or may not then be converted into the basic glycerol backbone given the experiment conditions. A specific enzyme for this was not supplied.

More glycerol was formed than expected in the high biomass experiment, and than is reasonably possible in the fermentation experiment (i.e. 8 mg/L of algae yielding 7 mg/L glycerol) (Fig. 4.5). Soluble phospholipids may have been present in the fresh algae slurry which contributed to additional glycerol. The dry weight determination procedure would not have captured these lipids or fatty acids if they were lost due to solubilization in the dilute acid. The untreated algae and algae treated with cellulase alone showed the formation of less than 1 g/L of glycerol. The algae treated with phospholipases showed glycerol formation of 4-8 g/L (Fig. 4.5). It is unclear whether the addition of cellulase enzymes in addition to pretreatment helps to increase the production of glycerol. Furthermore, products which may have also co-eluted with glycerol during HPLC analysis was not done. Likely culprits would have been glycerol phosphate or other organic acids.

4.3.2 Whole Cell Lysing Experiment

Due to the non-axenic growth of whole cell Chlorella vulgaris, glucose data proved unreliable as the culture was likely contaminated by glucose consuming organisms. The whole cell algae used in this experiment was not pretreated. After each sample

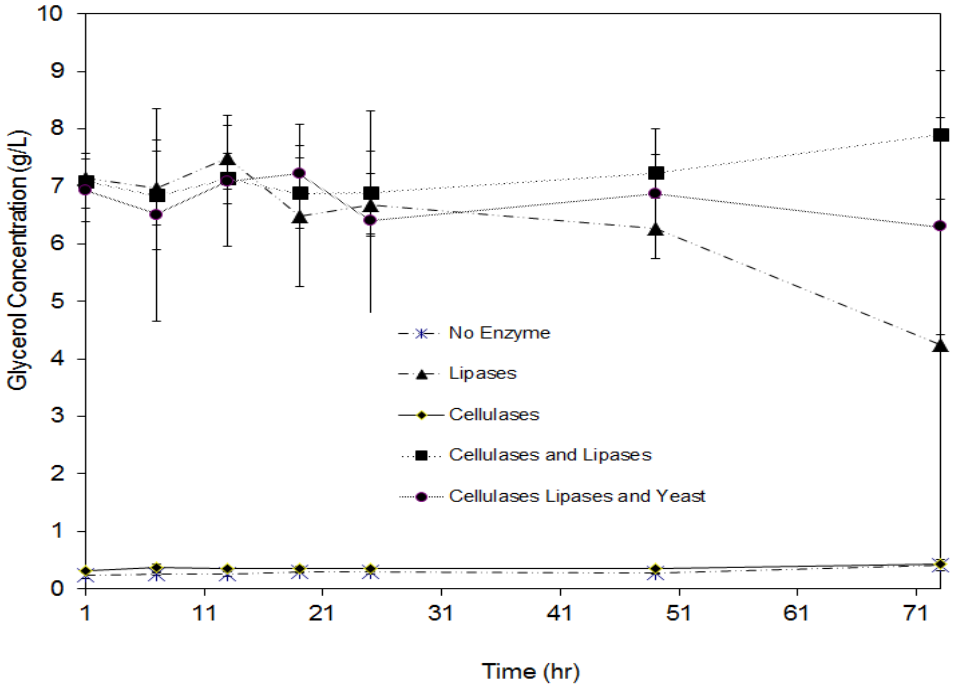

Figure 4.5: Time Course of Glycerol Production for the Fermentation Experiment

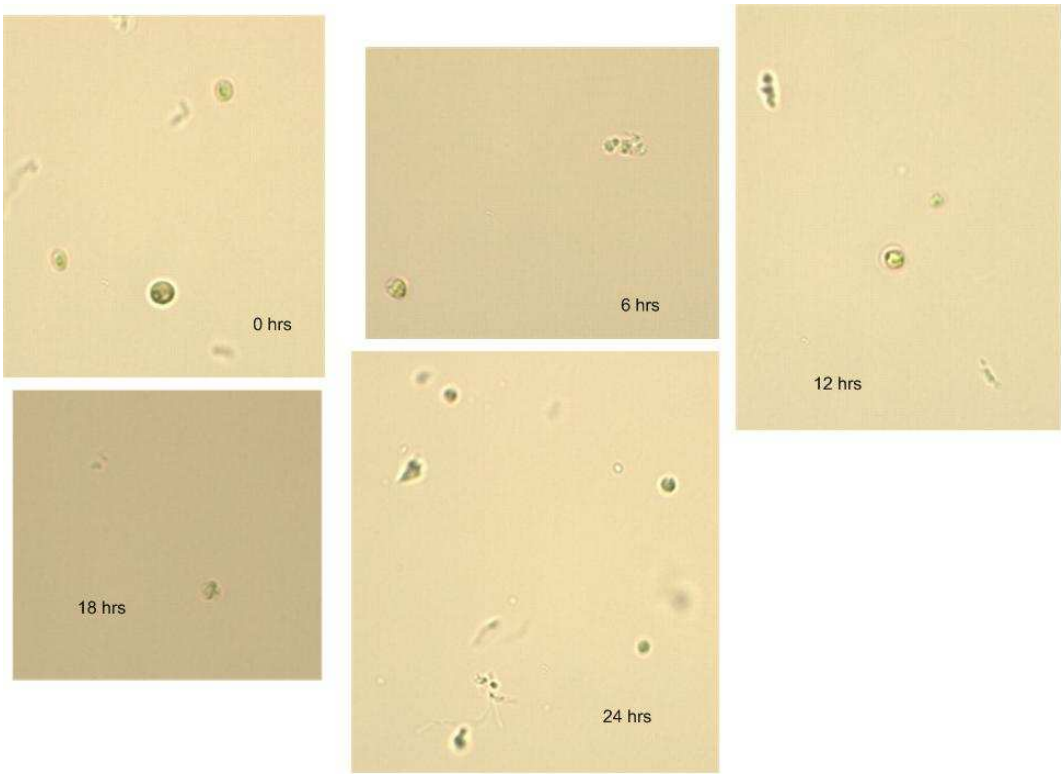

Figure 4.6: Chlorella Vulgaris Cells Treated with Cellulase and Lipase Enzymes

was taken, it was imaged at 1000X. None of the images showed obvious cell lysis. The cells were intact throughout the duration of the experiment. In the later data points imaged, some cells became mis-shapen and non-spherical (Fig. 4.2).

4.3.3 High Biomass Experiment

The high biomass experiment saw an increase in glucose converted compared to the fermentation experiment. This is to be expected as the initial solids content was almost five times larger. As the biomass was not fully characterized, yield

calculations cannot be done for cellulose hydrolysis or ethanol production. Final glucose concentration was found to reach 4.8 g/L. Curiously, it is hard to distinguish wether cellulase or lipase release more glucose alone. The previous result that cellulase and lipase are more effective when acting together than on their own is confirmed. All cellulase including trials also seemed to have a similar initial cellulose hydrolysis rate. Behavior consistent with SSF process was observed in trials which had yeast in them. The initial increase in glucose concentration before fermentation takes hold in the SSF trials is not seen in this data. This may be a sign of either slow hydrolysis in these samples or a quicker than usual start to fermentation. Glucose hydrolysis seems to be nearing completion after 25 hours.

Ethanol evolution was seen in only those samples which yeast and urea were added. A maximum ethanol concentration of 2.3 g/L was observed followed by a sharp decrease to a minimal concentration equivalent to those samples where not yeast was added. The point where ethanol concentration begins decreasing coincides with the time glucose concentraiton gradually decreases indicating less glucose available for the yeast to consume and the possibility of the yeast undergoing a diauxic shift from glucose to ethanol.

