

Computational Sustainability Assessment of Algal Biofuels and Bioproducts for
Commercial Applications

by

William James Barr

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved March 2016 by the
Graduate Supervisory Committee:

Amy Landis, Co-Chair
Paul Westerhoff, Co-Chair
Bruce Rittmann
Vikas Khanna

ARIZONA STATE UNIVERSITY

May 2016

ABSTRACT

To date, the production of algal biofuels is not economically sustainable due to the cost of production and the low cost of conventional fuels. As a result, interest has been shifting to high value products in the algae community to make up for the low economic potential of algal biofuels. The economic potential of high-value products does not however, eliminate the need to consider the environmental impacts. The majority of the environmental impacts associated with algal biofuels overlap with algal bioproducts in general (high-energy dewatering) due to the similarities in their production pathways. Selecting appropriate product sets is a critical step in the commercialization of algal biorefineries.

This thesis evaluates the potential of algae multiproduct biorefineries for the production of fuel and high-value products to be economically self-sufficient and still contribute to climate change mandates laid out by the government via the Energy Independence and Security Act (EISA) of 2007. This research demonstrates:

- 1) The environmental impacts of algal omega-3 fatty acid production can be lower than conventional omega-3 fatty acid production, depending on the dewatering strategy.
- 2) The production of high-value products can support biofuels with both products being sold at prices comparable to 2016 prices.
- 3) There is a tradeoff between revenue and fuel production
- 4) There is a tradeoff between the net energy ratio of the algal biorefinery and the economic viability due to the lower fuel production in a multi-product model that produces high-value products and diesel vs. the

lower economic potential from a multi-product model that just produces diesel.

This work represents the first efforts to use life cycle assessment and techno-economic analysis to assess the economic and environmental sustainability of an existing pilot-scale biorefinery tasked with the production of high-value products and biofuels. This thesis also identifies improvements for multiproduct algal biorefineries that will achieve environmentally sustainable biofuel and products while maintaining economic viability.

DEDICATION

This dissertation is dedicated to my fiancée Leeane Hamilton, and my parents, Elise Barr and Willie Barr and grandmother Elizabeth Tooson. Leeane has been patient and supportive over the years. My parents and grandmother were supportive of the long move from Pittsburgh to Arizona.

ACKNOWLEDGMENTS

I would like to acknowledge my advisor and co-chair, Dr. Amy Landis who has provided invaluable support and guidance throughout my Ph. D. career. I am very thankful to have had her for an advisor. I would also like to thank Dr. Paul Westerhoff who was my co-chair. He welcomed me to his group where I was able to expand on my knowledge, present my research to colleagues and make new friends. I would also like to thank Dr. Bruce Rittmann and Dr. Vikas Khanna who also served on my committee. It was a pleasure to work with them and their feedback made my thesis significantly better.

I would also like to thank Dr. Kristen Parrish and Dr. Oswald Chong who assisted me in my last year at ASU. I would like to thank Robert Stirling who was extremely supportive over the last two years and taught me how to perform an effective and thorough technoeconomic analysis.

I would like to thank my co-authors, in addition to Dr. Landis, Dr. Kullapa, Dr. Willie Harper, Matthew Weschler and Priscila Sanches Rodrigues.

I would also like to thank my fellow graduate students for their support including: Shakira Hobbs, Beki Burke, Dr. Troy Hottle, Dr. Claire Antaya Dancz, Xi Zhao, Dr. Scott Unger, Daina Rasutis, Cheyenne Harden, Evvan Morton, Habib Azarbadi, Tyler Harris, Alex Links, Priscila Sanches Rodrigues, David Hannigan, Anjali Mulchandani, Natalia Hoogesteijn, Neng Iong Chan, Levi Straka, Dr. Ben Wender, Dr. Susan Spierre Clark, Dr. Valentina Prado, Dr. Andrew Berardy, Janet Reyna, Dwarak Triplican, Andrew Fraser, Mindy Kimble and Lauren McBurnette.

I would like to thank ASU Lightworks and AzCATI, especially Gary Dirks, Dr. John McGowen, Bill Brandt, Travis Johnson, Jessica Cheng and Dr. Peter Lammers for funding and mentoring support as well as numerous opportunities to share my research with the wider algae community. I would like to thank Cellana LLC for working with me and assisting me in designing a thorough algae biofuel and bioproduct model, especially Martin Sabarsky, Avery Kramer, and Dr. Johanna Anton.

I would like to thank Debra Crusoe for her mentorship and support over the years. She has provided me a number of opportunities to share my graduate school journey on a number of different platforms.

I would like to acknowledge funding from the National Science Foundation (Award Number CBET 0932606/1241697), travel support from More graduate education at Mountain States Alliance (mge@msa), ASU lightworks for the technology research initiative funding (TRIF), GPSA travel grant, and Jennifer Cason and the ASU dissertation fellowship.

Finally I would like to thank my Lord and Savior Jesus Christ.

TABLE OF CONTENTS

	Page
LIST OF TABLES	viii
LIST OF FIGURES	x
CHAPTER	
1. INTRODUCTION	1
2. ENVIRONMENTAL IMPACTS OF ONSITE ANAEROBIC DIGESTION FOR ALGAE BIOFUEL RESIDUAL HANDLING AND CO-PRODUCT GENERATION.....	39
Introduction.....	39
Methods.....	41
Results & Discussion	47
Conclusion	56
3. ENVIRONMENTAL SUSTAINABILITY ASSESSMENT OF A COMMERCIAL-SCALE ALGAL MULTIPRODUCT BIOREFINERY	57
Introduction.....	57
Methods.....	64
Results & Discussion	76
Conclusion	84
4. ECONOMIC SUSTAINABILITY ASSESSMENT OF A COMMERCIAL- SCALE ALGAE MULTIPRODUCT BIOREFINERY	87
Introduction.....	87

CHAPTER	Page
Methods.....	91
Results & Discussion.....	99
Conclusion.....	108
 5. COMMERCIAL COMPUTATIONAL SUSTAINABILITY ASSESSMENT OF AN ALGAE MULTIPRODUCT BIOREFINERY FOR THE SIMULTANEOUS PRODUCTION OF BIOFUEL AND HIGH-VALUE PRODUCTS	111
Introduction.....	111
Methods.....	114
Results & Discussion	124
Conclusion	133
 6. CONCLUSION.....	137
 REFERENCES	144
 APPENDIX	
 A. CHAPTER 2 SUPPORTING INFORMATION.....	157
 B. CHAPTER 3 SUPPORTING INFORMATION.....	163
 C. CHAPTER 4 SUPPORTING INFORMATION.....	177

LIST OF TABLES

Table	Page
1. Summary of the Thesis	6
2. LCA/TEA Anaerobic Digestion Studies	23
3. Scenario Description.....	66
4. Existing Unit Process to Model Comparison	69
5. Amount of Products per Functional Unit	75
6. Financial Parameters.....	96
7. Break-even Selling Price:	100
8. Cultivation Module Parameters	117
9. Summary of Results.....	126
10. Required Selling Prices for Diesel and Omega-3 Fatty Acids	127
11. Summary of Thesis	136
12. Anaerobic Digestion Parameters	158
13. Downstream Processing Parameters.....	160
14. Landfill and Incineration Parameters	161
15. Electricity Mix Details.....	162
16. Algae LCI Inventory.....	164
17. Biomass Characteristics.....	165
18. Outdoor Cultivation 1	166
19. Outdoor Cultivation 2	167
20. Dewatering.....	168
21. Oil Handling Parameters	169

Table	Page
22. Diesel Production	170
23. Monte Carlo Analysis	171
24. Fish LCI Inventory	172
25. Fuel Consumption of Purse Seine Fisheries	173
26. Fish Biomass and Vessel Characteristics	174
27. Maintenance and Onboard Activities	175
28. Oil Handling	176
29. General Plant Parameters.....	178
30. Cost of Chemicals and Materials.....	179
31. Labor Estimates	180
32. Baseline Capital Costs.....	181

LIST OF FIGURES

Figure	Page
1. Algae Bioproducts Value Comparison	2
2. Algae General Process Flow.....	13
3. Algae Harvesting Process Energy Consumption.....	15
4. Potential Algal Bioproducts.....	17
5. DOE Baseline Algal Biofuel Production.....	32
6. Cellana’s Process Flow Diagram and Products.....	35
7. Conventional vs. Algae Omega-3 Fatty Acid Production	37
8. ADLCA System Boundaries	43
9. ADLCA Global Warming Potential	48
10. ADLCA Eutrophication Potential	51
11. Multiproduct Model Process Flow Diagram	68
12. Environmental Impact Comparison.....	78
13. Normalized GWP Impacts.....	82
14. Normalized GWP Tornado Plot for CPM Model.....	83
15. Normalized GWP Tornado Plot for MPM (MF) Model.....	84
16. Multiproduct Model (MPM) Process Flow Diagram	93
17. Multiproduct Model Cost of Production	101
18. Annual Operating Cost	102
19. Variable Costs Breakdown	103
20. Capital Cost Comparison.....	104
21. Sensitivity Analysis Annual Operating Cost	106

Figure	Page
22. Sensitivity Analysis Annual Operating Cost MPM MF	107
23. Algae Multiproduct Model System Boundary for the LCA.....	116
24. Energy Return on Investment Tornado Plots	128
25. Revenue and Environmental Impacts	130
26. Costs and Environmental Impacts	131
27. Sensitivity Analysis of GWP	133

CHAPTER 1

INTRODUCTION

Motivation:

To date, the production of algal biofuels is not economically sustainable due to the cost of production and the low cost of conventional fuels (Davis et al., 2011; Richardson & Johnson, 2015; USEIA, 2016). The US Department of Energy has conducted a number of Techno-Economic Analyses (TEA) to assess the economic potential of biofuels using lipid extraction and hydrothermal liquefaction (HTL) followed by hydroprocessing to produce renewable diesel (Davis et al., 2011; Davis et al., 2012; Davis et al., 2014b). Richardson and Johnson (2015) concluded that 40% reductions in both capital expenses (CAPEX) and operating expenses (OPEX) were needed for an algal biorefinery to have any chance of being financially feasible based on data from the harmonization models and large-scale cultivation facilities in Texas and at the University of Arizona. Interest has been shifting to high value products in the algae community to make up for the low economic potential of algal biofuels. Figure 1 shows the value of different potential algal bioproducts. Omega-3 fatty acid is an example of a product that has a much higher economic potential than biofuel but may also allow for the production of fuels as co-products from algae biorefineries. Co-product generation could be an important step to economic sustainability of algae as a feedstock for both biofuels and high-value products.

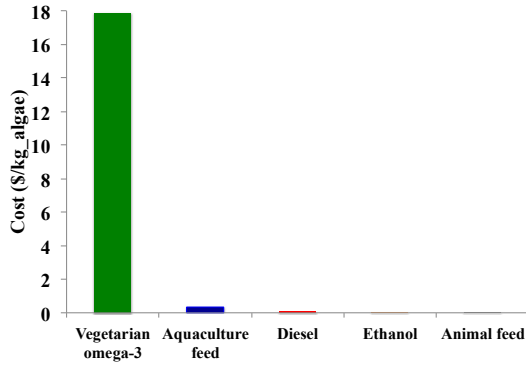


Figure 1: Algae Bioproducts Value Comparison
 Value of different bioproducts derived from algae as of March, 2016
 AFDW: Ash free dry weight.

The economic potential of high-value products does not eliminate the need to consider the environmental impacts of those products. Federally, the environmental goals of incentivizing algal biofuels are to reduce the CO₂ emissions compared to fossil fuels and provide sufficient energy to offset a significant portion of fossil fuel consumption (e.g. The Energy Independence and Security Act of 2007- EISA). In addition to greenhouse gases (GHGs) and energy independence, there are other environmental impacts of concern with respect to algal biofuels, including water consumption, land use, water quality impacts from wastewater, air quality impacts, and resource depletion (Mu et al., 2014; Soratana et al., 2014). The environmental impacts of high-value products should be compared to their conventional counterparts in a similar fashion that biofuels and petroleum-based fuels have been compared (Prabhu et al., 2009; Zaines & Khanna, 2013). Producing high-value products from algae with biofuels may negate the environmental benefits of algal biofuels if producing the high-value products has significantly higher environmental impacts than their conventional counterparts. Not quantifying these environmental impacts and potential tradeoffs in the early stages of

algae multiproduct biorefinery development that integrates high-value products could lead to unintended consequences related to other environmental factors not related to biofuels. This thesis explores the environmental impacts of high-value products compared to conventional production.

When considering the commercialization of algal biorefineries for biofuels and high-value products, both environmental impacts and economics must be considered. The United States Department of Energy (DOE) developed harmonized Life Cycle Assessment (LCA), TEA and resource assessment models across three different national labs (Argonne National Laboratories (ANL), National Renewable Energy Laboratory (NREL) and Pacific Northwest National Laboratory (PNNL) respectively). Studies from the harmonization models explored biofuel production at 5 to 10 billion gallons per year (BGY) of some form of diesel (i.e. green, bio or renewable; studies resulting from these models have assessed all three) from algae. Five BGY was selected as a minimum because that value represented 10% of the U.S. diesel consumption at the time the report was published (Davis et al., 2012). If multi-product biorefineries are commercialized to the degree indicated by the harmonization models this could greatly influence nutrient consumption, land use and the market of high-value products, which may not be produced at the same level as fuel. This thesis explores the effect that high levels of commercialization, comparable to what the harmonization models use, have on nutrient consumption, land use and the market for the specific high-value product.

There are numerous product output options for multi-product biorefineries. Selecting which method meets the most important needs is a critical step in the commercialization of algal biorefineries, and introducing other products may reveal other environmental

sustainability factors worth addressing. Being able to compare different bioproduct sets may reveal the potential economic and environmental sustainability issues not related to biofuels that are worth further consideration. For example, if the production of high-value if the global warming potential of the algal biorefinery for producing high-value products and fuel is less than the combined global warming potential for the conventional production of those two products, then that is something that should be quantified by algal biorefineries. Furthermore different multi-product scenarios will have different economic potential that may or may not be improved proportional to the environmental impacts. This thesis explores and compares the environmental impacts and economic potential of different algae multi-product scenarios, and identifies tradeoffs between environmental impacts and economic potential.

Research objectives:

This thesis evaluates the potential for economic and environmental sustainability for the simultaneous production of biofuels and high-value products that would allow a commercial-scale biorefinery to be economically self-sufficient and still contribute to climate change mandates laid out by the government. This work represents the first efforts to use LCA and TEA to assess the economic and environmental sustainability of an existing pilot-scale biorefinery tasked with the production of high-value products and biofuels. Insights from LCA and TEA point to improvements the biorefinery must achieve to produce environmentally sustainable biofuel while maintaining economic viability in the academic literature.

The research of this thesis was guided by four research questions

1. How do the environmental impacts of high value algal bioproducts compare to standard production of the same products?
2. How can high value algal bioproducts best support algal biofuel commercialization to meet government mandates and standards?
3. How do the environmental impacts and economic potential of two different multiproduct models that mix energy and high-value products compare to one another?
4. How can the environmental and economic performance of algal biofuels and bioproducts most effectively be improved and what are the tradeoffs between economic and environmental sustainability?

Organization of the thesis:

The thesis is organized around manuscripts that will be submitted to peer-reviewed journals, summarized in Table 1. Each chapter represents a stand-alone manuscript, and most chapters address several research questions. The thesis conclusion in Chapter 6 summarizes how the findings from the manuscripts address each research question and provides insight for future work.

Table 1: Summary of the Thesis

Shows how each chapter addresses the thesis research questions.

HVP: High value products

RQ: Research question

AD: Anaerobic digestion

LCA: Life Cycle Assessment

TEA: Techno-Economic Analysis

Paper	Chapter	RQ1: Environmental impacts of HVP	RQ2: HVP & Biofuel commercialization	RQ3: Comparing algae multi-product models	RQ4: Improvements and tradeoffs
ADLCA	2				o
Cellana LCA	3	o			o
Cellana TEA	4		o		o
Cellana LCA+TEA	5	o	o	o	o

Intellectual merit:

The comparative LCA and TEA of omega-3 fatty acid production will be one of the first large-scale LCAs for high value products based on data from a large-scale biorefinery; the analyses were conducted on Cellana, LLC operations. The research on Cellana operations was the product of the author; Cellana only provided data and information related to production. The work in this thesis also assessed improvements to production to support both economic and environmental sustainability. The LCA compares the environmental impacts to conventional production. To date, no one has published a pilot to commercial-scale analysis of an algae multi-product model coupling LCA and TEA, nor that has included and biofuels and high value products. LCA and TEA are rarely coupled together to determine if there are tradeoffs between economic profitability and environmental sustainability for biofuels and high-value bioproducts.

The combined LCA and TEA study will be one of the first studies examining the implications on a National scale of commercial algal biorefineries for both biofuels and high value products.

Broader impacts:

The results of this thesis will be useful to a number of different stakeholders. The pilot data from Cellana LLC allows them to contribute to the academic literature and advance their commercial goals, while addressing well-known sustainability challenges (e.g. climate change) and raising awareness of other important sustainability initiatives within the algae community (e.g. at risk fish stocks). Using pilot-data from a company demonstrates how industry can evaluate their processes and products via LCA and TEA. The multi-product model shows that there is potential for a biorefinery to be profitable for the production of high-value products and still produce renewable fuel. The improvements presented herein document a good starting point for the algae industry to move forward with commercialization high value multiproduct biorefineries in a sustainable manner.

This work was done in association with the Algae Testbed Public Private Partnership (ATP3) led by ASU's Arizona Center for Algae Technology and Innovation (AzCATI). ATP3 partners with other academic institutions and industrial partners to advance the knowledge of the algae community in producing sustainable algae-based products. The multi-product model will serve as an example that ATP3 can use to support other companies interested in transitioning into high-value products and using TEA and LCA services for assessing their products and processes as well as identifying areas of improvement. The tradeoffs between economic and environmental sustainability show

how industry stakeholders can work to find some balance between producing fuel for environmental sustainability and producing high-value products for economic sustainability.

In addition to the broader impacts of my research, outreach, service and mentorship were major parts of my graduate career. I mentored four undergraduate students during my time as a PhD student resulting in the following products (my undergraduate mentees are underlined):

- 1) Weschler, Matthew K., **Barr, William J.**, Harper, Willie F., Landis, Amy E. (2014). "Process energy comparison for the production and harvesting of algal biomass as a biofuel feedstock." *Bioresource Technology* **153**: p. 108-115
- 2) **Barr, William J.**, Rodrigues, Priscila S., Weschler, Matthew K., Harper, Willie F., Landis, Amy E. (2014) "Evaluation of the Environmental Impacts of Algae Harvesting and Production." Peer reviewed Conference Proceedings for the Life Cycle Assessment XIV conference. San Francisco CA.
- 3) Weschler, Matthew K., **Barr, William J.**, Harper, Willie F., Landis, Amy E. (2013). "Comparative assessment of energy requirements for microalgal biomass production." International Symposium of Sustainable Systems and Technology 2013. May 15-May 17 2013.

As shown in the article titles, the undergraduates contributed to algae research related to this thesis. In addition to advising undergraduate researchers, I participated on multiple panels for undergraduate students interested in graduate school including Gates Millennium Scholars and 1GPS, which is a program for first generation college students. I also mentored students in the National Society of Black Engineers

(NSBE), and I served as the southwest regional pre-college initiative (P.C.I.) chair in developing events and on how to effectively communicate with parents, students and administrators not directly affiliated with ASU. Finally, I volunteered for a number of outreach events for kids, such as ASU's Night of the Open Door from 2012-2014, where we introduced children and their families to engineering and energy research.

Literature Review:

Algae biofuels, policies and drivers

Global dependence on non-renewable liquid fuel, concerns over energy security, fossil fuel depletion and greenhouse gas emissions have led to the implementation of policies such as EISA 2007 and the associated Renewable Fuel Standards (RFS). The renewable fuel standards have called for one to two billion gallons per year of biomass-based diesel between 2010 and 2017 (EPA, 2015). In addition to volume standards, the RFS sets forth standards related to the types of renewable fuels. Advanced biofuels, of which biomass-based diesel is apart of, a minimum 50% life cycle GHG reductions are required. First generation biofuels were seen as a solution to climate change and U.S. energy security. However, the production of first generation biofuels led to a debate over the competition between the use of land and crops for food or for fuel. Algal biofuels meet the RFS advanced biofuels goals and had significant advantages over earlier generations of biofuels because algal biofuels use less land, does not have to compete with food, can use waste CO₂, waste nutrients, marginal land and sunlight for growth. Algae can also be used to produce a variety of biofuels.

Historical context

While algae have gained a great deal of attention in the last 20 years as a biofuel feedstock, the use of algae for useful products is not new. Large-scale cultivation of algae for food and for fuels dates back to the 1940s (Kim, 2015; Richmond & Hu, 2013). In the United States and Germany, large scale cultivation of *Chlorella sp.* began soon after World War II as a promising source of protein (Burlew, 1953). Lipid accumulation of algae under nitrogen-starved conditions was observed in 1952 (Richardson et al., 1969). Anaerobic digestion of algae for the production of methane and subsequent production of either a hydrocarbon fuel or electricity dates back to the late 1950s (Golueke & Oswald, 1959). Society has known about the benefits of algae for food, fuel and specific nutritional applications for over half a century.

The U.S. renewed its focus on algae for biofuels in response to the oil embargo of the 1970s, resulting in the development of the aquatic species program (ASP) (Sheehan et al., 1998). The program lasted from 1978 to 1996 and was funded by the US department of energy (DOE) for the production of algal biofuels from high lipid content algae. The program focused on algal biology, production systems, and resource availability. The algal biology work of the ASP resulted in the collection and assessment over 3,000 species of algae before narrowing the pool down to 300 species deemed feasible for biofuel production. The production systems research concluded that systems other than open ponds had limited chances of success due to the low cost of fuel production. The resource availability research concluded that there was sufficient land, CO₂, and water in

the U.S. to support algal biofuel production on a national-scale. The program was closed down in 1996 due to the decrease in fuel prices.

Research into algae for biofuels has again increased in the midst of increasing oil prices and renewed concerns over energy security (EISA, 2007). In 2008 the U.S. department of energy (DOE) held a workshop to provide guidance to a variety of stakeholders in the algae community on what they predicted would need to be done to commercialize algal biofuels. The report for that workshop became the DOE National Algal Biofuels Roadmap (DOE, 2010). The topics of the roadmap included algae cultivation, extraction and oil fractionation, infrastructure as well as public private partnerships and navigating regulations and policy. Prior to the workshop the DOE had recently renewed large investments in algal biofuels. The roadmap involves biofuel production from microalgae, macroalgae and cyanobacteria. In the years following the release of the DOE roadmap significant progress toward algal biofuel commercialization has been made with attention being given to environmental and economic sustainability of algal biofuels. Three DOE national laboratories, National Renewable Energy NREL, ANL, and PNNL came together and worked on unifying their algal biofuel models to ensure that the same or similar process models for resource assessment, life cycle assessment and techno-economic analysis are used across the laboratories to provide a benchmark for the research community.

Algal biofuel companies

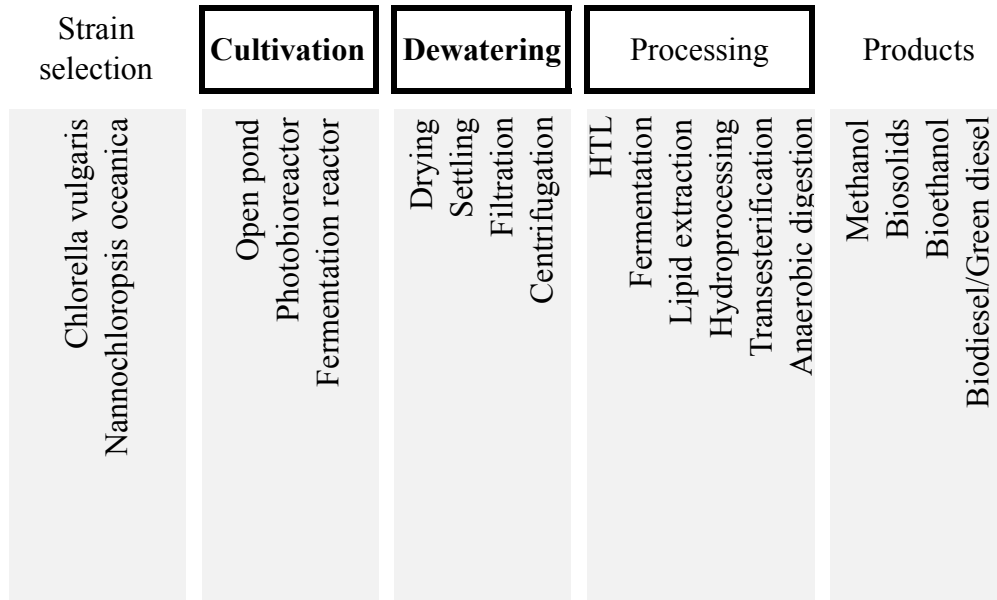
A number of companies have worked to make algal biofuels commercially viable. Some of the most prominent companies in algal biofuels to date include Algenol, Sapphire and Solazyme. Algenol was founded in 2006, and has a patented process for

producing bioethanol from a proprietary strain. In 2015 Algenol launched a demonstration facility in India co-located near a crude oil refinery owned by one of its partners, Reliance Industries. The U.S. Environmental Protection Agency (EPA) also approved Algenol's fuels as advanced biofuels (category D-5) of the renewable fuel standards. Sapphire Energy was founded in 2007 backed by venture capitalists for the production of a variety of products. In 2012, Sapphire Energy completed construction of a 100-acre algae farm projected to produce 1,600 metric tons of algal biomass for conversion to biofuels by 2017. In 2013, Teroso Inc. a Fortune 100 independent refiner purchased an "undisclosed amount of oil" produced at Sapphire's New Mexico based Algae Farm (Herndon, 2013). Solazyme was founded in 2003 with the general purpose of converting algae into useful products for society. Unlike the aforementioned companies, Solazyme uses heterotrophic algae in fermentation reactors to produce biofuels. In 2010, Solazyme provided the U.S. Navy with 100% algae-derived jet fuel (Solazyme, 2010). Algae biofuel companies have managed to develop and sell their fuels commercially. Despite these successes, the national-scale commercialization of algal biofuels remains elusive.

Production of algal biomass for fuels and bioproducts

Algae are unicellular (microalgae) or multicellular (macroalgae) autotrophic, photosynthetic eukaryotes. Cyanobacteria, also known as "Blue-green algae", are actually prokaryotes but can be used for the production of bioproducts as well. One of the major advantages of algae for bioproducts is that they can be grown using primarily CO₂, light and nutrients.

Photoautotrophic algal biomass for commercial production is produced in either PBRs or open ponds. Despite being photoautotrophs, algae are also grown under heterotrophic or mixotrophic (combination autotrophic and heterotrophic) conditions for the generation of bioproducts as well. In order to extract valuable products, once algae reach maturity they must be removed from their cultivation vessel and dewatered. Figure 2 shows the general steps of algal bioproduct generation and examples of the different processes, strains and products. A detailed description of each step in generating algal bioproducts from photoautotrophic algae follows.



Key:

Examples

Figure 2: Algae General Process Flow
 General process flow of algae for the production of biofuels and bioproducts
 Black boxes are the general processes. Examples of each step are in grey boxes
 HTL: Hydrothermal liquefaction

Cultivation of photoautotrophic algae can be done in open ponds and photobioreactors (PBR). Fermentation reactors are used for heterotrophic algae. Key issues related to cultivation systems include water sources, contamination and cost of production. While the work resulting from the ASP concluded that resource availability such as water and nutrients was not a risk for national-scale production, water consumption could be a serious issue depending on the region and the constraints already placed on freshwater (Lee, 2001; Pate et al., 2011; Rogers et al., 2014; Slade & Bauen, 2013a). Some algal species are capable of growing on non-freshwater such as seawater, wastewater and brackish water. Wastewater has the added benefit of potentially providing nutrients for algae cultivation (Chinnasamy et al., 2010; Wiley et al., 2011). However, using non-freshwater sources for algae cultivation may require some level of pretreatment for use to limit the risk of contamination (Jeong et al., 2015; Slade & Bauen, 2013b). Photobioreactors are closed systems for the growth of autotrophic micro algae and offer some protection from contamination. Photobioreactors can concentrate algae to a higher degree than in open ponds and this could affect the dewatering processes (Chisti, 2007; Rodolfi et al., 2009). However, photobioreactors are more expensive and energy intensive than open ponds (Sánchez Mirón et al., 1999), . Work has been done in recent years to reduce the cost and operational energy of open ponds (Quinn et al., 2012; Rodolfi et al., 2009). The lower cost of open ponds have made them more amenable to much larger-scale production as seen at locations such as Sapphire Energy's Algae Farm.

Harvesting is one of the biggest bottlenecks to commercial production and reaching environmental sustainability due to the high-energy intensity requirement (Barros et al., 2015; Gerardo et al., 2014; Lardon et al., 2009; Uduman et al., 2010). There are a wide

variety of harvesting technologies available. Thesis author William Barr worked with an undergraduate researcher, Matthew Weschler, to perform a comprehensive process energy analysis of algae harvesting technologies (Matthew K. Weschler et al., 2014). The process energy analysis considered different levels of harvesting so that the results would be applicable to other studies regardless of application. The concentration levels were 3 to 10% w/w (low), 10 to 30% w/w (high) and 90+% w/w (dry). Biofuel studies in the early part of the 21st century required 90% dry biomass for lipid extraction but advances in lipid extraction using wet biomass (~20%) eliminated the need for drying. Figure 3 depicts the results from the “high biomass concentration” which would be useable for wet lipid extraction for diesel production. The results from this study show that the open pond cultivation energy is significantly lower than the PBR results and that the energy consumption of the raceway pond is comparable to the energy consumption of centrifugation (4th bar from the left, labeled ‘RP, MS, DC).

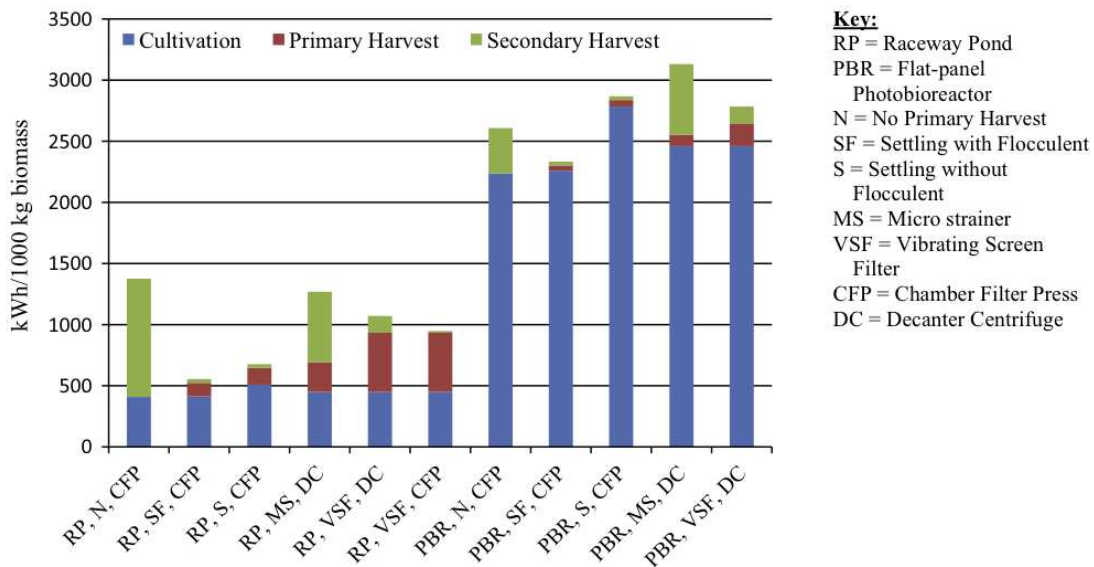


Figure 3: Algae Harvesting Process Energy Consumption
 Process energy consumption of different cultivation and harvesting scenarios. Taken from (Matthew K. Weschler et al., 2014)

Settling was one of the lowest energy harvesting methods, but lower efficiency than other unit processes and time consuming. Adding flocculant to enhance settling does not greatly change the energy consumption but improves efficiency and decreases the required settling time. However, the addition of chemical flocculants may degrade the quality of algal biofuels and bioproducts, and preclude the use of algae biomass or residuals in certain industries. Centrifugation is a mature technology used in wastewater treatment, and is very reliable, does not require chemical additives but is very energy intensive. The optimal dewatering depends on the desired products, resilience to chemical additives and commercial reliability.

There are a number of methods to convert algal biomass to energy. Diesel fuel can be produced from algae through multiple methods; these include lipid extraction followed by transesterification to produce biodiesel, lipid extraction followed by hydroprocessing to produce green diesel and hydrothermal liquefaction followed by hydroprocessing to produce HTL renewable diesel (Bidy et al., 2013; Huo et al., 2008; Soratana et al., 2014). Green diesel has the advantage over biodiesel of being more closely related to petroleum-based diesel than biodiesel (Kalnes et al., 2007). Renewable diesel is a general term for any oil product derived from a biomass that goes through a thermal depolymerization process. Green diesel is a sub-category of renewable diesel that uses the lipid fraction of a biomass, where as the HTL renewable diesel utilizes the entire algal biomass to produce HTL oil prior to hydroprocessing (Bain, 2006; Kalnes et al., 2007). Biodiesel is the diesel product resulting from the transesterification of the lipid fraction for the production of fatty acid methyl esters (FAME) (Bain, 2006). Besides diesel fuel, bioethanol can be produced through the saccharification and fermentation of the

carbohydrate portion of algae biomass (Park et al., 2012; Soratana et al., 2014). In addition to liquid fuels algae and the resulting algal biofuel residuals (ABR) can be used to produce biomethane from anaerobic digestion (Ras et al., 2011; Sialve et al., 2009).

There are a number of products that can be derived from different parts of the algal biomass. Figure 4 lists a few of the products that can be derived from each part of the biomass. In some cases, multiple products can be generated in tandem. Biodiesel and renewable diesel were described in previous sections. Polyunsaturated fatty acids (PUFA) are a general class of compounds within the lipids that have different health benefits. (Janssen & Kiliaan, 2014; Kaye et al., 2015). Certain species of algae are known to accumulate high amounts of proteins which are beneficial for animal feed and aquaculture feed (Spolaore et al., 2006). Algae can also accumulate target proteins beneficial to the growth of fish in aquaculture production (Maisashvili et al., 2015). Bioethanol can be produced from algae via saccharification and fermentation of the algal biomass (Demirbas, 2011; Liu et al., 2013). Algae can be used as a medium for bacteria to produce bioplastics through acid hydrolysis of the carbohydrates. (Miller et al., 2013). Both the protein and the carbohydrates can be used for animal feed (Mirsiaghi & Reardon, 2015; Spolaore et al., 2006; Wuang et al., 2016).

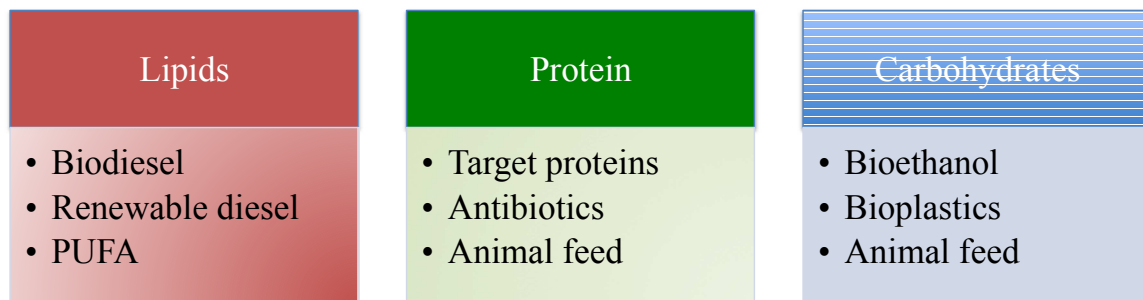


Figure 4: Potential Algal Bioproducts
 Products derived from various portions of the algal biomass
 PUFA: Polyunsaturated fatty acids

One key PUFA is omega-3 fatty acids. There is a high global demand for omega-3 fatty acids and demand continues to rise even as many of the world's water bodies are or at risk of being overexploited (Norse et al., 2012; Pérez-López et al., 2014; Rick & Erlandson, 2009). Alternative sources of omega-3 fatty acids need to be developed to assuage the burden on the limited and at risk wild fish population that currently represents the most common source of omega-3 fatty acids. Aquaculture production is increasing worldwide but as of 2012 remains a small percentage (15%) of US fish production. As a result a significant amount of research has gone into finding alternative sources of omega-3 fatty acids (Chen et al., 2013; Frankel et al., 1997; Ward & Singh, 2005). Algae biomass is a potentially sustainable alternative to fish for the consumption of omega-3 fatty acids that could support the necessary economic sustainability of algal biofuels while simultaneously addressing a second environmental issue.

Biodiesel research was done extensively during the lifespan of the DOE Aquatic Species program (Sheehan et al., 1998). Research into fuels faded in the 90s due to low fuel prices but was reinvigorated in the early 2000s due to rising fuel prices and concerns over energy security. Despite increased fuel prices, dewatering remained a major roadblock to biodiesel commercialization. Potential solutions that were explored included wet lipid extraction and supercritical extraction to allow for fuel conversion without the need to dry the algal biomass (Levine et al., 2010; Sathish & Sims, 2012). Biodiesel was also not as compatible with petroleum refineries like other renewable diesel products (Bain, 2006).

Renewable diesel is any diesel product derived from biomass that underwent thermal depolymerization. Green diesel is a renewable diesel product derived from the lipid portion of the biomass. This process is used to remove impurities from petroleum diesel and green diesel can be readily mixed with petroleum diesel (Huo et al., 2008; Kalnes et al., 2007). Green diesel was derived for use first with vegetable oils such as soybean, but has subsequently been applied to a number of algal species (Tran et al., 2010).

