

**LIFE CYCLE ASSESSMENT OF MICROALGAL BIODIESEL AND POLICY
IMPLICATIONS TO MINIMIZE UNINTENDED CONSEQUENCES**

by

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The overall goals of this research are to identify forms of environmentally friendly microalgal biodiesel production and to address the broader policy implications of the Renewable Fuel Standard 2 (RFS2) to avoid unintended environmental consequences from the production and the utilization of biofuels. Life cycle assessment (LCA) of microalgal biodiesel was conducted using a process LCA model with Monte Carlo Analysis (MCA) for uncertainty analysis. First, the study focused on environmental impacts from the production of microalgal mass. Twenty scenarios of microalgal cultivation with different sources of CO₂, nutrients and material used to construct photobioreactor (PBR) were evaluated. The results showed that the utilization of CO₂ from flue gas only reduces global warming potential (GWP), while the utilization of nutrients from municipal wastewater mainly reduces eutrophication potential (EP), and the selection of material used to construct PBR is important.

LCA of microalgal biodiesel was conducted to evaluate the major contributions to different environmental impacts and to identify the production condition with minimal impacts. Environmental impacts from four different microalgal biodiesel production scenarios to achieve the biomass-based diesel quantity required by the RFS2 were quantified. These four scenarios included lower and higher production efficiencies and different sources of resources, which are synthetic, and natural and waste. None of the four scenarios met the RFS's greenhouse gas emissions requirement. The emissions can be minimized by improving the energy efficiency of

harvesting process, since the GWP results are sensitive to energy consumption in harvesting process. Sensitivity of EP, ozone depletion potential and ecotoxicity potential to other parameters are also reported.

GWP, EP and photochemical smog formation potential (PSP) results from the scenario with minimal impacts were compared to the impacts from other diesels, petroleum diesel, soybean diesel and canola diesel. The tradeoffs between different types of diesel suggested that future RFS should include other environmental criteria such as EP and PSP to minimize unintended consequences. Possible strategies to setting life-cycle EP and PSP criteria are discussed.

Finally, a LCA on the co-production of microalgal biodiesel and bioethanol from microalgae and corn were conducted. CO₂ for microalgal cultivation was provided from flue gas and/or fermentation processes. The recovery of CO₂ from fermentation process decreases GWP, but slightly increases EP. The co-production of the microalgal biofuels can reduce GWP and EP by 54 and 52%, respectively, compared to the production of microalgal biodiesel alone. Lipid and carbohydrate contents of microalgae should be 24-36% and 44%, respectively, in order to produce the maximum energy content in algal biofuels with the minimum GWP and EP impacts.

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NOMENCLATURE

ASP	Algal Species Program
ASTM	American Society for Testing Material
BEES	The Building for Environmental and Economic Sustainability
BGY	Billion Gallons per Year
CF	Characterization Factor
DOE	Department of Energy
EISA	Energy Independence and Security Act
EP	Eutrophication Potential
EPA	The U.S. Environmental Protection Agency
Eq	Equivalent
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
GHG	Greenhouse Gas
GMM	Genetically Modified Microalgae
GREET	Greenhouse Gases, Regulated Emissions, and Energy use in Transportation
GWP	Global Warming Potential
HDPE	High-Density Polyethylene
LCA	Life Cycle Assessment

NOMENCLATURE (Continued)

LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LSD	Low-Sulfur Diesel
MCA	Monte Carlo Analysis
NER	Net Energy Ratio
NREL	National Renewable Energy Laboratory
PAN	Peroxyacetyl Nitrate
PBR	Photobioreactor
PC	Polycarbonate
PE	Polyethylene
PMMA	Polymethyl Methacrylate
PSP	Photochemical Smog formation Potential
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl Chloride
RFS	Renewable Fuel Standard
SVR	Surface area-to-Volume Ratio
TAG	Triacylglyceride or Triacylglycerol
TMDL	Total Maximum Daily Load

NOMENCLATURE (Continued)

TRACI	Tool for the Reduction and Assessment of Chemical and other environmental Impacts
TSS	Total Suspended Solid
ULSD	Ultra Low-Sulfur Diesel
US	United States
VOC	Volatile Organic Compound
w/w	Weight/weight

PREFACE

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1.0 INTRODUCTION

1.1 MICROALGAL BIODIESEL – A POTENTIAL BIOMASS-BASED DIESEL

Petroleum fuels are the main energy source for society and industrial activities. The world petroleum production in the year 2008 was approximately 166 EJ (one Exajoule = 10^{18} Joules), and increased to 189 EJ in 2009 (U.S. Energy Information Administration, 2011). Of the refined petroleum fuels used in the U.S., petroleum diesel accounted for 20%. Petroleum diesel has widely served society in the form of transportation or engine fuel. Its high density and ease of transport make it an ideal source of energy. However, our society's dependence on petroleum diesel has resulted in its depletion and environmental disruption results from its combustion which releases greenhouse gas (GHG) emissions at an approximate value of 92-95 g CO₂ Eq/MJ. GHG emissions such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), generated from the production and consumption of petroleum diesel, cause several environmental detrimental phenomena related to global warming (i.e. temperature changes, extreme weather conditions, sea level rising and lake level declining (Sheehan et al., 1998; Solomon & Miller, 2007). Another concern with petroleum diesel is its price, which continues to increase and affects industrial and global economies. One way to mitigate social, environmental and economical problems resulting from the unsustainable consumption of petroleum diesel is to

substitute petroleum diesel with a sustainable renewable energy source that has potential to be scaled up without significant environmental impact.

Oil crops such as soybean, palm, canola and sunflower can provide renewable sources of oil. The biodiesel that is obtained from such oil is often blended with petroleum diesel to meet international biodiesel fuel standards like the American Society for Testing Material (ASTM) D6751 standard (Moser, 2009). Biodiesel is considered as a renewable energy source because the production of oil crops uses the ultimate energy source, sunlight. Oil crops also have a shorter reproduction or photosynthetic process compared to petroleum which takes millions of years to reproduce. Nevertheless, renewable bio-based fuels are not necessarily sustainable simply because they can be quickly replenished. Apart from the high feedstock production cost, oil-crop diesel production leads to adverse effects of food-supply chain causing a scarcity of agricultural land and increasing prices of agricultural products (Bruce, 2008; Li et al., 2008b). The U.S. petroleum fuel consumption rate of 0.53 billion m³/year would require approximately 60% of the U.S. agricultural area to produce sufficient oil crops to substitute petroleum diesel production (Chisti, 2008). Oil crop production can result in adverse environmental impacts such as increasing water demand, water pollution, soil erosion and deforestation (Landis et al., 2007). Additional amount of nitrous oxide (N₂O) entering the atmosphere from synthetic nitrogen (N) fertilizer production could contribute as much or more global warming potential (GWP) than the emissions resulting from petroleum diesel production (Bruce, 2008; Li et al., 2008b). Furthermore, there are environmental tradeoffs from oil-crop diesel production, for example, the production of biodiesel from rapeseed reduces GHG emissions, but increases emissions contributing to eutrophication potential (EP) and photochemical smog formation potential (PSP) and energy consumption (Miller et al., 2007; Spirinckx & Ceuterick, 1996). Based on the

economic and environmental issues facing oil-crop diesel, a more sustainable form of biodiesel such as microalgal biodiesel becomes more appealing.