4.4 Conclusion

It was shown algal cell walls are susceptible to degradation using cellulase and lipase enzymes. Enzymatic degradation experiments were carried out on an industrially grown and harvested mixed-culture algal slurry as well as whole-cell dead *Chlorella*

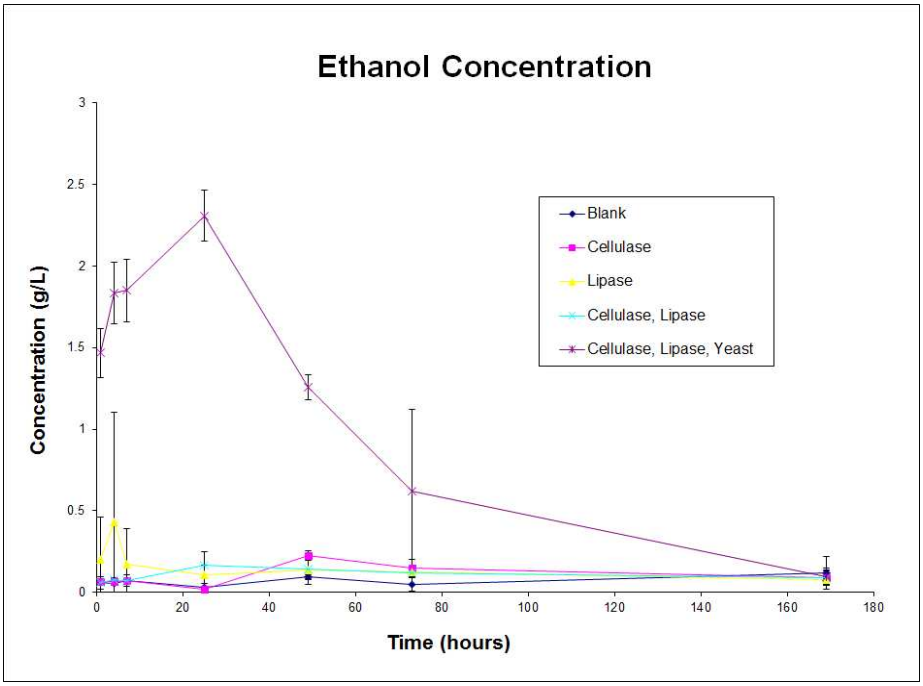

Figure 4.7: Time Course Ethanol Production; High Biomass Experiment

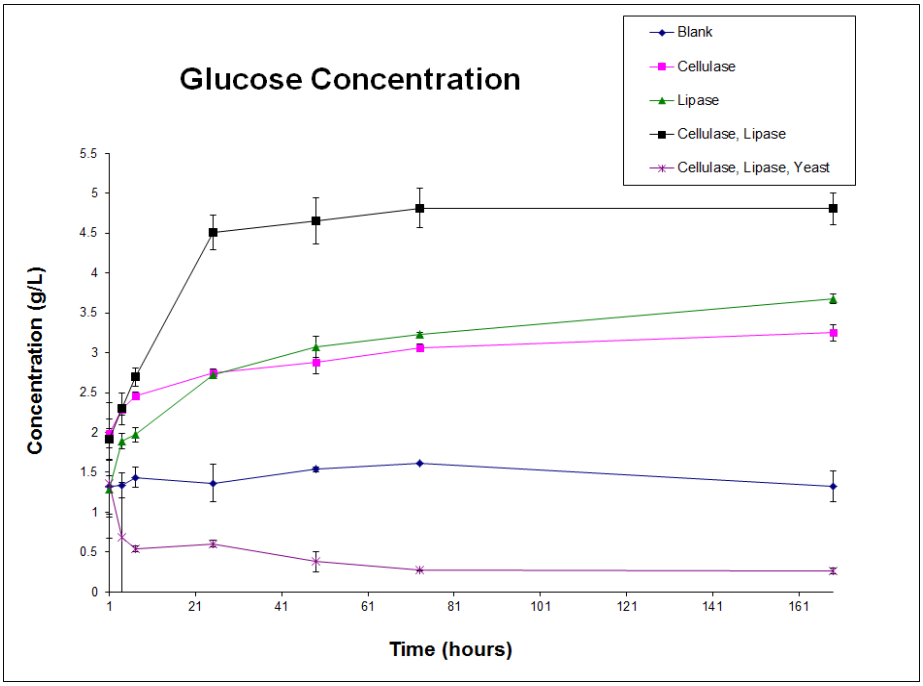

Figure 4.8: Time Course Glucose Production; High Biomass Experiment

vulgaris. The mixed culture cells had been previously disrupted while the *Chlorella vulgaris* were largely intact. Furthermore, the mixed culture was pretreated with 1.5% H_2SO_4 at 121^{o}C and 1 bar for 60 minutes prior to enzymatic treatment. Both feedstocks were then incubated with cellulase and lipase enzymes.

The fermentation experiment resulted in glycerol formation when incubated with the cellulases and phospholipases. Glucose was formed when the pretreated mixed culture was incubated with cellulases. Accurate mass balance could not be constructed and yields could not be calculated as the experimental feedstock was not well characterized. Further, one experimental unit was incubated with yeast but ethanol could not be observed presumably due to the low concentration, however a decrease in glucose concentration to 0 g/L was observed.

Chapter 5 – Life Cycle Inventory of A Combined Algal Biomass Biodiesel/Bioethanol Process

5.1 Introduction

Algae biofuels have positioned themselves as a third generation biofuel and promising candidate to meet liquid transportation fuel needs in the United States. Potential biofuels from algae are considered "third generation" because they do not compete with food resources by requiring arable land, nor do they necessarily require fresh water to grow. While algae do require macronutrients to grow, these can be found as a major constituent to many waste streams [Clarens et al., 2009, Tanticharoen et al., 1993, Chaumont, 1993]. Other requirements for algae growth are an appropriate pH and temperature, dissolved inorganic carbon and sunlight. Producing the quantities of biomass which would be needed to offset liquid fuel needs in the United States would require a scale of operation with millions of acres of algae ponds. Furthermore, a number of technical barriers still stand between an algae fuels dream and reality, notably a way to economically harvest a dilute (0.1% w/w) culture of algae and a way to separate cell components into their usable parts (lipids, protein and carbohydrates).

A great deal of analysis and simulation has been undertaken to investigate the numerous proposed methods for completing both of these tasks [Pienkos and

Darzins, 2009]. Also, there remains some debate as to what a full scale algae biofuels system would look like and how it would operate. Inherent to this debate is some concern over how sustainable such a system would be. There is a strong consensus amongst scientists, policy makers and citizens alike to ensure this next generation of energy conversion technology is as sustainable and renewable as possible, and certainly more so than fossil fuel based technology.

A tool for assessing sustainability which has emerged in recent years is Life Cycle Assessment (LCA). LCA is a method by which the sustainability of a process can be quantified by taking a holistic look at the resource demands and environmental impact of an entire process. Methods for accounting for other co-products which may be produced as a result of producing the so called "functional unit" have also been developed in LCA framework. Accompanying assumptions and process descriptions in LCA are critical to understanding the results. Differences in processing flows and assumptions can change the results and interpretations of an LCA vastly [Lundin et al., 2000, Tillman et al., 1994, Wang et al., 2007].