Hydrothermal liquefaction is a process that can be used to produce renewable diesel from the whole algal biomass without the lipids being extracted and without the high dewatering requirements of green diesel and biodiesel (Anastasakis & Ross, 2011; Biller & Ross, 2011; Valdez et al., 2012).

The production of biofuel does not consume the entire biomass and so there is potential for additional value to be derived from algal biofuel residuals (ABR). The production of biodiesel and green diesel result in lipid-extracted algae; an ABR consisting primarily of proteins and carbohydrates with a small amount of lipids left after lipid extraction. Co-products provided a promising method to capture the most value from the algal biomass while still achieving the goals of biofuels policy. Algal biomass can have a lipid content of up to 50% with many researchers using 30% as a baseline depending on the strain and cultivation condition (Lardon et al., 2009; Murphy & Allen, 2011; Razon & Tan, 2011; Richardson & Johnson, 2014, 2015; Richardson et al., 2014a; Xu et al., 2011). With up to 50% of the biomass being used, at least 50% remains unused. At commercial-scales, that percentage of unused biomass represents a significant waste stream that must be dealt with or a valuable stream of co-products that can be recovered in conjunction with algal biofuels. Common coproducts include biomethane from

anaerobic digestion (Collet et al., 2011; Harun et al., 2011; Lardon et al., 2009; Prajapati et al., 2014; Quinn et al., 2014; Wiley et al., 2011), biofertilizer (Wuang et al., 2016), bioethanol (John et al., 2011; Lam & Lee, 2015; Mehrabadi et al., 2015), and animal or aquaculture feed (Bichi et al., 2013; Spolaore et al., 2006). Making use of the LEA from algal biofuel production can improve the environmental performance of algal biofuels by generating additional energy or providing renewable feed products for agriculture and aquaculture applications.

There are a number of other factors that influence algal bioproducts development. One of the major decisions is the strain selection. The strain selection influences what products can be produced based on the biomass composition, what conditions the algae can be grown at (e.g. nutrient requirements, salinity and heat tolerance) and the potential for co-products. While the versatility of algae provides a variety of options for algal biofuel production, there was no unifying method for early algal biofuels research to follow. Due to this, results related to algal biofuels varied drastically based on a number of different factors. These factors include everything from CO₂ supply to water supply to co-product handling. Nutrients, CO₂ and light provisions can greatly influence the productivity and environmental impacts. Using waste streams as sources can offset the costs of wastes where the products would normally just go to the environment versus being used for something beneficial, but using synthetic sources of CO₂ and nutrients have impacts associated with both the use and the production. The decision to produce one product or multiple products is also an important decision; producing additional products can lead to added benefits but may also incur additional costs depending on additional unit process requirements.

Anaerobic digestion of Algae and ABR

One application that has been considered in many theoretical analyses of algal biofuels is anaerobic digestion. There have been a number of LCAs related to anaerobic digestion of different forms of algal biofuel residuals, and in some cases whole algal biomass (Table 2). Anaerobic digestion was a promising option for handling LEA and other ABR because it is a mature technology commonly used to treat the waste activated sludge resulting from secondary wastewater treatment. Anaerobic digestion of algae has been studied since the 1950's (Golueke et al., 1957) and has gained attention for co-product generation due to the emergence of national biofuel policies.

There are two main products of interest: methane and nutrient rich biosolids. The methane can be converted to electricity and heat that can be used to offset the electricity and heat consumption associated with algae cultivation and dewatering. A variety of substrates can be used for anaerobic digestion making it a versatile option for ABR but also for other biofuel feedstocks that result in unused biomass that needs handled. The nutrients in the digestate can be recycled to the cultivation phase to offset the consumption of synthetic nutrients while the biosolids can be used as a soil amendment.

In order to better understand the effectiveness of anaerobic digestion for improving algal biofuel residual performance, it is necessary to look at the key variables that impact methane production and nutrient recovery potential. The critical variables in determining the effectiveness of anaerobic digestion using algae and ABR as a substrate include methane yield, algal strain, organic loading rate and the effect of prior biofuel conversion processes on the substrate composition. The most common species analyzed is *Chlorella vulgaris*. A number of studies refer to the *C. vulgaris* composition presented in Lardon et

al. (2009). Not all studies selected specific species, instead focusing on the specific characteristics or a specific composition without identifying a species. Table 2 shows the species that each study selected. In biodiesel production high lipid accumulating species are selected leaving less biomass for digestion after biodiesel production. High lipid content is also ideal for anaerobic digestion because of the digestibility of lipids compared to the proteins and the carbohydrates. The strength and structure of the cell wall is also an important factor in anaerobic digestion. The cell wall can be difficult to break via hydrolysis, leading to long required digestion times (hydraulic retention time of 46 days (Collet et al., 2011)) and low overall digestibility of the algae (~50-60% as opposed to >70% for waste activated sludge (Clarens et al., 2011; Collet et al., 2011; Sialve et al., 2009)). There are technology options for reducing the digestion time and improving digestibility and subsequent methane production, but the impacts of implementing additional unit processes must be weighed against the added benefit (Fdez.-Güelfo et al., 2011; González-Fernández et al., 2012; Lee & Rittmann, 2011; Padoley et al., 2012).

Table 2: LCA/TEA Anaerobic Digestion Studies

Studies of anaerobic digestion of algal biofuel residuals (ABR) in the academic literature.

The columns identify what type of biomass was digested in each study.

LEA: lipid-extracted algae SSF: saccharification and fermentation residuals

Study	Algae	LEA	SSF	Species
Clarens 2011	o	o		Salt tolerant (<i>Phaeodactylum</i> , <i>Tetraselmis</i>)
Collet 2011	o			<i>C. Vulgaris</i>
Frank 2012	o	o		<i>C. Vulgaris</i> (from Lardon 2009)
Harun 2011	o	o	o	Multiple Species
Zamalloa 2012	o			<i>Scenedesmus obliquus</i> and <i>Phaeodactylum tricornutum</i>
Morken 2013		o		<i>C. Vulgaris</i> (from Lardon 2009)
Delrue 2012		o		Not specified based on Grobellar 2004
Ras 2011	o			<i>C. Vulgaris</i> (from Lardon 2009)
Mairet 2011	o			<i>C. Vulgaris</i>
Sialve 2009	o	o		<i>Chlorella sp.</i> (a good number)
Park, J. H. 2012			o	<i>Gelidium amansii</i>
Costa 2012	o			macroalgae w/ WAS
Ehimen 2011		o		<i>Chlorella sp.</i>
Migliore 2012	o			<i>G. longissima</i> and <i>C. linum</i>
S Park 2012		o		<i>N. Salina</i>
Vergara 2008	o			<i>Macrocystis pyrifera</i> , <i>Durvillea antarctica</i>

In addition to the key parameters of anaerobic digestion there are other risks to consider. The inputs required for co-product generation vs. the benefit provided by the co-products needs consideration to ensure that the generation of co-products does not lead to unintended consequences. Minimizing the number of required energy intensive unit processes for co-product generation will make this balance easier to achieve.

Methane utilization at commercial-scale is another important consideration. Existing industrial anaerobic digesters may not convert all of their methane to useful energy. Flaring is a common practice at wastewater treatment plants where the methane that is produced is burned instead of being converted to energy, thus the benefit of methane, as an energy product is lost. Methane may be converted to useful energy to a small degree by selling the energy from methane to the electricity grid at peak hours only, when digesters can attain the highest price for their electricity. Does ABR provide sufficient loading rates to justify commercial-scale digestion onsite and guarantee that the benefits are realized or will the fate of ABR depend on offsite digestion and potentially be lost to flaring?

Life cycle assessment

Life cycle assessment (LCA) is used to quantify the environmental impacts of different products and/or processes. LCA can be used to compare different products and processes in terms of a number of different impact categories related to climate change, water, air and soil quality as well as human health. EPA and NREL use LCA to assess and move policy forward related to specific environmental impacts. Companies use LCA to improve their environmental footprint, make environmental declarations related to their products and compare the impacts of their products to comparable alternatives. Life cycle assessment methodology is described by the international organization for standardization 14040 series (ISO, 2006). There are four major steps to LCA:

- 1) goal and scope definition defines what the purpose of the LCA will be and defines the boundaries between the system of interest and the ecosystem.

- 2) The life cycle inventory (LCI) is the data collection portion for inputs and outputs to and from the ecosystem and within the system interest
- 3) The life cycle impact assessment (LCIA) quantifies the actual affect that the LCI items have on the environment. Many of the environmental impacts of LCA are described in Bare (2002).
- 4) The final step of LCA is interpretation. This step involves scenario analysis, improvements, describing the relevance of the results and assessing uncertainty and variability.

LCA of Algal biofuels

The main goal of algal biofuel LCAs in general is to quantify the environmental impacts of algal biofuels compared to petroleum fuels and other biofuels. Usually, LCA studies either compare algal biofuels to other fuels or they use the LCA to identify 'hotspots' or areas in the algal biofuel production where environmental improvements could be achieved.

Life cycle assessment has been used extensively to assess the environmental impacts of algal biofuel production. The primary environmental impact category is GWP due to the promise of biofuels as an environmental alternative to CO₂ emissions from petroleum derive fuels. The range of GWP between studies depends on the numerous factors mentioned above. The GWP of petroleum ultra low sulfur diesel is 94.7 gCO₂e/MJ according to the California EPA results using the Argonne GREET model (Prabhu et al., 2009). GWPs ranging from 29 gCO₂e/MJ of fuel to 1880 gCO₂e/MJ (Clarens et al., 2011; Soratana et al., 2011; Woertz et al., 2014) were reported in algal biofuel studies. There were a number of reasons for the dramatic differences in GWP. Individual studies

selected different system boundaries; Jorquera et al. (2010) for example did not use dewatering as part of their LCA. Lardon et al. (2009) calculated the GWP of algal biofuel production from cultivation through dewatering and included co-product generation. Different cultivation systems have very different impacts and final concentrations (Chisti, 2007). Dewatering was also a key difference that was highly dependent on the selected unit process (centrifugation vs. settling vs. DAF). Different strains of algae require have varying dewatering requirements due to the size of the molecules and their tendency to aggregate. The harmonization studies and Lardon (Davis et al., 2014a; Lardon et al., 2009) used *C. vulgaris* in ponds concentrated to 0.5 g/L, while *Desmodesmus sp.* was identified as naturally settling to 10 g/L in pilot-scale studies (Beal et al., 2015).

In order to maintain fuel at an economic price, a minimum energy return on investment (EROI: calculated as the ratio of useable energy to society: energy consumed to get that energy) was calculated as 3 by Hall et al. (2009). EROIs ranging from 0.1 to 3.33 (Brentner et al., 2011; Clarens et al., 2010; Stephenson et al., 2010; Matthew K. Weschler et al., 2014) have been reported in the academic literature. Depending on the study, the EROI may include only the production of liquid fuel in some cases and in other cases includes an energy value of co-products including electricity and heat from anaerobic digestion but also an energy associated with animal feed products.

There have been multiple efforts to improve the environmental performance of algal biofuels. Reducing the consumption of virgin CO₂ represented a method capable of helping algal biofuel production and helping the conventional power industry by taking their waste CO₂ and recycling it. Depending on the dewatering unit processes used, CO₂

emissions due to the production of algae can already be high, but adding synthetic CO₂ as a resource that must be consumed despite being considered a readily available resource. As a result many studies have considered using CO₂ from flue gas or other source to recycle CO₂ from flue gas but also to have a low cost source of CO₂ (Chen et al., 2012; Rickman et al., 2013; Stephenson et al., 2010). Recycling flue gas is one method of recovering additional value from algal biofuels.

Another method to improve the environmental impacts of algal biofuels is to reduce the consumption of synthetic nutrients through the use of wastewater. Wastewater effluent may contain some nutrients left over from the wastewater treatment process and these nutrients can be used by algae to capture those nutrients before they reach the environment and potentially offset some of the nutrient requirements associated with algae (Fortier et al., 2014; Mu et al., 2014; Rickman et al., 2013; Rothermel et al., 2013).

Global warming potential and energy metrics (EROI or NER) are common to most algal biofuel LCA studies, but additional impacts need to be assessed as well, especially at commercial-scale. Nutrient related impacts are an important consideration due to the effect that algae and nutrients can have on bodies of water. Some LCA studies have assessed eutrophication potential (Brentner et al., 2011; Mu et al., 2014; Soratana et al., 2014). The cultivation and dewatering phases result in effluent that goes to the environment at some point and there may be traces of nutrients and algae left in these streams depending on cultivation conditions and harvesting efficiency. However, when wastewater was used as a nutrient source algal biofuels resulted in offsets to eutrophication due to the nutrients from wastewater effluent that were being used by

algae instead of being released to the environment (Mu et al., 2014; Soratana et al., 2014).

Water consumption at commercial scales may be very high and so some studies have considered water consumption and compared that to other fuels (Clarens et al., 2011; Harto et al., 2010). However, studies have shown mixed results with algae consuming more water than corn, canola and switchgrass derived fuels (Clarens et al., 2010) and in another case algae open systems consuming less water than corn ethanol, switchgrass with irrigation, and more water than soybean biodiesel while closed systems consumed less water than all of them but more water than switchgrass without irrigation (Harto et al., 2010). The assessment of water will depend on the system used and the associated evaporative losses associated with open ponds compared to closed PBR systems.

LCAs of pilot or commercial algae biorefineries

Many theoretical life cycle studies have been published for algal biofuel production; however, few are based on existing systems beyond lab scale data. The few studies that are based on real, non-lab scale systems tend to use inaccessible and private data protected by proprietary restrictions, where the authors are limited in the data they can present to the rest of the scientific community. LCAs of 3 pilot-scale facilities operated by Algenol, Sapphire and Cellana have been published in the academic literature. Incorporating data from real systems greatly improves model accuracy by reducing the need for certain assumptions, helping to identify key data points not commonly considered and parameters that are beyond the control of operators.

Luo et al. (2010), using publically available data for key points and private data from Algenol Biofuels, described a particular case of ethanol production from non-harvested cyanobacteria. They assessed the GHG emissions as a function of ethanol concentration in their non-harvested biomass. The results of their study revealed that reaching the GHG emissions of the RFS (36.5 gCO₂/MJ) was possible at most scenarios but that reaching the more ambitious DOE targets of 18 gCO₂/MJ required higher ethanol concentrations before distillation

Liu et al. (2013) also used private data from a pilot-scale facility from Sapphire Energy to compare hydrothermal liquefaction renewable diesel at lab-scale, pilot-scale data from Sapphire energy and full-scale industry forecasts. This study compared the greenhouse gas emissions and energy return on investment (EROI) of hydrothermal liquefaction diesel to petroleum diesel, gasoline, soybean biodiesel and corn ethanol. The algal HTL diesel had lower GHG emissions and EROI than both petroleum fuels and higher GHG emissions and EROI than soybean diesel and corn ethanol. To results of note were that pilot-scale EROI was lower than cellulosic and corn ethanol and had an EROI of less than 1. Besides cellulosic ethanol, corn ethanol and HTL diesel all other fuels had an EROI greater than 1.

Two companion studies were published based on Cellana's Kona Demonstration facility, which has a cultivation capacity of greater than 750 m³, for the production of biofuel and animal feed (Beal et al., 2015; Huntley et al., 2015). Huntley et al. (2015) demonstrated sustained production of metric tons of biomass using the KDF and used the data to model the scale-up of a 100-ha commercial facility for the production of biofuels. While other studies have identified the economic and scalability limitations of PBRs

(Sánchez Mirón et al., 1999), Huntley et al. (2015) reported that large diameter, large-volume PBRs were an economic method of maintaining inoculum for open ponds. Beal et al. (2015) is a combination LCA and technoeconomic analysis that assessed the potential for producing lipids for conversion to renewable or biodiesel under ten different cultivation and extraction scenarios. The first case was a default-case based on data from existing pilot-scale systems. Design changes are made to the first scenario to improve the environmental impacts and economic potential. The final scenario resulted in favorable environmental impacts and a fuel price of \$2/L that would be economically feasible to diesel prices in 2015.

While the work in the aforementioned paragraph was based off of Cellana LLC's KDF, similar to three of the chapters in this thesis, there are some key differences. The companion studies (Beal et al., 2015; Huntley et al., 2015) focus on biofuel as the main product, while the studies in this thesis focus on omega-3 fatty acid production coupled with biofuel production. This distinction changes the unit processes required due to the need to extract the oil from nearly dried (90% w/w) biomass in this thesis. The companion studies also use two diatoms for their study, while this study uses *Nannochloropsis oceanica*. In the companion studies they observed an in pond settling of up to 10 g/L for the diatoms, but that has not been observed for *N. oceanica* at Cellana, which leaves the ponds at 0.5 g/L. This greatly influences the required dewatering of the biomass.

Techno-economic analysis

Techno-economic analysis (TEA) is a method to quantify the entire cost of production for a product or process. TEA is often carried out to assess the economic

potential of a product or process by comparing it to market prices at specific moments in time, and to determine where improvements can be made to the financial feasibility of said products and processes. Companies use TEA to determine what the probability of success is for them for a given product. Government agencies use TEA to determine if the generation of products of interest to the government (such as biofuels) has the potential to be economically sustainable without government subsidies. Both entities also use TEA to find “hotspots” and potential areas for improvement. For this thesis, we propose four steps to TEA based on the various methods that exist in the public domain, including the DOE harmonization studies. Similar to LCA, TEA can be divided into four steps:

- 1) **Process concept:** Identifies the product or service and the necessary process steps to provide the product or service to customers. In the case of chemical production such as biofuels this includes the development of a process model. The process concept is also used to identify constraints based on the scale of production and optimization goals.
- 2) **Mass and Energy Balance:** The process model identifies mass and energy balances for all materials in a process concept based on data from literature and industry experts. Process flow estimates are created for intermediate steps and final production.
- 3) **Cost engineering:** Based on the process model and vendor quotes, utility prices and manufacturing costs the capital expenses (CAPEX) and operating expenses (OPEX) are determined

- 4) Financial analysis: Financial analysis is similar to the interpretation phase of LCA, where different scenarios can be explored and the conclusions are defined. Financial analysis also uses sensitivity analyses and Monte Carl analysis to assess the variability and uncertainty of the outputs (CAPEX, OPEX, total capital investment etc.).

TEA has been used for algal biofuel production by a number of different research groups to determine how improvements can reduce the cost of biofuel production and to determine when biofuel production might become economically feasible.

Harmonization studies

The U.S. department of energy (DOE) has noted the lack of consistency and the inability to compare across algal biofuel studies even among models developed by DOE national laboratories in terms of LCA, TEA and resource assessment (RA). As a result NREL, ANL and PNNL, came together to develop harmonized models for LCA, TEA and RA to identify the most promising locations for an algal biorefinery (Davis et al., 2012). The goal of the harmonization studies was to define a baseline algal biofuel production scenario with economic and environmental sustainability metrics. The baseline harmonization model quantifies the impacts of producing renewable diesel via lipid extraction. Additional studies by the DOE have modified the baseline by adding in hydrothermal liquefaction (HTL) of the entire biomass to improve the environmental performance as shown in Figure 5.

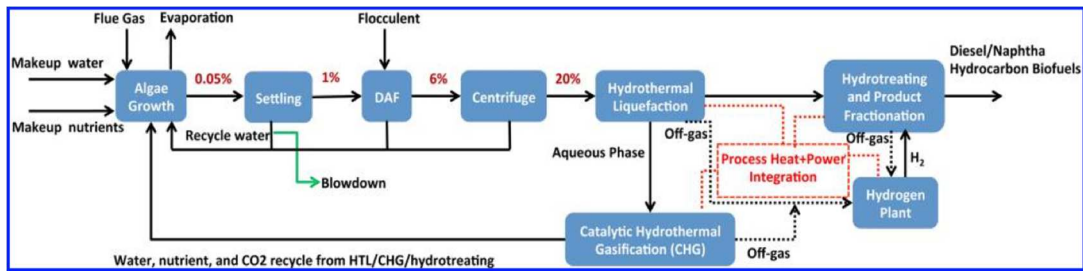


Figure 5: DOE Baseline Algal Biofuel Production
 *(Davis et al., 2014a)

The advantage of HTL over lipid extraction is that HTL can be done at 20% w/w (Biddu et al., 2013) alleviating the need for much of the required dewatering associated with lipid extraction, which is usually done around 90% w/w. The harmonization studies by the DOE represent a focal point for other algal biofuel and bioproduct studies to build around and compare to, that the algae community has previously lacked. Future work linking the harmonization studies to commercial-scale data could greatly improve the quality of computational sustainability models produced in the academic literature and further assist in reaching commercialization goals.

TEAs of algal biofuels

A number of TEA studies have been published related to algal biofuels. The aforementioned harmonization studies have resulted in a number of TEA analyses based on the baseline and updated scenarios (Davis et al., 2011; Davis et al., 2014a; Davis et al., 2014b). These studies focus on the production of renewable diesel from algae and base their cultivation data on existing systems for the commercial production of *Chlorella sp.*, *Spirulina sp.*, *Dunaliella sp.* and *Haemotococcus sp.* (Lundquist et al., 2010). The earlier TEA study, which was part of the harmonized models, uses lipid extraction followed by hydrotreatment for the production of green diesel based on the production of green diesel presented in Kalnes et al. (2007). The later studies related to the TEA section of the

harmonized models used similar assumptions but replaced drying and lipid extraction with hydrothermal liquefaction of the entire biomass to produce renewable diesel. The renewable diesel remained unfeasible at current economic prices and operational conditions but the fuel was near the RFS target GWP.

A number of studies associated with the National Association of Advanced Biofuels and Bioproducts (NAABB) have been done by researchers at Texas A&M (Bryant et al., 2012; Richardson & Johnson, 2014, 2015; Richardson et al., 2012; Richardson et al., 2014b). These studies used the baseline data from the DOE harmonization studies and measured their model against the DOE models. They calculated the cost of production and explored the potential for economic success via incremental cost reductions to both capital expenses (CAPEX) and operating expenses (OPEX). Based on their results, a cost reduction of 40% for both CAPEX and OPEX was identified as the turning point where more reductions in either expense would result in at least minimal economic success. They furthered their analysis by keeping cost reductions at 40% for both and calculating the economic success based on net present value as a function of biomass production and lipid content. Under the 40% cost reductions of both CAPEX and OPEX, most scenarios had a 95% probability of success, but without the cost reductions less than 10% of the scenarios had a probability of success. To date no studies have shown algal biofuels to be commercially viable at current technology conditions.

This thesis employs the use of a pilot-scale case study to address several research questions. Cellana LLC is located in Kona, HI on 6.8-acres of land. There are also six 1,000-L open ponds with a total cultivation capacity of 750,000 L. ASU has a history of collaboration with Cellana through ATP3; ASU already has a non-disclosure agreement

(NDA) in place. Cellana’s business model is built on a high value multiproduct model centered on Omega-3 fatty acids and is designed such that nutrients are a one-time input, and the algae deplete the pond of nutrients before harvest. Cellana produces four products; omega-3 fatty acids, biofuel, aquaculture feed and animal feed (Figure 6). Omega-3 fatty acids are a high value nutraceuticals compound that have benefits associated with the human heart and brain development (Campoy et al., 2012; Kris-Etherton et al., 2002; Simopoulos, 1991). The value of omega-3 fatty acids and the ability to produce biofuels simultaneously mean that there is potential for environmental and economic sustainability using this algae multi-product model production pathway.

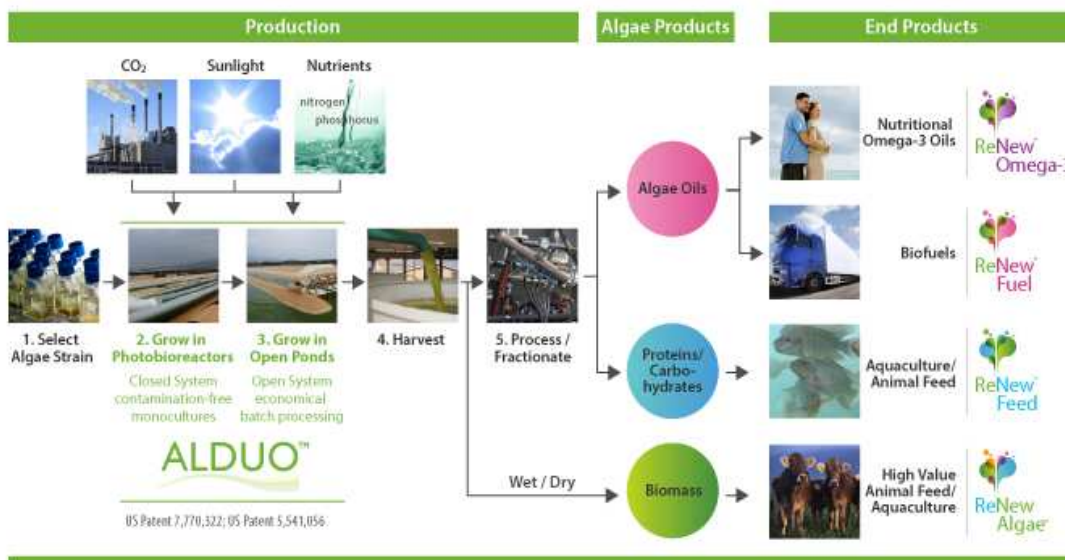


Figure 6: Cellana’s Process Flow Diagram and Products
Image taken from <http://cellana.com/>

A sustainability assessment of omega-3 fatty acids from algae must include the following; comparative environmental assessment of omega-3 fatty acids from fish and from algae, a cost evaluation of producing omega-3 fatty acids from both sources, and a thorough analysis of resource depletion resulting from omega-3 fatty acid production

from fish and algae. The environmental assessment should include not only global warming potential (GWP) but also water, air and land quality environmental impacts such as eutrophication potential (EP), smog formation potential (SFP) and acidification potential (AP). The cost analysis must consider the value of omega-3 fatty acids, the quality of the product, the co-products that can be developed simultaneously and if applicable, the additional unit processes required for co-product generation. The resource depletion analysis for algae should include fossil fuel depletion for energy consumption and phosphorus depletion in the US and globally. Resource depletion for omega-3 fatty acids production from fish should include fish stocks, harvesting and recovery.

Omega-3 fatty acid production from fish can be divided into two major processing stages also: fishing operations and oil processing. Fishing operations are fish production and transportation to processing. The two options for fish production are wild caught fish and aquaculture fish farming. Processing includes cooking, wet pressing method for oil extraction followed by oil refining. The refining process involves degumming the oils to remove phospholipids, neutralization to remove free fatty acids, bleaching to remove pigments and deodorizing to remove malodorous compounds. Figure 7 shows the process flow for omega-3 fatty acid production from fish and from algae.

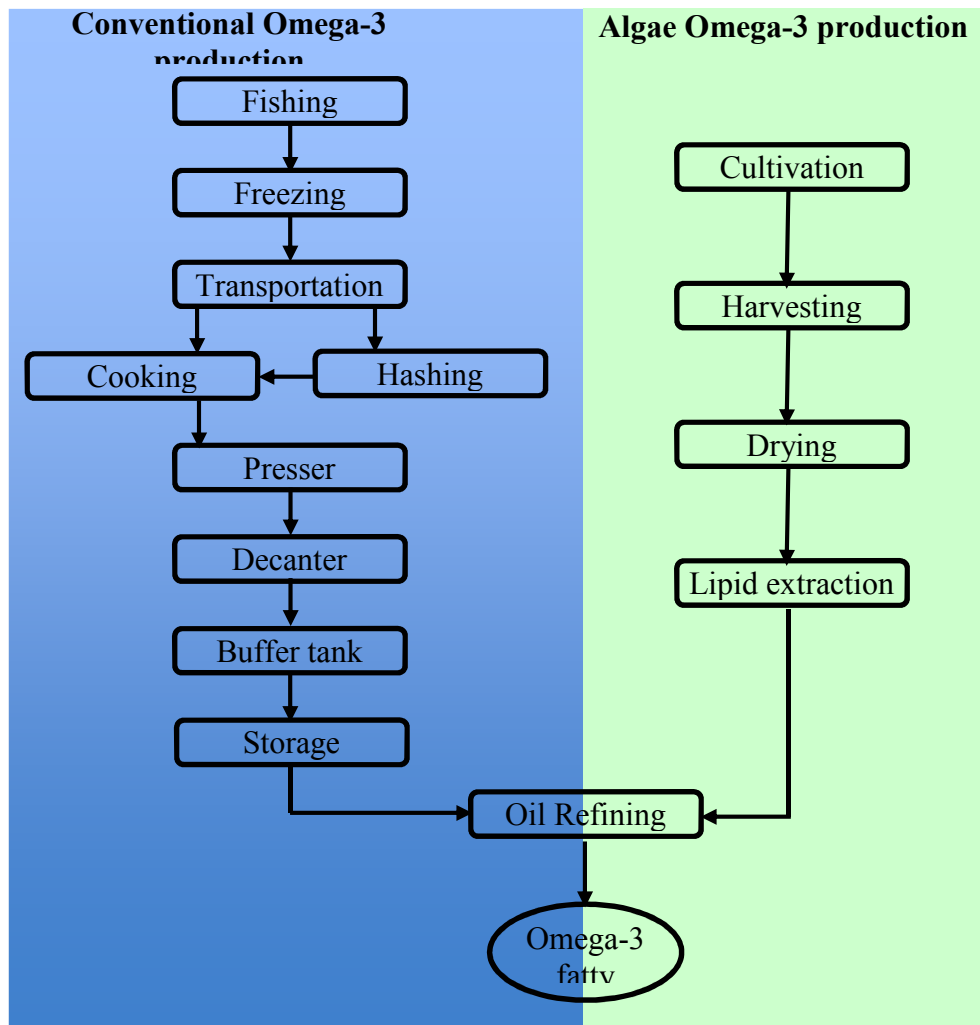


Figure 7: Conventional vs. Algae Omega-3 Fatty Acid Production

Omega-3 fatty acid production from algae can be divided into two major process stages: algae biomass production, and oil processing. Algae biomass production includes cultivation, primary harvesting, secondary harvesting and drying. With undergraduate researcher Matthew Weschler, I supervised a process energy analysis of over 100 different options for algae biomass production (M. K. Weschler et al., 2014). In addition, I contributed to LCA work related co-product generation with previous researchers in my group (Soratana et al., 2014). My research group has been developed a number of models

and resulting publications related to algal biofuel (Soratana et al., 2014; Soratana & Landis, 2011, 2013). Lipid extraction and omega-3 fatty acid refining follow algae biomass production. The Landis research group has also modeled algae lipid extraction in association with biodiesel production (Soratana et al., 2011).

This thesis applies the LCA and TEA methodology to a case study of Cellana's ALDUO process, which produces omega-3 fatty acids and renewable diesel from the lipid component as well as high protein feed from the LEA.

CHAPTER 2

ENVIRONMENTAL IMPACTS OF ONSITE ANAEROBIC DIGESTION FOR ALGAE BIOFUEL RESIDUAL HANDLING AND CO-PRODUCT GENERATION

This chapter addresses Research Question #4: How can the environmental and economic performance of algal biofuels and bioproducts most effectively be improved and what are the tradeoffs between economic and environmental sustainability?

Introduction

Algal biofuel production results in significant unused biomass, and creating valuable coproducts from this material can enhance the environmental footprint for algae biofuels. The lipids, for biodiesel production and carbohydrates for bioethanol production, can each represent less than 50% w/w (Lardon et al., 2009; Murphy & Allen, 2011) of the total algae biomass. Co-product generation from the remaining biomass can significantly improve the environmental performance of algal biofuels. Anaerobic digestion produces a methane rich biogas that can be converted to energy, and breaks the biomass down, converting the nutrients in the biomass to more bioavailable inorganic nutrients, and reduces the amount of waste biomass. The implementation of anaerobic digestion at an algae biorefinery however, may be difficult due to the high initial cost, difficulty of operation, and limited available algal biofuel residual (ABR) at individual biorefineries.

While onsite anaerobic digestion is a promising option for biofuel refineries, the reality is that onsite anaerobic digestion may not be feasible unless a significant amount of biomass load can be provided to ensure continuous operation. Offsite anaerobic

digestion is another option but it also has its own limitations due to the availability of commercial digesters at waste handling facilities and the likelihood that the methane will simply be flared. For example a 2011 report by the USEPA states that 104 combined heat and power (CHP) plants were in place at wastewater treatment plants; the report found that CHP is technically feasible at 1351 sites, and economically feasible at 257 to 662 sites (EPA, 2011). There are not only a limited number of places where transportation of ABR to an existing digester would not necessarily result in the methane being used but also that there are not a lot of sites available for this option. An EPA study on biogas operations at farms suggests that the amount of manure required for developing an economical onsite anaerobic digester for energy recovery is approximately 1000 tons of biomass annually (AgSTAR, 2011). Sapphire, an industry leader in algae biofuel commercialization efforts, produced almost 100 tons of algae between March and June of 2012 (Llewelyn & Piotraszewski, 2012). In order to meet the EPA's suggested biomass load for designing an onsite digester, algae biomass production would have to increase by at least one order of magnitude.

This study has two primary goals: to quantify the combined environmental benefits of energy and nutrient recovery using anaerobic digestion of algal biofuel residuals, and to compare onsite anaerobic digestion to offsite algal biofuel residual handling scenarios for co-product generation. This study considers eight main ABR handling scenarios; onsite anaerobic digestion of lipid extracted algae (LEA) from biodiesel production, onsite anaerobic digestion of saccharification and fermentation residuals (SFR) from bioethanol production, offsite anaerobic digestion of LEA and SFR, incineration of LEA and SFR and landfilling with methane capture of LEA and SFR. The offsite anaerobic digestion

scenarios were divided into 4 additional scenarios based on the amount of methane used, for a total of twelve scenarios (offsite peak and offsite flare for LEA and SFR). The twelve scenarios were selected for this study to compare the environmental impacts of onsite anaerobic digestion to offsite scenarios that would be less complex to operate, require less risk on the part of the biorefinery, less initial investment and less additional unit processes for capturing valuable co-products. Life cycle assessment was used, adapting the ISO 14040 framework, to quantify environmental impacts and benefits of the twelve handling scenarios.

Methods

A gate-to-gate life cycle assessment (LCA) was carried out to compare the environmental impacts of algal biofuel residual handling using four different methods: onsite anaerobic digestion, offsite anaerobic digestion, offsite incineration and offsite landfilling. The process model and inventory items, including databases, calculations and sources, are presented in the supporting information. Environmental impacts were quantified using TRACI 2.1 version 1.01 (Bare et al., 2012). The impacts considered for this study were global warming potential (GWP) and eutrophication potential (EP) to quantify water quality impacts.

Figure 8 shows the system boundaries for this study. The functional unit was the algal biofuel residual (ABR) resulting from 1 metric ton of algal biomass used to produce either biodiesel or bioethanol. Two types of ABR were considered based on Soratana et al. (2014); they were lipid-extracted algae (LEA) from biodiesel production and simultaneous saccharification and fermentation (SSF) residual from bioethanol production. The microalgal strain used for biofuel production was *Chlorella vulgaris* with

30% lipids, 37% carbohydrates and 33% protein content (Lardon et al., 2009; Soratana et al., 2014). The algal biomass underwent lipid extraction to separate the lipids from the remaining biomass (LEA); the LEA was composed of 4%, 51% and 45% of lipids carbohydrates and proteins respectively. The SSF residuals result from the saccharification and fermentation process of the algal biomass resulting in bioethanol and the residual biomass (SSF); the SSF was composed of 45% lipids, 6% carbohydrates, and 50% proteins. The environmental impacts of biofuel production were not considered in this study so that the results of this study could serve as an add-on to existing and future biofuel studies or any process that results in an output biomass where anaerobic digestion is an option.

Onsite anaerobic digestion was modeled for handling ABR at commercial scales, but it was also compared to the environmental impacts of sending the ABR to an offsite incinerator, anaerobic digester or landfill. For the anaerobic digestion, the substrate (ABR) was sent to the digester for the set hydraulic retention time (HRT –16 days), followed by centrifugation to separate the digestate (solid phase) from the centrate (liquid phase). The centrate was reused for cultivation of algae while the digestate was used for land application.