Microalgal biodiesel is produced from oil extracted small-sized algae, which contain higher oil content than macroalgae (Johnson & Wen, 2009; Li et al., 2008b; Sharif Hossain et al., 2008). Microalgal biodiesel is considered a viable option to reduce a society's dependence on petroleum diesel while lessening environmental impacts such as GWP compared to petroleum diesel. The potential of microalgal biodiesel to substitute petroleum diesel has been extensively discussed due to several characteristics of its feedstock, microalgae, and the quality of microalgal oil. Microalgae have high growth rates, productivity and photosynthetic efficiency which makes it possible to satisfy the massive energy demand (Avagyan, 2008; Greenwell et al., 2010; Huang et al., 2010; Li et al., 2008b; Vijayaraghavan & Hemanathan, 2009). Biomass productivity is greater than traditional oil crops; microalgae productivity is reported to be up to 50 times higher than that of switchgrass, the fastest growing terrestrial plant (Li et al., 2008b). For some particular microalgal strains, the maximum annual oil yield is approximately 135 times higher than soybean, 30 times higher than *Jatropha*, and 10 times higher than oil palm (Ferrell & Sarisky-Reed, 2010). Another ideal property of microalgae is that their mass can exponentially double in as little as 3.5 hours (Bruce, 2008). It was also reported that even microalgal cultivation in dry areas with little to no freshwater is possible. Microalgae harvesting can be conducted throughout the year since it depends little on seasonal variations (Lehr & Posten, 2009). The photosynthetic efficiency of microalgae ranges from 10-20% which is higher than that of switchgrass. Generally, switchgrass can convert solar energy to biomass energy no more than 1 W/m²/year (Watt per square meter per year) or less than 0.5% of the solar energy received at a typical mid-latitude region (200-300 W/m²) (Li et al., 2008b).

Microalgae also requires less cultivation area compared to oil crops, only 3% of the total agricultural area in the U.S. is required to produce the same amount of oil-crop diesel (Avagyan, 2008). The findings from the study under the Congressional funding program by the Aquatic Species Program, National Renewable Energy Laboratory, Department of Energy (ASP, NREL, DOE) suggested that microalgal oil can be obtained from microalgae with 60 to 70% lipid content at a growth rate of 19.5 kg/m²/year cultivated under optimal conditions by pressing process (Reising, 2007). Microalgal oil also meets the ASTM D6751 with a density of 0.864 kg/L, a flash point of 115 °C and a heating value of 41 MJ/kg (Huang et al., 2010; Moser, 2009). In summary, the characteristics of microalgae and the properties of microalgal oil meet the needs of the U.S. biomass industry which are that it is easy to grow, has a high yield and provides good-quality fuel (Bruce, 2008).

Microalgae has the potential to exhibit fewer environmental impacts than petroleum diesel and soybean-derived biodiesel since microalgae can convert air and water emissions to oil (Eriksen, 2008). They can be cultivated in diverse environments e.g. CO₂ abundant ambient air like flue gas, seawater, freshwater and wastewater (Carvalho et al., 2006a; Kadam, 2002; Pittman et al., 2011). Co-location of microalgal cultivation systems with flue gas sources can provide waste CO₂ as a source of carbon (C) for microalgal growth. Some microalgal strains can tolerate up to 5 to 15% of CO₂ concentration in flue gas; the typical CO₂ concentration in the atmosphere is only about 0.03 to 0.06%. The photobioreactor (PBR) cultivation system could reduce CO₂ from flue gas without having an effect from the residual nitrogen dioxide (NO₂) and nitric oxide (NO) in the flue gas on the productivity of microalgae, while the budget for pollution prevention instrument installation can be reduced (Reising, 2007). Microalgal cultivation can also be grown from the nutrients available in wastewaters. This form of industrial symbiosis minimizes

freshwater use and minimizes the chemical treatment of wastewaters. Microalgae consume N and phosphorus (P) and replace the need for wastewater treatment chemicals such as calcium carbonate (CaCO_3), calcium hydroxide (Ca(OH)_2) and ferrous sulfate (FeSO_4) often used in wastewater treatment process. Coupling the microalgal cultivation system and the wastewater treatment system can possibly achieve a mutually beneficial industrial symbiosis (Li et al., 2008b). It is evident that an algal wastewater treatment system is a potential environmentally friendly and a more economical option to remove nutrients and metals from wastewater than a conventional tertiary wastewater treatment system (Kong et al., 2010). Even though some microalgal strains can tolerate and also reduce heavy metals contaminated in wastewater, contamination is still a major concern when cultivating microalgae in wastewater. This contamination can be managed by a pre-treatment technology, sediment removal and wastewater stabilization.

This research focuses on the production of microalgal biodiesel from microalgal oil; however, microalgal mass and starch can also be used to produce bioethanol, bio-syngas and biohydrogen (Greenwell et al., 2010; Sivakumar et al., 2010; Yanqun et al., 2008). Compared to oil from oil crops, microalgal oil can be processed into a ready-to-use form biodiesel without any residues such as bark, stems, branches or leaves (Avagyan, 2008). Sediments from microalgal cultivation can be converted to biomethane, bioethanol, hydrogen and electricity (Bruce, 2008; Reising, 2007). The production of these biofuels coupled with other high-value chemicals can help microalgal cultivation and microalgal biodiesel production to be more cost-effective than oil-crop diesel production (Li et al., 2008b).

Although there seem to be several advantages that support the potential of using microalgae as a biodiesel feedstock, this technology has not yet reached industrial scale

production of microalgal biodiesel (Carvalho et al., 2006a). One of the limitations is the high price of valuable products from microalgae, excluding biodiesel. Other limitations are related to the cultivation system techniques, and the energy intensive harvesting and drying processes. The low concentration in the culture and the small cell size of microalgae with large water content of the harvested microalgal mass leads to a high harvesting cost and an energy intensive drying process (Li et al., 2008b). Microalgal cultivation can occur in open pond systems or in closed photobioreactors (PBRs). Open pond systems often provide lower yields of microalgal mass while PBRs typically have higher investment and operation cost compared to conventional agricultural activities. Despite these advantages and limitations of a microalgal biodiesel production, environmental impacts from microalgal biodiesel should be evaluated over its entire life cycle before developing to a large-scale operation in order to avoid unintended environmental consequences.