A number of studies have been done on algal biofuel systems, and these systems explicitly demonstrate the tie between LCA results and methods. Clarens et al. [2009] state in their base case assessment the harvest of algae alone requires more net energy than terrestrial crops and performs worse than terrestrial crops in all impact categories except land use. Their base case is assuming inputs of CO_2 and nutrients are not from waste streams but are instead manufactured. They go on to analyze the same process utilizing wastewater sources and show great improvement in impact categories. Lardon et al. [2009] also report the need to incorporate other

impact lowering technologies such as solar drying and wastewater culture.

This LCI dataset looks at another variant in algae processing. We propose a system where the useful constituents of algal biomass are utilized simultaneously at one facility to produce both biodiesel and ethanol, along with other coproducts. No coproduct allocations were considered in these LCI data, as was done previously [Sander and Murthy, 2010]. In this LCI dataset, as opposed to allocation strategies, one mass (algae) becomes two products, bioethanol and biodiesel. The basis for the study is a "combined" functional unit made up of proportional amounts of bioethanol and biodiesel leaving the proposed facility. The data will be for a well-to-gate process describing the "combined" functional unit of 227.8 MJ (210.2 MJ from biodiesel and 17.3 MJ from bioethanol). In this study, we strive to obtain accurate LCI data for a process utilizing an algae feedstock to simultaneously produce biodiesel and bioethanol at one facility.

The goal of this project is to establish baseline LCI data for this process and provide an LCI dataset for this process. Another goal in compiling this LCI is to guide process development in algal biofuels. Performing process simulations and computing LCI values will help to decipher which algal biofuels production method is best, from a process efficiency and sustainability perspective.

5.2 Materials and Methods

5.2.1 Process Flow

The process flowsheet was devised for a process which co-produces biodiesel and bioethanol from green algae feedstock. The main biomass conversion portion of the process was modeled using SuperPro Process Modeling Software [SuperPro, 2000] (Fig. 5.1). Each process step was modeled assuming an algae culture throughput of 95,644 kg/year of dry algae biomass (12 kg dry biomass per hour) which begins as 120 m^3 of culture medium (100 mg algae/L). The algae contain 50% lipids, 11% carbohydrates and the balance protein.

The first step in this proposed process is the growth of algal biomass in an open pond of volume 2,421 m^3. The pond is assumed to be six meters wide and 18 cm deep. The maximum length requirement and mixing power requirement were calculated from Shelef and Soder [1980]. This pond was assumed to operate as a continuous chemostat with a dilution rate of 0.05 h^{-1}. There is no CO_2 injection or nutrient addition into the pond. It is assumed the wastewater contains all components as a suitable substrate for algal growth. Further, there is assumed to be no artificial lighting and all of the photon energy driving photosynthesis is supplied naturally.

The next step in the processing of algae is a belt filtration step which concentrates the algae to 21% solids. This high solids slurry is sent to a pretreatment vessel and mixed with hot dilute acid to 0.7% wt. acid/ wt. algae. The pretreated algae mass is then neutralized and sent to a simultaneous saccarafication and fer-

mentation reactor to be hydrolyzed and fermented. Enzymes are dosed at a rate of 0.089 mg/g algae. This is adequate dosing assuming the enzyme broth being added has a total (cellulase and lipase) activity of 44 FPU/mg [Kumar, 2010]. Yeast was assumed pitched at a rate of 0.035 g yeast/g algae biomass (dry basis).

The hydrolyzed and fermented slurry is then sent to a centrifuge to separate the aqueous from the solid phase. The solid phase is sent to storage. It is presumed this protein rich solid phase product will have value as ruminant feed, although its value as a coproduct is not considered in this LCI dataset. The aqueous phase is sent to a series of distillation columns followed by molecular sieves to purify ethanol. remaining stillage is centrifuged to separate lipid phase which is sent to a biodiesel reactor. The lipids are mixed with potassium hydroxide catalyst (1% w/w lipids) and methanol (6 mol methanol/1 mol lipids), heated to 60^{o}C and reacted to form biodiesel and glycerol. The incoming lipid stream is assumed to contain 0.07% free fatty acid and 2.3% residual water. An actual algal lipid stream composition may contain much higher free fatty acid and residual water depending on lipase activity and centrifugation efficiency, respectively. For this simulation, the incoming lipid stream allows for a single base-catalyzed reaction with minimal catalyst destruction by free fatty acid or kinetic interference due to the presence of water. A more intense transesterification process (which might possibly include acid catalyzed transesterification and/or a multiple stage base catalyzed transesterification) may be necessary based on actual lipid stream constituents.

The products are sent to be centrifuged and separated into glycerol and a biodiesel phases. The biodiesel phase is sent to storage while the glycerol phase is

sent to another distillation column to recover and recycle methanol back into the process. The column bottoms are sent to a reactor to allow the residual potassium hydroxide to react with phosphoric acid to form potassium phosphate, a solid precipitate which is used as fertilizer.

In this dataset (Fig. 5.2) inputs and impacts are considered for transporting and distributing ethanol and biodiesel. The inventories regarding further handling of other products (algae cake, potassium phosphate, crude glycerol and other waste streams) are ignored.

This LCI is a "well-to-pump" LCI meaning the process flow which is analyzed stops at the fueling station. This is a popular style of LCA for transportation fuels because it does not account for the actual combustion of the fuel or vehicle life-cycles. The corollary to a well-to-pump LCA is the "well-to-wheel" LCA which does take into account fuel combustion and low-energy end products from combustion.

5.2.2 Secondary Processes

The RMEE described system boundary indicated the need to follow the processing chain up to five steps removed from the main process flow in some instances. This occurs mostly with regard to the resource intensive process of enzyme production. Other inputs which eclipsed the RMEE cut-off ratio were methanol and steam, both used in biodiesel-ethanol co-production. Fig. 5.2 displays the LCI process flow diagram and associated system boundary dictated by the RMEE calculations.

This is not a complete LCA defined process flow diagram [Curran, 2006, Bruijn et al., 2002] and those processes left out of the system diagram do not encompass all of the processes excluded from this LCI dataset.

The scope of this project is defined to be for a complete algae biofuel process taking place entirely inside the United States in the year 2010.

As there are no facilities currently producing algal biodiesel or algal ethanol, data specificity cannot be assigned to a facility or even an industry. The main processing data (excluding growth and transportation and distribution) came from the SuperPro model. Growth data came from a past LCA [Sander and Murthy, 2010]. The final transportation and distribution model came from the GREET model [GREET, 2008]. Much of the secondary processing data came from either the GREET model or the US LCI database [LCI, 2008]. Data sources are described more specifically in Table 5.1.

Woody biomass was assumed to originate from a high harvest timber stand in the Pacific Northwest.No coproduct allocations were assumed in this LCI. The protein rich algae cake may have value as ruminant feed. The glycerol stock and potassium phosphate also have value as products, but are not considered in this LCI. No specificity was required for this project as data was limited and only available from a few select sources. As mentioned above, some data was specific to regions in the United States, while other data was averaged across the entire United States while still other data did not have a geographic specificity associated with it.

The oldest data used was that describing inputs and design parameters for

growth, however it is not expected to change significantly over time. Pricing data (for RMEE boundary determination) and other LCI input values (mainly taken from either the GREET model or the US LCI database) were no older than 13 years. All data was gathered manually so as to observe the RMEE boundary as closely as possible.