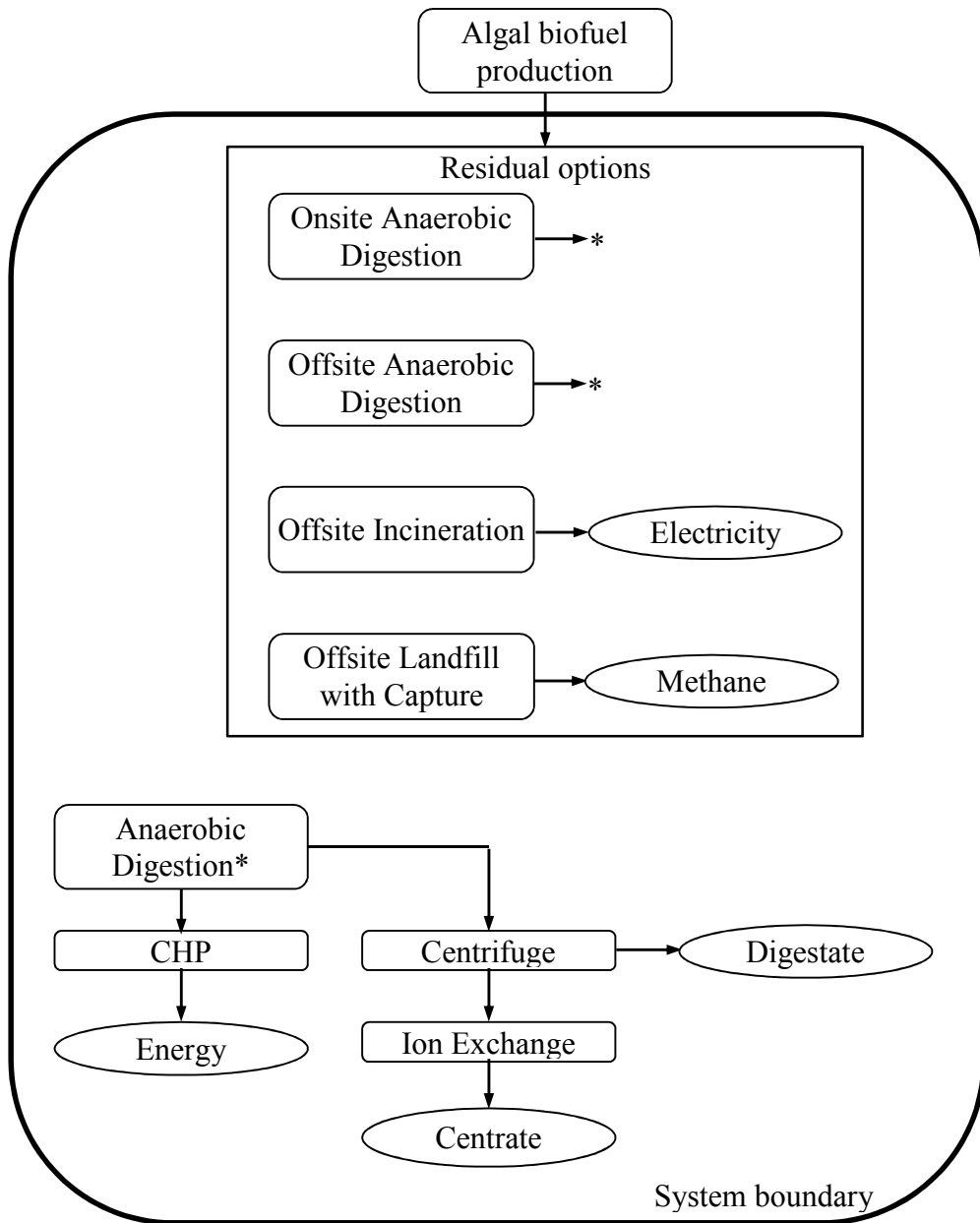


Figure 8: ADLCA System Boundaries

The algal biofuel residual (lipid extracted algae or simultaneous saccharification and fermentation residuals) was fed to one of the four residual options.

Digester mixing was done using rotary draft tube impeller mixers based on Massart et al. (2008) and scaled to the onsite and offsite digester. Alkalinity was maintained using hydrated lime. No nutrient inputs were required for anaerobic digestion; the methanogens

do require a small quantity of nutrients (Rittmann & McCarty, 2001) but those nutrients can be derived from the ABR and was subtracted from the nutrient offsets. Centrifugation of the digestate was based on an existing industrial decanter centrifuge with the capacity to handle the digester feed (Apex, 2000). The solid digestate handling and spreading was done in the same manner as solid organic fertilizer (Berglund & Börjesson, 2006).

Centrate can be recycled to any process requiring nutrients (cultivation for algae, back to the AD, WWTP head to meet nutrient demands) to represent a synthetic nutrient offset at the location where nutrients are recovered; however recycle of centrate back to the PBR was not included in this model.

The onsite anaerobic digestion handling method was modeled to include construction and operation of the digester. The parameters for the AD model are presented in the supporting information. The equations mentioned in the table are available in the supporting information. The digester was 50 m³. The model for onsite and offsite anaerobic digestion considered the load, the size of the digester, the nutrient content as well as the requirements for operating the digester (nutrients for microbes, alkalinity, heat, and electricity for mixing and pumping), and calculated the potential for methane generation for conversion to electricity and heat, and nutrient recovery for use as a land applicant and recycling to algae cultivation. Nutrient requirements for both on and offsite digesters were assumed to be the same as for an anaerobic digester for a wastewater treatment plant. The digestate contains the majority of the nutrients that were present in the algae during the cultivation phase. Some losses would take place due to biomass recovery inefficiencies (assumed to be 5% overall for biodiesel and bioethanol). The

digestate can be dewatered to provide a nutrient rich soil amendment for land application and a mineral nutrient rich liquid stream for recycling to the algae cultivation system.

Onsite anaerobic digestion requires initial investment and construction as well as time to stabilize the digester before benefits can be realized. However, onsite digestion gives the algal biorefinery the option to control co-product generation (e.g. recycling nutrients to cultivation, controlling how the methane is converted to useful energy and land application of digestate). Resource recovery from onsite and offsite anaerobic digesters was compared for different levels of energy and nutrient recovery at the offsite facility to represent the algae biorefinery's lack of control over co-products.

Combined heat and power was selected as the technology for converting the methane gas into electricity and heat. Combined heat and power was based on an industrial micro turbine (Turbec, 2011). Calculations and more detailed inventory information are provided in the supporting information. CHP was modeled based on the Turbec microturbine (2011) and has a thermal conversion efficiency of 47% and an electrical conversion efficiency of 30%.

The offsite handling methods include transportation to an existing incineration facility, transportation to an existing landfill with methane capture and transportation to an offsite anaerobic digester. The offsite anaerobic digester was modeled using the same model for the onsite digester to ensure consistency. The offsite digester was 3312 m³ based on an existing digester at Mesa Northwest Water Reclamation Facility (Personal Communication: 8-16-2012). Resource recovery at offsite anaerobic digestion has been divided into three different scenarios: 100% methane utilization, methane utilization at peak times (assumed to be 7 out of 24 hours per day) and no methane usage. While, these

numbers were determined based on the peak available hours other reasons for not using methane exist. Primarily the production of siloxanes when burning fuels in engines that can lead to significant damage, anaerobic digester operators may opt to avoid investing in the infrastructure to convert methane into useful energy with this risk. The financial aspect of biogas conversion was not within the scope of this study but certainly would need to be taken into account if onsite digestion was deemed to be a better option. This would include capital cost for methane storage potentially, additional biogas scrubbing and any maintenance for the energy conversion technology to minimize damage due to siloxane production. Sending the ABR to offsite handling facilities would be much simpler than designing an onsite ABR handling facility; an offsite facility would already be constructed and up and running, lower risk to the algal biorefinery and not require an initial setup and the subsequent environmental impacts. The incineration and landfill scenarios were modeled using data from peer reviewed literature sources (Cherubini et al., 2009; Damgaard et al., 2010; Levis & Barlaz, 2011; Wu et al., 2006). The environmental impacts of transportation, incineration and landfilling with methane capture were derived from Levis and Barlaz (2011), Cherubini et al. (2009) Damgaard et al. (2010) and Wu et al. (2006). The transportation distance for the offsite scenarios was calculated based on how far the biorefinery would have to send the ABR for the GWP of the onsite anaerobic digestion and the offsite anaerobic digestion to be equal.

Offsite incineration and landfill were also considered to provide a comparison of the environmental impacts of multiple offsite scenarios in addition to anaerobic digestion. The life cycle inventory data for most upstream processes was taken from USLCI v1.6 (NREL, 2012) where available and otherwise taken from ecoinvent v3.0.2 (Weidema B P

et al., 2013); the life cycle inventory data is presented in detail within the Supporting Information. Data for the inputs and resource consumption for operating and maintaining landfills and incineration facility, were used from Cherubini et al. (2009), which included construction materials for the landfill and incineration per kg of solids entering the facility. Energy consumption including natural gas, electricity and diesel fuel for transportation were also included from (Cherubini et al., 2009). Transportation impacts were derived from USLCI version 1.6. Environmental impacts were quantified using TRACI 2.1 version 1.01 (Bare et al., 2012). This study highlights global warming potential and eutrophication potential. Normalized impact assessment results for acidification, ozone depletion, and smog formation are presented in the supporting information. Consideration was also given to the aspect of what stakeholders would control co-product generation. Three different rates of methane utilization were considered for the offsite anaerobic digester to represent the lack of control that algal biorefineries would have when transporting algal biofuel residuals to offsite scenarios; the methane utilization rates were 100% methane usage (same as onsite), utilizing 1/6 of the total methane using combined heat and power (representing 4/24 peak hours per day for the most economical electricity distribution) and no methane usage.

Results & Discussion

Twelve algal biofuel residual handling scenarios were analyzed using a comparative life cycle assessment. The results of this study focus on the implications of implementing anaerobic digestion at commercial-scale for algal biofuel residual handling in terms of environmental impacts and commercial feasibility based on available feedstock. While ABR was considered for this study, any waste biomass where anaerobic digestion is an

option could apply these results to their study to determine the effect that different anaerobic digestion options have on their results.

Figure 9 shows the GWP impacts associated with ABR handling. The transportation was 123 miles and 90 miles for the offsite LEA and SSF scenarios respectively. Landfilling with methane capture had the lowest global warming offsets and was considered. Onsite and offsite AD scenarios had similar net GWP values as long as all of the methane was utilized. Electricity contributed significantly to GWP offsets. Nutrient recovery, which resulted in offsets due to avoided urea and superphosphate, contributed to the GWP offsets. The offsets to GWP were in the offsets in nutrient production. Nutrient consumption and recovery in this study included the amount of nutrients required for maintaining the anaerobic digester and the amount of urea and superphosphate that can be avoided through the recovery of nutrients from the liquid and solid digestate.

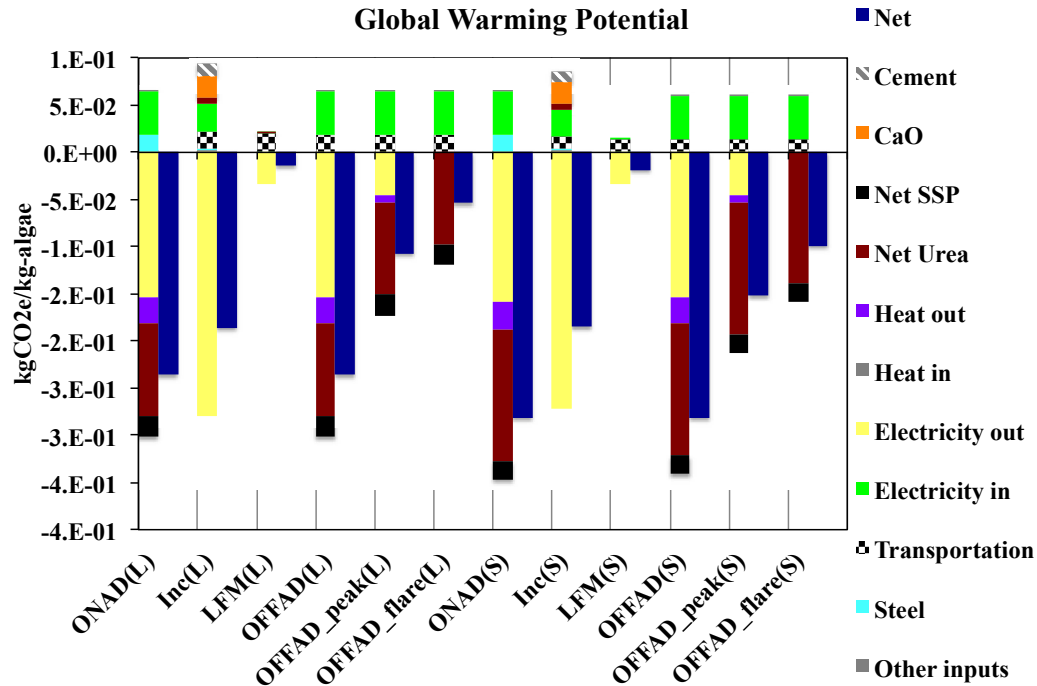


Figure 9: ADLCA Global Warming Potential
 Peak: Utilization of methane 30% of the methane representing the daily peak hours (noon – 7:00 pm) for Arizona Public Service electricity rates.
 Flare: all methane was flared, none converted to electricity and heat.
 ONAD: Onsite anaerobic digestion Inc: Incineration
 LFM: Landfill with methane capture OFFAD: Offsite anaerobic digestion
 (L): Lipid extracted algae (S): Sacharificaiton and fermentation
 residuals

The incineration GWP offsets were due 100% to electricity production (Figure 9). The amount of GWP offsets due to electricity out were lower than incineration for all scenarios but due to the nutrient consumption and heat, the anaerobic digestion scenarios resulted in greater net GWP offsets. The lowest net GWP was the onsite AD of saccharification and fermentation residuals (SFR) scenario at -2.8 kg-CO₂eq/kg-algae. Incineration had slightly higher GWP impacts from inputs than the other scenarios. Electricity to operate the anaerobic digester was the highest contributor to positive global warming impacts in all AD scenarios. Electricity production was the largest contributor

to GWP offsets, making up to 40-50% of the total offsets for AD scenarios. While incineration produces more electricity, AD allows for the recovery of electricity, heat and nutrients.

Offsite AD scenarios that did not use all of the methane had significantly lower GWP offsets. Today, industrial anaerobic digesters do not always utilize all of the methane they produce. Typically one of two practices takes place; methane is converted to electricity only when electricity prices are the highest during a given day, or all methane that is produced is flared and emitted primarily as CO₂. The former practice was termed 'peak' in the scenarios, while the latter was termed 'flare.' The peak and flare scenarios were included to illustrate the differences between current practices and an ideal case where 100% methane utilization occurs at locations that produce exceptionally high amounts of methane.

Figure 10 shows the eutrophication potential (EP) and net EP for the 12 handling scenarios. Only the eight AD scenarios resulted in net negative EP due to the nutrient recovery and reuse, which also avoids using virgin fertilizers for algae cultivation. The net negative AD scenarios were all between -3.2E-4 and -4.2E-4 kg-Neq/kg-algae_{total}. Anaerobic digestion scenarios where all of the methane is converted to electricity and heat resulted in similar amounts of EP offsets to offsite AD peak and flare scenarios. The landfill and incineration scenarios had negligible EP offsets. The nutrient recovery resulted in significant offsets to EP. The offsets to EP were due somewhat to the offset of production impacts but mostly due to the impacts associated with the use phase of the nutrients.

For the 2014 average US electricity mix shown in Figure 10, the non-anaerobic digestion scenarios had little contribution to EP. Landfilling scenarios resulted no net EP. Despite no nutrient recovery, a slight net negative EP was observed due to electricity production for incineration. The EP of each scenario was strongly dependent on the selected electricity mix. Coal is the major contributor to EP for electricity production. Greater uses of non-coal resources result in electricity having less influence on EP.

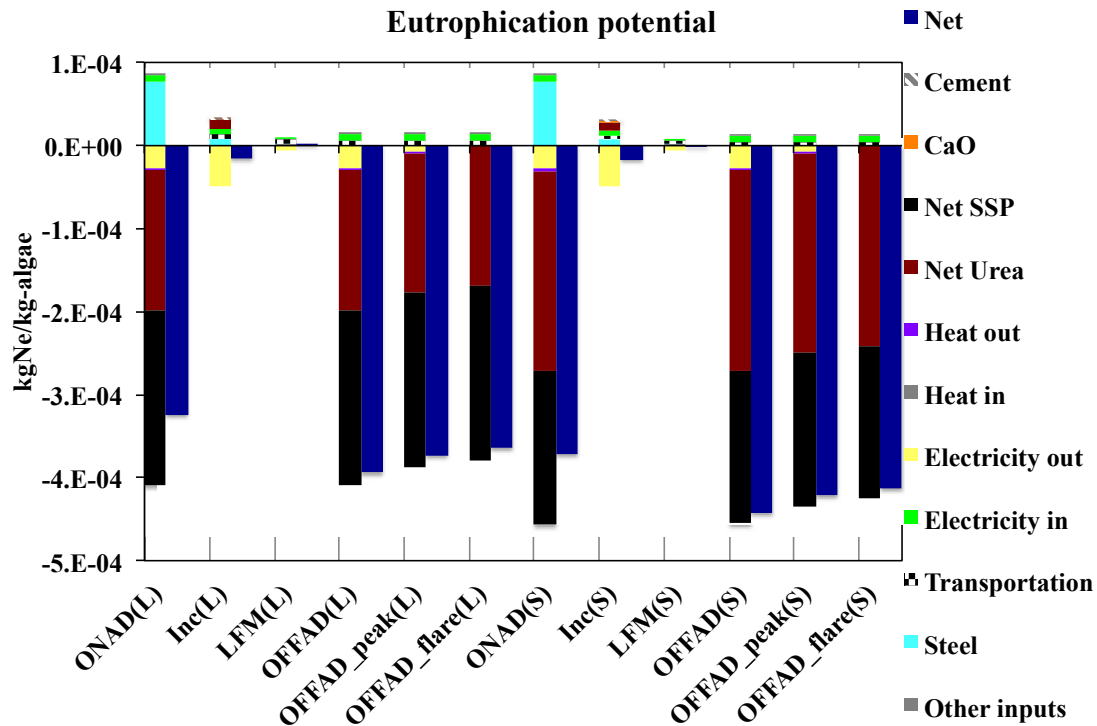


Figure 10: ADLCA Eutrophication Potential
 Peak: Utilization of methane 30% of the methane representing the daily peak hours (noon – 7:00 pm) for Arizona Public Service electricity rates.
 Flare: all methane was flared, none converted to electricity and heat.
 ONAD: Onsite anaerobic digestion Inc: Incineration
 LFM: Landfill with methane capture OFFAD: Offsite anaerobic digestion
 (L): Lipid extracted algae (S): Scharification and fermentation residuals

All AD scenarios resulted in a net negative EP, primarily due to nutrient recovery, which dominated the EP offsets. While phosphorus was the largest contributor to EP offsets, nitrogen also made a significant contribution (40-50% in all cases) to EP offsets due to nutrient recovery. The primary difference between onsite and offsite AD was the construction phase required for onsite AD and the transportation required for offsite AD.

However, transportation had negligible influence on EP compared to the other parameters of this model. All offsite AD scenarios had more net EP offsets. This was due to the environmental impacts associated with steel production required for constructing the onsite AD. Steel was the highest contributor to EP impacts. Mining waste and NO_x from furnace utilization contribute the most to EP due to steel. Steel contribution to environmental impacts may be reduced if the steel can be recycled at the end of the digester's life.

Onsite and offsite AD scenarios resulted in similar results when all of the methane was converted to electricity and heat. The offsite AD digested algal biofuel residuals (ABR) to a greater extent than onsite AD due to the fact that ABR was co-digested with the more readily digestible wastewater treatment sludge. Algae biomass has been shown to be more recalcitrant to digestion compared to municipal wastewater solids (Prajapati et al., 2014; Sialve et al., 2009). This model assumed that the volatile solids reduction was 65% for ABR. This volatile solids reduction lies between whole algal cell destruction (~40%) and primary sludge (87%) and was based on the fact that fractionation processes (lipid extraction for biodiesel, fermentation and distillation for bioethanol) make the ABR more degradable than whole algae biomass.

The digestibility of the substrate also affects the nutrient concentration in the digestate. A significant amount of the nutrients entering the digester were not bioavailable. The digestion process breaks down the biomass and converts the nutrients into a bioavailable form. The nutrients entering either onsite or offsite AD are the same concentration and amount, but the level of bioavailability of the nutrients leaving the digester is dependent on the amount of biomass that is digested. The higher extent of digestion of the ABR offsite due to co-digestion results in higher solids destruction and subsequent nutrient availability. Two types of nutrient recovery were considered in this model; nutrients in the centrate were recycled to the AD or to algae cultivation, while the digestate nutrients were land applied. The results of onsite and offsite nutrient recovery were a function of the processes for recovering the nutrients for land application and recycling. The differences in environmental impacts of nutrient recovery between onsite and offsite AD were minimal.

Significant environmental benefits from energy and nutrient recovery are possible using anaerobic digestion to manage algal biofuel residuals. Siting the AD in the same location as the algae cultivation systems (onsite AD) has lower net GWP than offsite AD scenarios that do not use all of the methane. Nearly all of the methane must be used (98%) for electricity to make the offsite scenarios have a net GWP comparable to the onsite scenarios. There was not a significant difference between any of the AD scenarios in terms of EP offsets. Anaerobic digestion can result in greater environmental benefits than other solids handling methods, because anaerobic digestion allows electricity, heat and nutrient recovery that other handling methods do not provide.

Nearly all scenarios had a net negative GWP and EP. Landfilling resulted in the least offsets to GWP and EP. Electricity production in all scenarios dominated GWP offsets. AD scenarios would still have a net negative EP and GWP without electricity and heat production indicating that nutrient recovery has the potential to offset GWP and EP impacts. The amount of electricity produced from incineration resulted in offsets that were comparable to the most promising AD scenarios that use electricity, heat and nutrient recovery for GWP offsets. Offsite scenarios that did not use all of the methane were more promising than landfilling scenarios but were much lower than the incineration and other AD scenarios in terms of GWP. Offsite AD scenarios must use nearly 100% of the methane to be comparable to onsite scenarios GWP offsets. All eight anaerobic digestion scenarios resulted in significant EP offsets. The other scenarios had negligible or no EP offsets.

Onsite anaerobic digestion is a promising method to make use of algae biofuel residuals but offsite options are also promising. Algae biorefineries would need to generate more than 1000 tons of algal biofuel residuals per year to justify the use of onsite anaerobic digesters. Sapphire Energy's 100 ha commercial-scale algae farm that is in development and expected to be online by 2017 has the potential to produce sufficient ABR once it reaches its 1,600 metric tonnes of whole algal biomass per year. Another option to improve the ability to use algae for anaerobic digestion is to decrease the capital, and operation and maintenance costs and chemical requirements associated with larger anaerobic digesters. Offsite scenarios also resulted in net negative GWP and EP. There are a number of major advantages to using offsite scenarios; onsite system design requires careful attention to the ABR load, offsite digesters do not require additional

capital or construction, the benefits of recovering energy and nutrients from ABR can be achieved immediately since offsite systems are already operational, and the size of the offsite facility is likely to benefit from economies of scale. Despite the environmental impacts of transportation, the results demonstrated that transporting ABR to offsite anaerobic digestion facilities within 125 miles resulted in comparable environmental impacts.

Conclusion

This study focused on the environmental sustainability of four different handling methods. For the onsite scenarios, the economic viability of designing an onsite AD needs to be considered. This model compares onsite anaerobic digestion to transportation to existing offsite facilities. This model examined the difference between onsite and offsite anaerobic digestion for energy and nutrient recovery. The use of the methane (i.e. for electricity and heat, or as a fuel) and the amount of methane converted or flared can affect the GWP and EP of different ABR handling systems. While ideally all of the algae biofuel residuals would be converted to valuable co-products, the reality is that offsite ADs rarely make use of all of their methane, if any, and while those scenarios still resulted in net negative GWP and EP impacts, in terms of GWP they are significantly lower (~40%) than the ideal scenarios that would use 100% of the methane. Four of the 12 scenarios considered involved anaerobic digestion of ABR and they showed a wide range of results and at least a few of the methods are worth considering in any analysis that considers anaerobic digestion as a potential unit process for co-product generation. While a 100-ha facility makes the use of onsite anaerobic digestion feasible from a load perspective, additional financial analysis and capital investment would be required to add

anaerobic digestion to the algal biorefinery. Algal biorefineries should consider sending their biomass offsite for digestion to get the most environmental benefit with the lowest risk to the algal biorefinery, and communicate clearly with offsite digester operators to determine if the ABR is used for energy generation, and be prepared to consider that their ABR may result in methane but does not result in useful energy. Regardless the ABR being used solely for the nutrient recovery does result in some offsets to both GWP and EP, and is a much lower risk to algal biofuel operation.

CHAPTER 3

ENVIRONMENTAL SUSTAINABILITY ASSESSMENT OF A COMMERCIAL-SCALE ALGAL MULTIPRODUCT BIOREFINERY

This paper will address research question #1: How do the environmental impacts of high value algal bioproducts compare to standard production of the same products?

Introduction

Energy independence and security has been one of the primary driving forces behind algal biofuels research in the United States, but the academic community has identified the need for multiple product pathways or industrial symbiosis to make algae biofuels environmentally sustainable (Dong et al.; Lardon et al., 2009; Quinn et al., 2014; Rothermel et al., 2013; Soratana et al., 2013). This is due in large part to environmental impacts associated with water use, nutrient consumption and high energy dewatering at commercial scale (Handler et al., 2012; Rogers et al., 2014). While biofuel has received the most attention from the academic literature due to concerns over climate change and energy security, a number of other products that can be produced from algae have been identified, including high-value products such as omega-3 fatty acids, but their influence on the overall life cycle of algae products has not been extensively quantified (Foley et al., 2011). This study quantified the environmental impacts of the simultaneous production of biofuel and high value products and compared those impacts to the impacts associated with conventional production of the same products.

Algae biomass can be synthesized into a wide range of products from different portions of the biomass simultaneously. Bioethanol can be synthesized from the carbohydrates, biodiesel can be synthesized from the lipids, and the remaining biomass after lipid extraction can be used as a feed or fertilizer product (Bryant et al., 2012; Halim et al.; Soratana et al., 2014; Tibbetts et al., 2015; Wuang et al., 2016). In addition, algae naturally synthesize a number of useful chemicals within the lipid fraction, such as omega-3 (n-3) fatty acids. A number of studies have assessed the production and environmental impacts of multiple algae products, but these studies tend to focus mostly on fuel, energy or feed products (Lardon et al., 2009; Soratana et al., 2014; Zaimes & Khanna, 2013). The environmental impacts of high value chemicals and nutraceuticals such as n-3s have not been evaluated in the literature and have not been compared quantitatively to the impacts of conventional production.

This study quantifies the life cycle environmental impacts of a commercial algae multiproduct biorefinery that produces renewable diesel from lipids, omega-3 fatty acids from lipids and high protein feed from lipid extracted algae (LEA). This is one of the first studies to assess the environmental impacts of such an algae multiproduct biorefinery and compare their impacts to conventional production. Biofuel and high-value products are derived from the lipid fraction of biomass simultaneously. This method will reduce the total amount of biofuels that can be produced from the biomass, but will increase the revenue potential of the multiproduct biorefinery compared to using biofuels alone. The use of the LEA for a high protein feed has been investigated in the past (Maisashvili et al., 2015; Mirsiaghi & Reardon, 2015). The environmental impacts of the three product algae biorefinery are compared to the environmental impacts of their counterpart

products: high protein fishmeal and omega-3 fatty acids, both produced from wild-caught fish and diesel fuel from the remaining oils after omega-3 fatty acid production.

1.1 Conventional Omega-3 fatty Acid Production

Global demand of omega-3 fatty acids (n-3) continues to rise due to their nutritional value in humans, while production of wild caught fish required to produce n-3 does not. The human body cannot sufficiently synthesize the primary n-3 fatty acids of interest, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and so humans must obtain these n-3 fatty acids from diet or supplements (Burdge & Calder, 2005; Plourde & Cunnane, 2007; Strobel et al., 2012). The majority of omega-3 fatty acid demand is met by consuming oily fish for food and nutrition supplements (Kaye et al., 2015). Fish oil also provides farmed fish with n-3 and aquaculture continues to increase as a source of fish for human food consumption (FAO, 2014; Strobel et al., 2012). Alternative oil feeds have been explored for aquaculture using terrestrial plant based oils, but those oil sources rarely contain n-3 and this reduces the n-3 content in the farmed fish fed via this method. Wild caught fish production has not increased significantly in the last decade and in some cases is in decline due to policy limits to prevent overfishing and protect fish stocks, and natural occurrences preventing fishing activity (e.g. El Niño phenomenon) (FAO, 2014). The increasing demand for omega-3 fatty acids in conjunction with the stagnant production of wild caught fish has inspired research and development in alternative sources of omega-3 fatty acids.

Microalgae are one alternative source of n-3 fatty acids. Algae are an important source of n-3 in the marine food chain and provide wild caught fish with n-3 (Fraser et al., 1989), and can be used in aquaculture (Tocher, 2015). Using algae to meet n-3 fatty acid demand instead of fish has the potential to assuage the burden of meeting n-3

demand that currently strains fisheries across the globe, but what are the environmental impacts of producing n-3 fatty acids from algae and how do those impacts compare to producing n-3 fatty acids from fish?

Wild caught fish are the primary source of both fish oil and fishmeal for aquaculture, (both oil and meal) and direct human consumption (fish oil only). In the United States, fish oil and fishmeal are sourced from the *Brevoortia patronus* (Gulf menhaden), which is native to the Gulf of Mexico. (Vaughan et al., 2007). The caught fish are transported to reduction factories that convert the whole fish to fish oil and fishmeal. The UN Food and Agriculture Organization (FAO) published a report on the production of fish oil and fishmeal using the wet press method (FAO, 1986). This method involves hashing the fish into even sizes followed by cooking. An oil press and decanter are then used to separate the oil from the remainder of the fish (meal). Once the oil is separated from the biomass, the fish oil undergoes a refining process to separate the n-3 from the remaining oils.

A number of life cycle studies have been carried out to evaluate the environmental impacts of fisheries. Avadi et al. (2014) assessed the environmental impacts of different processing methods for preparing anchoveta food products from Peru for direct human consumption with 1 kg of fish in human consumption product as the functional unit. They compared five different processing scenarios (fresh, frozen, salted, canned and cured). The fresh fishing method represents the first step in fish oil production while the processing stage shifts from preparing the fish for human consumption to reduction factories for meal and oil consumption. They found that fuel consumption for fishing activities dominated fresh fish production. Svanes et al. (2011) evaluated the environmental impacts of cod from autoline fisheries caught from the Northeast Arctic

stock. All fish that were caught were processed and utilized due to the fact that discards were not allowed in Norwegian Territorial waters. The system boundaries included fishing activities and transportation to retailer or professional user of the fish. The fuel consumption for onboard activities contributed the most to the environmental impacts of the autoline cod fishing whether that was for fishing fleet or transportation to retailers/wholesalers. Data was not available to distinguish between fuel for moving the vessel out to sea and back to port, and other onboard activities (freezing, and onboard processing equipment). Tyedmers et al. (2005) investigated the fuel efficiency of fishing fleets for many different species globally and found that as a global average, fishing fleets consumed 0.62 L diesel/kg fish landed. This value was for fuel consumption during the fishing and on board activities but does not distinguish between the fuel for powering the vessel and fuel for operating processing equipment on board. Thrane (2006) investigated the environmental impacts of Danish fish products and showed that not only did fuel consumption for fishing activities dominate global warming potential, but also all other impact categories except for ecotoxicity. The fuel efficiency of vessels in that study was 0.13 L diesel/kg caught mixed fish. Fuel consumption is a critical variable in analyzing fishery environmental impacts but there is a large range of fuel efficiencies that can vary the results tremendously. Fuel efficiency is highly dependent on the type of fishing fleet that is in use. Cappell et al. (2007) reported fuel efficiencies for a number of different vessel types and showed that fuel efficiencies ranged from 0.11 to 9 L/kg with all but the maximum value falling below 2.6 L/kg. The vessel type used for Peruvian anchoveta and Gulf menhaden were both purse seine vessels. Purse seine fishing involves hauling a large net (typically between two vessels) around a school of fish and drawing it

closed after filling. Cappell et al. (2007) reports fuel efficiencies for two purse seine fleets as 0.13 and 0.44 L/kg. González-García et al. (2015) investigated the eco-efficiency of Northern Portugal purse seining fishing vessels to determine the fuel and material consumption of these vessels. They found that there were differences in the efficiency of each vessel. While they were not certain why, they mentioned that other studies have noted a correlation between vessel efficiency and crew skill on seining vessels, due to the higher skill requirement for purse seine fishing compared to other types of fishing. Almeida et al. (2014) assessed the environmental impacts of purse seine fishing for sardines in Portugal. Fuel consumption represented more than 90% of every impact category they considered (acidification, eutrophication, global warming, ozone depletion and energy use). Each study therein shows a minimum lower than the minimum reported by Cappell et al. 2007 and a maximum that is close to the low end of the range of that same study. Purse seine vessels have been shown to have much better fuel efficiencies than the global average 0.62 L/kg reported by Tyedmers et al. (2005), but fuel consumption still dominates most or all the environmental impacts in many studies based on purse seine fleets.

Fish overexploitation is another major issue associated with increasing global consumption of fish and fish products. The State of the World Fisheries and Aquaculture 2014 report by the UN Food and Agriculture Organization (FAO, 2014) reported that in 2011, only 9.9% of the assessed marine fisheries were being fished below sustainable limits meaning the remainder of fisheries were either fully or over exploited. New LCA methods for assessing overfishing have been developed in recent years (Ziegler et al., 2015). Emmanuelsson et al. (2014) developed a midpoint indicator for measuring

overfishing quantified as lost potential yield (LPY) which is the average number of species catches due to overfishing using multiple fish mortality pathways. The aforementioned Avadi et al. (2014) study indicated that most stocks in Peru were at or beyond exploitation limits and proposed multiple methods to improve this. Those improvements include limiting allowable discard by direct human consumption (DHC) industries and ensuring vessels are properly equipped to maintain quality for DHC.

1. 2 Large-scale Algae LCAs

The peer-reviewed LCAs of algae systems are almost exclusively focused on algae biofuel production. Most of these studies are strictly theoretical in nature and don't utilize large-scale commercial algae data. Incorporating data from real systems greatly improves model accuracy by reducing uncertainty and variability, identifying key data points not commonly considered and improving the accuracy between increasing scales (lab-scale to commercial scale, vs. demonstration scale to commercial-scale). To date there have been few LCAs of algae bioproducts written based on data directly from large-scale facilities; Luo et al. (2010) developed a biofuel model for bioethanol production based on a planned pilot facility for Algenol LLC to be designed in Freeport TX, Liu et al. (2013) developed an LCA model for the production of biocrude via hydrothermal liquefaction, using existing data from Sapphire Energy's pilot plant in New Mexico, and two companion studies (Beal et al., 2015; Huntley et al., 2015) for the production of an oil fraction and protein rich fraction to be used for conversion into biofuel and animal feed respectively, were based off of Cellana's Kona demonstration facility (KDF) in Kona, HI. These studies all focused on fuel products primarily.

This study used LCA to quantify the environmental impacts of a commercial-scale algae multiproduct biorefinery based on Cellana's Kona demonstration facility.

Cellana has been operating the (KDF) in Kona, HI since 2009 for the purpose of collecting key data for improving the commercial potential of algae bioproducts. While Cellana's KDF has been focused on collecting key data, their future commercial goals include the production of three products; omega-3 fatty acids, biofuel, and high protein feed. The life cycle assessment was based on data recently collected from KDF and used to analyze the environmental impacts of a commercial-scale facility. Using the ISO 14040 series as a framework, a comparative LCA of an algae multiproduct biorefinery was carried out to compare the environmental impacts of algae based production to conventional production.

Methods

A comparative LCA of a three product algae multiproduct biorefinery and the conventional production of the three products was conducted to quantify their environmental impacts and identify potential areas of improvement based on Cellana's Kona demonstration facility (KDF). The environmental impacts were quantified using the U.S. Environmental Protection Agency (USEPA) Tool for the Reduction and Assessment of Chemical and other environmental impacts (TRACI 2.1) (Bare, 2002). The environmental impacts assessed in this study were ozone depletion (ODP), global warming potential (GWP), smog formation potential (SFP), acidification potential (AP) and eutrophication potential (EP). ODP, GWP, and SFP were selected as air quality impacts. SFP could be significant for the conventional omega-3 fatty acid product model due to the amount of time the fishing operators will be at sea and in direct contact with the SFP emissions. The AP and EP were selected as water and soil quality impacts.

Three algae multiproduct model scenarios were evaluated and compared to traditional products. Table 3 describes the four scenarios. The algae multiproduct model was designed based entirely on the Cellana LLC. KDF based on data from their system operations in 2015 and early 2016. Two additional scenarios were included to reduce the electricity consumption associated with dewatering the algae; the low energy centrifuge model using all of the same unit processes and the algae multiproduct model with a membrane filtration added as a primary harvesting step.

Table 3: Scenario Description

Name	Algae multiproduct model	Algae multiproduct model with Low Energy centrifuge	Algae multiproduct model with Membrane Filtration	Conventional product model
Description	Algae product model for the production of omega-3 fatty acids, renewable diesel and high protein feed	The algae multiproduct model with a low energy centrifuge to reduce the electricity consumption associated with dewatering the algae	The algae multiproduct model with membrane filtration as a primary harvesting step to reduce the electricity consumption	The conventional production of omega-3 fatty acids and fish meal with renewable diesel from excess oils
Membrane filtration	No	No	Yes	N/A
Centrifuge electricity consumption	8 kWh/m ³	4.3 kWh/m ³	8 kWh/m ³	N/A
Differences from Cellana KDF	None	Low energy centrifuge to replace conventional one	Membrane filtration as primary harvesting process	N/A

For the algae biorefinery, the strain used by Cellana was *Nannochloropsis oceanica*. This strain was selected because it is native to Hawaii, produces substantial amounts of omega-3 fatty acids and can also be used to produce biofuels and a high protein aquaculture feed. The composition of *N. oceanica* was modeled based on Razon & Tan (2011). For the conventional production, the fishery was assumed to use an industrial purse seine fishing fleet consistent with what would be expected in the US Gulf of Mexico for fish oil production (Vaughan et al., 2007) and included using the non omega-3 oil for biofuel and a portion of the remaining biomass for fishmeal. The functional unit

was the production of 1 metric ton of n-3, which was used as the basis to link the two models and balance the amount of biofuels and aquaculture feed. Conventional production resulted in a higher quantity of aquaculture feed per metric ton of omega-3 fatty acids. Additional aquaculture feed was added to the algae multiproduct biorefinery model to account for the differences in the two feed products.