1.2 RESEARCH GOALS AND OBJECTIVES

The overall goals of this research are to identify forms of environmentally friendly microalgal biodiesel production and to address the broader policy implications of the Renewable Fuel Standard 2 (RFS2) to avoid unintended environmental consequences from the production and the utilization of biofuels. This research aims to investigate the life-cycle environmental impacts of microalgal biodiesel using life cycle assessment (LCA) model with Monte Carlo analysis (MCA) for uncertainty analysis. Environmental impacts from different microalgal biodiesel production scenarios are quantified and compared to identify and minimize the major environmental impacts and tradeoffs. Further, the environmental impacts are compared to life-cycle environmental impacts from other diesels, petroleum diesel and biodiesels from soybean oil and canola oil to evaluate the tradeoffs between different types of diesel. The specific objectives are:

- 1) To quantify the life-cycle environmental impacts such as GWP, EP and PSP and energy consumption from microalgal biomass cultivation using an industrial symbiosis approach for providing nutrients and CO₂.
- 2) To identify the major environmental impacts over the entire life cycle of microalgal biodiesel using a process LCA model developed for microalgal biodiesel with MCA for uncertainty and sensitivity analysis.
- 3) To evaluate environmental tradeoffs and unintended consequences of meeting the RFS2 with different types of diesel (microalgal biodiesel, petroleum diesel and other biodiesels) and to assess how the RFS should be modified to address these unintended consequences.
- 4) To investigate the implications of co-production of ethanol and biodiesel to improve the environmental footprint of microalgal biofuels.

1.3 INTELLECUTAL MERIT

This dissertation provides the first complete LCA of microalgal biodiesel. The impact results over the entire life cycle of microalgal biodiesel identify processes that are responsible for the major impacts and thus identify areas of improvement for microalgal biodiesel. The existing RFS is assessed and modified based on the life-cycle impact results to minimize unintended consequences.

Currently, there is no comprehensive LCA of microalgal biodiesel evaluating impacts other than GWP. Since microalgal biodiesel contributes other impacts throughout its life cycle, this dissertation proposed criteria for other environmental impacts which should be included in future RFS. The results could alert the industries and the community to consider other impacts and tradeoffs.

2.0 BACKGROUND AND LITERATURE REVIEW

2.1 RENEWABLE FUEL STANDARD

The National Renewable Fuel Standard (RFS) program was established by Congress under the Energy Policy Act (EPAct) of 2005. The RFS program was revised by the U.S. Environmental Protection Agency (EPA) under the Energy Independence and Security Act (EISA) of 2007, which added Section 211(o) to the Clean Air Act (CAA) to ensure that the volumes of renewable fuel specified in the statute are enacted. The revised RFS is commonly known as the RFS2 program. The RFS2 program was effective since July 1, 2010, to establish specific annual volume standards for renewable fuels. Renewable fuels consist of advanced biofuels, which are cellulosic biofuel, non-cellulosic advanced biofuel and biomass-based diesel (e.g. biofuels produced from microalgae), and conventional biofuels (e.g. ethanol derived from corn starch). The annual volume standards in billion gallon per year (BGY) are calculated in terms of ethanol equivalent, despite some of the fuels being biodiesel. Biodiesel has an equivalence value of 1.5 ethanol equivalent gallons, while cellulosic biodiesel has an equivalence value of 1.7 ethanol equivalent gallons (Office of Transportation and Air Quality, 2011; U.S. Environmental Protection Agency, 2010b).

Apart from proposing projected annual volume standards for the renewable fuels, EPA is required to analyze life-cycle GHG emissions from increasing the use of renewable fuels. Listed

in Table 1 are percent reduction thresholds of life-cycle GHG emission for each type of renewable fuel set based on the emission from petroleum fuels distributed in 2005, which is approximately 90 g CO₂ Eq/MJ (Office of Transportation and Air Quality, 2010a; Office of Transportation and Air Quality, 2010b; U.S. Environmental Protection Agency, 2010b).

Table 1 Renewable Fuel Requirements of the Renewable Fuel Standard 2.

Year	Renewable Fuel (Billion Gallons per Year; BGY)				Total Advanced Biofuel (BGY)	Total Renewable Fuel (BGY)
	Conventional Biofuel	Cellulosic Biofuel	Biomass-Based Diesel	Non-Cellulosic Advanced Biofuel		
CO ₂ reduction*	20% reduction	60% reduction	50% reduction	50% reduction		
2008	9.0	n/a	n/a	n/a	n/a	9.0
2009	10.5	n/a	0.5	0.1	0.6	11.1
2010	12.0	0.1	0.65	0.2	0.95	12.95
2011	12.6	0.25	0.80	0.3	1.35	13.95
2012	13.2	1.0	1.0	0.5	2.0	15.2
2013	13.8	1.75	TBD	0.75	2.75	16.55
2014	14.4	3.0	TBD	1.0	3.75	18.15
2015	15.0	4.25	TBD	1.5	5.5	20.5
2016	15.0	5.5	TBD	2.0	7.25	22.25
2017	15.0	7.0	TBD	2.5	9.0	24.0
2018	15.0	8.5	TBD	3.0	11.0	26.0
2019	15.0	10.5	TBD	3.5	13.0	28.0
2020	15.0	10.5	TBD	3.5	15.0	30.0
2021	15.0	13.5	TBD	3.5	18.0	33.0
2022	15.0	16.0	TBD	4.0	21.0	36.0

n/a = Not Applicable, TBD = To Be Determined

*Percent life-cycle GHG emissions reduction threshold of the baseline which is approximately 90 g CO₂ Eq/MJ from petroleum transportation fuels distributed in 2005 (Office of Transportation and Air Quality, 2010a; Office of Transportation and Air Quality, 2010b).

2.2 MICROALGAL BIODIESEL PRODUCTION

Microalgal biodiesel production consists of six main processes, namely strain selection, cultivation, harvesting and dewatering, drying, extraction and conversion. Each process offers various options, thus one microalgal biodiesel production could be different from another resulting in different environmental impacts and tradeoffs.

2.2.1 Microalgal Strain Selection

Microalgae are prokaryotes, cyanobacteria, or eukaryotes. Most microalgae are photoautotrophic, depending on sufficient light, while some can be heterotrophic, depending on degradable organic substances (Carvalho et al., 2006a; Li et al., 2008b). They can be grown in seawater, brackish water and freshwater and on a marginal land (Li et al., 2008b; Tsukahara & Sawayama, 2005). The typical cells size is in the range of 2-20 μm in diameter. They have high growth rate and high photosynthetic efficiencies because of their simple structures. Their growth rate (μ) can be calculated using Equation 1. This equation is used to determine the specific growth rates of microalgae in the helical tubular reactor, or biocoil, based on the doubling time in exponential growth from semi-log cell density plots, where N_1 and N_2 are biomass at time 1 (t_1) and time 2 (t_2), respectively (Kong et al., 2010).

$$\mu = \ln (N_2/N_1)/(t_2-t_1) \quad \text{Equation 1}$$

Through photosynthesis with sufficient water, nutrients such as N, P and iron (Fe), and some trace elements, microalgae can convert CO_2 and solar energy to carbohydrates (starch and/or cellulose), proteins, nucleic acids and lipids (i.e. triacylglyceride or TAG). These

components of microalgal biomass can be extracted and used as for production of fuels, energy, and chemical feedstocks (Avagyan, 2008; Brennan & Owende, 2010; Gouveia & Oliveira, 2009; Janssen, 2002; Lehr & Posten, 2009; Rosenberg et al., 2008; Singh & Dhar, 2011; Tsukahara & Sawayama, 2005). Microalgae in microalgal biodiesel production can be categorized into two strains: indigenous strain and genetically modified microalgae (GMM) strain. The indigenous strains are more tolerant of the local environment, however their oil contents are lower compared to the GMM strains. One of the main objectives in microalgal genetic modification is to increase biomass productivity of microalgae (Posten, 2009). Generally, microalgal biomass is the expected yield from microalgal cultivation, whereas oil per gram microalgal biomass is the expected yield in microalgal oil production. Some microalgal species can accumulate lipid from 30 up to 50% of their dry weight biomass (Li et al., 2008b). Apart from lipid content, microalgal biomass productivity should also be considered when selecting microalgal strains for biofuel purposes (Eriksen, 2008; Greenwell et al., 2010; Shen et al., 2010).