The functional unit for this study is defined based on one hour's operation of the simulated process flow. In one hour, the process produces 5.104 kg of biodiesel and 0.583 kg of bioethanol. This fuel mix combines to 227.6 MJ and is assumed to have a market value of $7.67. The heat of combustion for biodiesel and bioethanol are assumed to be 41.2 [Akers et al., 2006] and 29.7 MJ/kg, respectively. The retail price of biodiesel is assumed to be $4.64/gallon while bioethanol is $2.44/gallon. Again, price calculations are only used in this study to determine the system boundary and have no effect on LCI data.

5.2.3 System Boundary

The RMEE system boundary determination method [Rayolds et al., 2000] was employed to draw the whole system boundary for this LCA. This method provides a way to objectively and completely describe an LCA system boundary whereby the system boundary is drawn before any LCI data is collected. RMEE boundary selection is performed by comparing the inputs of a process to the functional unit of an LCA on the basis of mass, economic value and energy content. If any of the three categories is found to have a ratio value higher than a predetermined

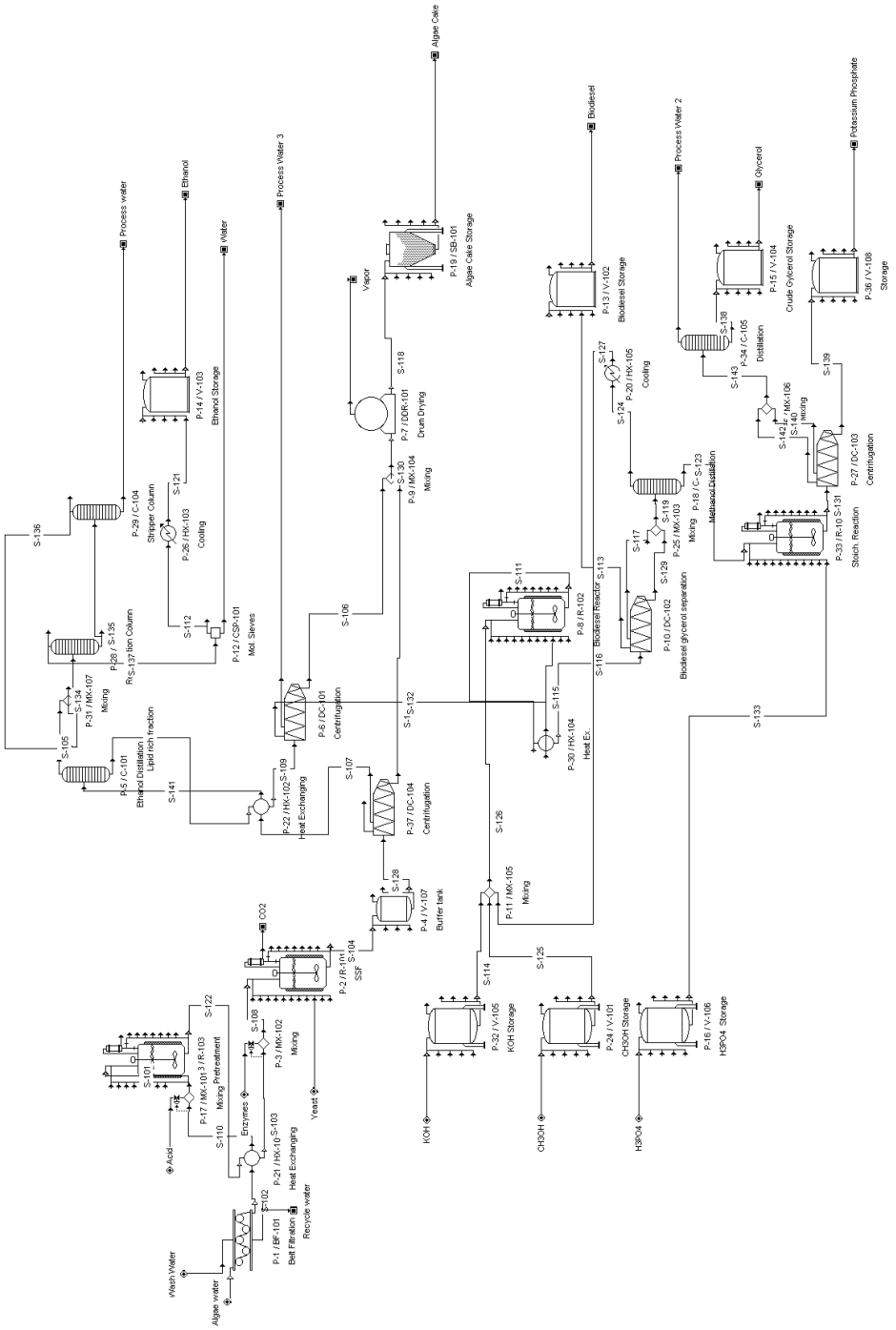

Figure 5.1: Combined Algal Biodiesel/Bioethanol Conversion Flowsheet

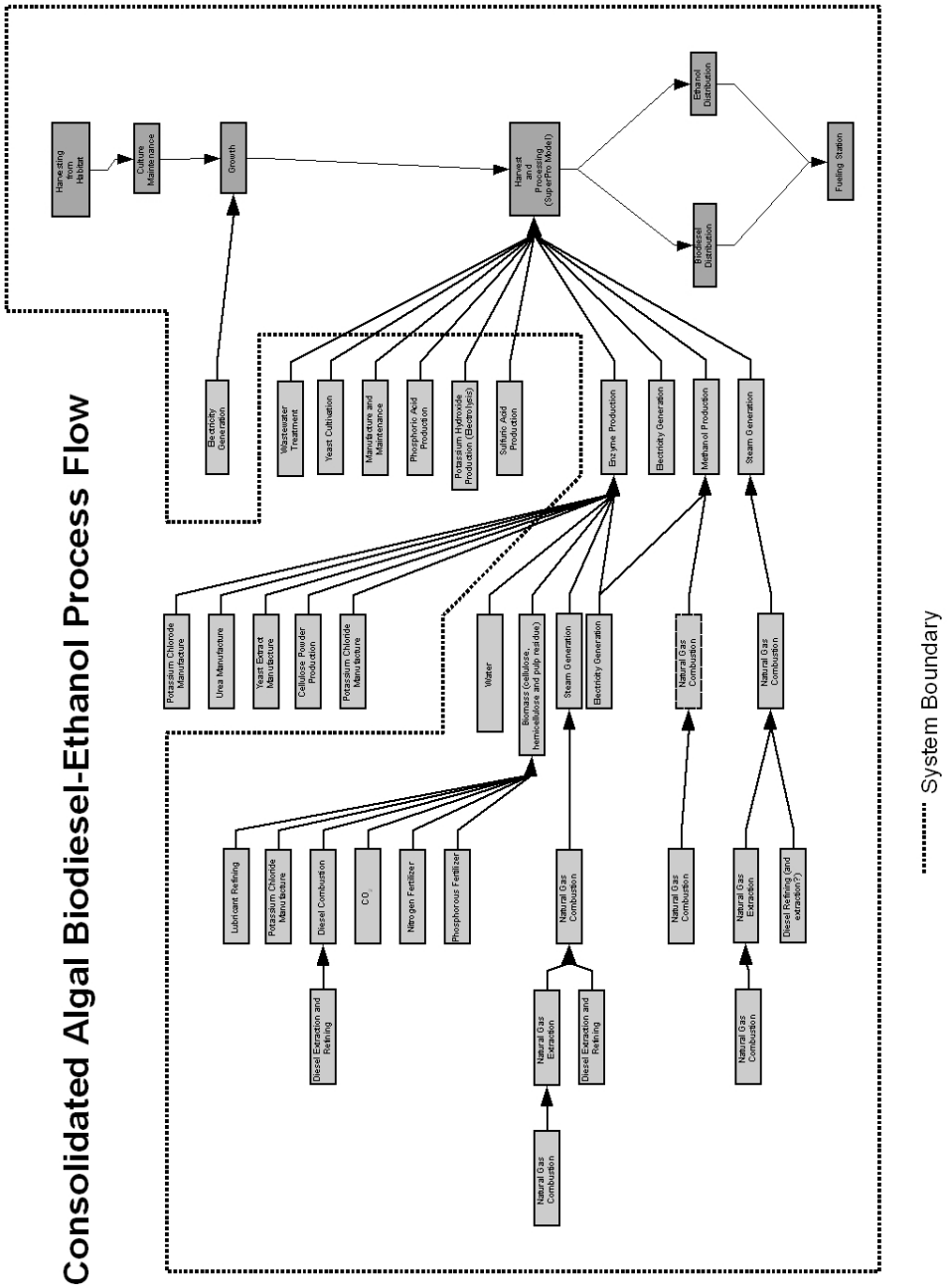

Figure 5.2: Combined Algae/Biodiesel Process System Boundary

cut-off ratio, the LCI values for that input are included in the LCA. The RMEE cut-off ratio chosen for this study was 0.05 (or 5%). Though this method was used and the resulting system boundary followed, four secondary unit processes were omitted due to lack of data though the RMEE boundary dictated they should be included in the LCI. These processes were the production of potassium chloride, cellulose powder, urea and yeast extract all used as media components in enzyme production. The general RMEE equation for deciding the inclusion or exclusion of a unit process is:

$$X_{\text{ratio}} = \frac{X_{\text{input}}}{X_{\text{functional unit}}} \tag{5.1}$$

The life cycle inventory was compiled in a Microsoft Excel$^{\text{TM}}$spreadsheet in a style similar to the GREET model datasheet [GREET, 2008]. Values which are key to computing highly sensitive values and those subject to change (e.g. the price of gasoline and others) were listed on the first worksheet and are integrated in a way which will re-compute LCI totals if a value on this page is changed. Although LCI values will be re-computed, changing input values will not change the system boundary and LCI totals will be calculated based on the original system boundary outlined above and shown in figure 5.2.

Each successive worksheet tabulates a unit process from the process flow path. The “electricity,” “natural gas,” “steam,” and “diesel” worksheets tabulate secondary inputs and emissions where the RMEE boundary dictated these LCI values

be included. Often, the RMEE boundary indicated LCI utility values be included from multiple processes. In these cases, total demand was calculated for the entire process and LCI values were computed from a total demand. This is considered accurate because while LCI values are being computed for a single "total" demand, none of these values would have individually been left out of the RMEE boundary.

All steam is assumed to be generated in a natural gas boiler at 80% combustion energy to steam energy conversion efficiency. As there was no LCI data for pipeline transport, cases which required pipeline transport were substituted with diesel truck transport. This included materials such as natural gas which is transported from a refinery to end-use facilities using a combination of truck, train and pipeline transport. In the following example, only the pipeline portion of transport was substituted for truck transportation, not the total transportation demand.