2.1 Kona Demonstration Facility Description

Figure 11 shows the production steps for the algae multiproduct biorefinery and the conventional product model. The steps for the algae multiproduct model were based off of the Kona demonstration facility that has been in operation since 2009. KDF employees collected data related to the cultivation and harvesting of the biorefinery operation for use in this LCA (Supporting information). Cultivation starts in closed controlled lab conditions before being sent outdoors. Indoor cultivation starts with 250 mL reactors followed by 2-L reactors followed by 20 L reactors. Outdoor cultivation begins with 220-L scale up bags that were seeded with 20-L carboys from the indoor cultivation. The scale-up bags were used to inoculate 24-m³ photobioreactors (PBRs). The PBRs were used to inoculate 60-m³ open ponds. The cultivation time for the ponds and PBRs was three days. The final concentration of the algae leaving the open ponds was 0.42 g/L. Following cultivation, algae was harvested using centrifugation followed by a ring dryer. The concentration of the biomass leaving the centrifuge and ring dryer was 20% and 95% respectively.

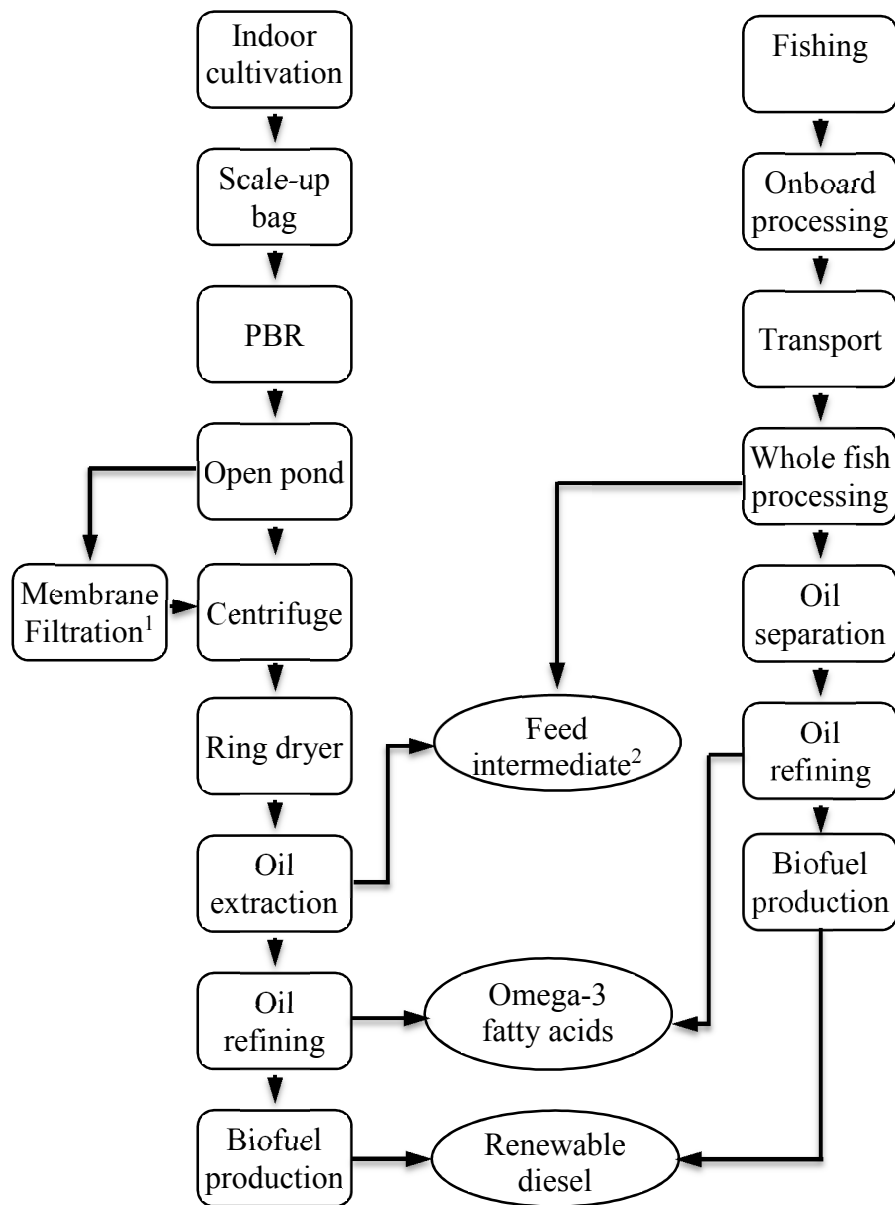


Figure 11: Multiproduct Model Process Flow Diagram

Process flow diagram for the algae multiproduct model and the conventional product model.

1: Membrane filtration is used only in the multiproduct model with membrane filtration scenario and not the other two.

2: The feed intermediate for the algae scenarios is lipid extracted algae (LEA) and the feed intermediate for the conventional product model is the fishmeal. Additional processing for both is outside of the system boundaries.

Since KDF does not currently operate at commercial-scale, (2.5 ha with 60 m³ ponds cultivation), the data collected from their unit operations were used to design a commercial-scale model (112 ha with 1,500 m³ ponds). Table 4 shows the comparison of the unit processes currently in use at the demonstration-scale facility, and the commercial-scale model that was designed based on the pond design Cellana LLC. plans for their commercial-scale production. The resulting commercial-scale model would result in the production of 120 MT of omega-3 fatty acids annually which would account for 10% of omega-3 fatty acid production from algae in 2012 according to the Global Organization for EPA and DHA (GOED) (Ismail, 2013).

Table 4: Existing Unit Process to Model Comparison

Cultivation process	Size at KDF	Commercial-scale model	Ratio of modeled units to existing units (v/v)
Scale up bags	220 L	220 L	1
Photobioreactor	24 m ³	51 m ³	2
Small pond	60 m ³	190 m ³	3.2
Medium pond	60 m ³	750 m ³	12.5
Large pond	60 m ³	1,500 m ³	25
Centrifuge capacity	70,000 gal/day	70,000 gal/day	1
Ring dryer	144 gal/day	1000 gal/day	7

2.2 Life Cycle Inventory

The data used for developing both the algae and conventional product models can be accessed in the supporting information accompanying this manuscript. Three primary tables were presented for both models; an LCI table including the database version and region, a parameters table that includes numbers and sources, and the table used for the Monte Carlo Analysis (MCA) including the minimum, maximum and best estimate with sources. An additional table was provided for the conventional products model to account

for the range of fuel efficiencies (L/tonne of fish) associated with purse seine fishing fleets. The functional unit for this study was the production of one metric ton of omega-3 fatty acids and the corresponding feed intermediate product and renewable diesel.

Cellana provided the majority of the life cycle inventory (LCI) data, based on their demonstration facility in Kona, HI. Beyond the data provided by Cellana, inventory data came from peer reviewed literature, government reports, industry reports, and LCI databases such as ecoinvent version 3. A life cycle assessment model for the production of algae n-3, aquaculture feed and biofuel was designed by expanding on previous algae biofuel work done by this research group (Soratana et al., 2014; M. K. Weschler et al., 2014). The data for conventional products model was collected from fishery LCAs, industry reports and existing LCI databases.

The model was designed by creating a modular commercial-scale facility where the input data was based on observed data at Cellana's existing KDF and the design was based on Cellana's expectations of their future commercial-scale facility (data shown in supporting information). The desired operating rate is 90%. The operating rate can decrease to as low as 80% to account for pond crashes and other technical difficulties. Any lower than 80% and operations would cease until operational issues can be resolved. The production from algae was based on the possible production from an eight module, 112-ha algae multiproduct biorefinery. This resulted in the production of 4,032 metric tons of algae/year.

The commercial-scale cultivation system modeled for this study involved a three-step indoor lab cultivation followed by a five-step outdoor large-scale cultivation based on Cellana's KDF system. The indoor cultivation steps were the same as described in the

Cellana KDF description section. The outdoor cultivation began with 220-L HDPE scale-up bags. The scale-up bags were fed from 20 L carboys located in the indoor cultivation area. The bags were used for one week and had a productivity of 0.06 g/L-day. After use the bags were then recycled for use as part of the construction of the main body of the PBRs. The use phase energy consumption was modeled based on previous work done by this research group (M. K. Weschler et al., 2014) assuming that the scale-up bags operated as PBRs. Nutrient data for all cultivation phases was collected from Cellana and aggregated with nutrient data from literature sources and stoichiometric nutrient requirements for *N. oceanica* found based on Razon and Tan (2011). The bags were harvested every 3 days. Each module contained 17 51-m³ PBRs made of HDPE that were fed from the scale-up bags. The life of the PBRs was 6 weeks and the HDPE used for the PBR was landfilled while the base of the PBR was maintained for future use. The energy data for the PBRs was handled in the same manner as the scale-up bags. The harvesting frequency of the PBRs was 3 days. PBR cultivation was followed by cultivation in 190-m³ open ponds (OP). Each module contained four of these ponds. 750-m³ open ponds followed the 190-m³ ponds. Each module contained 3 750-m³ open ponds. The final cultivation step utilized 1,500-m³ open ponds and each module contained 8 1,500-m³ open ponds. All ponds were lined with Hypalon. The open ponds had a life span of 20 years and a productivity of 16.08 g/m²-day. Energy data was modeled after the open ponds modeled by Lundquist et al. (2010) and Davis et al. (2012); these pond designs have been used for many of the US Department of Energy (DOE) harmonization studies for algae biofuels (Davis et al., 2014a; Davis et al., 2014b). The ponds were harvested every 5 to 7 days and sent to the centrifuge.

Three dewatering scenarios were considered to prepare the algae for valuable product recovery. The first two scenarios include two dewatering technologies; conventional centrifuge followed by the ring dryer and an energy efficient centrifuge followed by the ring dryer. Multiple scenarios were considered due to the effect that dewatering had on the overall environmental impacts. The difference between the centrifuges was the volumetric energy consumption where the conventional centrifuge (MPM scenario) and the energy efficient centrifuges (MPM (LE) scenario) had volumetric energy consumptions of 8 kWh/m³ ((Beal et al., 2015; Gerardo et al., 2015)) and 4.3 kWh/m³ (Evodos, 2013). The data for the first two scenarios were obtained from Cellana and peer reviewed literature. The final dewatering scenario added membrane filtration (MPM (MF) scenario) as a primary harvesting step. Data for the membrane filtration was collected from academic literature (Bhave et al., 2012; Gerardo et al., 2014; Gerardo et al., 2015). The initial concentration into dewatering was 0.42 g/L. The final concentrations of the membrane filtration, centrifuges, and ring dryers were 10, 200 and 950 g/L respectively. The centrifuge required 4 L/month of cleaning chemicals for maintenance. The centrifuges operated at 95% efficiency. The ring dryer had a maximum allowable flowrate of 144 gal/day. The ring dryer consumed 2.5 gal/hr of propane. The ring dryer had an efficiency of 95%. The dried biomass was then sent to oil extraction and refining following dewatering. The dewatering represented the end of what was being done at Cellana at the time of data collection. All operations up to oil refining would take place onsite at Cellana's future commercial-scale facility.

All of the steps following the dewatering step were modeled based on previous work conducted by this research group and data from the academic literature which were

applied to Cellana's model to simulate full commercial-scale production. Hexane oil extraction was modeled based on Soratana et al. (2014). Solvent loss during extraction was assumed to be between 1 and 5%. Oil refining was carried out to separate the omega-3 fatty acids from the remaining oil resulting in an omega-3 fatty acid product with 35% omega-3 fatty acid content and a biofuel product with 10% omega-3 fatty acid content due to inefficient separation.

2.3 Conventional Products Model

A conventional products model, where all products are derived from fish, was developed alongside the algae multiproduct model to compare the environmental impacts of the two. The same functional unit was used for the conventional product model inventory by setting the omega-3 fatty acid production of the conventional products model to the same omega-3 fatty acid production of the algae multiproduct model and making up the differences in feed product and fuel product through allocation using value allocation. Both product models were designed to handle two types of allocation, mass allocation and value allocation. Energy allocation was not considered because two of the three products were not useable energy products (feed and omega-3 fatty acids). Value allocation was the selected method for two reasons; the feasibility of a long term commercial algal biorefinery would be driven by the economic potential of the biorefinery, and the mass allocation would attribute 3% and 13% of the impacts to the omega-3 fatty acids and renewable diesel respectively which were the primary products of interest for the model. Conventional production resulted in a much greater production of feed product than the algae product and the impacts of the additional meal production only were added to the algae multiproduct model results by using value allocation. Using

mass allocation would result in 91% of the impacts of the total conventional product model being allocated to the fishmeal while the omega-3 fatty acids, which represent nearly all of the revenue for the conventional product model was less than 2% of the total mass for the conventional product model. Specific values for the conventional product model can be found in the supporting information accompanying this article. The conventional product model inventory came from a number of peer-reviewed literature sources and results were compared to the life cycle assessment results of other studies. The data for onboard activities, vessel construction, fuel consumption at sea for purse seine fisheries, fish preservation and processing was taken from Cappell et al. (2007), an industry report commissioned by European agency, Department for food and rural affairs (DEFRA), Almeida et al. (2014), and Fréon et al. (2014). The fuel consumption was modeled after purse seine fisheries because this type of fishery was one of the largest operating along the North American gulf coast (Vaughan et al., 2007) and studies showed that they had better fuel efficiency than the overall fuel efficiency for global fishing fleets. The EPA and DHA content of fish ranged from 12.8% to 15.4% and 6.9% to 9.1% the total fish oil (Yin & Sathivel, 2010) respectively. The oil extraction was done using an oil press method described by the UN Food and Agriculture Organization (FAO, 1986) a patent describing oil extraction from fish (Barrier & Rousseau, 2001) with the energy consumption based on a belt filter press (M. K. Weschler et al., 2014). The oil-refining step was the same as the algae oil refining to separate the omega-3 fatty acid product from the biofuel product. Fishmeal was produced simultaneously with the fish oil.

To compare the results between the two product models, both models had the same functional unit (1 MT of omega-3 fatty acids). The oil-refining phase was the same for both models so the renewable diesel production was also the same. The difference between the two models stemmed from the amount of total biomass required to achieve the functional unit. Achieving the functional unit with the conventional product model resulted in significantly more feed product than the algae multiproduct model. This was accounted for by attributing the environmental impacts of that additional feed to the algae using value allocation. The method for producing the fishmeal product (wet press method) also produces fish oil so attributing the entire impacts of the method to the algae model would also add to the omega-3 fatty acid produced. Value allocation was selected over mass allocation because value allocation is more likely to drive decision making in whether or not to operate either system for the production of all three products. Table 5 shows the amount of each product produced per functional unit for both models including the required additional biomass to balance the algae multiproduct model and the conventional product model.

Table 5: Amount of Products Per Functional Unit

	Algae multiproduct model	Conventional product model
Omega-3 fatty acids	1 MT/FU	1 MT/FU
Renewable Diesel	1,444 gal/FU	1,444 gal/FU
Feed intermediate	23 MT/FU	57 MT/FU
Additional feed requirement	34 MT/FU	N/A

2.4 Monte Carlo Analysis

There was a significant amount of variability and uncertainty in both models.

Monte Carlo Analysis (MCA) was carried out to determine the 95% CI for the GWP.

MCA was carried out with the @risk excel add-in software using 500,000 trials to achieve reproducible results for both the algae multiproduct model and the conventional product model. The number of trials was determined by running each model in triplicate at different number of trials starting at 50,000 trials and increasing the number of trials until consistent results were achieved across all three attempts for both models. This was significantly more trials than has been used in many algae biofuel LCAs due to the high variation of fuel efficiencies in the conventional product model and the interaction between the dewatering impacts and the biomass productivity which were both key input variables to the algae multiproduct model. All input variables were defined with triangular distribution. For the two models with the best results the variability was further explored to determine which variables had the greatest influence over the GWP impact category, using tornado plots to determine the difference from the baseline for each individual input variable.

Results & Discussion

The environmental impacts of three algae multiproduct model (MPM) scenarios were compared to the combined impacts of the conventional fish three product model (CPM). The base case MPM scenario was a commercial-scale model based on data from the KDF with standard centrifuge energy consumption based on the academic literature (Beal et al., 2015; Gerardo et al., 2015; Matthew K. Weschler et al., 2014). The MPM scenario with a low energy centrifuge (MPM(LE)) was based on using a commercially available centrifuge with a lower volumetric energy consumption than what was used for the base case MPM. The MPM scenario with the membrane filtration (MPM(MF)) was based on introducing membrane filtration to the base case as a primary dewatering step to alleviate

some of the burden on the centrifuge with a harvesting method with a much lower energy consumption. Figure 12 shows the environmental impacts for all four models normalized to the highest contributor of each category. The “additional fishmeal” category in each chart represents the impacts from the added fishmeal required to balance out the differences in feed products between the algae multiproduct models and the conventional product model as described in Table 5. The conventional product model had the lowest ODP. The ODP of the conventional product model was dominated by the processing of fish oil and fishmeal at the reduction facility (76%), and impacts related to onboard operations including vessel construction and ice production (24%). Changing the dewatering strategy from conventional centrifugation to membrane filtration followed by centrifugation changed the ODP by less than 10%. ODP of the multiproduct model scenarios was dominated by carbon dioxide production for algae cultivation (55-60% of the total ODP) and the production of solvent for lipid extraction (31-35%). Improvements to the ODP for the multiproduct scenarios can be achieved most effectively through minimizing the use of virgin CO₂ for cultivation.

Normalized Environmental Impacts

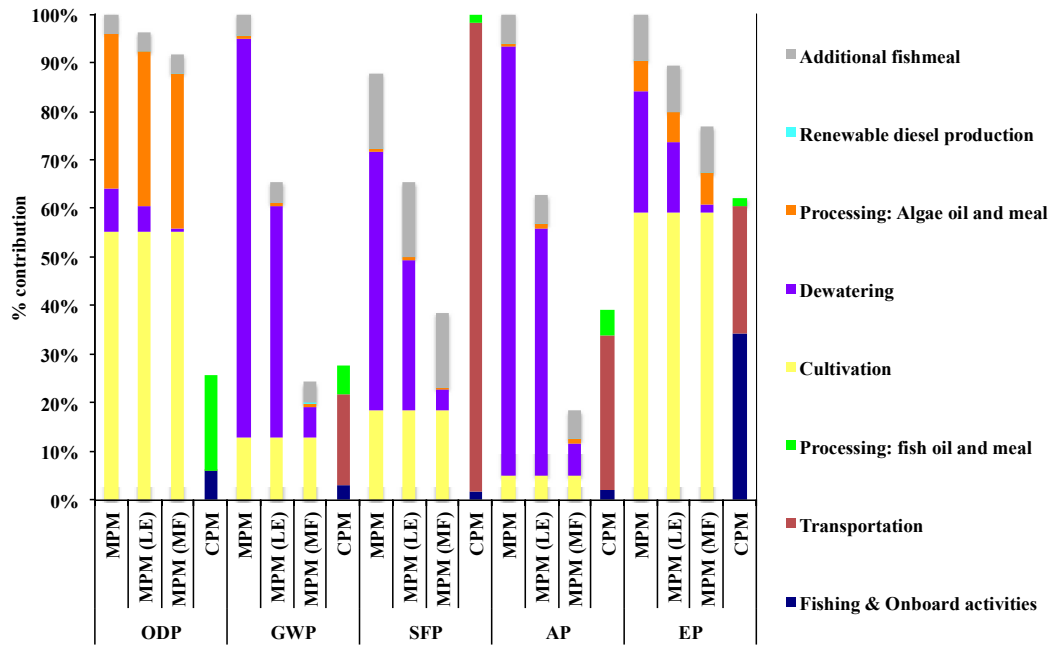


Figure 12: Environmental Impact Comparison
Compares omega-3 fatty acid production for both models. Results normalized to the highest impact in each category.

MPM: Multiproduct Model LE: Low energy centrifuge
MF: Membrane Filtration

The multiproduct model using membrane filtration had the lowest GWP. The top three contributors to GWP for the MPM (MF) scenario were cultivation (54%), dewatering (26%) and additional fishmeal to balance the models (18%). The cultivation phase contribution to GWP was due to the CO₂ and electricity consumption during the cultivation phase with the CO₂ consumption being 88% of the GWP contribution due to cultivation. Reduction in GWP for this scenario can be achieved through reducing the virgin CO₂ consumption and reusing waste CO₂. The conventional product model had the second lowest GWP. The top three contributors to GWP for the conventional product model were fuel consumption for transportation during the fish capture phase (67%), Processing fish oil and meal (21%), and onboard and vessel construction impacts (11%).

The other two multiproduct model scenarios had GWP more than three times higher (for the multiproduct model) and two times higher (for the multiproduct model with low energy centrifuge) than the GWP for the conventional product model. This was due entirely to the centrifuge energy consumption, which was used to dewater algae from 0.5 to 200 g/L in those two scenarios (MPM and MPM(LE)) versus 10 to 200 g/L for the membrane filtration scenario (MPM(MF)). Dewatering represented more than 75% of the GWP for both of those scenarios with 99% of that being due to electricity consumption. Cultivation represented 13 to 20% of the GWP for the multiproduct model scenarios without membrane filtration.

The multiproduct model with membrane filtration had the lowest SFP. The conventional product model had the highest SFP. Cultivation, additional fishmeal and dewatering were the highest contributors to SFP at 48%, 40% and 11% respectively. Cultivation impacts were due to the production of virgin CO₂, dewatering impacts were due to electricity consumption and the additional fishmeal impacts were due to the significantly higher total SFP for the conventional product model compared to the MPM(MF) model. The SFP of the conventional product model was more than double the SFP for the multiproduct model with membrane filtration. The SFP of the conventional products model was dominated by fuel consumption for transportation during fish capturing activities (97%).

The multiproduct model with membrane filtration had the lowest AP. Dewatering (36%), additional fishmeal (33%) and cultivation (27%) dominated AP for this scenario. Cultivation impacts were due mostly to CO₂ production (68%). The conventional product model had an AP more than two times higher than the multiproduct model with

membrane filtration. However, it was less than half of the AP of the multiproduct model base line scenario. Fuel consumption during the transportation for fish capture contributed more than 80% to the AP.

The conventional product model had the lowest EP. Fishing and onboard activities (55%) and transportation for fish capture (43%) dominated the EP. The EP due to fishing and onboard activities was the result of wastewater produced and the production of materials for vessel construction and maintenance. The EP of the conventional product model was 81% of the multiproduct model with membrane filtration. The multiproduct model with membrane filtration was dominated by cultivation (77%) followed by additional fishmeal (13%). Cultivation also dominated the other two multiproduct models (60-66%) but dewatering was the second largest contributor (16-25%) due to the much larger electricity consumption for dewatering. Despite the large increase in dewatering electricity consumption, the EP for the multiproduct model with membrane filtration scenario was 77% of the MPM base case scenario.

Dewatering and cultivation dominated the algae impacts, while fuel consumption during fishing activities dominated the conventional products model. Centrifugation was the largest contributor to dewatering impacts in most categories for MPM scenarios without membrane filtration. The exception was for ODP where the production of propane was the largest contributor to dewatering in that category. CO₂ production, consumption and electricity consumption were the primary contributors to impacts related to cultivation. Membrane filtration as a primary harvesting step reduced the environmental impacts related to GWP, SFP and AP by at least 25% but had little influence on ODP and EP. The low energy centrifuge significantly reduced the impacts of

all categories except ODP when compared to the baseline MPM scenario. In order for the environmental impacts of the algae multiproduct biorefinery to be competitive with conventional production, membrane filtration or some other primary harvesting would be necessary to reduce the required electricity consumption for centrifugation.

Environmental impacts due to cultivation were largely the result of CO₂ production and consumption for algae cultivation where CO₂ consumption was the primary contributor to GWP, the production of virgin CO₂ was the largest contributors to EP and ODP. The CO₂ consumption for cultivation can be reduced by reusing CO₂ from waste resources such as flue gas from power plants (Soratana et al., 2013) instead of using virgin CO₂. This strategy would require the algae biorefinery to acquire such an agreement with a nearby power plant to provide sufficient quantities of flue gas. For the CPM model fuel consumption was the highest contributor to GWP (67%), SFP (97%) and AP (82%), and the second highest contributor to EP (42.5%).

Normalized GWP impacts

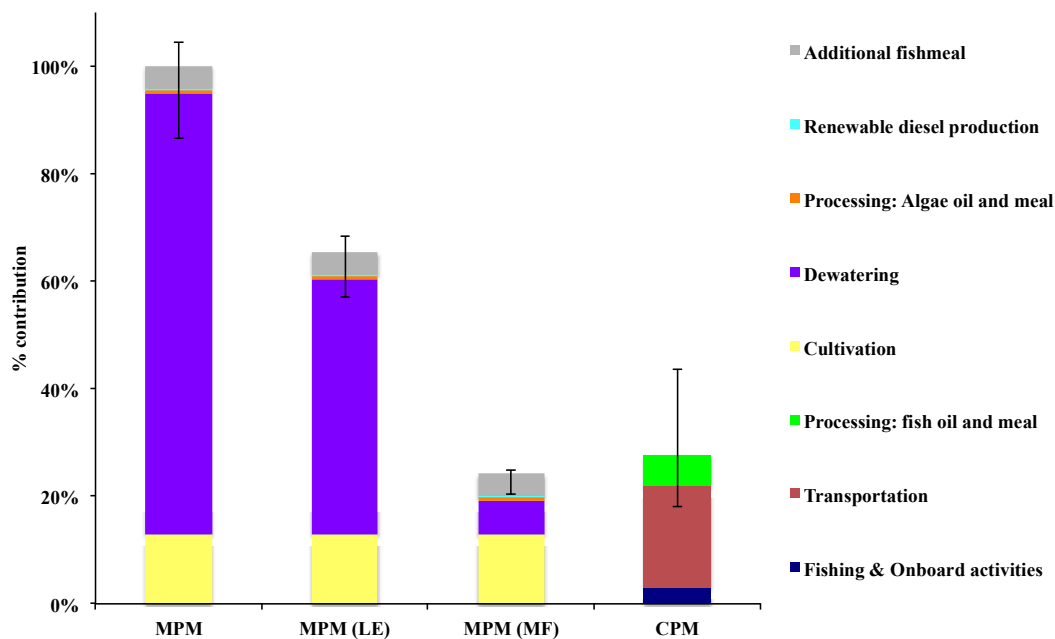


Figure 13: Normalized GWP Impacts
 MPM: Multiproduct Model LE: Low energy centrifuge
 MF: Membrane Filtration

Figure 13 shows the global warming potential normalized to the highest contributor with the 95-percentile results for each scenario. The conventional product model had the highest variability. Figure 14 shows the sensitivity analysis for the normalized GWP for the conventional products model. The fleet fuel consumption can cause up to an 80% change in the GWP from its baseline. The fuel consumptions from a number of fishery studies were presented in the supporting information. The fleet fuel consumption was based only on purse seine fishing fleets; the type most likely to be used for the capture of fish for fish oil and meal production in the U.S. from the Gulf Menhaden and Peru from the Peruvian Anchoveta and also one of the most fuel efficient fishing methods (Avadí et al., 2014; Vaughan et al., 2007). The range of fuel consumption depends on a number of

factors, some of which are beyond the control of human operators such as fish availability in a specific region and weather conditions.

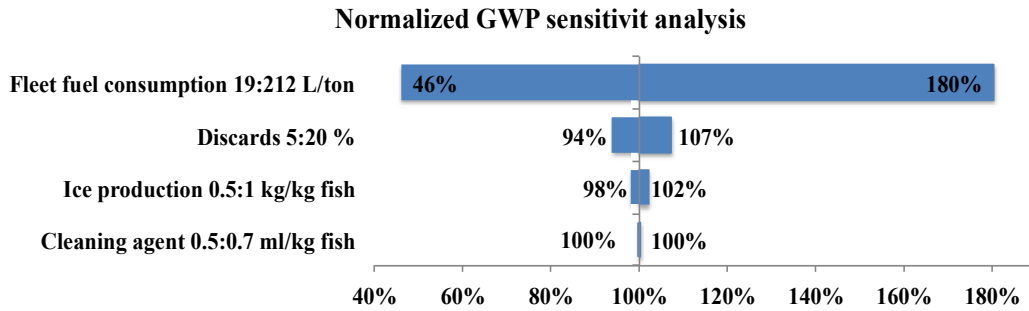


Figure 14: Normalized GWP Tornado Plot for CPM Model

The membrane filtration scenario of the multiproduct model had the lowest variability and was primarily the result of the membrane filtration electricity and the algal biomass productivity. Figure 15 shows the sensitivity analysis of the membrane filtration scenario and the conventional product model. At the lowest fuel consumption requirements and the lowest membrane filtration energy consumption the CPM and the MPM (MF) scenarios were nearly equal. Membrane filtration was identified as a promising dewatering method for commercial-scale applications by the National Alliance for Advanced Biofuels and Bioproducts (NAABB). Dissolved air flotation was another option for reducing the energy consumption for dewatering; this method was used by the DOE harmonization studies (Davis et al., 2014a) but was not considered here due to the unknown influence a chemical flocculant required for dissolved air flotation would have on the omega-3 fatty acid quality or the oil refining process.

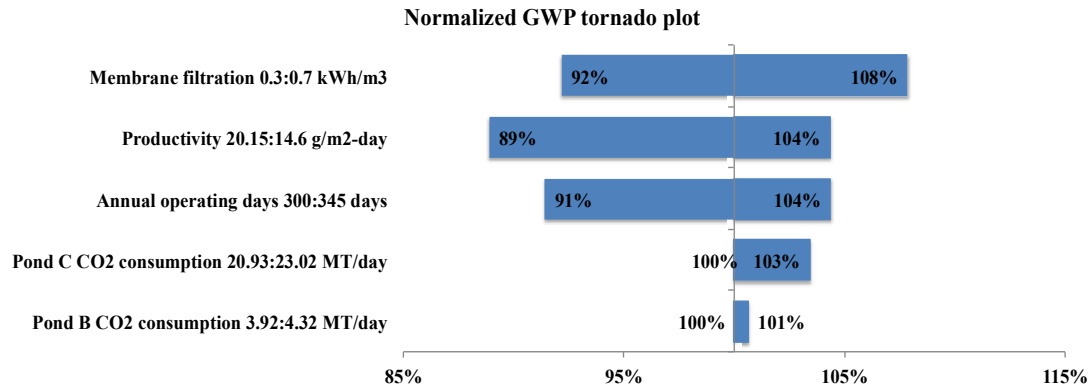


Figure 15: Normalized GWP Tornado Plot for MPM (MF) Model

The MPM scenarios involving only centrifugation for algae harvesting (MPM and MPM (LE)) had higher variability than the membrane filtration scenario but lower variability than the conventional product model. The variability in these scenarios was due to the algal biomass productivity and the CO₂ consumption in the ponds for cultivation. The lowest GWP of the two centrifuge only scenarios would not be as low as the baseline GWP for the conventional product model. Future endeavors for achieving a commercial algae multiproduct biorefinery should focus on membrane filtration, algae biomass productivity and CO₂ uptake efficiency. Aspects of the membrane filtration that require consideration include the electricity consumption, membrane fouling, cleaning and replacement, and maximum attainable final concentration.

Conclusion

The algae multiproduct model biorefinery that employ membrane filtration as a primary harvesting step prior to centrifugation have equivalent or better environmental impacts than the same suite of conventional products derived from fish. However, if centrifugation is the only harvesting step then the GWP and AP can be more than double the impacts of the conventional products. The dewatering differences in dewatering for

the multiproduct model scenarios does not have enough impact on ODP or EP to reduce them to the level of the conventional product model. The top three contributors to the environmental impacts associated with the algae multiproduct model were the dewatering method, the algae biomass productivity and the CO₂ consumption for algae cultivation. The electricity consumption of the centrifuge alone makes using that unit process as the first harvesting step more environmentally burdensome than conventional fish production. There are many dewatering options available for algae bioproducts but the centrifuge has consistently been one of the most commonly cited methods because of its reliability, the commercial maturity of the technology (i.e. it is used extensively in wastewater treatment) and high dewatering capability without the need for additives. The DOE harmonization studies utilize a centrifuge for downstream dewatering after dissolved air flotation, but the use of a chemical flocculant may affect the quality of n-3 and so this dewatering method was not considered in this study (Davis et al., 2014a). As an alternative, membrane filtration was a harvesting technology that does not require a chemical flocculant that could serve as a sufficient primary harvesting technique; the use of membrane filtration reduced the GWP of the algae multiproduct model by 79% of the base case algae multiproduct model. The algae biomass productivity was also a key variable influencing environmental impacts. Exploring options for capturing and refining waste CO₂ from flue gas can reduce CO₂ consumption for algae cultivation (Rickman et al., 2013; Venteris et al., 2014). The results of this study have identified a number of key variables for exploring improvements to the environmental impacts of the algae multiproduct model; including the need for a dewatering method without chemical additives, alternative CO₂ sources and biomass productivity. The results of this study

have further indicated that the environmental impacts of an algae multiproduct biorefinery have the potential to be comparable to the production of their conventional counterparts.

The ability to enhance the lipid, EPA and DHA content of the algae could also greatly improve the overall environmental impacts. It has already been noted in the academic literature that lipid content of algae can be increased through nutrient deprivation, but nutrient deprivation has a significant decrease in the overall biomass production (Lardon et al., 2009), and omega-3 content (Guihéneuf & Stengel, 2015). While the dewatering method had the largest influence on the results, operators should consider methods to increase the productivity and accurately determining how nutrient loading impacts both total lipid content and omega-3 fatty acid content.

Based on the results of this model, a commercial algae multiproduct model has the potential to have similar environmental impacts to conventional non-renewable production as long as a suitable low energy dewatering technology can be implemented. Algae n-3 represents an alternative source to the growing global n-3 market that would alleviate the pressure on the already highly stressed, and in some cases overexploited fisheries.

CHAPTER 4

ECONOMIC SUSTAINABILITY ASSESSMENT OF A COMMERCIAL-SCALE ALGAE MULTIPRODUCT BIOREFINERY

This chapter addresses research question #2: How can high value algal bioproducts best support algal biofuel commercialization to meet government mandates and standards?

Introduction

Algae based omega-3 fatty acids (n-3) have the potential to be an economically viable product, while simultaneously alleviating the pressure on at risk wild caught fisheries globally to meet increasing n-3 demands. Omega-3 fatty acids most commonly referred to as fish oils, are a class of long-chain polyunsaturated fatty acids (LC-PUFA) that medical research has shown to have numerous health benefits including cardiovascular and brain structure health (Campoy et al., 2012; Diaz-Castro et al., 2015; Janssen & Kiliaan, 2014; Kris-Etherton et al., 2002; Simopoulos, 1991). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the two primary n-3s that have significant benefits to human health (Simopoulos, 1991). Wild caught fish are rich in n-3 and provide most of the n-3 globally for both human and aquaculture consumption (FAO, 2014).

Though algae has been used for aquaculture feed (Chauton et al., 2015), there is also potential to use algae as a direct source of n-3 for human consumption. A number of different algal strains can be used to grow omega-3 fatty acids including *Nannochloropsis*

sp., *Phaeodactylum* sp. and *Porphyidium* sp. In phototrophic algal species the n-3s are present as a part of the membrane lipids, while in heterotrophic species the n-3s accumulate in the triglycerides (Ward & Singh, 2005). The share of aquaculture in world fish production increased by nearly 20% from 2006 to 2012 and represented ~50% of fish for human consumption globally. This is projected to increase to 62% by 2030 (FAO, 2014). Wild caught fish fulfill global omega-3 fatty acid demands for both direct human consumption and aquaculture. The goal of this study was to assess the economic potential of a commercially relevant algal biorefinery designed for the production of omega-3 fatty acids for direct human consumption.

Algal omega-3 fatty acids have been used for direct human consumption for years but in limited situations. There are significant differences between the consumption of fish based and algae based omega-3 fatty acids that influence the commercial application and price of both. According to the Global Organization for EPA and DHA (GOED) (Ismail, 2013), based on a report commissioned by Frost and Sullivan in 2012, 75% of algal DHA was used for infant formula. The GOED also reported that algae's global contribution to omega-3 fatty acids for human consumption was 0.2%, most of which used algae grown under heterotrophic conditions. Algal omega-3 fatty acids have the potential to support global omega-3 fatty acids to a greater degree and provide omega-3 in a preferred form to conventional n-3 for some consumer segments. Some consumers complain of fishy burps when taking fish based omega-3 fatty acid supplements. Vegetarian and vegan consumers represent a niche market for algae where conventional fish products and even other alternatives such as Krill do not compete. There is significant market potential for algae-derived omega-3 fatty acids.

The use of algae for aquaculture is a mature technology and has become more important in recent years due to increases in fish demand coupled with stagnant wild-caught fish production (Camacho-Rodríguez et al., 2015; Chen et al., 2016; Maisashvili et al., 2015; Sukenik et al., 1993). One advantage of using algae for aquaculture over direct human consumption is that less unit processes are required than in other applications that require energy intensive dewatering and other downstream processing (Chauton et al., 2015). However, using algae for aquaculture precludes the capture of other potentially environmentally beneficial products such as biofuels. After recovering omega-3 fatty acids, a significant portion of the biomass can still be used as a concentrated feed product for both aquaculture and agriculture applications. The successful development of high-value algae products could support the development of other algae products that do not have the same economic potential such as greater quantities of high protein aquaculture feed and biofuel.

A number of techno-economic analyses (TEA) have been done for algae biorefineries that produce biofuels as the main product and in some cases include agricultural feed as a co-product but no studies include omega-3 fatty acids for direct human consumption as part of their analyses. Beal et al. (2015) and Huntley et al. (2015) are companion LCA/TEA studies based on Cellana's Kona Demonstration Facility (KDF) in Kona, HI for the production of algal biofuels. These studies explored the potential for the production of diatoms based on an existing facility and multiple theoretical cases that represented improvements in the environmental impacts and reductions in the total cost of production. While these studies were also based on Cellana LLC's KDF, there are some key differences between those studies and this study; the

species they explored do not produce significant quantities of omega-3 fatty acids but they do settle naturally providing for much different dewatering requirements. Their studies focused solely on biofuel intermediate and agriculture feed co-product generation, while the study presented here focuses on omega-3 fatty acids but also includes biofuel, aquaculture feed and downstream fuel processing.