2.2.2 Cultivation of the Microalgal Biomass

The two main microalgal cultivation systems are open ponds and PBRs. The typical design of open ponds are circular and raceway, while the common designs of PBRs are vertical, horizontal and helical tubular, flat-plate and fermenter (Chisti, 2008). Open pond systems can be coupled with PBR systems, like the successfully operated coupling cultivation system of *Haematococcus pluvialis* and *Chlorella* sp. The constant and contamination-free conditions of the first-stage PBR system benefit the continuous cell division of microalgae, whereas the second-stage open pond system exposes microalgal cells to nutrient and other environment to stimulate metabolites

production of microalgae (Huntley et al., 2007; Li et al., 2008b). However, the coupled system is not further discussed in this research.

Open pond systems are the most conventional microalgal cultivation configuration in industrial-scale operation for the production of valuable bioproducts from microalgae, but not for the production of microalgal biomass for microalgal biodiesel production. The systems typically consist of a circular or raceway pond constructed from concrete with plastic liner and piping system, and a rotating arm or paddle wheel used to agitate the culture (Huntley et al., 2007; Lehr & Posten, 2009; Posten, 2009; Reijnders & Huijbregts, 2009), as depicted in Figure 1. The main constraint of open pond systems is the difficulty in controlling culture conditions such as a microbial contamination, weather fluctuation and water loss from evaporation. Microbial contamination can be avoided by selecting microalgal strains that can tolerate an extreme condition e.g. high salinity or high pH media, which is not a suitable avoidance for most microalgal species. Examples of high-tolerant strains are *Spirulina platensis*, *Chlorella* sp. and *Dunaliella salina* (Posten, 2009; Reijnders & Huijbregts, 2009).

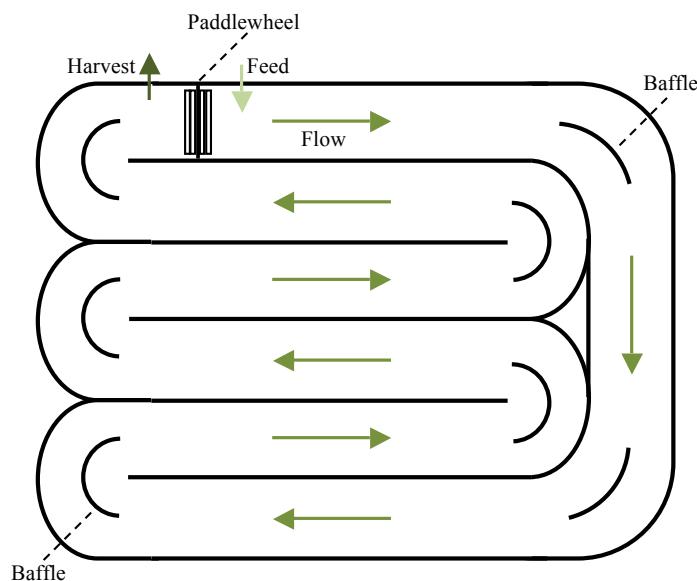


Figure 1 Basic design of open pond system.

A better control of cultivation conditions can be obtained by switching from an open pond system to a PBR system (Li et al., 2008b; Posten, 2009). Table 2 summarizes the design criteria for the different types of PBRs including light harvesting efficiency, CO₂ supply, control degree, land use, and scale-up possibility to maintain a constant condition throughout temporal and spatial aspects of the PBR system. The productivity of microalgal biomass depends on light penetration of a PBR, which relies directly on a surface area-to-volume ratio (SVR) of a PBR. Therefore, to increase the productivity, a high SVR must be reached via the configuration design of PBR (Carvalho et al., 2006a). The productivity of microalgal biomass in PBRs is approximately three times higher than that in open ponds; hence harvesting cost can be significantly lower. However, the major constraint of using PBR in an industrial-scale microalgal biomass production is their high cost of operation (Carvalho et al., 2006a; Posten, 2009).

Table 2 Design criteria of closed photobioreactor (PBR) (adapted from (Carvalho et al., 2006a)).

Type of PBR	Light Harvesting Efficiency	Degree of Control	Land Use	Scale-up	Productivity (g/L/d); Species
Vertical Tubular	Medium	Medium	Medium	Possible	0.5; <i>P. cruentum</i>
Horizontal Tubular	Good	Medium	Poor	Possible	0.25; <i>S. platensis</i> 0.7; <i>Nannochloropsis</i> sp.
Helical Tubular	Medium	Good	Excellent	Easy	0.4; <i>S. platensis</i>
Flat-plate	Excellent	Medium	Good	Possible	0.85; <i>Nannochloropsis</i> sp. 2.15; <i>S. platensis</i>
Fermenter	Poor	Excellent	Excellent	Difficult	0.03-0.05; Several

The two main types of PBRs investigated in this study are (1) tubular, vertical and horizontal, and (2) flat-plate PBRs (Carvalho et al., 2006a; Posten, 2009). Tubular and flat-plate PBRs are widely researched because of their advantages in harvesting natural light, which is abundant at no cost, and their scale-up possibility. These two types of PBRs consist of two main systems, a light-harvesting unit and a gas-exchanging unit. The light-harvesting unit is made of

small diameter tubes to provide a high SVR, while CO₂ is supplied and biomass is harvested in a gas-exchanging unit. The culture is circulated between the two units by pumping under a limited shear force condition to prevent cell damage (Carvalho et al., 2006a; Chisti, 2008; Posten, 2009).

The most common design for microalgal mass production is a tubular PBR. A vertical tubular PBR is an airlift or bubble column reactor, shown in Figure 2 (a). It has several configurations such as tubes, bags and flexible plastic film in a metal frame chamber. Typical PBRs are made out of polyethylene (PE) or glass to allow sufficient light penetration. Each configuration has its advantages and limitations. For example, PE bags are inexpensive, highly transparent and easy to operate and sterilize via a high temperature in film extrusion process. The limitations of vertical tubular PBR are that its productivity decreases when the PBR's volume is increased; in addition, energy loss often results from a high-reflected solar radiation due to the angle of tube layout (Carvalho et al., 2006a; Sorgeloos et al., 1977). Another configuration of tubular PBR is a horizontal tubular PBR, as depicted in Figure 2 (b). The light-harvesting unit of horizontal PBR is usually made of transparent materials such as polycarbonate (PC) or glass. Horizontal tubular PBRs can be expanded to larger culture volumes because of their high contamination tolerance. Unlike vertical tubular PBRs, the scale-up process of horizontal tubular PBRs to an industrial scale of 5-10 m³ is simply achieved by adding more tube sets, while maintaining an adequate linear velocity at the typical rate of 30 cm/s, and avoiding increasing O₂. Moreover, horizontal tubular PBRs are efficient and cost-effective if operated under a low shear stress condition with an appropriate mixing rate. However, the major problems of horizontal tubular PBRs are the temperature control and relatively high operation cost. It requires costly temperature controls for cooling purposes since the temperature can be increased up to 20°C within a day due to the light infiltration. Consequently, to assure a low temperature level,

large quantities of water are required for water spraying and cooling. Another method to maintain the temperature involves submerging the cultivation system in water, which is not applicable for a glass horizontal tubular PBR due to the fragility of the glass (Carvalho et al., 2006a).