Hydrolyzing enzymes were dosed based on laboratory measured values [Kumar, 2010] of Accelerase 1500^{TM}activity (found to be 44 FPU/mL). Hydrolysis demand per functional unit was calculated based on the assumption that algae biomass contained 11% carbohydrates and 10% membrane lipids (both w/w dry basis). Phospholipase activity, density and economic value were assumed to be the same as Accelerase 1500^{TM}, and thus the total dosing requirements were calculated based on a 30 hour hydrolysis time. Zhuang [2006] developed a SuperPro process simulation from which input and emission data was used in computing LCI data for cellulase enzyme production.

The production of fertilizer grade MKP (mono-potassium phosphate) was assumed to take place according to the following reaction:

Table 5.1: Data Sources

Unit Process	Data Source	Data Gathered from Source
Growth	[Borowitzka and Borowitzka, 1988]	Mixing Correlation, Electricity Demand
Biodiesel/Ethanol Production	SuperPro Process Simulation	Material Inputs, Water and Steam Demand, Functional Unit Throughput, Emissions
Enzyme Production	[Zhuang, 2006]	Material Inputs, Water and Steam Demand, Electricity Demand
	[Kumar, 2010]	Accelerase 1500^{TM}Activity
Transportation and Distribution	[GREET, 2008]	Energy Inputs and Emissions
Methanol Production and Distribution	[LCI, 2008]	Material and Energy Inputs, Emissions
Natural Gas Processing, Refining and Transportation	[LCI, 2008]	Material and Energy Inputs, Emissions
Biomass Growth, Harvest, Transport and Reforesting	[LCI, 2008]	Material and Energy Inputs, Emissions
Diesel Extraction and Transport	[LCI, 2008]	Material and Energy Inputs, Emissions
Electricity Generation and Transmission	[GREET, 2008]	Electricity Generation Mix, Emissions
Steam Generation	[LCI, 2008]	Material and Energy Inputs, Emissions
Potassium Chloride Production	No Data	
Cellulose Powder Production	No Data	
Urea Production	No Data	
Yeast Extract Production	No Data	

$KOH + H_3PO_4 \rightarrow KH_2PO_4(s) + H_2O$

This reaction forms a precipitate which is assumed to require negligible processing resources to collect and prepare for use. Also, this precipitate is assumed to form at a rate faster than the production facility can provide potassium hydroxide.

5.3 Results and Discussion

The net energy balance of this process is 14.7 meaning it takes 14.7 MJ of main process energy to create one MJ well-to-gate of combined algal biofuels. This is substantially larger than has been reported in previous algal biofuel well-to-pump LCA's [Sander and Murthy, 2010].

Enzymes are by far the most life cycle intensive input in this process. For example, the production of 1 kg of enzyme slurry requires 1,976.2 kg of growth media and 143.4 kg of woody biomass hog fuel. Furthermore 447 kWh of electricity is needed in the production of 1 kg of enzyme slurry. 76.8% of the electricity demand in this study stems from the production of cellulases. Every material input which goes into the production of cellulases eclipsed the RMEE boundary cut-off ratio. If the enzyme production step were removed from the process flow of this LCI, the net energy ratio would drop to 3.45. CO_2 emissions would remain unchanged as no CO_2 is emitted directly from the enzyme production step. If enzymes are going to be a staple input in any bioenergy conversion system, no matter the feedstock, it is clear a less life-cycle intensive production process is needed to reduce life cycle impact.

Table 5.2: LCI Emissions

Output	Amount (g)
VOC's	28.19
CO	311.28
NO_x	1,076.37
PM-10	65.93
PM-2.5	27.93
SO_x	1,944.99
CH_4	1,671.43
N_20	11.45
CO_2	1,055,459.61
Other	68,392.65

Another option for making the use of enzymes more life-cycle friendly would be the use of an immobilized enzyme system allowing for the re-use of enzymes over many batches. Barriers to this technology are increased cost, loss of enzyme activity upon immobilization, and additional processing steps needed to clean and re-charge an immobilization substrate which might potentially lead to additional down time. Another life-cycle improvement may come naturally as demand for bioprocessing enzymes grows and forces companies to employ larger economies of scale for enzyme production. The model enzyme production system used in this study utilized a batch throughput of 10,010 kg enzyme slurry per batch [Zhuang, 2006]. This volume of production is by no means a technical ceiling in bioproduction [Lewis and Young, 2002].