A number of TEA studies have been produced using the Farm-level Algae risk model developed at Texas A&M (Richardson & Johnson, 2014, 2015; Richardson et al., 2014a; Richardson et al., 2014b). These studies focused on a head to head financial comparison of open pond systems to photobioreactor (PBR) systems, reducing costs through the use of alternative harvesting technologies and extraction techniques, and identifying scenarios where algal biofuels had a high probability (greater than 95%) of success based on biomass production rate, lipid content, capital expenses (CAPEX) and operating expenses (OPEX). The U.S. Department of Energy (DOE) has also published a number of studies based on their harmonization models and the accompanying report (Davis et al., 2012) to standardize and link LCA, TEA and resource assessment (RA). (Davis et al., 2011; Davis et al., 2012; Davis et al., 2014a; Davis et al., 2014b). While there have been a number of studies related to algal biofuels to date, the only TEA focused on algal omega-3 fatty acid production was Chauton et al. (2015) and that study was concerned with algae as a source of n-3 for aquaculture and not for direct human consumption.

Numerous theoretical TEA studies have been conducted on algal biofuels and their co-products. Despite a plethora of advances in algae production, commercial viability of algal biofuels has yet to be achieved due to the energy consumption, cost of nutrients and the low cost of conventional fuels. This has led to greater interest in producing high value

products from algae some of which are already commercially available such as DHA from heterotrophic algae and astaxanthin, and other products with market potential such as omega-3 fatty acids from phototrophic algae (DOE, 2010). Commercial success of high-value algae bioproducts could open up new opportunities for algal biofuels and other products that have positive environmental benefits.

This study used TEA to assess the commercial viability of producing algal n-3 for direct human consumption based on Cellana's KDF using *N. oceanica*. The KDF is a pilot-scale facility capable of 750 m³ of cultivation. In addition to the production of omega-3 fatty acids, the remaining biomass can be used for the production of 3 other products: biofuel, aquaculture feed and animal feed which were included in the TEA. This study represents one of the first TEAs of algal omega-3 fatty acid production based on data at a large-scale facility.

Methods

This study used TEA to quantify the costs of producing omega-3 fatty acids, green diesel and high-protein feed from autotrophic microalgae based on Cellana LLC's KDF. The TEA herein was performed in a similar manner to previous TEAs used for the DOE algae harmonization studies (Davis et al., 2011). The first step in the TEA was to create a process model for the commercial algal biorefinery system. Next, capital costs, labor and operating costs were collected for the input variables in the process model based on data provided by Cellana, data from the academic literature and prices from online vendors. After all of the costs and revenue items were collected a financial analysis was carried out to calculate the total capital expenses (CAPEX), operating expenses (OPEX) and the required selling price to meet the total cost of production for the algal biorefinery. After

the costs were calculated a sensitivity analysis and Monte Carlo Analysis were carried out for each scenario to account for variability and uncertainty.

2.2 Process model and KDF description

The KDF has been in operation since 2009 and uses their patented ALDUO system (Huntley et al., 1996; Huntley & Redalje, 2010). In the ALDUO system, PBRs were first used for quickly concentrating the biomass while minimizing contamination, followed by open ponds for large-scale production using short runtimes to limit contamination. Following cultivation, lipid extraction using hexane separates the lipids from the remaining biomass. Further refining takes place to separate the omega-3 fatty acids from the total fatty acids based on Chakraborty and Joseph (2015).

Cellana's KDF operates at pilot-scale; it is on 2.5 ha with 750 m³ of cultivation capacity. However the KDF pilot was designed as a modular system. So a commercial-scale model was designed by scaling up versions of their cultivation and dewatering modules. The commercial-scale facility was assumed to be on a 112 ha site with a production of 120 MT of omega-3 fatty acids annually. This size commercial facility alone could produce 10% of the omega-3 fatty acid production from algae in 2012 based on data from the GOED (Ismail, 2013). Figure 16 shows the process flow diagram for the commercial-scale model. The detailed assumptions for the scaled-up model can be found in the supporting information. Most unit processes were scaled-up less than 10 times with the exceptions being the medium ponds (12.5 times scale-up from KDF) and the large ponds (25 times scale-up).

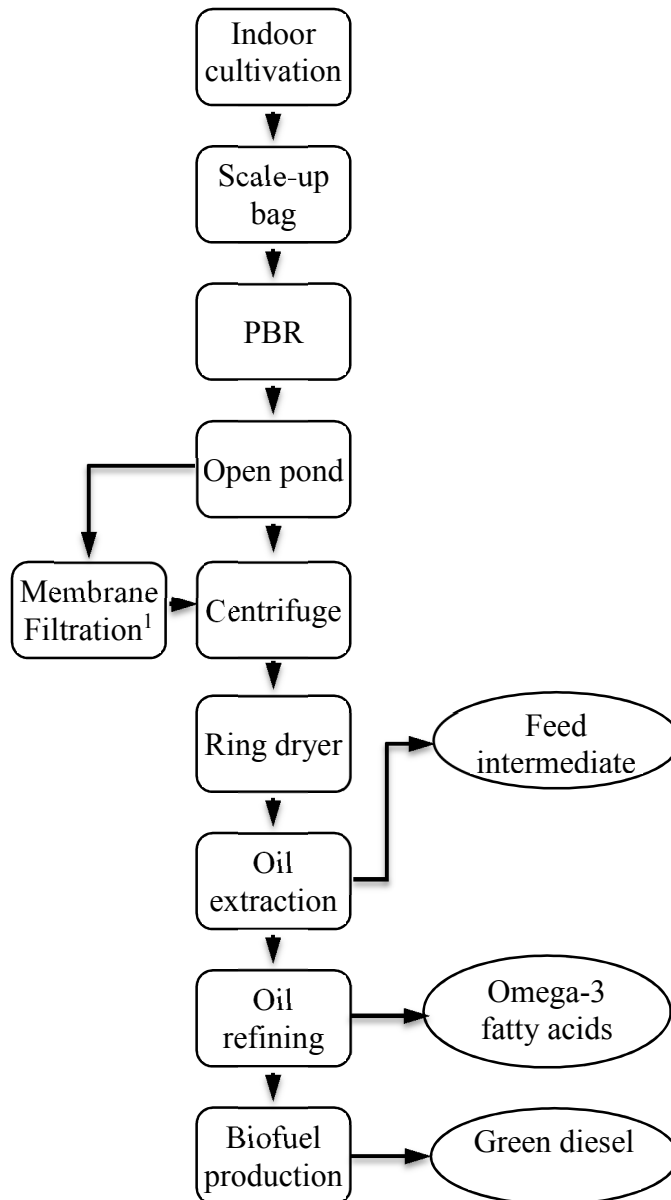


Figure 16: Multiproduct Model (MPM) Process Flow Diagram

1: Membrane filtration is used in the membrane filtration scenario of the commercial scale model only and was not in use at the KDF.

The commercial production facility was assumed to be 112-hectares and contained eight of the KDF cultivation modules. Each module contained seventeen 51-m³ PBRs, four 188-m³ open ponds, three 750-m³ open ponds and eight 1,500-m³ open ponds.

The strain that Cellana uses for their cultivation is *N. oceanica*. This strain was selected because it is native to Hawaii and has been known to accumulate lipids, specifically

omega-3 fatty acids. Cultivation began in a laboratory setting. The consumption of water, electricity and nutrients were negligible at that stage. The first step of outdoor production was 220-L scale-up bags used to inoculate the 51 m³ PBRs to begin large-scale cultivation using the ALDUO system. The facility productivity ranged from 14.6 to 20 g/m²-day with a best estimate of 16 g/m²-day. The minimum productivity was the average productivity in the harmonization model (Davis et al., 2014a), the maximum productivity was the value that resulted in the final pond concentration of 0.5 g/L, which was identified by Cellana as the maximum pond concentration during data collection, and the best estimate was the average productivity at the KDF.

Cellana used a two-step dewatering process involving centrifugation to 20% w/w biomass and drying to 90% w/w biomass. Centrifuge data from Cellana was aggregated with data based on a commercial-scale case study of decanter centrifuges (FSA consulting, 2002). Each centrifuge had a capacity of 267,000 gallons per day and efficiency range from 95 to 99%. The electricity requirement of the centrifuges was 8 kWh/m³. Propane consumption for drying was 0.05 kg/kg sludge based on the propane consumption at Cellana's KDF and the electricity consumption of the dryer was 0.27 MJ/kg of solids (Beal et al., 2015).

The downstream processing was not carried out at the KDF, but at commercial-scale, Cellana expects to perform downstream processing onsite. Downstream processing- including oil extraction, n-3 recovery and production of green diesel- were modeled based on other studies published by the authors and other peer-reviewed literature. Oil extraction was carried out using hexane extraction followed by phase separation and solvent recovery via distillation. The extracted lipids undergo further refining to recover

n-3, and the remaining lipid extracted algae (LEA), which was sold as a high-protein aquaculture feed. Oil refining involved separating the omega-3 fatty acids from the TFA. The remaining TFA was deemed suitable for green diesel production which was modeled based on Huo et al. (2008).

2.2 Data Collection

Authors collected data from Cellana for the development of the process model and the TEA model. Cellana provided data from onsite measurements, manufacturers and vendors. Additionally for each data point a confidence level was provided from one to five with one representing a guess and five representing an exact quote or measurement. The data included sources such as manufacturers, vendors and onsite measurements, a confidence level based on how often the data was recorded or when estimations were required. For data points where there was variability in the measurements, Cellana provided a range of data points using the aforementioned confidence level system. The data collected from Cellana was incorporated into each phase of the TEA model development and provided in the supporting information. Data collection was an iterative process, which aimed to collect only high quality, accurate data; by the end of the data acquisition all onsite and offsite data collected from Cellana that was used in the TEA had a confidence rating of five. In order to protect proprietary information, some Cellana datasets were aggregated with other data collected from peer-reviewed literature, technical reports and vendors; these are noted in the SI with both Cellana and the source that it was aggregated with. The cost of individual chemicals, utilities, capital expenses and labor estimates were also collected from vendors and the academic literature. All input data for the process model and financial model is presented in the SI.

2.3 Cost Engineering

The financial parameters for the TEA model are presented in Table 6. The outside battery limits (OSBL) costs were assumed to be 15% of the inside battery limits (ISBL). The Project costs were assumed to be 15% of the ISBL+OSBL. The depreciation rate was calculated based on the life expectancy of the plant (20 years), which is the life expectancy of the open ponds and the dewatering unit processes in use at the KDF. The insurance and property tax, and the maintenance and materials were identified from a publication resulting from the DOE harmonization studies (Davis et al., 2014a) so that the results of presented here can be easily compared to that model future algal biofuels and bioproducts studies that use the DOE harmonization studies versus using a variable that is highly dependent on highly variable and potentially negotiable values. The working capital was assumed to be 4 months of fixed operating costs and came out to 3% of the plant capital. Cellana validated assumptions for the financial parameters for all financial parameters presented in Table 6. The values not taken from the harmonization model were similar to the values used in the harmonization model.

Table 6: Financial Parameters

ISBL: Inside battery limits	OSBL: Outside battery limits
OSBL	15% of ISBL
Project costs	15% of the ISBL+OSBL
Depreciation rate	5% per year
Insurance and property Tax	0.7% of total capital investment
Maintenance and materials	3% of total capital investment
Working capital	3% of total capital investment

Cellana provided the capital costs for many of the unit processes based on the unit processes at the KDF and the remaining capital costs were collected from the academic literature and online vendors. The specific values for capital costs are available in the

supporting information. Land costs and pond construction were taken from the academic literature for algal biofuels (Beal et al., 2015; Davis et al., 2014a) best estimate was the average of those two sources. The capital costs of cultivation included Hypalon™ (pond liner) and pond installation can be found in the SI. The centrifuges had a 20-year life and the capital cost was based on data for commercial-scale centrifuges found in the academic literature and industry case studies (FSA, 2002). The ring dryers also had a 20 year life and the capital cost was based on Beal et al. (2015). Cellana provided estimates for the capital costs of oil extraction and refining equipment at commercial scale. Details for the process model and the capital costs are available in the supporting information.

The operating costs for each unit process was based on costs available from vendors. The operating costs for cultivation included the cost of nutrients, electricity, synthetic CO₂, water, and HDPE for the PBR and scale-up bags. The HDPE was included in the operating costs because the low life expectancy of the PBRs (6 weeks) and scale-up bags (3 months). The HDPE for the PBRs was recycled for use in the scale-up bags. The operating costs for the dewatering were electricity, propane (ring dryer) and chemicals for maintenance. The operating costs for the oil extraction and refining was electricity and chemicals. The cost of electricity was the average cost of electricity for industrial use in 2015. Data for all operating costs are presented in the SI of this manuscript with citations for the academic literature and vendors.

Labor requirements were collected from Cellana and salaries for each position were collected from the Bureau of Labor Statistics (BLS). Authors evaluated Cellana's current labor at the KDF, and interviewed Cellana employees to determine how many positions would be required to operate the commercial-scale facility. Based on those interviews,

the operational staff requirements were determined to be 1.02 ha/operator, 1.78 ha/non-operations staff and 0.65 ha/total number of employees. The salary of each position is presented in the SI of this manuscript.

2.4 Financial analysis

Monte Carlo Analysis (MCA) was carried out to determine the 95% CI for the annual cost of production and the capital cost. The @risk excel add-in software with 150,000 trials was used to achieve reproducible results for all scenarios. All input variables were defined with triangular distribution (min, best estimate, max). The parameters for the sensitivity analysis are provided in the SI of this manuscript. A sensitivity analysis was carried out for each individual input parameter used in the MCA to generate tornado plots for the total cost of production to assess the influence each individual variable had on the best estimate for the total cost of production

Authors hypothesized based on previous research, that the two largest contributors to variable operating costs were likely to be CO₂ consumption and electricity consumption. CO₂ was predicted to be a high cost because many studies have noted the significant cost and cost variance of CO₂ depending on the source of CO₂ (flue gas with a low CO₂ % or other industries such as cement plants that provide nearly pure CO₂ gas) (Beal et al., 2015; Davis et al., 2011; Richardson et al., 2012). The high electricity consumption for algae has been identified as a major contributor to operating costs as well (Beal et al., 2015; Harun et al., 2011). Dewatering was considered by designing three dewatering scenarios to quantify the potential for improvements to reduce the variable operating costs. The first scenario was the base case scenario and used unit processes currently in use at the KDF. The second scenario, called the low energy centrifuge multiproduct

model, evaluated improvements to the base case scenario by reducing the electricity consumption of the centrifuge from 8 kWh/m³ to 4.3 kWh/m³. This improvement to electricity consumption was based on capabilities of an existing low energy pilot-scale centrifuge (Evodos, 2013). The base case and low energy centrifuge multiproduct models use all of the same processes with the energy consumption being the only modification. This modification would lead to a change in the variable cost of production that would overshadow the influence of all other sensitivity analysis parameters, and so was considered as a separate scenario. The third scenario, called the membrane filtration multiproduct model, improved on the base case scenario by introducing membrane filtration as a primary harvesting step before centrifugation. The membrane filtration material was polyvinylidene fluoride (PVDF). The lifespan of the membrane filtration units was 10 years (Cote et al., 2012). Capital and operating costs for the membrane filtration were derived from an industry report for a membrane filtration system installed by the Pall corporation for water treatment in Ingleside, Texas (1998). A scaling factor of 0.6 was used to scale the membrane filtration for this model down to account for the difference between the capacity required for this model and the capacity of the membrane filtration system for the case study.

Results & Discussion

3.1 Technoeconomic analysis

To determine the economic feasibility of producing n-3, algal biorefineries would have to obtain a selling price that can meet their required return on capital (RRC). Omega-3 fatty acid selling prices were calculated as a function of the RRC with all operational variables and other financial parameters set at the baseline. Table 7 shows the omega-3 fatty acid

selling price for all scenarios that would meet the different RRCs from 0 to 30%. 0% is not a realistic RRC but was used to look at the minimum price where the annual operating cost was equal to the selling price. The selling price of n-3 produced using the membrane filtration scenario was lower than the centrifuge scenarios at all RRCs. The retail selling prices in April 2016 of omega-3 fatty acids was \$256/kg of omega-3 (EPA+DHA only) for Nature Made™ fish oil from Walgreens.

Table 7: Break-even Selling Price:

The prices calculated were the break even selling price to meet each required return on capital (RRC) in \$/kg omega-3 fatty acids

MPM: Multiproduct Model LE: Low energy centrifuge

MF: Membrane Filtration

RRC	MPM \$/kg	MPM (LE) \$/kg	MPM (MF) \$/kg
0%	\$133 (113-141)	\$113 (99-124)	\$102 (87-112)
10%	\$189 (164-209)	\$170 (143-188)	\$160 (136-179)
20%	\$245 (215-276)	\$226 (188-243)	\$218 (180-245)
30%	\$302 (2268-344)	\$251 (232-306)	\$275 (233-312)

Figure 17 shows the annual cost of production for each required return on capital (RRC). The CAPEX was higher than the OPEX whenever there was a required return on capital greater than 10%. The CAPEX and OPEX were slightly lower for the membrane filtration scenarios than the centrifuge scenarios. The variability of each scenario

increased as the RRC increased, indicating that CAPEX had a greater influence on variability than OPEX. The variability in CAPEX was represented by variability in capital costs and the capacity of different unit processes. The variability in the OPEX was governed by the variability in material and electricity consumption and the variability in the cost of consumables. While the membrane filtration system as a whole had a life of 10 years replacing the membrane material was considered within OPEX.

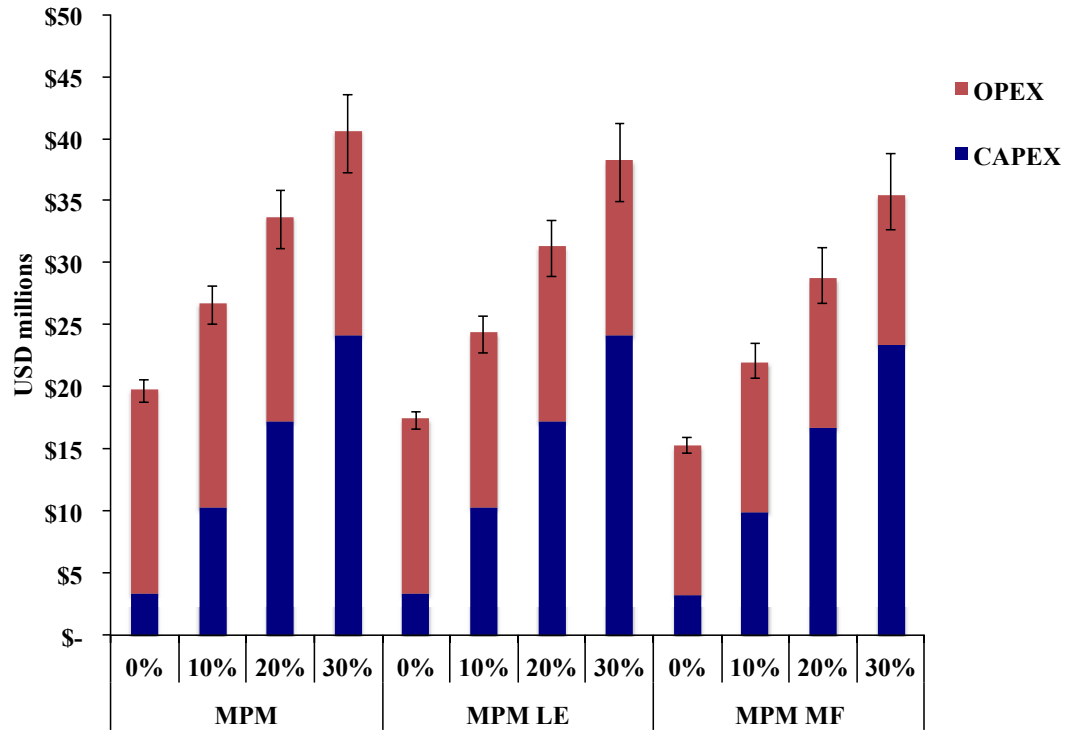


Figure 17: Multiproduct Model Cost of Production Required Return of Capital. The error bars show the 95% CI for each scenario
 MPM: Multiproduct Model LE: Low energy centrifuge
 MF: Membrane Filtration

The annual operating cost can be described in terms of variable operating expenses (raw materials and utilities), fixed operating expenses (labor, overhead, taxes), depreciation on capital equipment and non-depreciable items (land and working capital). Figure 18 shows the annual operating costs normalized to the highest cost scenario. The base case commercial-scale multiproduct model (MPM) was the closest scenario to the existing KDF and had the highest operating cost. The MPM with a low energy centrifuge (MPM (LE)) and with membrane filtration (MPM (MF)) were two improvements based on commercial technology available today and resulted in cost reductions of 9 and 23% respectively. Total fixed costs, which included labor and overhead, represented 49% of each scenario. The variable costs (utilities and raw materials) represented 32, 21, and

11% of the MPM, MPM (LE) and MPM (MF) scenarios respectively. The total fixed costs were the largest contributors to all scenarios. The utilities (water and electricity) were the second largest contributor in the MPM scenario, the third largest contributor in the MPM (LE) and MPM (MF) scenarios. The low energy centrifuge (MPM (LE)) and membrane filtration (MPM (MF)) scenarios both represent cost reductions to the base case multiproduct model (MPM) annual production costs. Membrane filtration represented a significantly greater cost reduction to the annual operating costs than the low energy centrifuge.

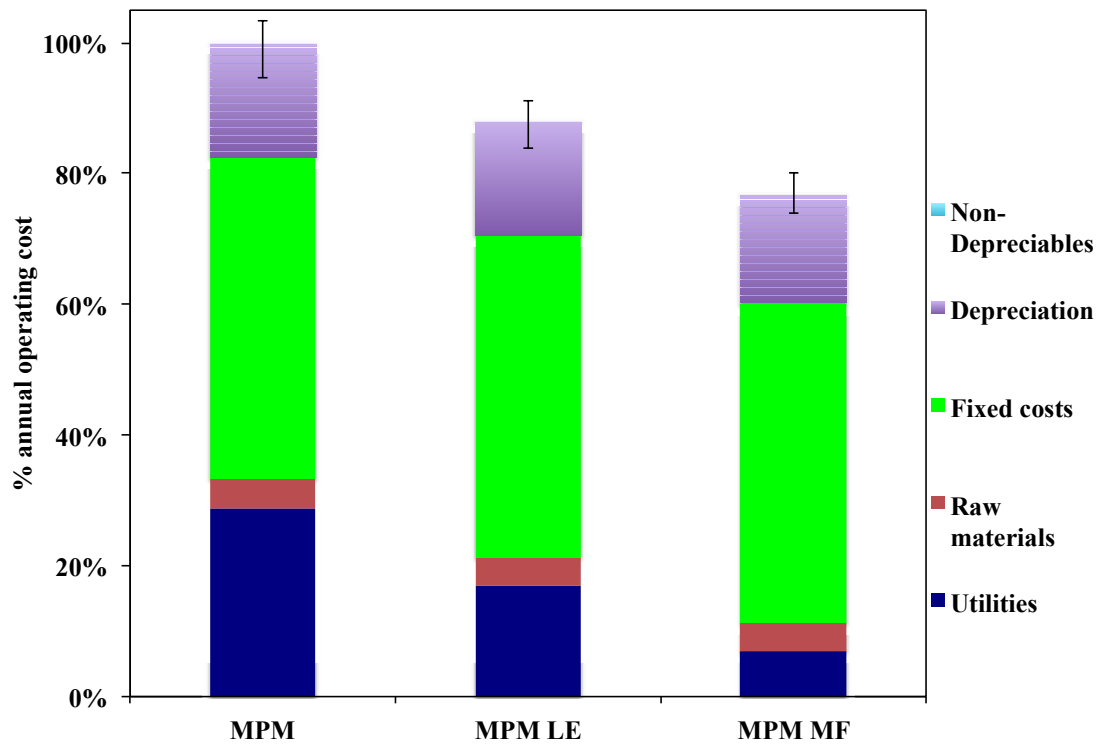


Figure 18: Annual Operating Cost
 MPM: Multiproduct Model
 LE: Low energy centrifuge
 MF: Membrane Filtration

Figure 19 shows the details for the variable operating costs (utilities and raw materials). The Centrifuge electricity was the largest contributor to variable costs for the base case scenario but was just over 10% of the variable costs for the membrane filtration scenario. CO₂, hexane and seawater were significant costs in both models but contributed more to the total variable costs of the membrane filtration scenario than the base case scenario. Improvements in water consumption/recycling and chemical consumption for extraction would have a significant impact on reducing costs in the membrane filtration scenario, but these changes would be less noticeable for the centrifuge only scenarios.

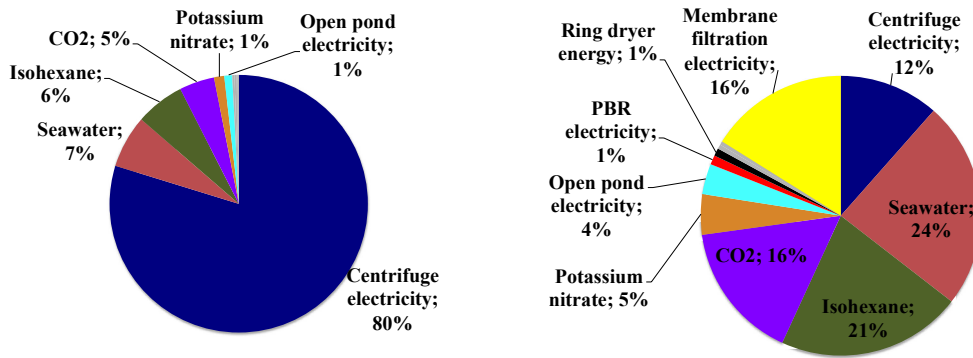


Figure 19: Variable Costs Breakdown
 Base case MPM on the left and MPM-MF on the right
 Does not include labor, depreciation and non-depreciable costs.

Figure 20 shows the capital costs for each scenario. MPM (MF) had the lowest capital cost while the other two scenarios had the same capital cost. Introducing membrane filtration reduced the total capital investment by 4%. Pond liner was the largest contributor to the capital cost at 31% of the total capital cost followed by the oil processing. The pond liner was considered separately from the ponds and installation because while the pond liner improves performance, the ponds could be operated without the pond liner. Oil processing included oil extraction and refining to separate omega-3

fatty acids from the total lipids. The total capital investment related to the ponds overall was 44% of the total capital investment for all scenarios. In the centrifuge only scenarios, the centrifuge represented 16% of the capital cost while but decreased to 1% for the membrane filtration scenarios. This was due to the membrane filtration handling the majority of the volumetric load and reducing the number of required centrifuge units significantly. The membrane filtration represented 11% of the scenario where it was the primary harvesting step. While membrane filtration represented a small decrease in capital cost, this result compounds with the operating costs where membrane filtration also resulted in a significant decrease.

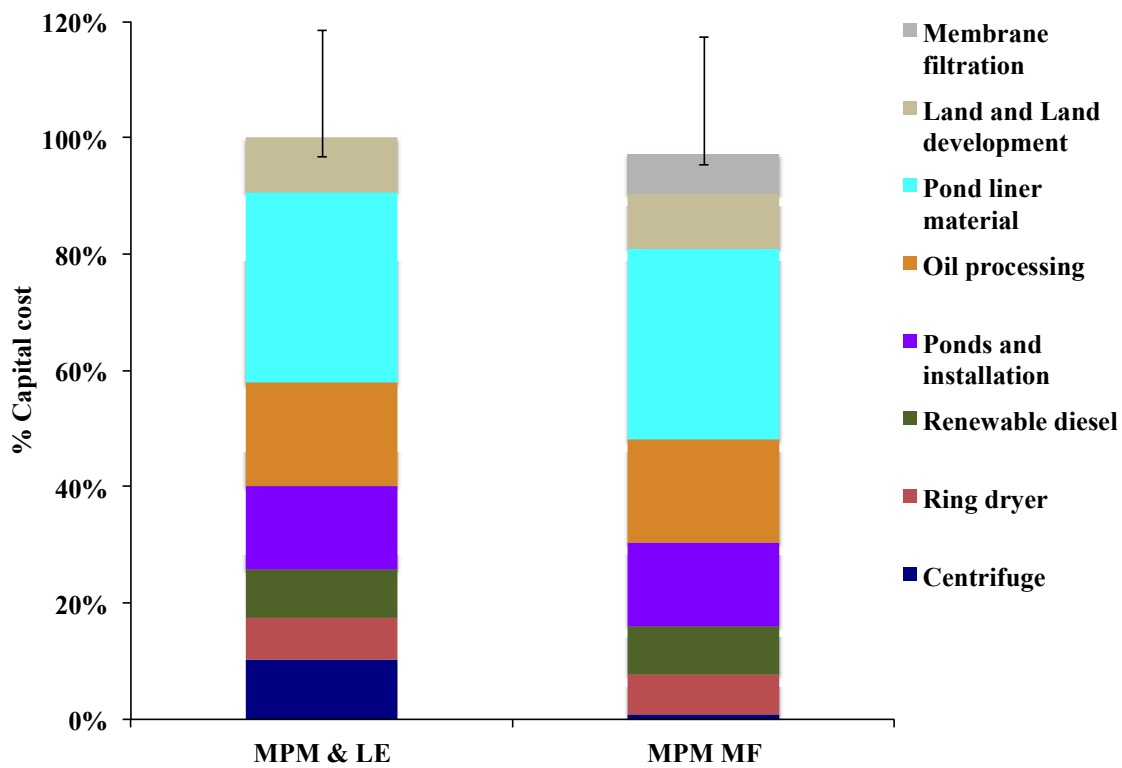


Figure 20: Capital Cost Comparison
 MPM: Multiproduct Model LE: Low energy centrifuge
 MF: Membrane Filtration

3.2 Sensitivity analysis

Figure 21 shows the tornado plot for the total annual operating cost (\$/MT) for the base case scenario (a) and the low energy centrifuge scenario (b). The results were normalized to the highest annual operating cost between the three scenarios (base case scenario). The trends for the results were nearly the same for both scenarios. Productivity affected the results the most. Higher productivity resulted in lower cost of production in \$/MT of algae. Beyond the productivity no variable shifted the results more than 10% for either scenario. The next largest contributor was the cost of the Hypalon™ pond liner. Despite being a capital cost, this variable affected the operating cost due to the materials and maintenance being a function (3%) of the capital cost as well as the depreciation being included in the annual operating cost. The third largest contributor was the annual operating days. As the operating days increased, both the biomass produced and the materials consumed increase. While those two aspects of production affected the annual operating costs (\$/MT) in opposite ways, the increased biomass production had a greater impact than the increased consumption of materials, thus the maximum, 345 days per year resulted in the minimum cost of production per metric ton of biomass and the minimum, 300 days per year resulted in the maximum cost of production. Overall site preparation was another capital cost that affected the annual cost of production for the same reason as the Hypalon mentioned above. The price of the potassium nitrate for pond cultivation was the fifth largest contributor but represented a less than 1% change despite a change of almost \$250/MT. Cost savings related to performance include increasing productivity further, recycling the nutrients (potassium nitrate) or reducing the nutrient consumption, and minimizing the down time to ensure the most possible annual operating

days. Cost savings related to the market include algal biorefineries getting discounts for large purchases for nutrients and pond liner.

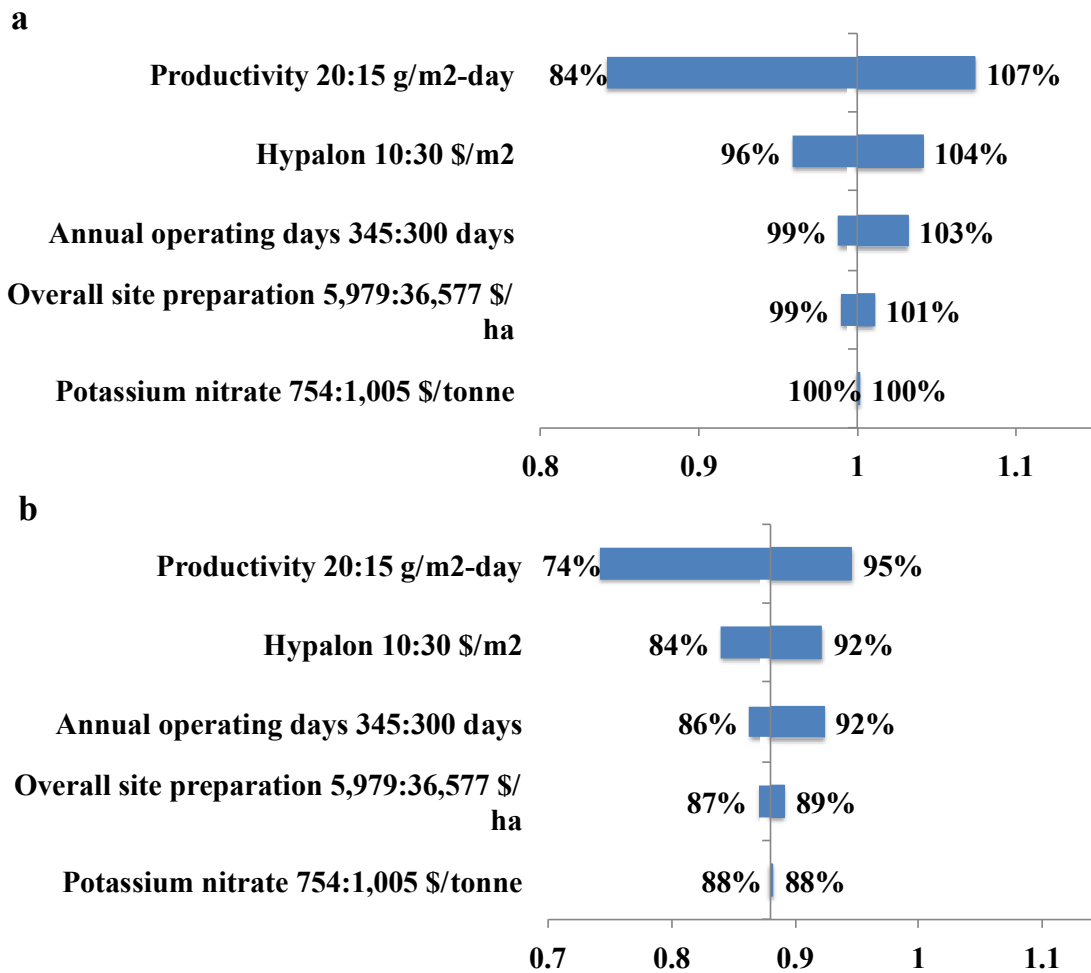


Figure 21: Sensitivity Analysis Annual Operating Cost
 a. MPM base case
 b. MPM low energy centrifuge
 100% represents the baseline of the base case scenario

Figure 22 shows the sensitivity analysis of the total annual operating cost for the membrane filtration scenario. This scenario was normalized in the same manner as the sensitivity analyses in Figure 21. Productivity again had the largest influence on the cost, though it had a slightly lower effect on the results than in the scenarios without membrane filtration. The second largest contributor to variability was the annual

operating days, with Hypalon moving to the third largest contributor for the membrane filtration scenario. Increasing the annual operating days increased the total biomass produced without changing the cost of labor, and maintenance and materials, which now represent a larger portion of the annual operating cost (see Figure 18). The next two largest contributors were the capital costs, pond liner (Hypalon™) and overall site preparation. The fifth largest contributor to variability was the membrane filtration electricity. Based on the results from the sensitivity analysis the productivity has the greatest potential for achieving cost reductions regardless of the dewatering method.

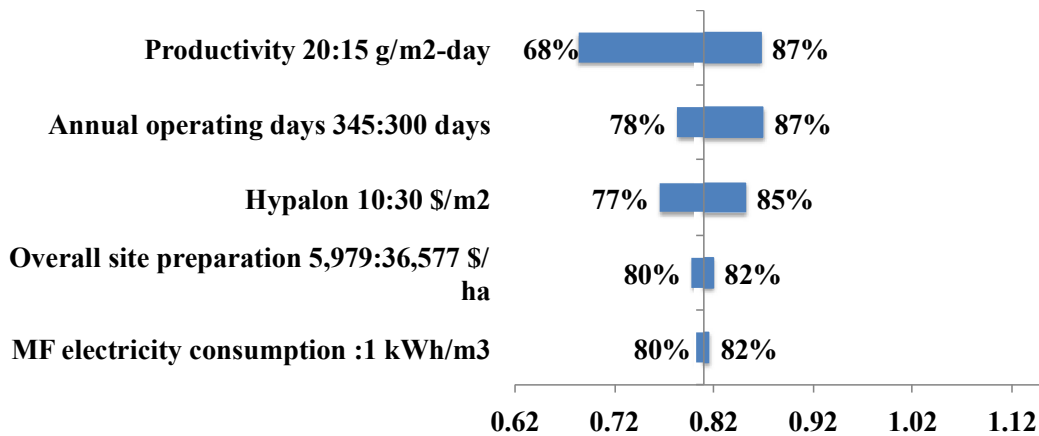


Figure 22: Sensitivity Analysis Annual Operating Cost MPM MF
100% represents the baseline for the base case multiproduct model scenario.