Another type of PBR discussed in this study is the flat-plate PBR. This type of PBR consists of narrow panels intended to increase SVR for a higher light-harvesting efficiency. A gas-exchanging unit is at the bottom through perforation plastic tubes, as presented in Figure 2 (c). The main advantages of flat-plate PBR are the high biomass productivity, uniform light distribution and high light-harvesting efficiency when oriented toward the sun. On the other hand, the main disadvantage is the small diameter of a flat-plate PBR, with a high photosynthetic activity, can result in oxygen buildup (Carvalho et al., 2006a).

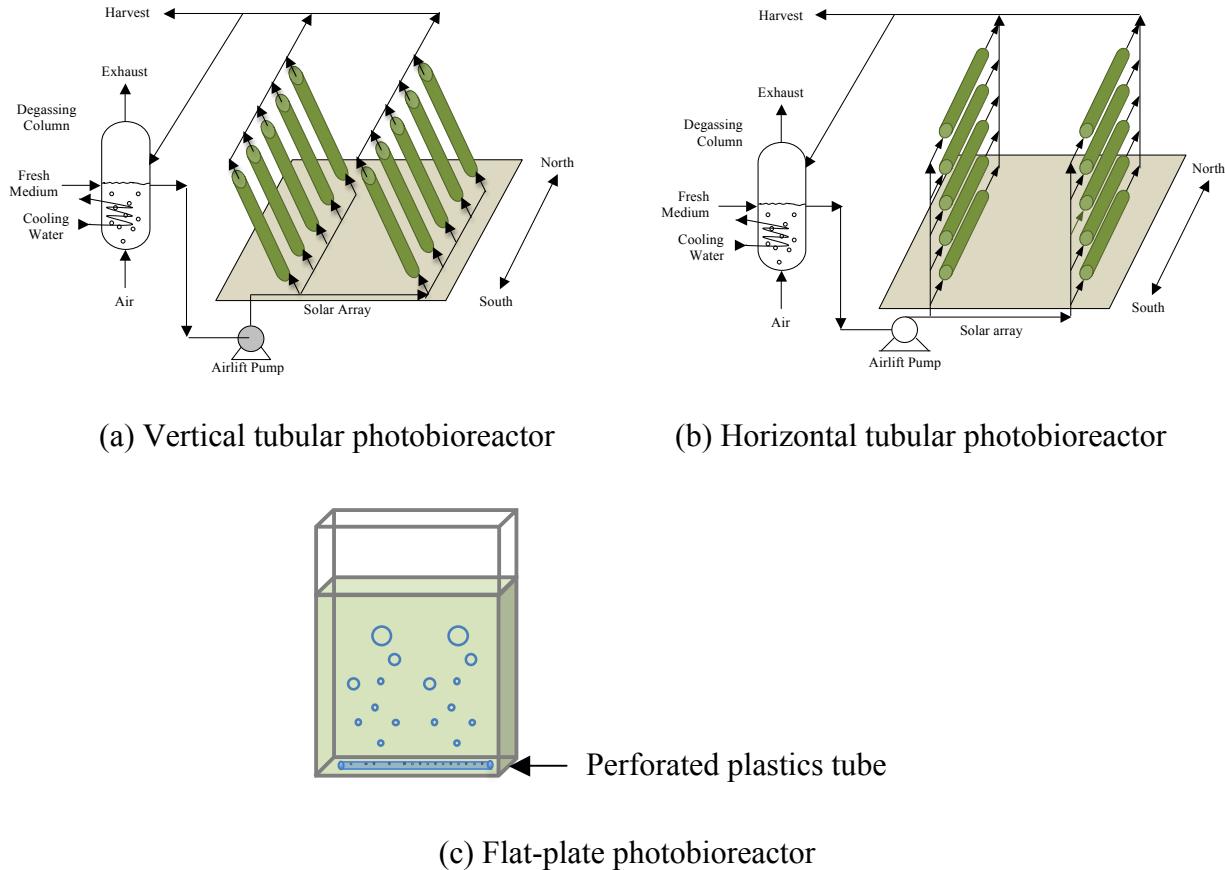


Figure 2 Schematic flows of photobioreactors.

2.2.3 Harvesting of the Microalgal Slurry

The third process of microalgal biodiesel production is harvesting and dewatering. Microalgal biomass in a microalgal suspension with 0.02 to 0.06% of total suspended solid (TSS) can be harvested in a one- or two-step harvesting process for microalgal slurry with 2 to 7% of TSS. The slurry can be further dewatered to achieve microalgal paste with 15 to 25% of TSS (Shelef et al., 1984). Due to a relatively low microalgal mass concentration in the cultivation system (from 0.001 to 0.005 g/m³), and the small cell size of microalgae (from 2 to 20 µm in diameter), the operation cost and energy consumption during the harvesting process are a significant

concern (Carvalho et al., 2006a; Li et al., 2008b; Shelef et al., 1984). Researchers have found that additional difficulties in microalgal harvesting process also result from microalgae's negatively charged surfaces and possibly from their high mobility (Shelef et al., 1984).

There are several harvesting methods and each one offers different benefits and limitations. For chemical and biological flocculations, their operation cost is low, however, they require a long processing time and the harvested biomass also has a risk of bioreactive product decomposition. Alternatively, other common harvesting methods are sedimentation, centrifugation, filtration, additional flocculation and combination of flocculation and flotation; however, they are more costly (Lee et al., 2009; Mata et al., 2010; Shelef et al., 1984).

A suitable harvesting and dewatering method should be selected based on the value of the main products, the concentration of microalgal biomass in the cultivation systems and the size of microalgal cells to be harvested (Li et al., 2008b). The three most common harvesting and dewatering methods for microalgal biodiesel production are filtration, flotation and sedimentation (Lee et al., 2009; Shelef et al., 1984; Weissman, 1987). Filtration (e.g. microstraining and belt filtering) is simple and viable for large microalgal suspension volumes. A microstrainer is a rotary drum cover by a straining material, such as fabric, stainless steel or polyester. A belt filter typically consists of a belt made of rubber, polytetrafluoroethylene (PTFE) or terylene covered with cloth which is moved toward the stationary vacuum unit for drainage. Filtration is suitable only for larger sizes of microalgae, for example Coelastrum and Spirulina grow as long as 10 µm and 0.5 mm, respectively (Sheehan et al., 1998; Shelef et al., 1984). Moreover, the method requires flocculation as a preceding process, filter or membrane replacement, and a pumping system which would make it more expensive (Weissman, 1987).