The wastewater input in this study is used in two processing steps. Wastewater is used as a growth medium for the algae, and as wash water in the initial belt filtration concentrating step. The remaining water demand was that of cooling wa-

Table 5.3: LCI Material Inputs

Material Input	Amount (kg)
Wastewater	121,265.40
Process Water	14,821.79
Woody Biomass (Hogged Fuel)	126.23
Potassium Chloride	47.43
Carbon Dioxide	27.87
Yeast Extract	9.88
Enzymes	1.58
Methanol	l0.99
Sulfuric Acid	0.047

Table 5.4: Energy Inputs

Material	Demand (kg)
Natural Gas	95.92
Diese	l2.39
Coal	223.38
Liquefied Petroleum Gas	0.05
Gasoline	0.08
Residual Fuel Oil	7.01
Biomass	15.7
Total Electricity Demand (kWh)	921.5
Main Process Energy (MJ)	3,352.12
Primary Energy (MJ)	11,375.78

ter. This water did not include water used in boilers or boiler feed makeup water. Depending on the apparatus used for cooling, different additives may need to be added to this water to prevent corrosion in metal cooling equipment. These additives or any additional water treatment of this "process" water was not accounted for in this LCI dataset. Research in algae processing has been wrought with challenges regarding water use, the volumes of water potentially demanded, water losses to evaporation and the eventual removal of water from culture [Pienkos and Darzins, 2009, Sander and Murthy, 2009, 2010]. A sustainable water use solution in algal biofuels is necessary to make algal biofuels sustainable.

Fig. 5.3 cites two total energy values. Primary energy refers to energy demand in generating steam, electricity and the extraction, refining and transport of natural gas and diesel. Main process energy is energy demand from all other processes quantified in figure 5.3 not listed above. It would be inappropriate to incorporate one complete value as "total energy" because this would account values such as the electricity used in a process, and the energy (in the form of raw inputs) which is used to generate electricity, as two separate values. This would be redundant, counting "electricity" energy twice.

There is a need for data specific to the biofuels industry. This data will make biofuel and transportation fuel LCA's more accurate and useful. This may also alleviate the problem of missing or incomplete data, as was encountered in this study. As more full scale cellulosic and bioenergy plants are coming online, data from these plants may be useful in conducting future LCA's and building a stronger case for biofuels as petroleum replacements.

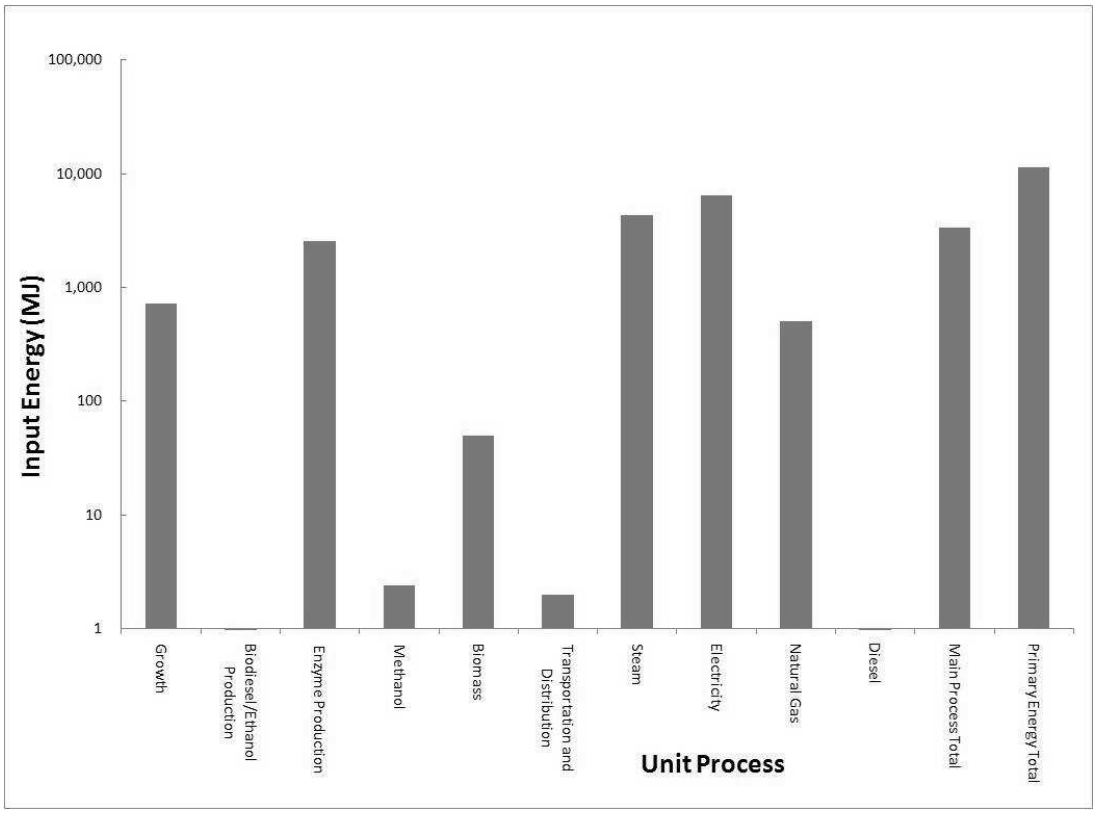

Figure 5.3: Input Energy Demand for each LCI Unit Process

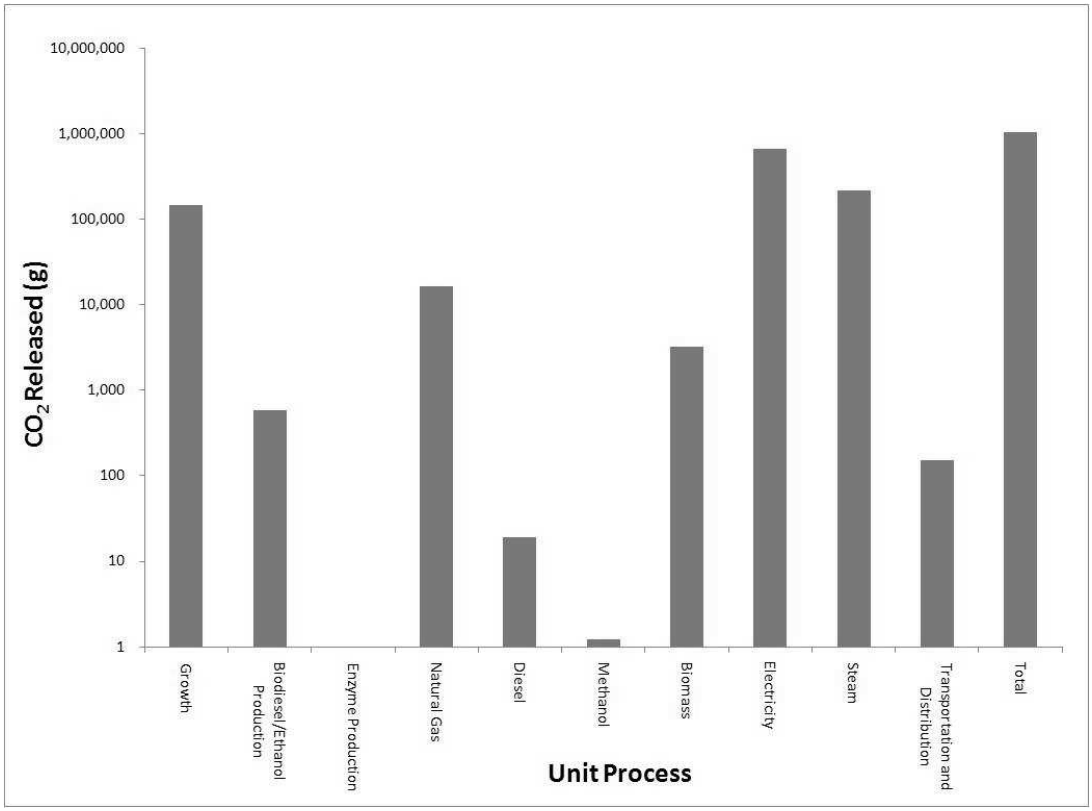

Figure 5.4: CO_2 Emissions from ach LCI Unit Process

5.4 Conclusion

This study was undertaken to collect baseline data for a hypothetical combined biodiesel-bioethanol production process utilizing algal biomass as a feedstock. LCI data was collected for a "well-to-pump" process and organized in a Microsoft Excel^{TM}spreadsheet.