3.3 Reducing the cost of production

There are a number of options for reducing the costs associated with algal omega-3 fatty acid production involving both capital costs and operating costs. Reducing the capital costs at the start of production may or may not be within the control of the operators of the biorefinery. Effectively selecting a dewatering technology is a key step that is within the operators control but will require quality control to ensure that the dewatering options do not have a negative impact on the algal biomass or the resulting

products. Adding membrane filtration reduced the electricity requirement, the number of centrifuges required the annual operating costs and the capital costs. The capital costs for the membrane filtration were based on an existing water treatment membrane filtration system. Flocculation is commonly cited as a dewatering method for algae (Davis et al., 2014a; Weschler et al., 2014) for use with settling and dissolved air flotation as in the DOE harmonization studies. However, a chemical flocculant may negatively influence the quality of omega-3 fatty acids and before commercial implementation can be achieved that would need to be assessed. Other potential improvements include supercritical extraction, which has applications for both algae dewatering and omega-3 fatty acid extraction from lipids for algal omega-3 and fish omega-3 (Mishra et al., 1993; Quinn et al., 2014; Rubio-Rodríguez et al., 2010), and ultrasonic harvesting which can be used to induce flocculation without a chemical additive and break cell walls for easier lipid extraction (Bosma et al.; Coons et al., 2014).

The productivity was the largest contributor to the variability in the annual operating cost. Increased productivity resulted in increases in material consumption and increases in biomass production. The cost decrease associated with higher biomass production significantly outweighed the cost increase associated with higher material consumption.

Conclusion

Membrane filtration was able to reduce the cost of production significantly through reductions in capital costs and operating costs. The technology has already been used commercially for wastewater treatment and while further research is necessary to determine the product quality, final achievable concentration and energy consumption at commercial-scale, membrane filtration appears to be a promising solution to the high

energy associated with centrifugation. Other dewatering options that do not require a chemical additive or damage the biomass before the desired products can be captured should be identified and tested as well. This is important for the production of omega-3 fatty acids for human consumption and is likely to be important to any products that are produced for human consumption from algae. Operators should also work to continue increasing productivity as that reduced the cost of production regardless of the dewatering strategy.

Marketing the algal omega-3 fatty acid products as a non-fish based omega-3 fatty acid product has a number of benefits; it is available to vegetarian and vegan consumers, it eliminates the “fishy” after taste that many users of fish-based omega-3 fatty acids have complained about, and it has the potential to reduce global reliance on fish derived omega-3 fatty acids. Fish derived omega-3 fatty acids come from wild-caught fish, which in some parts of the world are at risk of overexploitation. The algae multiproduct model has the potential to be profitable if the production parameters modeled here can be achieved and the omega-3 fatty acids can be sold at retail prices observed in April, 2016. The omega-3 fatty acids represent nearly 90% of the annual operating revenue.

The omega-3 fatty acid market is very volatile in terms of retail prices and that greatly influences the interpretation of the results of this study. This study focuses on the production of algae based omega-3 fatty acids, which represent a vegetarian alternative to conventional fish oils. Observed retail prices for fish oil and vegetarian omega-3 fatty acids in April 2016 were \$256 and \$895 per kg of omega-3s, respectively for products of the same brand (Nature Made™) and retailer (Walgreens). Nature Made™ vegetarian omega-3s sold for \$1,700/kg of omega-3's in 2015 which was two times the observed

price in April 2016 due to the retailer having a buy 1 get 1 free sale at the time of the April 2016 observation (the same sale was in place for the previous observations in February and March 2016). The results presented in this study allow readers to use the results in association with future market research regardless of how the prices of vegetarian omega-3 fatty acids change.

CHAPTER 5

COMMERCIAL COMPUTATIONAL SUSTAINABILITY ASSESSMENT OF AN ALGAE MULTIPRODUCT BIOREFINERY FOR THE SIMULTANEOUS PRODUCTION OF BIOFUEL AND HIGH-VALUE PRODUCTS

This chapter addresses all research questions

Introduction

Research interest in the algae community has been shifting to high-value products and other applications in the wake of declining fuel prices and improvements in the production of high-value non-energy algal products. Algal biofuels have yet to reach commercialization alone, but the value that can be derived from generating multiple algal bioproducts simultaneously has been cited as a potential market driver (Dong et al.; Foley et al., 2011; Lardon et al., 2009). The higher economic potential of niche-products such as nutraceuticals like omega-3 fatty acids may justify the development of a commercial algae multiproduct biorefinery. However, producing high-value products from the lipid portion of algae, which is most often used to produce fuels, may limit the potential for biofuel production. In addition, researchers in the algae community have identified benchmarks for algal biofuel production compared to conventional fuel (Clarens et al., 2010; Davis et al., 2014) but work needs to be done comparing high-value algal products to their conventional counterparts. There is a need to quantitatively assess and compare the sustainability of algae for biofuels as a primary product and algae biorefineries that produce high-value products as well as other products, such as biofuels.

No LCAs have been conducted on multi-product algae biorefineries that include the production of omega-3 fatty acids for direct human consumption as a part of their analysis. Only one TEA study was found that assessed algae as a source of omega-3 fatty acids (Chauton et al., 2015) but that study focused on using the whole algal biomass for aquaculture to avoid the costly and energy intensive dewatering but precluding the use of algae for multiple products. Most of these LCAs and TEAs focus on theoretical algae systems. Very few studies integrate data from actual algae production facilities, either from the pilot scale or commercial facilities (Beal et al., 2015; Huntley et al., 2015; Liu et al., 2013; Luo et al., 2010; Richardson & Johnson, 2014). To date no studies have combined LCA and TEA to assess algal multiproduct biorefineries for the production of high-value products and biofuels.

The algae community has studied algal biofuels extensively and the U.S. Department of Energy (DOE) has worked to advance algal biofuel production in a unified manner. The national algal biofuels roadmap produced by the DOE based on their 2008 workshop, provided researchers with a framework to advance algae for the production of biofuels and bioproducts (DOE, 2010). The DOE developed harmonized LCA, TEA and resource assessment models for algal biofuels production (Davis et al., 2012; Davis et al., 2014). The harmonization studies have provided a well-established benchmark for other algal biofuels studies. There is a need in the algae community to explore high-value algae products in a similar fashion to the harmonization studies to ensure that comparisons can be made between studies effectively. The harmonization studies as well as the National Algal Biofuels Roadmap can also support high-value product research in a similar

manner and be used as a starting point to compare algae for high-value products versus algae for biofuels.

In order to model a commercial-scale algal biorefinery, data from systems beyond lab-scale production needs to be included in models to avoid excessive assumptions about scale-up across too many orders of magnitude. Cellana LLC's Kona Demonstration Facility (KDF) has been operating since 2009, and provides pilot and commercial-scale data for this study. Cellana's production model, using their patented ALDUO system (Huntley et al., 1996; Huntley & Redalje, 2010) operates using photobioreactors to prevent contamination before open pond cultivation with short run times to maximize production while minimizing the risk of contamination. KDF has primary data related to the cultivation and harvesting of algal biomass using large outdoor systems with a cultivation capacity of greater than 750 m³ and additional estimates for commercial-scale downstream processing.

The goal of this study is to quantify the environmental impacts and economic potential of a commercial-scale biorefinery model for two different product sets; 1) green diesel and high protein feed for agriculture and aquaculture and 2) the high value nutraceutical omega-3 fatty acids, green diesel and high protein feed for agriculture and aquaculture.

The results of this study will evaluate the tradeoffs between environmental impacts and economic potential for algae as a feedstock and provide a framework by which the results for the high value products can be compared to their conventional counterparts.

Methods

This study used TEA and LCA to assess the environmental and economic impacts of a commercial-scale algal multiproduct biorefinery capable of producing multiple sets of products. LCA was performed using the ISO 14040 standards as a framework (ISO, 2006). TEA was performed using methods described in a previous TEA publication related to the harmonization studies (Davis et al., 2011). The four steps of an LCA are typically iterative: 1) goal and scope definition, 2) life cycle inventory (LCI) which includes process model development, and 3) life cycle impact assessment (LCIA) and 4) interpretation and improvement. Techno-Economic Analysis combines production process modeling (biological and downstream) with financial analysis to provide decision-making insights. We define four steps of a TEA, which are also iterative: 1) develop process concept, 2) calculate mass and energy balances, 3) cost engineering, and 4) financial analysis. Steps 1 and 2 of the TEA and LCA overlap significantly. The same process model including mass and energy balances was used for both the TEA and LCA. As such, LCA and TEA steps 1 and 2 are described together in the methods. The difference between the two methods occurs at step 3; in TEA additional inventory items include costs related to utility, chemicals and materials, revenue inventory items, as well as capital expenses are collected for the variables in the LCI. In TEA, a financial analysis is performed to calculate the cost of production, capital expenses (CAPEX), operating expenses (OPEX) and revenue. In LCA, LCIA is conducted to translate the inventory data into meaningful environmental impact categories. Monte Carlo Analysis (MCA) is a critical step in step 4 of both LCA and TEA (interpretation and financial analysis respectively). Conducting LCAs and TEAs with MCA enable analysis of parameter

tradeoffs, sensitivity, and alternative scenarios analysis, which aids management decision-making. Triangular distribution (min, max, and best estimate) was applied to all input parameters where there was variability or uncertainty.

Figure 23 illustrates the system boundary for this study. *Nannochloropsis oceanica* was the algal species used for product generation, because this strain is native to Hawaii where the KDF is located, and is known to accumulate omega-3 fatty acids. The algal biomass was used to generate two sets of products; 1) the high-value set which includes omega-3 fatty acids, green diesel and high protein feed for aquaculture and animal feed and 2) the biofuel set which includes green diesel and the same high protein feed. The functional unit was 1 gallon of green diesel produced. The production system was divided into three phases: cultivation, dewatering and processing.

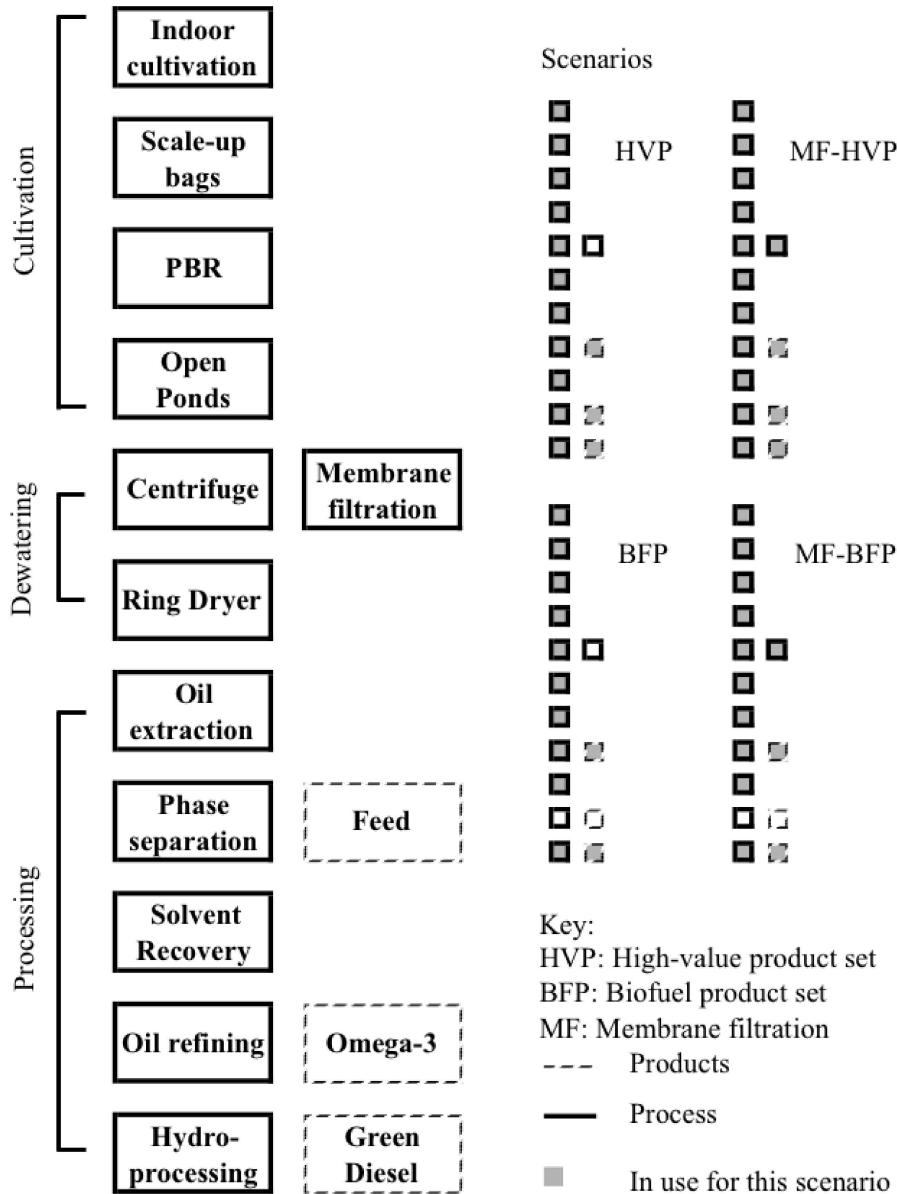


Figure 23: Algae Multiproduct Model System Boundary for the LCA. Right side shows the for scenarios with the grey boxes identifying the products and processes in use for each

While the KDF utilizes a centrifuge as the only dewatering step before drying, it is common practice in other field studies and in the academic literature to have a primary dewatering step before centrifugation, due to the high electricity consumption of centrifugation. The harmonization studies, for example, employ settling and dissolved air

flotation as primary harvesting steps. However, dissolved air flotation and settling use a chemical flocculant and the effect of that flocculant on omega-3 fatty acid quality for direct human consumption is unknown, so membrane filtration was selected as an alternative primary dewatering step that can still reduce the electricity consumption but does not require a chemical flocculant. As a result the high-value product set and biofuel product set were assessed for two different dewatering scenarios resulting in four total scenarios. The first scenario used dewatering exactly as it appears at the KDF while the second dewatering scenario added membrane filtration to reduce the electricity consumption.

The commercial-scale model assumed eight outdoor cultivation modules were used based on Cellana’s patented ALDUO system described above (Huntley et al., 1996; Huntley & Redalje, 2010). Prior to outdoor cultivation lab-scale cultivation and culture maintenance takes place indoors. After cultivation in 20-L bioreactors the first outdoor step was 220-L scale-up bags. The scale-up bags were used to inoculate the photobioreactors, which represent the beginning of the commercial-scale modules. Table 8 shows the details for one module of production in the commercial scale model.

Table 8: Cultivation Module Parameters

Module unit description	Volume (m ³)	# of units per module	Algal biomass Production capacity (MT/yr.)
Photobioreactor	51	17	170
Small pond	190	4	390
Medium pond	750	3	1,020
Large pond	1,500	8	4,370

To explore the effect of productivity on the results two additional productivities were considered in the statistical analysis. The productivity ranged from 14.6 to 20 g/m²-day

with 16 being the best estimate. The best estimate was the average productivity at Cellana. The minimum productivity was 14.6 g/m²-day; the average productivity in a publication resulting from the harmonization studies (Davis et al., 2014). The maximum productivity was 20 g/m²-day; the productivity required for the large ponds to reach the maximum observed concentration at the KDF for the open ponds (0.5 g/L).

Three unit processes were used for dewatering, membrane filtration, centrifugation and drying. Cellana's KDF uses centrifugation and drying. Membrane filtration was added to each scenario as a potential improvement in terms of cost and environmental impacts. For membrane filtration data for the electricity consumption came from Bhave et al. (2012), which used membrane filtration to dewater *N. oceanica*. Data related to the life of the digester came from Cote et al. (2012) and data for the capital cost of membrane filtration came from the estimates of the Pall corporation (1998) for a commercial-scale membrane filtration system installed for water treatment in Texas. For the remaining dewatering unit processes, data provided by Cellana included capacity, efficiency, life expectancy, initial concentration, final concentration, maintenance, and propane consumption for the ring dryer. Data for electricity consumption of the centrifuge came from the academic literature including data from the KDF provided by two companion studies done for algal biofuels (Beal et al., 2015; Gerardo et al., 2014; Huntley et al., 2015; Weschler et al., 2014). The centrifuge electricity consumption ranged from 8 to 9 kWh/m³ with 8 being the best estimate due to that being the electricity from the previous studies based on the KDF. Data for the electricity consumption of the ring dryer came from Beal et al. (2015).

Downstream processing was not handled at the KDF and so the majority of the data for the process model description was derived from the academic literature with Cellana providing data related to the final omega-3 fatty acid and biofuel products. The oil extraction was common to all scenarios and was modeled using hexane extraction followed by phase separation and distillation to recover the solvent (Huo et al., 2008; Soratana et al., 2014). The results of the oil extraction were the total lipids and the lipid extracted algae that would be used for high-protein feed. Further oil refining was unique to the omega-3 fatty acid scenarios and was carried out on the total lipids to separate out the omega-3 fatty acids from the remaining oils (Chakraborty & Joseph, 2015). The final omega-3 fatty acid product contained 35% omega-3 fatty acids while the remaining oils that would be used for biofuel contained 10% omega-3 fatty acids. The conversion of the oils to green diesel was the same for both the total lipids for the green diesel and feed only scenarios and for the remaining lipids from the omega-3 fatty acid scenario after oil refining. The conversion to green diesel was modeled after Huo et al. (2008).

B) Life Cycle Inventory (LCA), and Cost Inventory (TEA)

At the beginning of the project, authors requested data from Cellana for the development of all aspects of the model (mass and energy balance, LCA, TEA and statistical analysis) and repeated when necessary to get a full understanding of the operation at Cellana's KDF and commercialization scale-up potential. Sources of the data included manufacturers, vendors, reports, and onsite measurements. Cellana was able to provide authors with both individual data points and ranges where uncertainty and variability were inherent to the parameter. Data that Cellana provided was aggregated with data from peer-reviewed sources, technical documents and government reports. Data

provided by Cellana was presented in the supporting information with the data it was aggregated with. The authors devised a simple method for Cellana to provide data and assess the quality and their confidence in the data and sources. By the end of the project, Cellana was able to provide highly accurate data and data ranges for all items requested by the authors.

Details for the life cycle and cost inventory are available in the SI. The cultivation inventory items used for both LCA and TEA consisted of seawater, synthetic CO₂ and nutrients for growing the algae, HDPE for PBR and scale-up bag construction, electricity for pond mixing and pumping, and Hypalon™ for pond construction. For dewatering inventory items included electricity, propane and cleaning chemical consumption. The inventory items used for both LCA and TEA for the downstream processing phase were the hexane consumption for extraction, energy for phase separation and solvent recovery, chemical and energy consumption for the oil refining to recover n-3 (high-value product set only) and the energy and chemical inputs and outputs for the green diesel production.

The life cycle assessment process contributions were collected from ecoinvent 3.1 or USLCI 1.6 databases (Norris, 2004; Weidema B P et al., 2013). Preference was given to USLCI data because the default was for North American or US based production for that database, but where USLCI data was not available ecoinvent data was used with preference given to data representing North America for all three phases of production.

The cost inventory was divided into capital expenses (CAPEX) and operating expenses (OPEX). The OPEX consisted of the cost of chemicals, materials and utilities mentioned above. Those costs were collected from online vendors, USEIA (2016a) for average industrial electricity costs for 2015, and Cellana. The HDPE for the PBR and

scale-up bags were considered as OPEX because the life of the HDPE in those systems was three months or less. CAPEX included the cost of Hypalon™ (pond liner), pond, pipe and pump installation, and site preparation. The cost inventory for the dewatering included cost of electricity, propane, and cleaning chemicals for maintenance (OPEX), as well as the cost of each centrifuge and ring dryer unit (CAPEX). The cost inventory for downstream processing included the revenue potential of the all products, the average annual spot price for petroleum diesel in 2015 in the United States (USEIA, 2016b), costs of all chemicals and electricity (OPEX) as well as the estimated cost of the capital equipment for the oil extraction and refining (provided by Cellana) and the green diesel processing (Davis et al., 2014).

C) Cost Engineering (TEA)

The material and energy consumption and production from the process model was used to evaluate a total capital cost and operating cost for each scenario. Labor requirements for the commercial-scale model were validated with Cellana and supplemented with salary data from the Bureau of Labor Statistics (BLS). The labor requirements were 1.02 ha/operator. Labor details are presented in the SI. Labor requirements were factored into the fixed operating expenses and included overhead. Additional financial parameters related to the capital and operating expenses are presented in the SI and were the same values as the parameters used by the harmonization studies (Davis et al., 2014) where appropriate, with the exception of the labor overhead, which used overhead data collected from the BLS.

The overall cost of production was used to calculate the selling price of omega-3 fatty acids to meet the required return on capital investment (RRC). The RRC is an

unknown quantity that depends on negotiations between a commercial-scale biorefinery and a bank providing the loan for the capital investment to agree upon an expected rate of return. In order to represent this, the RRC was considered as a range from 0 to 20% with 0% representing the breakeven selling price on an annual basis without consideration of capital cost. For all scenarios the price of the feed products was based on available prices from online vendors. Details for the cost of each chemical with the vendor is available in the SI. The revenue was analyzed in three different ways depending on the scenario:

- 1) For the HVP scenarios, the diesel price was kept at the price mentioned above so that the results could be based solely on the price of omega-3 fatty acids and those prices were compared to the retail prices available at online vendors.
- 2) For the BFP scenarios, where the analysis was for break-even prices, the diesel price was calculated in \$/gal to meet the break-even point for required return on capital (RRC).
- 3) Where the revenue of all scenarios was compared, the diesel price was held constant at the spot price in 2015 so that the diesel price would be the same for all scenarios.

E) Monte Carlo Analysis (LCA and TEA)

Variability and uncertainty were evaluated using Monte Carlo Analysis (MCA) for LCA and TEA results. All input parameters (for LCA and TEA) where variability or uncertainty was present were assigned a triangular distribution (min, best estimate, max). The best estimate was selected based on the following criteria in order of priority; if Cellana was the only source of data the average was considered the best estimate, and for data sets aggregating Cellana data with data from reports and the academic literature, data

from Cellana was considered the best estimate. In the absence of data from Cellana, the best estimate was the average of the input data from the DOE harmonization models and Beal et al. (2015) (previous Cellana biofuel study). For vendor prices (not provided by Cellana), average was considered the best estimate in most cases except for when all but one suppliers had the same price, then the price of the majority was the best estimate and the outlier was considered the min or the max as appropriate. MCA was carried out using 250,000 trials to ensure reproducibility for every scenario for each output (LCA and TEA) of interest. The 95% confidence interval was then calculated for all output parameters using normal distribution and the mean. The results to the MCA for LCA and TEA outputs are included as error bars in the results to show the possible range of output parameters.

Further analysis was carried out to determine the influence each individual input parameter had on the outputs. Up to the top five most influential inputs to each output were presented in tornado analyses for the EROI and GWP. Any of the top five inputs that resulted in 0% change to the output parameters were omitted from the results section.

D) Life cycle impact assessment (LCA)

The LCIA was quantified using the US Environmental Protection Agency (USEPA) Tool for the Reduction and Assessment of Chemical and other environmental Impacts (TRACI 2.1) (Bare, 2002). Two impact categories were considered; global warming potential (GWP) to account for climate change impacts, and eutrophication potential (EP) to account for water quality impacts related to algae production at commercial-scale. The energy return on investment (EROI) was calculated as the ratio of the energy content of the fuel produced (Energy out) to total energy in (electricity including the renewable

fraction, natural gas and fuel consumption) required for producing the fuel for all scenarios. The EROI included all electricity consumption and the energy content of propane consumed for drying for the input, and the energy content of the green diesel produced. The EROI included both renewable and non-renewable energy for the input but does not include embodied energy.

To explore the impact on resource depletion, the production of 5 billion gallons of green diesel per year (BGY) from the required number of identical commercial-scale facilities producing only diesel and high protein feed was considered; resource depletion included land use and nutrient consumption. This volume was selected so that it would be readily comparable to the harmonization studies, which also considered 5 BGY due to that being 10% of annual diesel consumption in the US, when the harmonization model was developed. The land use was compared to the amount of available land suitable for algal biofuel production identified by resource assessment studies used for the harmonization models (Venteris et al., 2013). Nutrient consumption was compared to the nutrient consumption for agriculture purposes in 2011 (USDA, 2013) to determine if the nutrient consumption for this much algae production would have a significant affect on nutrient consumption for agricultural purposes. This assessment did not consider the co-products in this analysis. Additionally the amount of omega-3 fatty acids produced from the same number of facilities was compared to global omega-3 fatty acid production from 2010 to 2012 (FAO, 2014).

Results & Discussion

The EROI, summarized in Table 9, was less than 1 for all multiproduct scenarios. For biofuel production to be sustainable the EROI needs to at least be greater than one. The

EROI was closer to zero for the scenarios without membrane filtration. Furthermore, producing omega-3 fatty acids reduced the EROI because it reduced the amount of green diesel that could be produced.

The national implications of commercial algal biorefineries for the production of 5 billion gallons of green diesel per year, could impact nutrient consumption, land use and the omega-3 fatty acid market, which may only be able to support a fraction of the possible omega-3 fatty acid produced from algae. Nitrogen and phosphorus consumption from the national-scale facility represented 3% and 1% of 2011 nutrient consumption in the U.S.; nutrient consumption would not have a negative influence on national nutrient consumption practices. The land requirement in the Davis et al. (2014) publication related to the harmonization studies to produce 5 BGY was 810,000 ha (1,671 sites at 485 ha each), which was 41% of the land requirement required for 5 BGY in this study. This was expected because the diesel production from the harmonization study was higher per metric ton of algae due to using hydrothermal liquefaction (HTL) of the entire biomass, instead of lipid extraction. The land required for this study was then compared to the land availability as identified in the resource assessment component of the harmonization studies. The land required to produce 5 BGY based on the results of this study represented less than 30% of the total number of seawater sites identified (Venteris et al., 2013). The production of fish oil was 980,000 MT/yr. from 2010 to 2012 and is expected to increase to over 1 million MT/yr. in 2022 (FAO, 2014). The amount of omega-3 fatty acids produced at the national-scale required for 5 BGY would be more than double the amount of total fish oil, of which omega-3 fatty acids is just a fraction of.

This would be too much fish oil to put on the market but would allow for a certain quota of n-3 to be produced before all of the remaining algal biomass was tasked for biofuel production.

Table 9: Summary of Results

MF: membrane filtration

HVP: High value product set

BFP: Biofuel product set

EROI: Energy return on investment

Highlighted cell: 5 BGY of renewable diesel

National scale results were calculated by calculating the number of facilities for the BFP scenario to produce 5 BGY of green diesel.

88-ha Commercial Facility	HVP	MF-HVP	BFP	MF-BFP
Algae (MT/yr)	4,033	3,831	4,033	3,831
Omega-3 fatty acids (MT/yr)	123	117	-	-
Green Diesel (gal/yr)	180,00	171,000	282,000	268,000
Animal Feed (MT/yr)	168	160	168	160
Aquaculture feed (MT/yr)	2,850	2,703	2,846	2,703
EROI	0.08	0.56	0.12	0.89
National Scale	HVP	MF-HVP	BFP	MF-BFP
Algae (MMT/yr)	71.5	67.9	71.5	67.9
Omega-3 fatty acids (MMT/yr)	2.2	2.1	-	-
Green diesel (BGY)	3.2	3	5	4.8
Animal Feed (MMT/yr)	3	2.8	3	2.8
Aquaculture feed (MMT/yr)	50.5	47.9	50.5	47.9
Land required (ha)	1,900,000			
Nitrogen fertilizer (KNO ₃)	2,450,000			
Phosphorus fertilizer (K ₃ PO ₄)	112,000			

Table 10 shows the required selling price for both of the main products (green diesel and omega-3 fatty acids) for each scenario to cover the cost of production. The prices for the membrane filtration scenarios were lower than the non-membrane filtration scenarios in all cases. This indicates that the capital cost and the operating cost of the membrane filtration were lower than the non-membrane filtration scenarios. Introducing membrane filtration had a positive effect on the EROI and on the overall cost of production.

The retail price of omega-3 fatty acids was \$256/kg of omega-3 (EPA+DHA only) for the Nature Made™ fish oil product and \$895 of omega-3 (EPA+DHA only) for the Nature Made™ 100% vegetarian omega-3 fatty acid supplements (observed April 2016 from Walgreens. Vegetarian omega-3 was 50% off in February, March and April 2016). The prices of omega-3 fatty acids is extremely volatile due to factors that can reduce omega-3 fatty acid production from wild-caught fish, such as weather patterns that could limit the amount of wild-caught fish caught in a given season and environmental restriction in place to prevent over-exploitation and shifts in demand. In June 2015, the Nature Made™ fish oil was \$308/kg, the Nature Made vegetarian fish oil was 1,790 \$/kg. The retail price of omega-3 fatty acids is very volatile and changes not only due to availability but also depending on the individual retailer. Despite this variability the cost of production for the algae omega-3 fatty acids was still lower than both the vegetarian omega-3 fatty acids and the conventional fish oils even at a 20% RRC. Achieving these omega-3 fatty acid selling prices would allow for diesel to be sold at a price competitive with petroleum diesel but without omega-3 fatty acids the diesel price would be far too high to compete with petroleum diesel.

Table 10: Required Selling Prices for Diesel and Omega-3 Fatty Acids

HVP: High-value product set

BFP: Biofuel product set

MF: Membrane filtration

n-3: omega-3 fatty acids

Required return on Capital		HVP	MF-HVP	BFP	MF-BFP
0%	n-3 price (\$/kg)	\$133	\$102	-	-
	Diesel price (\$/gal)	\$1.63	\$1.63	58.96	45.54
10%	n-3 price (\$/kg)	189	160	-	-
	Diesel price (\$/gal)	\$1.63	\$1.63	83.53	70.67
20%	n-3 price (\$/kg)	\$246	218	-	-
	Diesel price (\$/gal)	\$1.63	\$1.63	108.11	95.80

Figure 24 shows the sensitivity analysis for the EROI. Only the membrane filtration-biofuel product set had an EROI that could possibly exceed 1 based on the input parameters. Only two or three variables affected the EROI for each scenario. EROI was affected by the productivity followed by the centrifuge electricity consumption for the HVP and BFP scenarios and by the membrane filtration electricity consumption, followed by the productivity and the centrifuge electricity consumption of the MF-HVP and MF-BFP scenarios. Changing from centrifugation to membrane filtration significantly improves the EROI, but the EROI only exceeds 1 for the MF-BFP scenario.

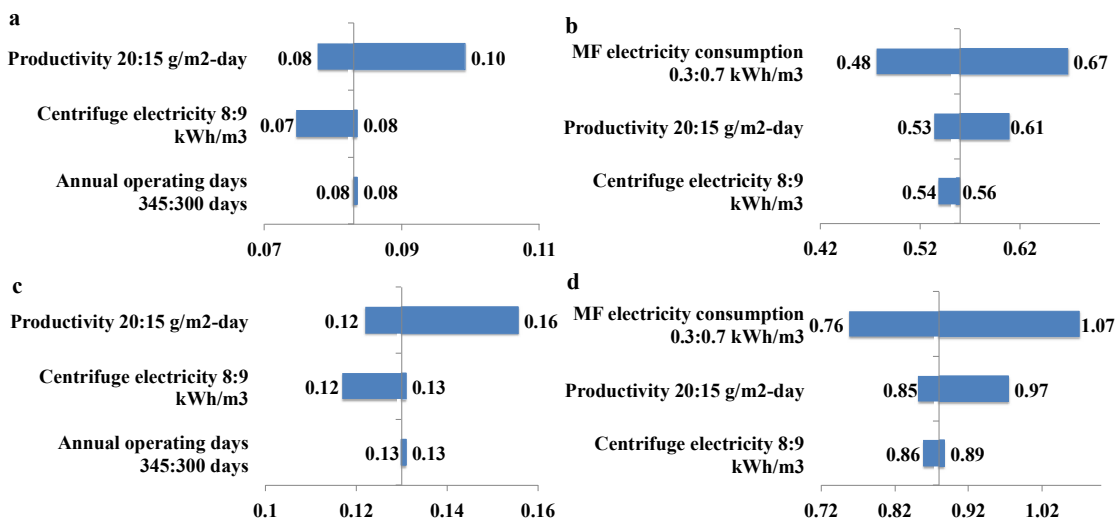


Figure 24: Energy Return on Investment Tornado Plots

a: HVP b: MF-HVP c: BFP d: MF-BFP HVP: High-value product set

BFP: Biofuel product set MF: Membrane filtration

The numbers next to the input parameters represent the inputs corresponding to the min and max respectively.

Figure 25 compares the environmental impacts to the revenue of each production scenario and to the total mass that was produced by product. For the revenue the value of

the diesel price was set at \$1.63/gal (2015 spot price previously mentioned) instead of being set at the price presented in

Table 10, so that all scenarios were calculated using the same individual prices for the products for Figure 25. The omega-3 fatty acids made up most of the revenue in all cases despite being a nearly insignificant portion of the total biomass. The only other product with a statistically significant contribution to the total revenue was the aquaculture feed, even in the BFP scenarios where the green diesel was the main product of interest.

Introducing membrane filtration (HVP to MF-HVP and BFP to MF-BFP) had a statistically insignificant affect on the total production on a mass basis. The environmental impacts decrease significantly when membrane filtration was introduced, but the revenue did not change significantly. The biomass produced per gallon of diesel decreased from the HVP product set to the BFP product set because the amount of diesel increased. The difference in the total biomass produced and the total mass of the products was due to the inefficiencies in downstream processing. The total biomass presented in Figure 25 was the total biomass leaving the final dewatering step meaning that inefficiencies related to cultivation and harvesting was already accounted for.

The 95% confidence interval was calculated to determine the range of environmental impacts, production, and cost and revenue (Figure 25). The cost and revenue had small variability while the variability in environmental impacts was much higher. In terms of environmental impacts the membrane filtration scenarios had lower variability than their counterparts. This was due to the sensitivity of the centrifuge electricity playing a much larger role in the overall variability. This affected GWP more than it affected EP.

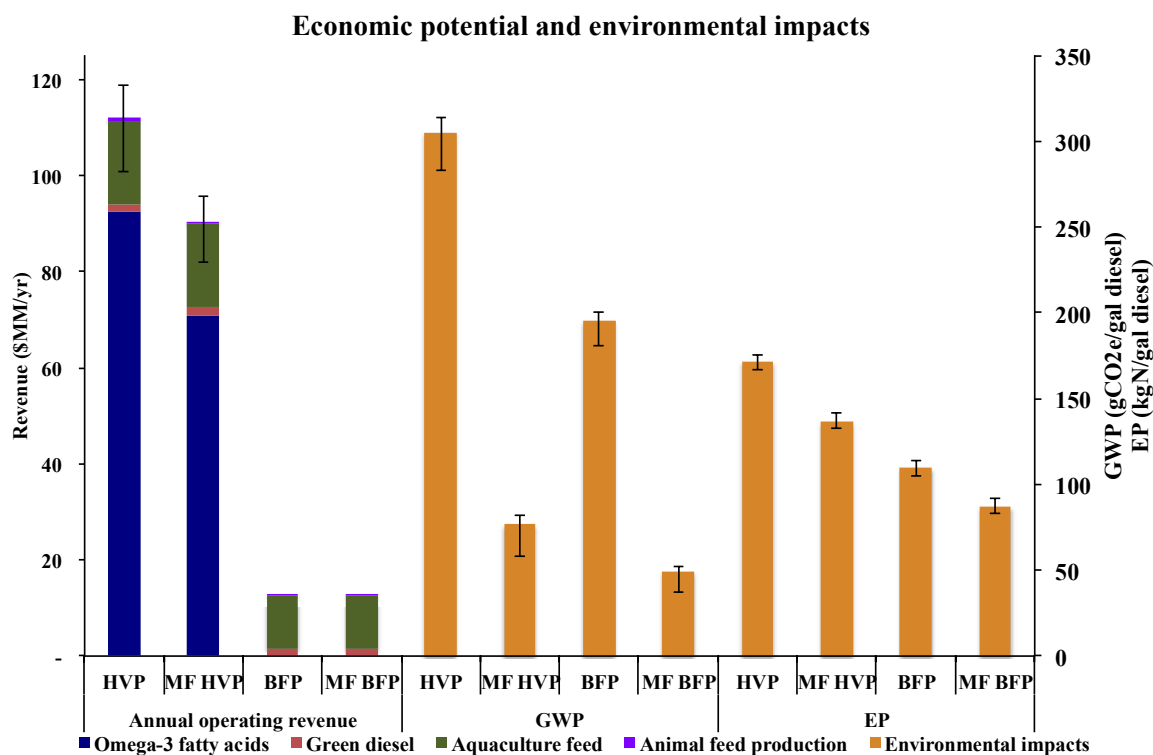


Figure 25: Revenue and Environmental Impacts

Error bars represent the 95% confidence interval

HVP: High-value product set

MF: Membrane filtration

BFP: Biofuel product set

GWP: Global warming potential

EP: Eutrophication potential

The MF scenarios were developed to reduce the electricity consumption by introducing membrane filtration as a primary harvesting step before centrifugation. This unit process was selected because it did not have the drawbacks associated with using a chemical flocculant. In all categories for scenarios with membrane filtration, the cultivation impacts were higher than the dewatering impacts due to the consumption of nutrients and the production of synthetic CO₂ (EP), due to the cost of nutrients and electricity (OPEX), and due to the electricity and synthetic CO₂ consumption (GWP) (Figure 26).

Transitioning from the HVP scenario to the MF-HVP scenario reduced GWP by 75%, the EP by 20%, the total capital investment by 4%, and the OPEX by 19%. Further improvements to dewatering for the environmental impacts and OPEX would require reducing the electricity consumption further. Improvements to dewatering in the CAPEX would require reducing the cost of the dewatering unit processes, finding higher capacity dewatering units, or finding another primary harvesting strategy that could meet the same criteria as the membrane filtration with a lower capital cost.