2.2.4 Drying of the Dried Microalgal Biomass

Drying of microalgal cake is optional depending on the product and extraction process. Dried microalgal biomass is more desirable for dry extraction since moisture content in the microalgal biomass interrupts chemical reaction during the extraction process (Shelef et al., 1984). Moisture contents of 12 to 15% or 18% w/w can be achieved in typical drying processes. With respect to energy consumption, solar drying is the cheapest drying method, and with modern solar drying technologies can be cost effective, e.g. a solar dryer consisting of a solar air heater and a drying chamber, a small fan feature and a tunnel dryer. However, a long drying time and a large drying surface area are usually required (Kadam, 2002; Lutz et al., 1987; Shelef et al., 1984). Another low-cost drying method is a low-pressure shelf, yet its efficiency is limited. In contrast, drum drying, spray drying, fluidized bed drying, freeze drying, and refractance window dehydration technologies offer higher drying efficiency with higher investment and operation costs (Li et al., 2008b; Sander & Murthy, 2010).

2.2.5 Extraction of the Microalgal Oil

The extraction process releases metabolites of interest from the algal biomass. For microalgal biomass, the four major metabolites are carbohydrates, proteins, nucleic acid and lipids (TAG and fatty acids). TAG is the main substrate for microalgal biodiesel production (Ferrell & Sarisky-Reed, 2010; Kyndt, 2010).

Extraction can be divided into mechanical methods (e.g. pressing and ultrasonic-assisted extraction) and chemical methods (e.g. supercritical CO₂, Soxhlet and solvent extractions) (Kyndt, 2010). For lipid recovery, a solvent extraction is a quick and efficient extraction method

(Mata et al., 2010). There are several organic solvents that can be employed, e.g. hexane, ethanol (96%) or a hexane-ethanol (96%) mixture. The solvent should be chosen based on its ability to penetrate through layer or cell of biomass and separate the lipid material (Batan et al., 2010a; Demirbas, 2009a; Ferrell & Sarisky-Reed, 2010; Mata et al., 2010; Mulbry et al., 2009; Stephenson et al., 2010; Vijayaraghavan & Hemanathan, 2009). The lipid yield from the extraction process can also be increased by employing cell disruption methods before chemical extraction. Examples of cell disruption methods are cell homogenizers, bead mills and osmotic shock (Ferrell & Sarisky-Reed, 2010; Lee et al., 2009; Mata et al., 2010).

2.2.6 Conversion to the Microalgal Biodiesel

Biomass can be converted to valuable chemicals and biofuels by a variety of conversion technologies. As illustrated in Figure 3, the biomass energy conversion processes can be divided into biochemical, thermochemical and physicochemical conversions. Biochemical conversion can be subdivided into fermentation, anaerobic digestion, bioelectrochemical fuel cells, and other fuel producing processes utilizing the metabolism of organisms. Thermochemical conversion is the thermal decomposition of organic contents in biomass to yield fuel products by gasification, pyrolysis and liquefaction (Tsukahara & Sawayama, 2005). Physicochemical conversion, also known as alcoholysis or transesterification of alcohol and TAG, is used in biodiesel production (Moser, 2009).

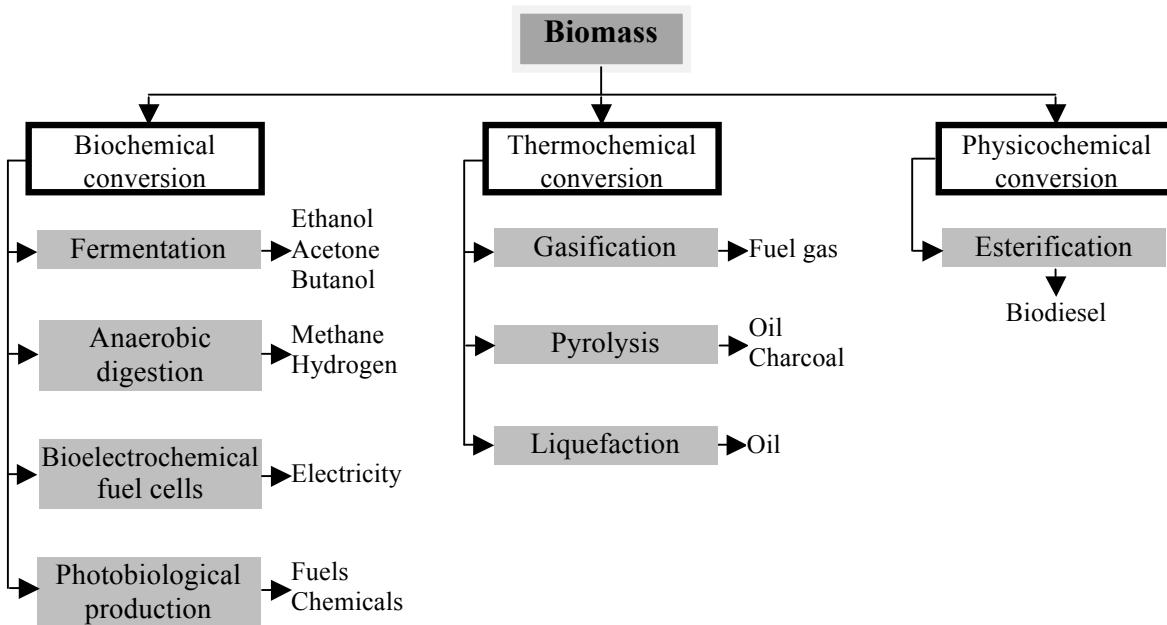


Figure 3 Energy conversion processes of biomass.

Adapted from (Jenkins & Williams, 2006; Tsukahara & Sawayama, 2005)

The chemical reaction of the transesterification process occurs when one molecule of TAG reacts with three molecules of mono-alcohol, generally methanol or ethanol, within the presence of catalysts such as alkali, acids, or enzymes. The products of the transesterification process are a molecule of glycerol and three molecules of fatty acid methyl ester (FAME) or biodiesel, as illustrated in Figure 4 (Li et al., 2008b; Moser, 2009). In the transesterification reaction, one molecule of TAG and three molar equivalents of methanol in the presence of an alkali catalyst 0.5% by weight react at 60°C for one hour to produce glycerol and FAME (Moser, 2009).