Process modeling was done using SuperPro process modeling software.

It was found a net energy of 14.7 was required for the production of the studied "combined" functional unit (biodiesel and bioethanol). Further, 1,052.5 kg of CO_2 is released per functional unit. Enzyme production was by far the most life-cycle intensive unit process. Also, there exists a need for more specific LCI data pertaining to the biofuels industry.

Chapter 6 – Conclusion

In this thesis, downstream processing technology of algae is explored from a life cycle perspective.

A well-to-pump life cycle assessment was first performed to assess the sustainability of a modern day algal biofuel system and to identify, if any, process technologies which might be improved to reduce the life cycle impact of such a process. Dewatering of algal cultures was identified as a critical process in need of technological advancement. Dewatering of the algal cultures is done to facilitate the further downstream component separation. Two technologies were then investigated which might remove the need for algal dewatering yet still allow for cost effective biodiesel production.

The first technology investigated was the application of rapid sand filters as a first-pass algal dewatering technology, with the hope of concentrating an algae slurry 10-50 times. As mentioned before, algal processing technology is currently constrained by economics and must rely on low cost, low tech processing. Rapid sand filters have been used in wastewater processing to aid primary settling (and other processes) in suspended solids removal to below enforcement levels. They are not only low cost and relatively simple, but are also industrially proven. Rapid sand filters (which typically operate under 1-3 m of available head pressure) pass water much quicker than do slow sand filters (which are gravity fed). A typical

cycle consists of backflushing after a period of operation to clean the filters. After backflushing the filters, they are returned to the operational cycle.

Typically many units are run in parallel to buffer against process disruptions. The investigation carried out involved designing and operating three sand filters, each utilizing a filter media (sand) of different "effective particle size." The removal efficiencies were evaluated for each sand filter. Some general conclusions (such as smaller sand sizes better remove algae than do larger sand sizes) were able to be drawn, but the research question asked was not answered because of an inability to close mass balances around the sand filters during operation. A brief exercise in modeling was also performed using the tank in which the feedstock algae was grown.

The next technology explored borrows elements from cellulosic biomass processing. After an acid pretreatment, cellulases and phospholipases are used to enzymatically degrade the algal biomass into its components. The presence of these components was then observed using HPLC. The carbohydrate portion was simultaneously fermented into ethanol. An experiment was performed investigating the feasibility of such a process. Time course evolution of both glucose and glycerol was confirmed and fermentation activity was observed. A high biomass loading experiment was performed at a solids loading of 5%. Hydrolysis products were observed and fermentation products were observed at higher solid loading rates.

Finally a life cycle inventory was compiled for a hypothetical well-to-pump algal process whereby both biodiesel and bioethanol are manufactured simultaneously

utilizing algae as a feedstock.

This promotes a more modern idea of a multi-product system where the low cost of the final product from a traditional algal system (biodiesel) might be buffered by making additional value products from the same biomass in order to increase its overall value and feasibility. Such a process might also free algae processing from the drying constraints. This life cycle inventory pointed out the immense life cycle impact of the commercial production of enzymes. As was the case for drum drying in the previous LCA performed, one process in particular demanded the most energy and emitted the most CO_2.

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APPENDICES

Appendix A – Modified Materials and Methods for Sand Filter Experiment

A.0.1 Bioreactor Growth

Chlamydomonas reinhardtii is grown in 1.5 L of culture volume in a 2 L bioreactor (Biostat Corporation) in a modified TAP medium. The reactor was operated at 30^{o}C. The culture was agitated at 180 rpm. A one meter long fluorescent fixture with two bulbs was affixed six inches from the front of the reactor. The reactors were grown in batch mode with growth cycles lasting from four to seven days.

A.0.2 Dilution

Each bioreactor growth culture (1 g/L) is to be diluted to a biomass concentration of 100 g/L (about 10-15 times) with DI water. This diluted culture is then to be used as input to the sand filters. Dilution is done in a 19 L plastic bucket and biomass was kept agitated using a stirbar and stirplate or a small lab scale agitatot. Filtration is to be performed immediately after the growth culture was diluted so as to filter the culture before it has had time to settle or naturally flocculate. Because the original culture broth will be diluted to its final working concentration, the effects of algal extracellular organic matter (EOM) [Sakevich

and Usenko, 2008] might be underrepresented in this study.

A.0.3 Uniform Media Rapid Sand Filter Design

The algal sand filters are designed using the principles of rapid sand filtration. Rapid sand filtration is most commonly used in wastewater and stormwater treatment to remove suspended particles and reduce turbidity [Sincero and Sincero, 2003]. The three predominant and interrelated parameters in uniform media rapid sand filter design are particle size, bed height and pressure drop across the sand bed. Typically, large scale uniform grade rapid sand filters are designed to operate at about 30 psig available head pressure and about 1 meter in depth [Degremont, 1973]. An increase in bed particle effective size (d_e) will require a larger bed depth to achieve the same solids removal of a bed with smaller effective particle size. Generally, a larger bed depth will be characterized by a larger pressure drop across the bed, necessitating more pressure to push culture through it.

ASTM grade C-144 grade pavers sand was sieved into three grades and used as sand bed material. The three sieve sizes used were 150-300 μm, 300-600 μm and 600-840 μm and, ssuming uniform particle size distributions, correspond to effective particle sizes (d_e) of 165 μm, 330 μm and 624 μm. The three filter beds were designed as uniform media sand filters meaning all media in the bed was the same size and material type. A design rule for sand filter bed heights states the L/d_e should be 1000-2000 [Tchobanoglous et al., 2003]. For this study the corresponding bed heights (L) were chosen such that the L/d_e for all beds was

1500.

The available pressure was chosen as 2 m H_2O. This was a design value mentioned as the the practical limit for wastewater treatment plants uperating rapid sand filters [Tchobanoglous et al., 2003]. Head pressure is kept constant using a pump (see fig. A.1) to pump diluted culture into the inlet (top) of the sand filter. An overflow is to be installed at a height 2 m above the top of the sand bed. The inlet culture was pumped at a higher rate than flow through the sand bed. This ensured constant head pressure at all times during the filter runs.

Filter bed flowrate was calculated using the Carman-Kozney equation [Tchobanoglous et al., 2003]. This equation incorporates the porosity of the bed (in this case assumed to be 0.525), headloss across a clean filter bed (h), bed height (L), and other factors to determine the flow velocity through a clean, unclogged sand bed. The flow velocity was chosen such that the clean filter headloss across the filter bed (h) was 0.5 m H_2O. The flowrate will be controlled with a flowmeter affixed directly to the filter outlet. This was chosen so the filter can be operated at constant flowrate. As a filter gets clogged with sand, the flowmeter valve is gradually opened so as to keep flowrate constant through the filter bed. The value for "h" was chosen so the available head pressure (2.0 m H_20) was significantly larger. This ensured the flowrate chosen could be held constant throughout the filter run.