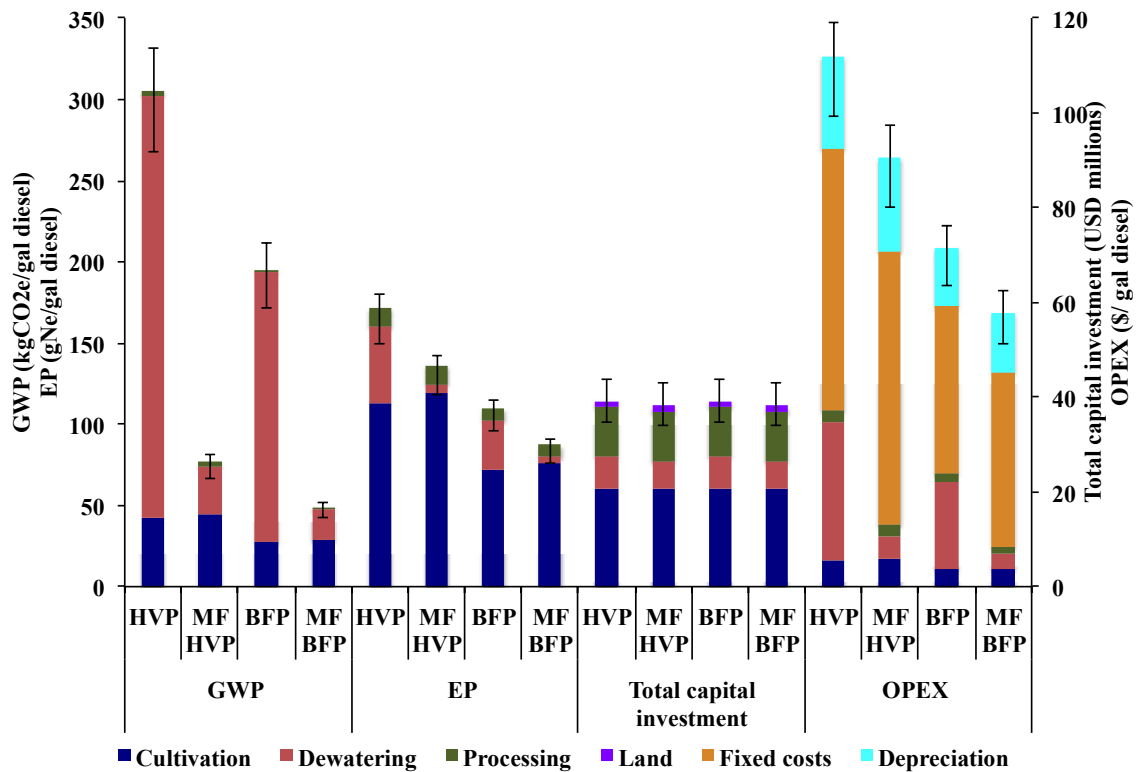


Figure 26: Costs and Environmental Impacts
 Error bars represent the 95% confidence interval
 GWP: Global warming potential EP: Eutrophication potential
 MF: Membrane filtration CAPEX: Capital expenses
 HVP: High value product set OPEX: Operating expenses

Figure 27 shows the sensitivity analysis of the GWP in gCO₂e/MJ. Productivity affected the GWP the most in all scenarios followed by either the operating days per year

for the base case or the membrane filtration electricity for the membrane filtration scenarios. The change in CO₂ consumption did not have a significant contribution to GWP based on the range used here. The life cycle GWP of petroleum diesel is 95g CO₂e/MJ (Davis et al., 2014; Prabhu et al., 2009). None of the GWPs for this study were lower than petroleum diesel. Further analysis indicated that for the MF-BFP scenario, 95 gCO₂e/MJ could be reached if the following three conditions were met; eliminate the drying phase, replace all synthetic CO₂ with recycled CO₂ and increase the productivity to at least 25 g/m²-day. The algae community has already demonstrated that alternatives to high energy dewatering can reduce the GWP of algal biofuels below the GWP of petroleum diesel (Davis et al., 2014), but further research needs to be done to allow for high-value product generation using those same lower energy dewatering strategies and simultaneous fuel production.

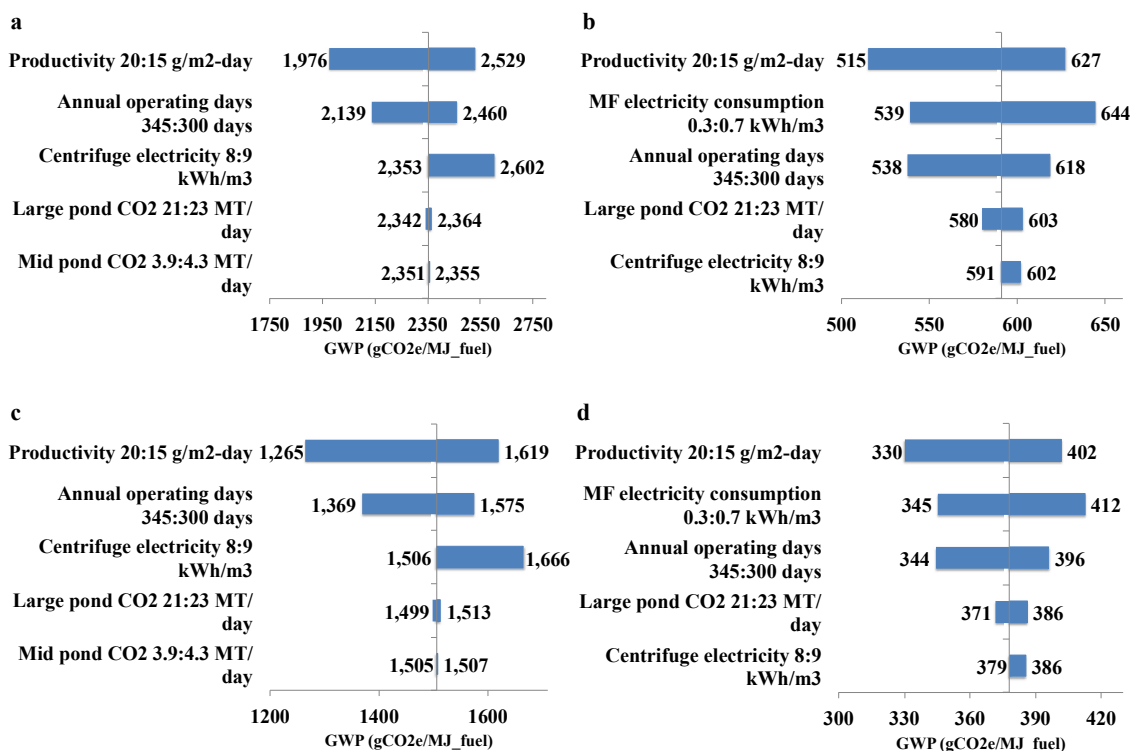


Figure 27: Sensitivity Analysis of GWP

a: HVP

b: MF-HVP

c: BFP

d: MF-BFP

HVP: High-

value product set

BFP: Biofuel product set

MF: Membrane filtration

N3: omega-3

fatty acids

GD: Green diesel

The numbers next to each input parameter represent the value of that parameter at the min and max respectively.

Conclusion

This study demonstrated the potential for an economically sustainable algae multiproduct biorefinery for the production of biofuel and high-value products. Based on the model, the required omega-3 fatty acid selling price for 20% required return on capital (\$217/kg n-3) would be competitive with 2016 retail prices of conventional fish oil (\$256/kg n-3) and vegetarian omega-3 fatty acids (\$895/kg).

The production of omega-3 fatty acids could serve to support the algal biorefineries from a financial perspective, enabling the co-production of biofuels. However, the EROI of the

HVP scenarios was lower than their BFP counterparts, due to the reduction in green diesel production for the HVP scenarios (Table 9). The results of this study were presented normalized to the volume of diesel produced to compare to how diesel prices are commonly presented (\$/gallon) and for the GWP results were additionally normalized to the energy content of the fuel to compare to the renewable fuel standards and conventional diesel metrics (gCO_{2e}/MJ).

When attempting to improve the environmental footprint, GWP for both product models was highly influenced by dewatering energy consumption. High-value products intended for human consumption, like omega-3s, may limit the alternative unit processes that can be used for dewatering but membrane filtration was deemed to be a suitable alternative because it does not require a chemical additive and has been used commercially for water treatment. Other promising technology options include supercritical extraction and ultrasonic harvesting for not only improving dewatering, but also allowing simultaneous lipid extraction, cell disruption and separating omega-3 fatty acids from the total fatty acids (Bosma et al.; Coons et al., 2014; Quinn et al., 2014; Rubio-Rodríguez et al., 2010). Unit processes that require chemical additives may affect the quality of the final product if residual traces of these additives, such as flocculants for dewatering, are present in the final product. The amounts of these chemicals may be small compared to the total lipid fraction (lipids=30% of algal biomass), but may be significant compared to the omega-3 fatty acid content of the final product (omega-3 fatty acids=4% of the original algal biomass entering lipid extraction). Membrane filtration resulted in global warming potential and eutrophication potential reduction of 75% and 20% respectively compared to not using membrane filtration for primary harvesting.

The results of this model show that the cost of production for omega-3 fatty acids was comparable to observed retail prices and would allow green diesel that was co-produced with omega-3 fatty acids to be sold at the same price as petroleum diesel. However, significant work needs to be done to ensure that those fuels meet standards and government mandates in terms of EROI and CO₂ emissions. An ideal case where energy reduction is achieved through removing the drying phase and a significant portion of the centrifugation dewatering, indicated that with progress in advanced oil extraction and refining techniques it may be possible to generate omega-3 fatty acids and produce fuel that has comparable or lower emissions to petroleum diesel and an EROI greater than 1.

CHAPTER 6

CONCLUSION

This thesis evaluated the potential for economic and environmental sustainability for the simultaneous production of biofuels and high-value products that would allow a commercial-scale biorefinery to be economically self-sufficient and still contribute to climate change mandates laid out by the government. This work represents the first efforts to use LCA and TEA to assess the economic and environmental sustainability of an existing pilot-scale biorefinery that produces high-value products and biofuels. Insights from LCA and TEA point to improvements the biorefinery must achieve to produce environmentally sustainable biofuel while maintaining economic viability. Table 11 shows the research questions and the chapters that they correspond to.

Table 11: Summary of Thesis

Shows how each chapter addresses the thesis research questions.

HVP: High value products

RQ: Research question

AD: Anaerobic digestion

LCA: Life cycle assessment

TEA: Techno-economic analysis

Paper	Chapter	RQ1: Impacts of HVP	RQ2: Supporting biofuel commercialization	RQ3: Resource consumption and depletion	RQ4: Improvements and tradeoffs
ADLCA	2				0
Cellana LCA	3	0			0
Cellana TEA	4		0		0
LCA+TEA resource consumption	5	0	0	0	0

Findings related to the first research question (How do the environmental impacts of high value algal bioproducts compare to standard production of the same products?) demonstrate that high-value production of algal omega-3 bioproducts has the potential to have similar environmental impacts to conventional non-renewable products produced from fish oils as long as a suitable low energy algae dewatering technology can be implemented. Algae n-3 represents an alternative source to the growing global n-3 market that would alleviate the pressure on the already highly stressed, and in some cases overexploited fisheries. Using the Cellana LLC production model as a case study, this study shows that the life cycle GHG emissions of the green diesel produced in conjunction with omega-3 fatty acids do not meet RFS mandates. Reducing the dewatering energy consumption and reducing the use of synthetic CO₂ will help improve both algae n-3 compared to conventional n-3 and reduce the life cycle GHG emissions of the green diesel. In the event that biofuels become economically feasible on their own, reducing the amount of n-3 produced could also improve the life cycle GHG emissions by increasing the total amount of fuel produced.

Findings related to the second research question (How can high value algal bioproducts best support algal biofuel commercialization to meet government mandates and standards?) show that the required selling price to meet the cost of production for omega-3 fatty acids was competitive with conventional fish oils and vegetarian omega-3 fatty acids already being sold on the market. However, the n-3 market is very volatile and the price can change rapidly depending on everything ranging from fish availability, fishery management impositions on n-3 production and weather patterns shortening fishing seasons. That price volatility may make this a risky market over the entire life of

an algal multi-product biorefinery despite the fact that there is significant economic potential at retail both retail prices observed here. Selling n-3 at the retail prices observed in April 2016 under the modeled production conditions could support the commercial production of biofuel. The omega-3 fatty acids represent the majority of the economic potential but a nearly negligible portion of the biomass. The majority of the excess biomass could be used for aquaculture feed, animal feed, or some other useful product of lipid-extracted algae (i.e. bioplastics medium). Aquaculture feed represents a small percentage of the economic potential when n-3 is produced, but represents all of the economic potential when biofuel is produced at today's prices without n-3. This research shows that improving key bottlenecks in the algae biorefinery, such as dewatering, would improve the economic potential. Reducing the dewatering energy consumption would improve the economic potential but replacing the sale of n-3 to produce more diesel fuel would reduce the economic potential.

Findings related to the third research question (How do the environmental impacts and economic potential of two different multiproduct models that mix energy and high-value products compare to one another?) indicate that omega-3 fatty acids represent less than 5% of the biomass but more than 90% of the potential revenue. The energy return on investment of the high-value product set was lower than the energy return on investment of the biofuel only product set. Furthermore, the CO₂ emission level of the biofuel produced using this model does not meet the RFS standards of 48 gCO₂e/MJ (50% of conventional diesel) for either high-value products or biofuel product combinations.

Findings related to the fourth research question (How can the environmental and economic performance of algal biofuels and bioproducts most effectively be improved

and what are the tradeoffs between economic and environmental sustainability?) identify three key tradeoffs between economic and environmental sustainability:

- Introducing membrane filtration as a primary dewatering step reduced the global warming potential (77%) and operating cost (9%), but increased the required capital investment by 23%.
- Producing high-value products and biofuels together results in greater economic potential than producing just biofuels, but lower energy return on investment and increased global warming potential for the overall multiproduct refinery.
- In relation to anaerobic digestion, onsite digestion provides better control and consistently higher offsets to GWP but increased capital investment, while GWP offsets from offsite digestion depend on the operators of the offsite digester but does not require additional capital investment for the algal biorefinery

There were also a few key improvements that were without tradeoff:

- Increasing the productivity did not affect the capital investment but it increased the amount of product in all forms and that led to an increase in revenue potential and a decrease in environmental impacts
- Using a low energy centrifuge reduced the environmental impacts and the cost of production without affecting the capital costs compared to using conventional high-energy centrifugation.

This thesis demonstrates the potential of algae for commercial high-value production and lays out some key steps for existing biorefineries moving forward. Results show that there is potential for algal biorefineries to be economically profitable with the sale of high-value products while still producing biofuels, but there are tradeoffs

between the environmental impacts and economic potential. Additional work needs to reduce the life cycle greenhouse gas emissions to meet the renewable fuel standards.

Future work

To simultaneously produce high-value products and biofuels, algal biorefineries will have to meet a number of metrics for both products. For biofuel production the target should be the RFS standard of 50% reduction of GHG emission reductions for biomass-based fuels compared to petroleum diesel. The second target should be to produce high-value products with environmental impacts lower or at least comparable to conventional production of those same targets. The third metric should be to produce fuel with an energy return on investment greater than three or at the absolute lowest greater than one. A number of steps need to be taken to meet these three targets.

Dewatering remains a bottleneck for algae production in all forms, especially when centrifugation and drying are required. Membrane filtration has been identified as one potential solution but there are many others. Chamber filter press (CFP) is a potential alternative to centrifugation. The electricity consumption is less than 1 kWh/m³ (compared to 8 kWh/m³ for centrifuge) and the final concentration can be up to 270 g/L (compared to 200 g/L for centrifuge) (Bhave et al., 2012; Gerardo et al., 2015; Weschler et al., 2014). The capital cost for other filter presses was between 1-2 million dollars for handling more than 1 MGD; more than sufficient capacity for an algal biorefinery of the scale modeled for this dissertation. However, there is concern that this method may not be feasible with small algae such as *N. oceanica* (Molina Grima et al., 2003). Field results for CFP were from the dewatering of large algae (>70 µm) but *N. oceanica* is only 2 to 5 µm (Rodolfi et al., 2009). At this size, significant biomass losses could occur

across the filter. If a company wishes to CFP, then they should start with lab-scale tests for *N. oceanica* as a proof of concept for dewatering. These tests should be used to determine the maximum reliable final concentration of algae leaving the press, the efficiency and the energy consumption. If based on these tests reliable and acceptable results are achieved, pilot studies should be the next step.

Significant improvements to the dewatering were identified in this thesis but the results still showed that the fuel product did not meet RFS GHG emission standards of 48 gCO_{2e}/MJ standards and the EROI was too low to be feasible. In order to further reduce the dewatering impacts it is necessary to consider alternative methods that would allow for handling moist biomass for the extraction of products. Supercritical CO₂ extraction is a potential method to extract lipids from algae and possibly begin the oil refining process to produce omega-3 fatty acids (Rubio-Rodríguez et al., 2010; Soh & Zimmerman, 2011). This method is also of interest to the conventional fish oil community and could serve as an area to build a partnership with the conventional fish oil community.

Contributions to the field

The work of this dissertation serves as one of the earliest works to combine LCA and TEA of algae high-value products. This combination is beneficial for viewing the tradeoffs between environmental and economic sustainability within the same system. Literature to date primarily consists of separate LCAs and TEAs; it is difficult to compare the results from two different systems where critical differences such as dewatering method, species used, location of the biorefinery can all change the process model.

This work also serves as a template for how LCA and TEA can be conducted simultaneously by incorporating and combining steps from each method to produce a

single model capable of producing results for both assessments. The LCA methodology is governed by the ISO 14040 standards and TEA methodology is fairly consistent across practitioners but with no single governing body. However, the requirements for both methods, when considering the production of chemicals or other products, allows for a great deal of overlap. Both methods require process model development, and while there are some extra inventory requirements for each individual method, the majority of the modeling work can be done for both simultaneously. The process model developed for this thesis was conducted mostly in spreadsheets with the Monte Carlo Analysis requiring additional programming or software such as @risk. Going forward it would be beneficial for practitioners of each method to produce models that can simultaneously produce LCA and TEA results. This dissertation can serve as a template for that.

Finally, this modeling work will help provide AzCATI with an example that they can provide to other industry stakeholders interested in commercial production of algae for producing multiple products. AzCATI is a large-scale research facility at Arizona State University that partners with industry partners and other universities to advance research in algal biofuels and bioproducts. AzCATI frequently tests pilot-scale dewatering and cultivation systems for industry partners and was the lead university on the \$15 million DOE Algae Testbed Public Private Partnership (ATP3). An example of this is the research presented in this thesis that was conducted in collaboration with Cellana LLC. The Cellana LCA and TEA includes large-scale data that can be well aggregated to protect proprietary data but still providing meaningful results to the academic, industrial, and government communities. Going forward companies can benefit this work for three main reasons. Using the LCA and TEA to identify tradeoffs

between the environmental and economic sustainability allows companies to quantitatively decide how much of a loss of performance they are willing to take in either category. Furthermore this type of work can identify future research opportunities and the metrics they need to reach to achieve the improvement they are looking for. Finally, this work can help companies by serving as a framework for quantifying the environmental impacts of algal bioproducts and their conventional counterparts to address multiple environmental impacts including fossil fuel consumption and GHG emissions but also resource depletion, agriculture and food production.

REFERENCES

- AgSTAR. (2011). *Market Opportunities for STARBiogas Recovery Systems at U.S. Livestock Facilities*.
- Almeida, C., Vaz, S., Cabral, H., & Ziegler, F. (2014). Environmental assessment of sardine (*Sardina pilchardus*) purse seine fishery in Portugal with LCA methodology including biological impact categories. *The International Journal of Life Cycle Assessment*, 19(2), 297-306.
- Apex. (2000). Decanter Centrifuge Datasheet.
- Avadí, A., Fréon, P., & Quispe, I. (2014). Environmental assessment of Peruvian anchoveta food products: is less refined better? *The International Journal of Life Cycle Assessment*, 19(6), 1276-1293.
- Bare, J., Young, D., & Hopton, M. (2012). TRACI User Manual.
- Bare, J. C. (2002). Traci. *Journal of Industrial Ecology*, 6(3-4), 49-78.
- Barrier, P., & Rousseau, J.-Y. (2001).
- Beal, C. M., Gerber, L. N., Sills, D. L., Huntley, M. E., Machesky, S. C., Walsh, M. J., Greene, C. H. (2015). Algal biofuel production for fuels and feed in a 100-ha facility: A comprehensive techno-economic analysis and life cycle assessment. *Algal Research*, 10(0), 266-279.
- Benemann, J., R., & Oswald, W. J. (1996). *Systems and Economic Analysis of Microalgae ponds for conversion of CO₂ to biomass (D. o. Energy, Trans.)*. Pittsburgh: Pittsburgh Energy Technology Center.
- Berglund, M., & Börjesson, P. (2006). Assessment of energy performance in the life-cycle of biogas production. *Biomass and Bioenergy*, 30(3), 254-266.
- Bhave, R., Kuritz, T., Powell, L., & Adcock, D. (2012). Membrane-Based Energy Efficient Dewatering of Microalgae in Biofuels Production and Recovery of Value Added Co-Products. *Environmental Science & Technology*, 46(10), 5599-5606.
- Bichi, E., Frutos, P., Toral, P. G., Keisler, D., Hervás, G., & Loor, J. J. (2013). Dietary marine algae and its influence on tissue gene network expression during milk fat depression in dairy ewes. *Animal Feed Science and Technology*, 186(1-2), 36-44.

- Biddy, M., Davis, R., Jones, S., & Zhu, Y. (2013). *Whole algae hydrothermal liquefaction technology pathway*: National Renewable Energy Laboratory.
- Biorad. (2000). Immun-Blot PVDF membrane.
- Brentner, L. B., Eckelman, M. J., & Zimmerman, J. B. (2011). Combinatorial Life Cycle Assessment to Inform Process Design of Industrial Production of Algal Biodiesel. *Environmental Science & Technology*, 45(16), 7060-7067.
- Bryant, H. L., Gogichaishvili, I., Anderson, D., Richardson, J. W., Sawyer, J., Wickersham, T., & Drewery, M. L. (2012). The value of post-extracted algae residue. *Algal Research*, 1(2), 185-193.
- Burdge, G. C., & Calder, P. C. (2005). Conversion of α -linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reproduction Nutrition Development*, 45(5), 581-597.
- Camacho-Rodríguez, J., Cerón-García, M. C., Fernández-Sevilla, J. M., & Molina-Grima, E. (2015). The influence of culture conditions on biomass and high value product generation by *Nannochloropsis gaditana* in aquaculture. *Algal Research*, 11, 63-73.
- Campoy, C., Escolano-Margarit, M. V., Anjos, T., Szajewska, H., & Uauy, R. (2012). Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. *British Journal of Nutrition*, 107(SupplementS2), S85-S106.
- Cappell, R., Wright, S., & Nimmo, F. (2007). Sustainable production and consumption of fish and shellfish: Environmental Impact Analysis. United kingdom: Royal Haskoning.
- Chauton, M. S., Reitan, K. I., Norsker, N. H., Tveterås, R., & Kleivdal, H. T. (2015). A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: Research challenges and possibilities. *Aquaculture*, 436(0), 95-103.
- Chen, C.-Y., Chang, Y.-H., & Chang, H.-Y. (2016). Outdoor cultivation of *Chlorella vulgaris* FSP-E in vertical tubular-type photobioreactors for microalgal protein production. *Algal Research*, 13, 264-270.
- Chen, C.-Y., Chen, Y.-C., Huang, H.-C., Huang, C.-C., Lee, W.-L., & Chang, J.-S. (2013). Engineering strategies for enhancing the production of eicosapentaenoic acid (EPA) from an isolated microalga *Nannochloropsis oceanica* CY2. *Bioresource Technology*, 147(0), 160-167.
- Cherubini, F., Bargigli, S., & Ulgiati, S. (2009). Life cycle assessment (LCA) of waste management strategies: Landfilling, sorting plant and incineration. *Energy*, 34(12), 2116-2123.

- Choe, J. K., Mehnert, M. H., Guest, J. S., Strathmann, T. J., & Werth, C. J. (2013). Comparative Assessment of the Environmental Sustainability of Existing and Emerging Perchlorate Treatment Technologies for Drinking Water. *Environmental Science & Technology*, 47(9), 4644-4652.
- Clarens, A. F., Nassau, H., Resurreccion, E. P., White, M. A., & Colosi, L. M. (2011). Environmental Impacts of Algae-Derived Biodiesel and Bioelectricity for Transportation. *Environmental Science & Technology*, 45(17), 7554-7560.
- Clarens, A. F., Resurreccion, E. P., White, M. A., & Colosi, L. M. (2010). Environmental Life Cycle Comparison of Algae to Other Bioenergy Feedstocks. *Environmental Science & Technology*, 44(5), 1813-1819.
- Collet, P., Helias, A., Lardon, L., Ras, M., Goy, R.-A., & Steyer, J.-P. (2011). Life-cycle assessment of microalgae culture coupled to biogas production. *Bioresource Technology*, 102(1), 207-214.
- Cote, P., Alam, Z., & Penny, J. (2012). Hollow fiber membrane life in membrane bioreactors (MBR). *Desalination*, 288, 145-151.
- Damgaard, A., Riber, C., Fruergaard, T., Hulgaard, T., & Christensen, T. H. (2010). Life-cycle-assessment of the historical development of air pollution control and energy recovery in waste incineration. *Waste Management*, 30(7), 1244-1250.
- Davis, R., Aden, A., & Pienkos, P. T. (2011). Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy*, 88(10), 3524-3531.
- Davis, R., Fishman, D. B., Frank, E. D., Aden, A., Coleman, A. M., Pienkos, P. T., . . . Wang, M. (2012). Renewable Diesel from Algal Lipids: An integrated Baseline for Cost, Emissions and Resource Potential from a Harmonized Model:
- Davis, R., Fishman, D. B., Frank, E. D., Johnson, M. C., Jones, S. B., Kinchin, C. M., . . . Wigmosta, M. S. (2014a). Integrated Evaluation of Cost, Emissions, and Resource Potential for Algal Biofuels at the National Scale. *Environmental science & technology*.
- Davis, R., Kinchin, C. M., Markham, J., Tan, E. C. D., & Laurens, L. M. L. (2014b). Process Design and Economics for the Conversion of Algal Biomass to Biofuels: Algal Biomass Fractionation to Lipid- and Carbohydrate-Derived Fuel Products. Golden, CO: NREL.
- Diaz-Castro, J., Moreno-Fernández, J., Hijano, S., Kajarabille, N., Pulido-Moran, M., Latunde-Dada, G. O., . . . Ochoa, J. J. (2015). DHA supplementation: A nutritional strategy to improve prenatal Fe homeostasis and prevent birth outcomes related with Fe-deficiency. *Journal of Functional Foods*, 19, Part A, 385-393.

DOE. (2010). *US DOE, 2010 National Algal Biofuels Technology Roadmap U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program.*

Dong, T., Knoshaug, E. P., Davis, R., Laurens, L. M. L., Van Wychen, S., Pienkos, P. T., & Nagle, N. Combined algal processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts. *Algal Research*.

EISA. (2007). *Energy Independence and Security Act of 2007.*

Emanuelsson, A., Ziegler, F., Pihl, L., Sköld, M., & Sonesson, U. (2014). Accounting for overfishing in life cycle assessment: new impact categories for biotic resource use. *The International Journal of Life Cycle Assessment*, 19(5), 1156-1168.

EPA. (2011). Opportunities for Combined Heat and Power at Wastewater Treatment Facilities.

Evodos. (2013). Evodos 50 Technical Data. Netherlands: Evodos.

FAO. (1986). *The production of Fishmeal and fish oil.* Rome: Retrieved from <http://www.fao.org/docrep/003/X6899E/X6899E00.HTM>.

FAO. (2014). The State of World Fisheries and Aquaculture. In FAO (Ed.). Rome: Food and Agriculture Organization of the United Nations.

Fdez.-Güelfo, L. A., Álvarez-Gallego, C., Sales Márquez, D., & Romero García, L. I. (2011). Biological pretreatment applied to industrial organic fraction of municipal solid wastes (OFMSW): Effect on anaerobic digestion. *Chemical Engineering Journal*, 172(1), 321-325.

Foley, P. M., Beach, E. S., & Zimmerman, J. B. (2011). Algae as a source of renewable chemicals: opportunities and challenges. *Green Chemistry*, 13(6), 1399-1405.

Frank, E. D., Elgowainy, A., Han, J., & Wang, Z. (2013). Life cycle comparison of hydrothermal liquefaction and lipid extraction pathways to renewable diesel from algae. *Mitigation and Adaptation Strategies for Global Change*, 18(1), 137-158.

Frankel, E. N., Satué-Gracia, T., Meyer, A. S., & German, J. B. (1997). Oxidative Stability of Fish and Algae Oils Containing Long-Chain Polyunsaturated Fatty Acids in Bulk and in Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry*, 50(7), 2094-2099.

Fraser, A. J., Sargent, J. R., Gamble, J. C., & Seaton, D. D. (1989). Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. *Marine Chemistry*, 27(1), 1-18.

Fréon, P., Avadí, A., Vinatea Chavez, R., & Iriarte Ahón, F. (2014). Life cycle assessment of the Peruvian industrial anchoveta fleet: boundary setting in life cycle inventory analyses of complex and plural means of production. *The International Journal of Life Cycle Assessment*, 19(5), 1068-1086.

Gerardo, M. L., Oatley-Radcliffe, D. L., & Lovitt, R. W. (2014). Minimizing the Energy Requirement of Dewatering *Scenedesmus* sp. by Microfiltration: Performance, Costs, and Feasibility. *Environmental Science & Technology*, 48(1), 845-853.

Gerardo, M. L., Van Den Hende, S., Vervaeren, H., Coward, T., & Skill, S. C. (2015). Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. *Algal Research*, 11, 248-262.

Golueke, C. G., Oswald, W. J., & Gotaas, H. B. (1957). Anaerobic digestion of algae. *Applied Microbiology*, 5, 8.

González-Fernández, C., Sialve, B., Bernet, N., & Steyer, J. P. (2012). Thermal pretreatment to improve methane production of *Scenedesmus* biomass. *Biomass and Bioenergy*, 40(0), 105-111.

González-García, S., Villanueva-Rey, P., Belo, S., Vázquez-Rowe, I., Moreira, M., Feijoo, G., & Arroja, L. (2015). Cross-vessel eco-efficiency analysis. A case study for purse seining fishing from North Portugal targeting European pilchard. *The International Journal of Life Cycle Assessment*, 20(7), 1019-1032.

Guihéneuf, F., & Stengel, D. B. (2015). Towards the biorefinery concept: Interaction of light, temperature and nitrogen for optimizing the co-production of high-value compounds in *Porphyridium purpureum*. *Algal Research*, 10, 152-163.

Halim, R., Webley, P. A., & Martin, G. J. O. The CIDES process: Fractionation of concentrated microalgal paste for co-production of biofuel, nutraceuticals, and high-grade protein feed. *Algal Research*.

Hall, C. A., Balogh, S., & Murphy, D. J. (2009). What is the minimum EROI that a sustainable society must have? *Energies*, 2(1), 25-47.

Handler, R. M., Canter, C. E., Kalnes, T. N., Lupton, F. S., Kholiqov, O., Shonnard, D. R., & Blowers, P. (2012). Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: Analysis of the prior literature and investigation of wide variance in predicted impacts. *Algal Research*, 1(1), 83-92.

Harto, C., Meyers, R., & Williams, E. (2010). Life cycle water use of low-carbon transport fuels. *Energy Policy*, 38(9), 4933-4944.

Harun, R., Davidson, M., Doyle, M., Gopiraj, R., Danquah, M., & Forde, G. (2011). Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. *Biomass and Bioenergy*, 35(1), 741-747.

Henkanatte-Gedera, S. M., Selvaratnam, T., Caskan, N., Nirmalakhandan, N., Van Voorhies, W., & Lammers, P. J. (2015). Algal-based, single-step treatment of urban wastewaters. [Research Support, Non-U.S. Gov't

Research Support, U.S. Gov't, Non-P.H.S.]. *Bioresour Technol*, 189, 273-278.

Huntley, M. E., Johnson, Z. I., Brown, S. L., Sills, D. L., Gerber, L., Archibald, I., . . . Greene, C. H. (2015). Demonstrated large-scale production of marine microalgae for fuels and feed. *Algal Research*, 10(0), 249-265.

Huntley, M. E., Niiler, P. P., & Redalje, D. (1996). 55410156.

Huntley, M. E., & Redalje, D. G. (2010). 7770332.

Huo, H., Wang, M., Bloyd, C., & Putsche, V. (2008). Life-Cycle Assessment of Energy and Greenhouse Gas Effects of Soybean-Derived Biodiesel and Renewable Fuels (O. o. S. a. T. I. U.S. Department of Energy, Trans.). Argonne Illinois: Argonne National Laboratory.

Ismail, A. (2013). *Trends in the Omega-3 market*. Presentation. Global organization for EPA and DHA omega-3s. Retrieved from <http://micnorway.com/wp-content/uploads/2013/10/3-B1-Adam-Ismael.pdf>

Janssen, C. I. F., & Kiliaan, A. J. (2014). Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: The influence of LCPUFA on neural development, aging, and neurodegeneration. *Progress in Lipid Research*, 53, 1-17.

John, R. P., Anisha, G. S., Nampoothiri, K. M., & Pandey, A. (2011). Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresource Technology*, 102(1), 186-193.

Kadam, K. L. (2002). Environmental implications of power generation via coal-microalgae cofiring. *Energy*, 27(10), 905-922.

Kaye, Y., Grundman, O., Leu, S., Zarka, A., Zorin, B., Didi-Cohen, S., . . . Boussiba, S. (2015). Metabolic engineering toward enhanced LC-PUFA biosynthesis in *Nannochloropsis oceanica*: Overexpression of endogenous $\Delta 12$ desaturase driven by stress-inducible promoter leads to enhanced deposition of polyunsaturated fatty acids in TAG. *Algal Research*.

Koller, M., Muhr, A., & Brauneegg, G. (2014). Microalgae as versatile cellular factories for valued products. *Algal Research*, 6, Part A, 52-63.

- Kris-Etherton, P. M., Harris, W. S., Appel, L. J., & Committee, f. t. N. (2002). Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Circulation*, *106*(21), 2747-2757.
- Lam, M. K., & Lee, K. T. (2015). Chapter 12 - Bioethanol Production from Microalgae A2 - Kim, Se-Kwon *Handbook of Marine Microalgae* (pp. 197-208). Boston: Academic Press.
- Lardon, L., Helias, A., Sialve, B., Steyer, J.-P., & Bernard, O. (2009). Life-Cycle Assessment of Biodiesel Production from Microalgae. *Environmental Science & Technology*, *43*(17), 6475-6481.
- Lee, I.-S., & Rittmann, B. E. (2011). Effect of low solids retention time and focused pulsed pre-treatment on anaerobic digestion of waste activated sludge. *Bioresource Technology*, *102*(3), 2542-2548.
- Levis, J. W., & Barlaz, M. A. (2011). Is Biodegradability a Desirable Attribute for Discarded Solid Waste? Perspectives from a National Landfill Greenhouse Gas Inventory Model. *Environmental Science & Technology*, *45*(13), 5470-5476.
- Liu, X., Saydah, B., Eranki, P., Colosi, L. M., Greg Mitchell, B., Rhodes, J., & Clarens, A. F. (2013). Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction. *Bioresource Technology*, *148*(0), 163-171.
- Llewelyn, B., & Piotraszewski, A. (2012). Sapphire Energy's Commercial Demonstration Algae-to-Energy Facility Now Operational. Columbus, NM: Sapphire Energy.
- Lundquist, T. J., Woertz, I. C., Quinn, N. W. T., & Benemann, J., R. (2010). *A realistic Technology and Engineering Assessment of Algal Biofuel Production*. Digital Commons @ Cal Poly. California.
- Luo, D., Hu, Z., Choi, D. G., Thomas, V. M., Realff, M. J., & Chance, R. R. (2010). Life cycle energy and greenhouse gas emissions for an ethanol production process based on blue-green algae. *Environmental science & technology*, *44*(22), 8670-8677.
- Maisashvili, A., Bryant, H., Richardson, J., Anderson, D., Wickersham, T., & Drewery, M. (2015). The values of whole algae and lipid extracted algae meal for aquaculture. *Algal Research*, *9*, 133-142.
- Manikan, V., Nazir, M. Y. M., Kalil, M. S., Isa, M. H. M., Kader, A. J. A., Yusoff, W. M. W., & Hamid, A. A. (2015). A new strain of docosahexaenoic acid producing microalga from Malaysian coastal waters. *Algal Research*, *9*, 40-47.
- Massart, N., Doyle, J., Jenkins, J., Rowan, J., & Wallis-Lage, C. (2008). *Anaerobic Digestion-Improving Energy Efficiency with Mixing*. Paper presented at the WEFTEC.

Mehrabadi, A., Craggs, R., & Farid, M. M. (2015). Wastewater treatment high rate algal ponds (WWT HRAP) for low-cost biofuel production. *Bioresource Technology*, 184, 202-214.

Mirsiaghi, M., & Reardon, K. F. (2015). Conversion of lipid-extracted *Nannochloropsis salina* biomass into fermentable sugars. *Algal Research*, 8, 145-152.

Mitra, M., Patidar, S. K., George, B., Shah, F., & Mishra, S. (2015). A euryhaline *Nannochloropsis gaditana* with potential for nutraceutical (EPA) and biodiesel production. *Algal Research*, 8, 161-167.

Mohn, F. H. (1980). Experiences and strategies in the recovery of biomass from mass cultures of microalgae. . In G. Shelef & C. J. Soeder (Eds.), *Algae biomass production and use*. Amsterdam: Elsevier/ North Holland.

Mohn, F. H. (1988). Harvesting Microalgal biomass. In M. A. Borowitzka & L. K. Borowitzka (Eds.), *Micro-algal Biotechnology*. New York: Cambridge University Press.