The diameter of each filter was chosen last to accomidate a constant volumetric flowrate through all filters of 3.0 L/hr. This number was chosen out of practicality. "Rapid" sand filtration is often defined by the superficial velocities (m/hr) they operate at. Typically, volumetric flowrates are chosen to match demand in a

wastewater treatment facility. For this study, a volumetric flowrate of 3 L/hr was chosen for all filters. The diameter of each filter was chosen to accomodate this volumetric flowrate. The diameter of the filters for the filters of d_e 165 μm, 330 μm and 624 μm were 6.4 cm, 4.5 cm and 3.3 cm, respectively.

The filters are to be constructed of cast acrylic tubing 9 total feet in length. The filters should be comprised of two pieces of tubing flanged together. An underdrain consisting of a acrylic disc with many holes drilled in it and a piece of 63 micron mesh can be glued to the bottom of the underdrain to retain any sand particles but allow algae and other particulate matter to pass. All tubing and connections are 1/4" vinyl tubing. A rotameter will be affixed to the bottom of the filter followed by an online microphotometer. The filtrate will then be emptied into a 1 L graduated cylinder.

A.0.4 Uniform Media Rapid Sand Filter Operation

Filters were operated by first filling the filter beds with diluted algae with the valve at the filter outlet closed. Flow was then continued into the filter and cycled back to the inlet through the overflow. The valve on the bottom of the filter bed is then opened and the desired flowrate set.

After 3 L has passed through the filter, the bed was then backflushed and cleaned. This is accomplished using a combined water/air scour method [Tchobanoglous et al., 2003]. A 19 L bucket was placed 2 m above the top of the sand filter. This bucket is connected to a flowmeter and check valve and eventually to the bottom

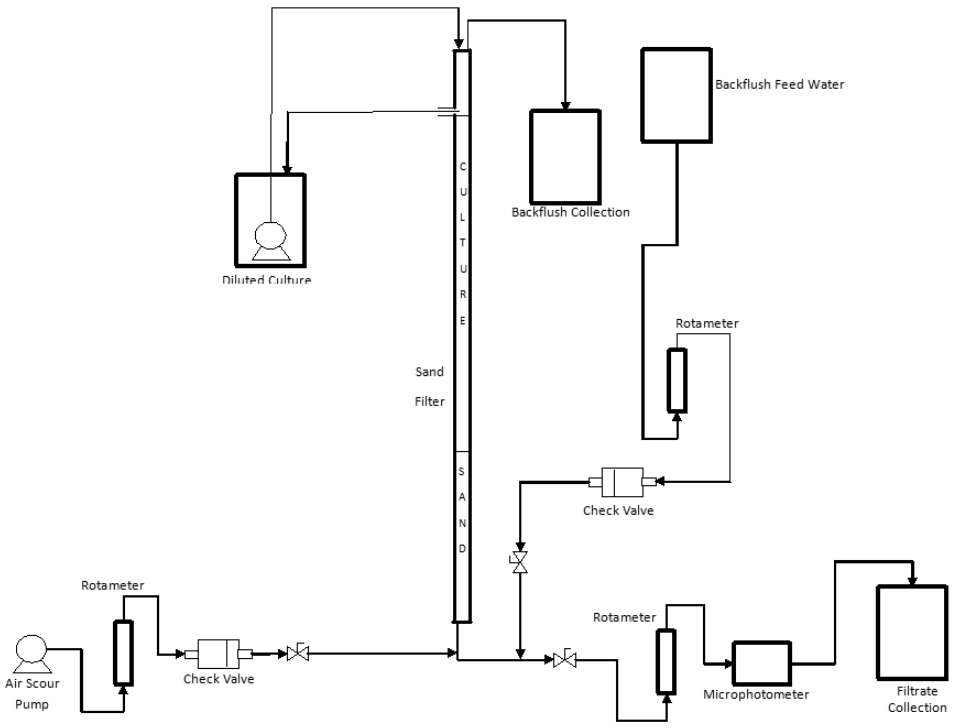

Figure A.1: Modified Experimental Filter Apparatus

of the sand filter. An air scour pump is also to be attached to a filter for backflush. Air and water flowrates are calculated by extrapolating data of typical air flowrates based on filter d_e. The water backflush flowrates for the 0.624, 0.33 and 0.165 mm d_e filters are calculated to be 8.5, 10.8 and 15.6 mL/sec, respectively. Air flowrates are calculated to be 269, 336 and 479 mL/sec. Backflush is to be performed until the filter ran clear and was collected into 500 mL segments for analysis.

A.0.5 Design of Experiment and Analysis

This study hopes to determine which (of three particle sizes) filter type is best for capturing algae biomass and recovering it for further processing. As mentioned, sand filters might be a low-cost and low-tech option as a first step in concentrating and dewatering algal biomass cultures. Each "run" consisted of filtering three liters of diluted culture medium through each filter bed with the hopes of capturing the ripening stage of each filter, its effective filtering length of time/volume and signs of breakthrough. The bed is then immediately backflushed, samples collected and analyzed. Each filter was subjected to three separate runs from three different initial culture growths.

Parameters which were sought to either calculate or otherwise elucidate from this study, and are also vital operating parameters of rapid sand filters [Tchobanoglous et al., 2003] were the filtration rate (rate of removal), the duration and magnitue of the ripening period, effluent turbidity at different times of filtration, time and volume through to filter breakthrough and filter headloss before and after filtering.

Turbidity samples should be collected of the influent and effluent to determine removal performace. Filter headloss cannot be measured directly during a run. Ripening can be elucidated from the volume course measurement of outlet turbidity. A "clean" filter, or filter with no solids entrained in it will remove less solids than a "ripe" filter, as they will be allowed to pass through the spaces within the filter easier.

Full scale rapid sand filters will typically have some form of continuous real-time measurement of turbidity, filtration rate and filter bed headloss. As this experimental system will be used intermittantly, periodic manual sampling will be used in place of online sensors for some measurements. Further, use of a manual flowmeter will require manual flow adjustment to maintain constant superficial velocity.

Data which is to be collected are the biomass concentrations in the filter outlet, pressures across the filter bed, and readings of online instruments taken during test runs and backflush. A volume-course graph of sand filter outlet is very indicative of its performace as is the pressure across the bed. Wastewater treatment plants must operate sand filters to provide water which meets turbidity standards. In the case of biomass recovery, collecting and subsequently backflushing with the greatest effectiveness is also important. The overall recovery effectiveness is calculated by dividing the mass of algae recovered from backflushing by the mass of algae inputted into the filter. This calculation was coupled with an overall mass balance to ensure completeness and closure.

A.0.6 Analysis

Absorbance readings were taken at 680 nanometers to assess the biomass concentration using UV-Vis Spectrophotometer (Shimadzu PharmaSpec UV-1700, Shimadzu Corporation, Columbia, MD). A biomass correlation curve was created for each run by filtering three dilutions of backflush, encompassing the range of all samples taken, through preweighed and dried glass fiber filter papers (#934-AH 42.5mm, Whatman Ltd., Madistone, Kent, UK). 100 mL of each dilution was filtered in triplicate and averaged to give a three point biomass-absorbance correlation.

The sand filter inlet from each run was characterized by centrifuging 2 mL of culture and observing the algal mass under a microscope (Motic BA300, Motic Ltd., Richmond, British Columbia). 100 cells are to be chosen from the observation and their shortest dimension are measured. These measurements are to be grouped by size.