Murphy, C. F., & Allen, D. T. (2011). Energy-Water Nexus for Mass Cultivation of Algae. *Environmental Science & Technology*, 45(13), 5861-5868.

Norse, E. A., Brooke, S., Cheung, W. W. L., Clark, M. R., Ekeland, I., Froese, R., . . . Watson, R. (2012). Sustainability of deep-sea fisheries. *Marine Policy*, 36(2), 307-320.

NREL. (2012). *U.S. Life Cycle Inventory Database*. Retrieved from: <https://http://www.lcacommons.gov/nrel/search>

Padoley, K. V., Tembhekar, P. D., Saratchandra, T., Pandit, A. B., Pandey, R. A., & Mudliar, S. N. (2012). Wet air oxidation as a pretreatment option for selective biodegradability enhancement and biogas generation potential from complex effluent. *Bioresource Technology*, 120(0), 157-164.

Park, J.-H., Yoon, J.-J., Park, H.-D., Lim, D. J., & Kim, S.-H. (2012). Anaerobic digestibility of algal bioethanol residue. *Bioresource Technology*, 113(0), 78-82.

Pérez-López, P., González-García, S., Allewaert, C., Verween, A., Murray, P., Feijoo, G., & Moreira, M. T. (2014). Environmental evaluation of eicosapentaenoic acid production by *Phaeodactylum tricornutum*. *Science of The Total Environment*, 466–467(0), 991-1002.

- Petrusevski, B., Bolier, G., Van Breemen, A. N., & Alaerts, G. J. (1995). Tangential flow filtration: A method to concentrate freshwater algae. *Water Research*, 29(5), 1419-1424.
- Plourde, M., & Cunnane, S. C. (2007). Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Applied Physiology, Nutrition, and Metabolism*, 32(4), 619-634.
- Prabhu, A., Pham, C., Glabe, A., & Duffy, J. (2009). GREET pathway for California reformulated gasoline blendstock for oxygenate blending (CARBOB) from average crude refined in California, Version 2.1: California Air Resources Board: Sacramento, CA.
- Prajapati, S. K., Kumar, P., Malik, A., & Vijay, V. K. (2014). Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: A closed loop bioenergy generation process. *Bioresource Technology*, 158(0), 174-180.
- Quinn, J. C., Smith, T. G., Downes, C. M., & Quinn, C. (2014). Microalgae to biofuels lifecycle assessment — Multiple pathway evaluation. *Algal Research*, 4, 116-122.
- Ras, M., Lardon, L., Bruno, S., Bernet, N., & Steyer, J.-P. (2011). Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. *Bioresource Technology*, 102(1), 200-206.
- Razon, L. F., & Tan, R. R. (2011). Net energy analysis of the production of biodiesel and biogas from the microalgae: *Haematococcus pluvialis* and *Nannochloropsis*. *Applied Energy*, 88(10), 3507-3514.
- Richardson, J. W., & Johnson, M. D. (2014). Economic viability of a reverse engineered algae farm (REAF). *Algal Research*, 3, 66-70.
- Richardson, J. W., & Johnson, M. D. (2015). Financial Feasibility analysis of NAABB developed technologies. *Algal Research*, 10, 16-24.
- Richardson, J. W., Johnson, M. D., Lacey, R., Oyler, J., & Capareda, S. (2014a). Harvesting and extraction technology contributions to algae biofuels economic viability. *Algal Research*, 5, 70-78.
- Richardson, J. W., Johnson, M. D., & Outlaw, J. L. (2012). Economic comparison of open pond raceways to photo bio-reactors for profitable production of algae for transportation fuels in the Southwest. *Algal Research*, 1(1), 93-100.
- Richardson, J. W., Johnson, M. D., Zhang, X., Zemke, P., Chen, W., & Hu, Q. (2014b). A financial assessment of two alternative cultivation systems and their contributions to algae biofuel economic viability. *Algal Research*, 4(0), 96-104.
- Rick, T. C., & Erlandson, J. M. (2009). Coastal Exploitation. *Science*, 325(5943), 952-953.

- Rickman, M., Pellegrino, J., Hock, J., Shaw, S., & Freeman, B. (2013). Life-cycle and techno-economic analysis of utility-connected algae systems. *Algal Research*, 2(1), 59-65.
- Rittmann, B. E., & McCarty, P. L. (2001). *Environmental Biotechnology: Principles and Applications*. New York: McGraw Hill.
- Rogers, J. N., Rosenberg, J. N., Guzman, B. J., Oh, V. H., Mimbela, L. E., Ghassemi, A., . . . Donohue, M. D. (2014). A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. *Algal Research*, 4, 76-88.
- Rossignol, N., Vandanon, L., Jaouen, P., & Quéméneur, F. (1999). Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. *Aquacultural Engineering*, 20(3), 191-208.
- Rothermel, M. C., Landis, A. E., Barr, W. J., Soratana, K., Reddington, K. M., Weschler, M. K., . . . Harper, W. F. (2013). A Life Cycle Assessment Based Evaluation of a Coupled Wastewater Treatment and Biofuel Production Paradigm. *Journal of Environmental Protection*, 4, 1018.
- Shelef, G., Sukenik, A., & Green, M. (1984). *Microalgae Harvesting and Processing: A Literature Review*.
- Sialve, B., Bernet, N., & Bernard, O. (2009). Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances*, 27(4), 409-416.
- Simopoulos, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *The American Journal of Clinical Nutrition*, 54(3), 438-463.
- Soratana, K., Barr, W. J., & Landis, A. E. (2014). Effects of co-products on the life-cycle impacts of microalgal biodiesel. [Research Support, U.S. Gov't, Non-P.H.S.]. *Bioresour Technol*, 159, 157-166.
- Soratana, K., Harper Jr, W. F., & Landis, A. E. (2011). Microalgal biodiesel and the Renewable Fuel Standard's greenhouse gas requirement. *Energy Policy*, 46, 498-510.
- Soratana, K., Khanna, V., & Landis, A. E. (2013). Re-envisioning the renewable fuel standard to minimize unintended consequences: A comparison of microalgal diesel with other biodiesels. *Applied Energy*, 112, 194-204.
- Soratana, K., & Landis, A. E. (2011). Evaluating industrial symbiosis and algae cultivation from a life cycle perspective. *Bioresour Technol*, 102(13), 6892-6901.

- Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 101(2), 87-96.
- Stephenson, A. L., Kazamia, E., Dennis, J. S., Howe, C. J., Scott, S. A., & Smith, A. G. (2010). Life-Cycle Assessment of Potential Algal Biodiesel Production in the United Kingdom: A Comparison of Raceways and Air-Lift Tubular Bioreactors. *Energy & Fuels*, 24(7), 4062-4077.
- Strobel, C., Jahreis, G., & Kuhnt, K. (2012). Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products. *Lipids Health Dis*, 11(1), 144-144.
- Sukenik, A., Zmora, O., & Carmeli, Y. (1993). Biochemical quality of marine unicellular algae with special emphasis on lipid composition. II. *Nannochloropsis* sp. *Aquaculture*, 117(3), 313-326.
- Svanes, E., Vold, M., & Hanssen, O. (2011). Environmental assessment of cod (*Gadus morhua*) from autoline fisheries. *The International Journal of Life Cycle Assessment*, 16(7), 611-624.
- Thrane, M. (2006). LCA of danish fish products. New methods and insights (9 pp). *The International Journal of Life Cycle Assessment*, 11(1), 66-74.
- Tibbetts, S. M., Whitney, C. G., MacPherson, M. J., Bhatti, S., Banskota, A. H., Stefanova, R., & McGinn, P. J. (2015). Biochemical characterization of microalgal biomass from freshwater species isolated in Alberta, Canada for animal feed applications. *Algal Research*, 11, 435-447.
- Tocher, D. R. (2015). Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. *Aquaculture*.
- Turbec. (2011). Technical description: T100 Natural Gas Retrieved 5/25/14, 2014, from [http://www.newenco.co.uk/file_upload/T100 Detailed Specifications.pdf](http://www.newenco.co.uk/file_upload/T100%20Detailed%20Specifications.pdf)%13
- Tyedmers, P. H., Watson, R., & Pauly, D. (2005). Fueling Global Fishing Fleets. *AMBIO: A Journal of the Human Environment*, 34(8), 635-638.
- Uduman, N., Qi, Y., Danquah, M. K., Forde, G. M., & Hoadley, A. (2010). Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. *Journal of Renewable and Sustainable Energy*, 2(1), 012701-012715.

UN. (2015). *Framework Convention on Climate change*. France: United Nations.

USEIA. (2016). *Electric Power Monthly*. Retrieved from http://www.eia.gov/electricity/monthly/epm_table_grapher.cfm?t=epmt_1_1.

Vaughan, D. S., Shertzer, K. W., & Smith, J. W. (2007). Gulf menhaden (*Brevoortia patronus*) in the U.S. Gulf of Mexico: Fishery characteristics and biological reference points for management. *Fisheries Research*, 83(2–3), 263-275.

Venteris, E. R., Skaggs, R. L., Wigmosta, M. S., & Coleman, A. M. (2014). Regional algal biofuel production potential in the coterminous United States as affected by resource availability trade-offs. *Algal Research*, 5, 215-225.

Wakeman, R. J. (2007). Separation technologies for sludge dewatering. *Journal of Hazardous Materials*, 144(3), 614-619.

Ward, O. P., & Singh, A. (2005). Omega-3/6 fatty acids: Alternative sources of production. *Process Biochemistry*, 40(12), 3627-3652.

Weidema B P, Bauer C, Hirschier R, Mutel C, Nemecek T, Reinhard J, . . . G, W. (2013). *Overview and methodology. Data quality guideline for the ecoinvent database version 3. ecoinvent Report 1(v3)*.

Weschler, M. K., Barr, W. J., Harper, W. F., & Landis, A. E. (2014). Process energy comparison for the production and harvesting of algal biomass as a biofuel feedstock. *Bioresour Technol*, 153(0), 108-115.

Wiley, P. E., Campbell, J. E., & McKuin, B. (2011). Production of Biodiesel and Biogas from Algae: A Review of Process Train Options. *Water Environment Research*, 83(4), 326-338.

Woertz, I. C., Benemann, J. R., Du, N., Unnasch, S., Mendola, D., Mitchell, B. G., & Lundquist, T. J. (2014). Life Cycle GHG Emissions from Microalgal Biodiesel – A CA-GREET Model. *Environmental Science & Technology*, 48(11), 6060-6068.

Wu, M., Wu, Y., & Wang, M. (2006). Energy and Emission Benefits of Alternative Transportation Liquid Fuels Derived from Switchgrass: A Fuel Life Cycle Assessment. *Biotechnology Progress*, 22(4), 1012-1024.

Wuang, S. C., Khin, M. C., Chua, P. Q. D., & Luo, Y. D. (2016). Use of Spirulina biomass produced from treatment of aquaculture wastewater as agricultural fertilizers. *Algal Research*, 15, 59-64.

Xiao, Y., Zhang, J., Cui, J., Yao, X., Sun, Z., Feng, Y., & Cui, Q. (2015). Simultaneous accumulation of neutral lipids and biomass in *Nannochloropsis oceanica* IMET1 under high light intensity and nitrogen replete conditions. *Algal Research*, *11*, 55-62.

Xu, L., Brilman, D. W. F., Withag, J. A. M., Brem, G., & Kersten, S. (2011). Assessment of a dry and a wet route for the production of biofuels from microalgae: Energy balance analysis. *Bioresource Technology*, *102*(8), 5113-5122.

Yin, H., & Sathivel, S. (2010). Physical properties and oxidation rates of unrefined menhaden oil (*Brevoortia patronus*). *Journal of food science*, *75*(3), E163-E168.

Zaimes, G. G., & Khanna, V. (2013). Environmental sustainability of emerging algal biofuels: A comparative life cycle evaluation of algal biodiesel and renewable diesel. *Environmental Progress & Sustainable Energy*, *32*(4), 926-936.

Ziegler, F., Groen, E., Hornborg, S., Bokkers, E. M., Karlsen, K., & de Boer, I. M. (2015). Assessing broad life cycle impacts of daily onboard decision-making, annual strategic planning, and fisheries management in a northeast Atlantic trawl fishery. *The International Journal of Life Cycle Assessment*, 1-11.

APPENDIX A

CHAPTER 2 SUPPORTING INFORMATION

Table 12: Anaerobic Digestion Parameters

Anaerobic digestion				
Parameters	Units	Value	Data Sources	Database
Steel	Kg	3500	(ISSF, 2012)	Chromium Steel at plant ecoinvent 2.2 RER
Volume (V)	m ³	50	Selected to maintain loading rate	
Hydrated lime	mg/L	518	(Rittmann & McCarty, 2001)	Lime hydrated loose at plant ecoinvent 2.2 CH
Volumetric Flowrate	m ³ /day	3	Calculation	
HRT	Days	16.67	(Tchobanoglous et al., 2003)	
Operating Temperature	°C	35	(Tchobanoglous et al., 2003)	Natural gas combusted in industrial equipment USLCI 1.6 US
Concentration in digester	%	6%	("Northwest Water Reclamation Plant Mesa, AZ tour," 2012)	
%VS stabilized	%	75-90	(Tchobanoglous et al., 2003)	
Digester mixing energy	kWh/kg	0.8	(Massart et al., 2008)	US electricity mix 2014
Digester pumping energy	kWh/day	4.0	(Choe et al., 2013)	US electricity mix 2014

Anaerobic digestion (con)				
Parameters	Units	Value	Data Sources	Database
Nitrogen Requirements	mg _N / g _{COD}	5-15	(Rittmann & McCarty, 2001)	Urea at regional storehouse ecoinvent 2.2 RER
Phosphorus Requirements	mg _P / g _{COD}	0.8-2.5	(Rittmann & McCarty, 2001)	Single superphosphate at regional storehouse ecoinvent 2.2 RER
Methane Yield	L _{CH₄} / kg-VS	50-540	(Clarens et al., 2011; Collet et al., 2011; Ehimen et al., 2011; Frank et al., 2012; Migliore et al., 2012; Park & Li, 2012; Ras et al., 2011; Sialve et al., 2009)	
COD: Biomass	kg _{COD} / kg _i		Calculation based on biomass composition	
VS:biomass	kg _{VS} / kg _{ABR}	0.76	("Northwest Water Reclamation Plant Mesa, AZ tour," 2012)	
VSLR range (high rate digester)	kg _{VS} / m ³ -day	0.6-4.8	(Rittmann & McCarty, 2001)	

Table 13: Downstream Processing Parameters

Centrifuge				
Parameters	Units	Value	Data Sources	Database
Capacity	m ³ /day	2736	(Apex, 2000)	
Initial Concentration	% w/w	3	(Apex, 2000)	
Exit Concentration – DC	% w/w	24	(Apex, 2000)	
Process Loss – DC	%	5	Assumption	
Electricity Consumption – DC	kWh/m ³	5.08	(Apex, 2000)	US electricity mix 2014
Nutrient Recovery				
Parameters	Units	Value	Data Sources	Database
Energy For land application	MJ/tonne	28	(Berglund & Börjesson, 2006)	US electricity mix 2014
Combined heat and Power				
Parameters	Units	Value	Data Sources	
Heat conversion efficiency	%	47	(Turbec, 2011)	Natural gas combusted in industrial equipment USLCI 1.6 US
Electricity conversion efficiency	%	30	(Turbec, 2011)	US Electricity mix 2014

Table 14: Landfill and Incineration Parameters

Landfill with methane capture and incineration (Top line LFM, bottom line Inc)				
Parameters	Units	Value	Data Sources	Database
Natural gas	g/g	n/a 6.01×10^{-5}	(Cherubini et al., 2009)	Natural gas combusted in industrial equipment USLCI 1.6 US
US electricity mix 2014	kWh/ g	5.31×10^{-7} 6.68×10^{-5}	(Cherubini et al., 2009)	US electricity mix 2014*
Diesel equipment	m3/g	6.24×10^{-4} 1.57×10^{-4}	(Cherubini et al., 2009)	Diesel combusted in industrial equipment (USLCI 1.6) US
HDPE	g/g	1.86×10^{-4} 1.33×10^{-5}	(Cherubini et al., 2009)	HDPE PIPES (Industry data 2.0) RER
Clay	g/g	4.47×10^{-2} 9.8×10^{-5}	(Cherubini et al., 2009)	Clay plaster market (ecoinvent 3.0.2) GLO
Concrete	g/g	n/a 6.87×10^{-4}	(Cherubini et al., 2009)	Aerated concrete block type P4 05 reinforced density, 485 kg/m3 (ELCD 2.0.0) RER
Steel	g/g	4.2×10^{-7} 5.62×10^{-4}	(Cherubini et al., 2009)	Chromium Steel at plant (ecoinvent 2.2) RER
Urea	g/g	n/a 3×10^{-3}	(Cherubini et al., 2009)	Urea at regional storehouse (ecoinvent 2.2) RER
Hydrated lime	g/g	n/a 3.2×10^{-3}	(Cherubini et al., 2009)	Lime hydrated loose at plant (ecoinvent 2.2 CH)
Quicklime	g/g	n/a 2.5×10^{-2}	(Cherubini et al., 2009)	Quicklime at plant (USLCI 1.6) US
Cement	g/g	n/a 1.35×10^{-2}	(Cherubini et al., 2009)	Portland Cement, at plant (USLCI 1.6) US

Table 15: Electricity Mix Details
 All data from USEIA (2016)

US Electricity Mix 2014			
Parameters	Units	Value	Database
Coal	%	40	Bituminous coal at power plant (USLCI) US
Nuclear	%	19	Nuclear at power plant (USLCI) US
Natural gas	%	25	High voltage, production at conventional power plant (ecoinvent 3.0.2) US
Hydroelectric	%	7	High voltage, production at hydro reservoir (ecoinvent 3.0.2) US
Hydroelectric at pumped storage	%	0.3	Hydropower at pumped storage power plant (ecoinvent 2.2) US
Wind	%	2.4	High voltage, wind > 3MW turbine onshore (ecoinvent 3.0.2) US
Waste	%	0.2	Municipal waste incineration plant (ecoinvent 2.2) CH
Geothermal	%	0.2	High voltage geothermal (ecoinvent 3.0.2) US
Wood	%	0.19	Onsite boiler, softwood mill average NE-NC (USLCI 1.6) RNA
Photovoltaic	%	0.15	Low voltage photovoltaic, 3kWp (ecoinvent 3.0.2) US

APPENDIX B

CHAPTER 3 SUPPORTING INFORMATION

Table 16: Algae LCI Inventory

Table includes database region and what unit process each item in the inventory relates to. Lab cultivation includes 250 mL, 2 L, and 20 L

Cultivation systems. Large-scale cultivation includes scale-up bags, PBRs and ponds.

Cultivation includes both lab and large-scale cultivation

Name	Database	Region	Description
Sodium nitrate	Ecoinvent 3.1	Global	Lab cultivation
Sodium phosphate	Ecoinvent 3.1	Europe	Lab cultivation
HDPE	Industry 2.0	Europe	Large scale cultivation
Potassium nitrate	Ecoinvent 3.1	Europe	Large scale cultivation
Sodium nitrate	Ecoinvent 3.1	Global	Lab cultivation
Sodium phosphate	Ecoinvent 3.1	Europe	Lab cultivation
Monopotassium phosphate	Ecoinvent 3.1	Global	Large scale cultivation
Carbon dioxide	Ecoinvent 2.2	Europe	Cultivation
2 butoxy-ethanol	Ecoinvent 3.1, USLCI	Europe, North America	Centrifuge cleaning
Propane	Ecoinvent 3.0.2	Global	Dryer operation
Hexane	Ecoinvent 2.2	Europe	Oil extraction
Electricity	2014 US electricity mix	USA	Various unit operations
Phosphoric acid	Ecoinvent 3.1	Global	Oil refining
NaOH	USLCI 1.6	North America	Oil refining
Fuller's Earth	USLCI 1.6	USA	Oil refining
Acetic acid	USLCI 1.6	North America	Oil refining

Cultivation systems. Large-scale cultivation includes scale-up bags, PBRs and ponds.

Cultivation includes both lab and large-scale cultivation

****Tables 17 to 23 were used for chapters 3,4 and 5 of this dissertation****

Table 17: Biomass Characteristics

Biomass characteristics and lab scale cultivation. Nutrient quantities were based on the biomass composition from Razon and Tan 2011 and the Redfield ratio.

Biomass characteristics			
Required CO ₂	2.11	gCO ₂ /g	Razon and Tan 2011
Nutrient uptake efficiency	0.75	%	Soratana 2014
Lipids	0.3	glipid/gbiomas s	Cellana and NREL aggregation
Redfield ratio	16	gN/gP	Metcalf and Eddy
N required	4.85	mgN/galgae	Razon and Tan 2011
P required	.303	mgP/galgae	Based on redfield ratio
Lipid density	920	kg/m ³	NREL
250 mL cultivation			
Total volume	250	mL	Cellana
250 mL Maximum biomass concentration	0.5	g/L	
Nitrogen source	NaNO ₃		
Phosphorus source	Na ₃ PO ₄		
Cultivation time	1	week	
2 L cultivation			
Total volume	2	L	Cellana
2 L Maximum biomass concentration	0.5	g/L	
Nitrogen source	NaNO ₃		
Phosphorus source	Na ₃ PO ₄		
Cultivation time	1	week	
To scale up	1.6	L	
20 L cultivation			
Total volume	20	L	Cellana
20 L Maximum biomass concentration	0.5	g/L	
Nitrogen source	NaNO ₃		
Phosphorus source	Na ₃ PO ₄		
Cultivation time	1	week	
To scale up	16	L	

Table 18: Outdoor Cultivation 1

Includes scale-up bag and PBR parameters. Nutrient requirements met based on biomass characteristics in the same manner as the small-scale cultivation.

Scale up bag			Cellana
Volume per bag	220	L	
Material	HDPE		
Material density	950	kg/m ³	
Volumetric productivity	0.06	g/L-day	
Inoculum volume	16	L	
Biomass initial concentration	0.05	g/L	
Biomass final concentration	0.5	g/L	
Nitrogen source	KNO ₃		
Phosphorus source	KH ₂ PO ₄		
Algae losses	0.025	w/w	
Scale up bag harvesting frequency	3	days	
Lifetime	90	days	
# of scale up bags required to inoculate PBR	6	bags	
Photobioreactor (model)			Cellana
Final concentration	0.46	g/L	
Number per module	17		
Volume	867	m ³ /module	
Cultivation time	3	days	
Productivity	16.1	g/m ² -day	
CO ₂ consumption	1.1	MT/day	
Photobioreactor (KDF)			Cellana
Final concentration	0.5	g/L	
Volume	24	m ³ /module	
Cultivation time	1	week	
Productivity	16.1	g/m ² -day	

Table 19: Outdoor Cultivation 2
Open ponds

Pond parameters (model)				
Pond A	Final concentration	0.59	g/L	Cellana
	Number per module	4		
	Volume	750	m ³ /module	
	Cultivation time	3	days	
	Productivity	16.1	g/m ² -day	
	CO ₂ consumption	1.4	MT/day	
Pond B	Final concentration	0.52	g/L	
	Number per module	3		
	Volume	2250	m ³ /module	
	Cultivation time	3	days	
	Productivity	16.1	g/m ² -day	
	CO ₂ consumption	4.3	MT/day	
Pond C	Final concentration	0.52	g/L	
	Number per module	3		
	Volume	2250	m ³ /module	
	Cultivation time	3	days	
	Productivity	16.1	g/m ² -day	
	CO ₂ consumption	4.3	MT/day	
Pond parameters (KDF)				
	Final concentration	0.5	g/L	Cellana
	Volume	60	m ³	
	Cultivation time	3	days	
	Productivity	16.1	g/m ² -day	

Table 20: Dewatering

Membrane filtration	Value	Unit	
Initial concentration	0.419	g/L	Bhave et al., 2012
Final concentration	10	g/L	
Efficiency	95%	%	
Electricity consumption	0.5	kWh/m ³	
Lifetime	10	years	
Capacity	7.8	MGD	
Centrifuge	Value	Unit	
Centrifuge capacity	300,000	gal/day	Aggregated Cellana, Weschler et al. 2014, Beal et al. 2015, FSA consulting
Biomass initial concentration	0.46	g/L	
Biomass final concentration	200	g/L	
Centrifuge efficiency	95-99	%	
Cleaning agent		Simple Green	
Amount of cleaning agent required	4	L/month	
Cleaning chemical		2 butoxy-ethanol	
Density of chemical cleaner	900	kg/m ³	
Centrifuge lifetime	20	years	
Disposal		Landfill	
Centrifuge cost	\$235,000	\$/centrifuge	
Power requirement	8.9	kWh/m ³	
Ring Dryer	Value	Unit	
Initial concentration	200	g/L	Cellana, Beal et al. 2015
Final concentration	950	g/L	
Propane consumption	0.05	kg/kg sludge	
Propane density	493	kg/m ³	
Efficiency	95	%	
Dryer lifetime	20	years	

Table 21: Oil Handling Parameters

Oil extraction			Soratana et al. 2014, Huo 2008
Oil yield	0.89	w/w	
Biomass moisture content	0.05		
Extraction solvent	Isohexane		
Solvent feed	5.17	kg solvent/kg	
Solvent losses	0.024	kg	
Biomass flowrate to extraction	100	kg/hr.	
Hours of operation	10	hr.	
Algae meal	0.7942	kg/kg algae	
Lipids	0.2058	kg/kg algae	
Oil refining			Chakraborty 2015
Phosphoric acid	0.0085	mL/g oil	
Electricity centrifuge	8	kWh/m ³	
Efficiency	0.86	w/w crude oil	
NaOH	1	meq/L	
NaOH volume	0.0003	L/g degummed oil	
NaOH	12	µg/g of degummed oil	
Yield	0.804	w/w crude oil	
Activated carbon	0.000225	g/g crude oil	
Fuller's earth	0.00075	g/g crude oil	
Efficiency	0.95	w/w neutralized oil	
Acetic acid	0.25	meq/L	
Yield	0.73	w/w crude oil	
EPA	21	% fatty acids	
DHA	24.5	% fatty acids	

Table 22: Diesel Production

Renewable Diesel		
Oil input	1.174	lboil/lb diesel
Hydrogen consumed	0.032	lb/lb diesel
Steam consumed	0.0329	lb/lb diesel
CO2 emitted	0.082	lb/lb oil input
Propane produced	0.059	lb/lb oil input
Wastewater generated	0.0971	lb/lb oil input
Natural gas consumed	84.05	BTU/lb diesel
Electricity consumed	0.0275	kWh/lb diesel
Cooling water consumed	27.11	lb/hour
Green diesel energy content	18925	BTU/lb diesel

Soratana et al.
2014, Huo 2008

Table 23: Monte Carlo Analysis

Triangular distribution was applied to all inputs. Where aggregated data with Cellana and other sources was used, Cellana was always selected as the best estimate. Beal 2015 was a biofuels study based on Cellana’s system, so Beal 2015 was selected as best estimate where data directly from Cellana was not provided.

Parameter	Min	Best	Max	Unit	
PBR CO ₂ consumption	1.07	1.13	1.18	MT/day	Aggregated Cellana, Davis et al., 2014
188-m ³ pond CO ₂ consumption	1.31	1.37	1.44	MT/day	Cellana
750-m ³ CO ₂ consumption	3.92	4.12	4.32	MT/day	
1500-m ³ CO ₂ consumption	20.93	21.97	23.02	MT/day	
Productivity	14.60	16.08	20.15	g/m ² -day	
MF electricity	0.3	0.5	0.7	kWh/m ³	Bhave et al., 2012
Pond biomass losses	1%	3%	5%	%	Selected
Annual operating days	300	330	345	days	Aggregated Beal et al. 2015, Weschler et al., 2014
Centrifuge efficiency	95%	99%	99%	%	

Table 24: Fish LCI Inventory

Fishing operation includes all onboard activities and transportation to fishing locations and back to port. Processing is the reduction of the whole fish to fish oil and fishmeal.

Name	Database	Region	Description
Steel	Ecoinvent 2.2	Europe	Vessel construction
Lubricant oil	LCA food	n/a	Vessel maintenance
Electric and coils: copper	ELCD 2.0.0	Europe	Vessel maintenance
Fishing net: nylon	Ecoinvent 3.1	Europe	Fishing operation
Fishing net: bronze	Ecoinvent 3.1	Global except Europe	Fishing operation
Fishing net: steel	Ecoinvent 2.2	Europe	Fishing operation
Fishing net: HDPE	Industry 2.0	Europe	Fishing operation
Hydraulic oil	Ecoinvent 3.1	Global except Europe	Vessel maintenance
Wood	USLCI	USA	Vessel construction
HDPE boxes	Industry 2.0	Europe	Onboard storage
Ice production	LCA food	n/a	Onboard storage
Fuel consumption	USLCI 1.6	USA	Fishing operation
Water	Ecoinvent 3.1	Switzerland	Fishing operation
Electricity	2014 US electricity mix	USA	Various unit operations
Fuel oil	USLCI 1.6	USA	Processing
Natural gas (heating)	USLCI 1.6	USA	Processing
Phosphoric acid	Ecoinvent 3.1	Global	Oil refining
NaOH	USLCI 1.6	North America	Oil refining
Fuller's Earth	USLCI 1.6	USA	Oil refining
Acetic acid	USLCI 1.6	North America	Oil refining

Table 25: Fuel Consumption of Purse Seine Fisheries

Includes a range of fuel consumptions from studies in the last decade. Freon 2014 includes fuel consumptions from many different studies. The average of these fuel efficiencies was used as the best estimate for the fish oil model. The min and the max were used for the MCA.

Fuel consumption (L/tonne fish)	Source
211.54	Freon 2014
155.05	
108.17	
99.76	
97.36	
90.14	
86.54	
206.73	
84.13	
42.07	
21.63	
151.44	
21.63	
118.99	
20.43	
18.75	
70	Ellingson 2006
110	Almeida 2007
111	

Table 26: Fish Biomass and Vessel Characteristics
 Fish biomass and vessel characteristics

Fish biomass			
EPA content	0.141	kg/kgoil	Yin and Sathivel 2010
DHA content	0.08	kg/kgoil	
EPA density	0.943	g/mL	MSDS from Sigma Aldrich
DHA density	0.95	g/mL	
Discards	13	%	Cappell 2007 (pelagic like menhaden and cod for oil)
Fish oil content	0.0292	kg/kg fish	Murilo et al 2014 (average)
Construction			
Concrete (ballasts)	100	g/tonne fish	Freon 2014
Batteries (lead)	0.3	g/tonne fish	
Batteries (sulfuric acid)	0.6	g/tonne fish	
Coils: copper wire	1.1	g/tonne fish	
Copper	5.3	g/tonne fish	
Engine: Steel	23	g/tonne fish	
Fishing net: nylon	21.175	g/tonne fish	
Fishing net: bronze	21.175	g/tonne fish	
Fishing net: steel	21.175	g/tonne fish	
Fishing net: HDPE	21.175	g/tonne fish	
Hull and structure: Steel	713.4	g/tonne fish	
Propeller: bronze	1.6	g/tonne fish	
Wood	172.6	g/tonne fish	
Zinc	1	g/tonne fish	

Table 27: Maintenance and Onboard Activities

Maintenance			
Lubricant oil	80.6	g/tonne fish	Cappell 2007
Lubricant oil density	1040	kg/m ³	
Electric and coils: copper	13.3	g/tonne fish	Freon 2014
Engine: Steel	23	g/tonne fish	
Fishing net: nylon	190.675	g/tonne fish	
Fishing net: bronze	190.675	g/tonne fish	
Fishing net: steel	190.675	g/tonne fish	
Fishing net: HDPE	190.675	g/tonne fish	
Hoses: Rubber	7	g/tonne fish	
Hull: Steel	1.5	kg/tonne	
Hydraulic oil	34.2	g/tonne fish	
Paint	43.1	g/tonne fish	
Wood	164.3	g/tonne fish	
Storage			
Fish boxes	HDPE		Cappell 2007
HDPE utilization	0.003	kg/kg fish	
Box uses	50	#	
Onboard preservation			
Ice production	0.75	kg/kg fish	Cappell 2007
Cooling agent	0.01	g/kg fish	
Cleaning agent (simple green)	0.6	ml/kg fish	
Fleet fuel consumption	96.07	L/tonne	Average from Table B2
Processing			
Water	0.0205	m ³ /kg fish	Cappell 2007
Electricity	0.032	kWh/kg fish	
Fuel oil (diesel)	0.049	L/kgfish	
Fish cooker heating power	34	kW	
Fish cooker electric power	35	kW	
Fish cooker capacity	60	kg/hour	Industrial cooker
Fish cooker capacity	1440	kg/day	

Table 28: Oil Handling

Oil extraction: Oil press			
Power (expeller)	100	hp	Modeled after a belt filter press: Weschler et al. 2014
Power (expeller)	74.63	kW	
Power (kettle)	20	hp	
Power (kettle)	14.93	kW	
Capacity	60	tons/day	
Oil press efficiency	89	%	US patent 6214396: BFP modeled after Weschler et al. 2014
Capacity	12.5	tons/hr.	Zhoushan Xinzhou fishmeal equipment factory LW500-2000
Power requirement	30	kW	
Oil decanter efficiency	95	%	
Oil refining			
Phosphoric acid	8.50E-03	mL/g oil	Chakraborty 2015
Electricity centrifuge	8	kWh/m ³	
Efficiency	0.86	w/w crude oil	
NaOH	1	meq/L	
NaOH volume	0.0003	L/g degummed oil	
NaOH	1.2E-05	g/g of degummed oil	
Yield	0.804	w/w crude oil	
Activated carbon	2.25E-04	g/g crude oil	
Fuller's earth	7.50E-04	g/g crude oil	
Efficiency	0.95	w/w neutralized oil	
Acetic acid	0.25	meq/L	
Yield	0.73	w/w crude oil	
EPA	0.21	% fatty acids	
DHA	0.245	% fatty acids	

APPENDIX C

CHAPTER 4 SUPPORTING INFORMATION

**Tables 29 to 31 apply to chapters 4 and 5. There are no additional tables for chapter 5.

Table 29: General Plant Parameters

Name	Value	Unit	
Required return on capital	0 to 30%	%	Selected
Operating life	20	Years	Cellana
OSBL + OPC depreciation period	20	Years	Based on operating life
OSBL + OPC depreciation rate	5%	per year	
ISBL depreciation period	20	Years	
ISBL depreciation rate	5%		
Insurance, property tax	0.7%	% of Total Plant Capital	
Maintenance & materials	3%	% of ISBL	Davis et al., 2014
Operating rate	80-95%		Selected
Electricity	\$0.069	\$/kWh	US EIA 2015 national average
Outside Battery Limits %	15%	% of ISBL	Selected (comparable to literature)
Project Costs	15%	% of ISBL + OSBL	
Working Capital	3%	% of Total Plant Capital	
Facility size	88	ha	Cellana

Table 30: Cost of Chemicals and Materials

Name	Value	Unit	Source
CO ₂	\$39	\$/tonne	(Davis et al., 2011)
Electricity	0.069	\$/kWh	(USEIA, 2016)
Water	0.20	\$/1000 gal	Utility price (agriculture water rate)
Propane	\$3.29	\$/gal	Cellana
Diesel	\$3.39	\$/gal	
Polypropylene	\$9,800.00 to 12,060	\$/tonne	
Potassium nitrate	\$750 to 1,000	\$/tonne	
Monopotassium phosphate	\$1,010.00 to 1,100	\$/tonne	
Sodium nitrate	\$200 to 500	\$/tonne	
Sodium phosphate	\$360 to 430	\$/tonne	
HDPE	\$1,100.00 to 1,200	\$/tonne	
Hypalon	\$10 to 30	\$/m ²	
Simple green	\$2,220	\$/m ³	
Isohexane	\$1,005	\$/tonne	
Phosphoric acid	\$850	\$/tonne	
Sodium hydroxide	\$430	\$/tonne	
Acetic acid	\$620	\$/tonne	

Table 31: Labor Estimates

Labor estimates Salaries were provided on an FTE basis. The total overall cost per employee (including overhead) was presented as the Total personnel cost. Total operational staff requirement was 1.07 ha/FTE. Salaries for all personnel were collected from the Bureau of labor statistics (BLS).

Position	Salary (\$/per FTE)
<u>a. Admin</u>	
i. Security	\$44,040.00
ii. Custodian	\$22,320.00
iii. Clerical	\$30,650.00
iv. Purchasing	\$40,520.00
v. IT	\$64,710.00
vi. HSSE	\$41,400.00
vii. HR	\$64,900.00
viii. Quality Control	\$44,420.00
ix. Site Manager	\$99,230.00
<u>b. Laboratory and Culture Maintenance</u>	
i. Scientist	\$64,690.00
ii. Lab Technicians	\$40,970.00
<u>c. Engineering / Maintenance</u>	
i. Managers	\$72,970.00
ii. Master mechanics	\$47,520.00
iii. Instrument technicians	\$36,020.00
iv. Programmers	\$74,080.00
v. Electrical	\$46,880.00
<u>d. Operations</u>	
i. Unit Operators	\$32,390.00
ii. Harvest Operators	\$32,390.00
iii. PBR Operators	\$32,390.00
iv. Operations Manager	\$73,160.00
v. Operations Office Support	\$31,840.00
vi. Extraction Operators	\$37,580.00
Total Personnel Cost	\$4,434,093.88

Table 32: Baseline Capital Costs

	Cost per parameter	Source
Ponds and installation	\$31,959	(Beal et al., 2015; Davis et al., 2014)
Gas and water pipes	\$11,134	(Davis et al., 2014)
Water pumps	\$3,299	
Pond liner material	\$10/m ²	(Alibaba, 2015)
Membrane filtration	\$2,274,000	Pall corporation
Cost of centrifuge	\$235,000/unit	Cellana, (Beal et al., 2015)
Cost of ring dryer	2,750,000	
Cost of oil extraction equipment	\$6,950,256	