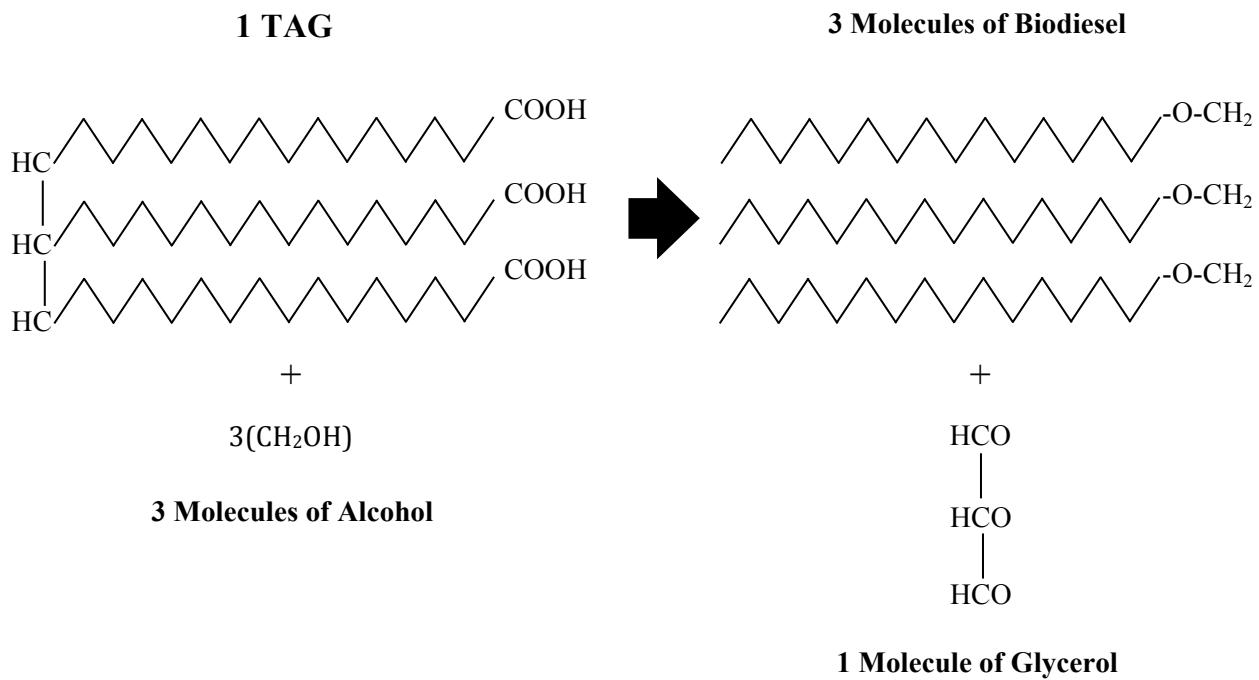


Figure 4 Transesterification process.

The properties of TAGs depend on feedstock, type of catalyst and process to be used in transesterification (Moser, 2009). Each feedstock yields different free fatty acid (FFA) contents; therefore, an appropriate catalyst should be employed to avoid unwanted side reactions. An example of an unwanted side reaction is the reaction of a feedstock with $\geq 3\%$ of FFA and catalysts such as sodium hydroxide (NaOH), potassium hydroxide (KOH) or methoxide (CH_3O^-) to form soap, resulting in an irreversible catalyst. Unlike other homogeneous base catalysts, sodium methoxide (CH_3ONa), which is currently used in an industrial biodiesel production, does not form water, which can be a problem. In the presence of remaining catalyst, water can react with biodiesel to produce FFA and alcohol. Calcium ethoxide ($\text{Ca}(\text{OC}_2\text{H}_5)_2$) is also an effective base catalyst in biodiesel production, though more alcohol and catalyst are required. Other advantages of homogenous base catalysts are a faster reaction, about 4,000 times faster, a more complete reaction, and less corrosive to industrial equipments compared to acid catalysts. Moreover, the reaction is operated under lower temperature, pressure and time conditions. Base-

catalyzed transesterification is limited by the FFA content in feedstock, which in contrast, is not a problem when employing acid catalyst in the process. Therefore, acid catalysts are more appropriate for the production of biodiesel from high FFA-containing feedstock (Moser, 2009).

Generally, a homogeneous catalysis reaction requires less alcohol, shorter reaction times and more complicated purification processes than a heterogeneous catalysis reaction in transesterification. Moreover, enzymes such as heterogeneous lipases are commonly used in ethyl or higher esters production. Another biodiesel production method is a non-catalytic reaction called supercritical fluids transesterification. The major advantages of supercritical fluid transesterification are a short reaction time, e.g. 4 minutes, and a simple purification for product since there is no catalyst to be removed. However, this method requires more energy, alcohol, and investment and maintenance costs due to the pressurized reaction vessels and discontinued process. Methanol can be used in this reaction under a very high pressure (45-65 bar), temperature (350°C) and amount of alcohol (42:1 molar ratio) condition (Moser, 2009).

Apart from TAG and catalysts, another important component in transesterification reaction is alcohols. Methanol is the most commonly used alcohol in commercial biodiesel production since it is inexpensive; other mono-alcohols, for instance, ethanol, propanol, iso-propanol and butanol, can also be used depending on the cost and availability. Ethanol is used in the regions like Brazil where ethanol is cheaper than methanol. Moreover, ethanol becomes more of interest since it is less expensive than methanol and the product can be a complete biodiesel, if the ethanol is a bioethanol, while methanol, propanol and iso-propanol are petroleum-based chemicals e.g. methanol is obtained from methane which is a natural gas (Moser, 2009).

Various types of alcoholysis reaction are named according to the alcohol employed in the reaction. The reaction depends on several parameters, which are type and quantity of catalyst,

time and temperature of the reaction, quantity of alcohol and/or rate of agitation. The conventional reaction conditions using different types of alcohols are summarized in Table 3 (Moser, 2009). The typical biodiesel yield that can be recovered is up to 98% by weight (Mata et al., 2010; Moser, 2009).

Table 3 Summary of the conventional transesterification conditions.

Process	Conventional Reaction Conditions					Products
	R-OH : Oil	Alkali catalyst (wt.%)	Agitation Rate (rpm)	Temperature (°C)	Reaction Time (Hours)	
Methanolysis	6:1	0.5	600+	60	1	Fatty acid methyl esters and glycerol
Ethanolysis	6:1	0.5	600+	75	1	Fatty acid ethyl esters and glycerol
Butanolysis	6:1	0.5	600+	114	1	Fatty acid butyl esters and glycerol

2.3 LIFE CYCLE ASSESSMENT

LCA is a tool to quantify life-cycle resource consumption and environmental impacts from a product or service; it is commonly used to compare alternative products, such as petroleum derived diesel, microalgal diesel, and soybean-derived biodiesel. LCA can also be used to identify processes or emissions that should be targeted in order to reduce the environmental footprint of the product. The life-cycle stages and processes generally involved with the manufacture of a product are: raw material acquisition, production, transportation, use and end-of-life. Life-cycle thinking is also referred to as cradle to grave (McDonough & Braungart, 2002). LCAs are defined by the International Organization for Standardization (ISO) 14040

series (International Organization for Standardization, 2006). ISO describes the four main steps of an LCA as:

- 1) **Goal and Scope Definition** is where objectives of the LCA study i.e. a process LCA or a comparative LCA, system boundaries and a functional unit are identified and established.
- 2) **Life Cycle Inventory** (LCI) collects inventory data from literature and life-cycle databases, such as BUWAL, ecoinvent, ETH-ESU, Franklin and IDEMAT (Delft University of Technology, 2001; Frischknecht & Jungbluth, 2004; Frischknecht et al., 2007; Norris, 2003; Spriensma, 2004). Inventories are collected according to the system boundaries, and all necessary inventories required in order to achieve the defined goal. This is a comprehensive and critical phase since LCA results rely on the quality of LCI.
- 3) **Life Cycle Impact Assessment** (LCIA) presents the LCI data in terms of understandable and quantifiable environmental impacts, such as global warming and smog formation. Three steps to conducting the LCIA include impact category definition, classification and characterization. The LCIA tool used in this study is the Tool for Reduction and Assessment of Chemical and other environmental Impacts (TRACI) developed by the US EPA. TRACI is an LCIA tool developed particularly for the U.S. It provides nine environmental impact categories, which are GWP, acidification, carcinogenic, non-carcinogenic, respiratory effects, EP, ozone depletion, ecotoxicity and PSP. Each impact is calculated on a midpoint basis, which can avoid an estimation or forecasting in the LCIA and can still reflect the stressors and the effects of the contaminants (Bare et al., 2006; Bare et al., 2003). Midpoint characterizations are presented in kg Eq of a reference substance. Midpoint impact categories can be allocated into one or more damage categories or endpoint. An LCIA tool, IMPACT 2002+ allocated midpoint impact categories into four endpoint damage categories (human health, ecosystem quality, climate

change and resources), which allows different impact categories to be directly compared (Jolliet et al., 2003). Another LCA tool that uses damage-oriented approach is the Building for Environmental and Economic Sustainability (BEES). The BEES tool combines different impact category performance by multiattribute decision analysis and defines the relative contribution of each impact category to the environment by weighting (Lippiatt, 1999). Furthermore, the LCIA results can be normalized, grouped, weighed and analyzed to improve quality of the results.

4) **Interpretation and Improvement Analysis** is where the LCI and LCIA results are correlated, interpreted and improved to present meaningful information and to enable decision-making consistent with the defined goal and scope. Interpretation should deliver results and explain limitation to inform industries and decision makers (International Organization for Standardization, 2006; Udo de Haes, 2006; Udo de Haes & van Rooijen, 2005).

Two LCA methods that deal with the production system with multiple final products are an attributional LCA and a consequential LCA (Thomassen et al., 2008). Attributional LCA is an LCA method for an isolated system where all impacts are attributed to each of the system's products by allocation or displacement. The method presents impacts from a life cycle and its sub-systems. On the other hand, consequential LCA focuses on the effects of changes made within the life cycle (Ekvall & B.P. Weidema, 2004). Consequential LCA, which can be called a change-oriented or prospective LCA, considers a much broader system boundary and normally deals with co-products by system expansion. Consequential LCA is also applicable to prospectively indicate impacts from policy (Kaufman et al., 2010). Evidently, low-carbon renewable fuel regulations such as RFS2 and California's Low-Carbon Fuel Standard conducted consequential LCA to include either direct or indirect impacts over life cycle of biofuels

(Assessment and Standard Division Office of Transportation and Air Quality, 2010; University of California, 2007).

In the case where the production system results in several products, one product is defined as a primary product, while the other remaining products are defined as co-products. The original system boundary is then expanded to include the production of a displaced co-product. The avoided environmental impacts from the production of the co-products are credited to the primary product. In this study, attributional LCA with system expansion is conducted to avoid allocation issues.

2.3.1 Development of LCA Model for Microalgal Biodiesel

Various studies used LCA to quantified environmental impacts, mainly GWP and EP, and water and energy consumption from the production of microalgal biodiesel (Batan et al., 2010b; Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2010; Collet et al., 2011; Jorquera et al., 2010; Kadam, 2002; Lardon et al., 2009; Sander & Murthy, 2010; Stephenson et al., 2010; Yang et al., 2011). The system boundaries for each study were different, which makes it difficult to compare among them. Other studies focused on particular stages of microalgal biodiesel production, while others evaluated the overall production. For example, Clarens et al. employed the use of LCA to evaluate microalgae conducted a comparative LCA of algal biomass to other biofuel feedstocks; however this study only evaluated cultivation (Clarens et al., 2010). Other studies investigated the impacts from utilizing different sources of resources such as CO₂ and nutrients from synthetic and waste streams (Batan et al., 2010a; Evans & Wilkie, 2010; Lardon et al., 2009; Sander & Murthy, 2010; Soratana & Landis, 2011; Yang et al., 2011).

Because of the multiple options available for producing microalgal biodiesel, Aresta et al. developed an LCA model within *Excel* spreadsheets that would allow users to adjust input values according to their designs of microalgal biodiesel production. The concept was implemented and resulted in a computer software, COMPUBIO, on *Excel* using Visual Basic 6.0 to create the user interface (Aresta et al., 2005). The software provides different options for macroalgal and microalgal biofuels production e.g. supercritical CO₂, organic solvents and pyrolysis methods were the options for extraction process. However, the software is limited to only energy consumption during the production and does not evaluate any other emissions or LCIA environmental impact categories.

Other LCA models for biofuels exist; one of the most well known is the Greenhouse gases, Regulated Emissions, and Energy use in Transportation (GREET) model developed by Argonne National Laboratory. Other LCA tools and models exist that do not focus on biofuels, for example the Economic Input-Output (EIO-LCA) model by Carnegie Mellon University and the SimaPro version 7.1 by PRé Consultants take two very different approaches to LCA. Impacts from the EIO-LCA are evaluated based on economic values of the related industries. Compared to the price of petroleum diesel, using the existing uncompetitive price of microalgal biodiesel production as an input to obtain the impact results from the EIO-LCA model would result in an expectedly high impact results. Impacts from the process LCA approach used in SimaPro are evaluated based on resources consumed during the production. However, none of these LCA tools have algal biofuel production systems built in. At the time of this thesis, GREET was in its development phase for microalgal fuels, while EIO-LCA and SimaPro do not provide specific inventories or calculation for microalgal biodiesel (Argonne National Laboratory, 2010; Carnegie Mellon University Green Design Institute, 2008; Goedkoop et al., 2008). These

existing LCA tools contain data limited to fossil fuels and conventional biofuels such as soybean biodiesel.

An LCA model specifically for microalgal biodiesel was developed in this research to aid in the green design of biodiesels and to evaluate a wide range of impacts resulting from microalgal biodiesel production and consumption. This model quantifies the impacts of microalgal biodiesel production over its entire life cycle, also commonly termed ‘pond to wheel’.

The LCA model of microalgal biodiesel in this study was developed mainly to evaluate environmental impacts over the life cycle of microalgal biodiesel. The model was developed on *Excel* spreadsheets, like GREET and COMPUBIO, therefore no special software was required, and inventories can be easily updated. The model consists of microalgal biodiesel production input parameters (e.g. lipid content of microalgae and processes efficiencies), inventories from life cycle databases, and the ability to compute for quantity of resources required and environmental impacts contribution from one functional unit over the life cycle of microalgal biodiesel. The model also offers a wide range of options for each process of microalgal biodiesel production. Nevertheless, how the model was developed herein is certainly not the only way to structure a model that evaluates microalgal biodiesel production and consumption. Moreover, the development of the model is not the focus of this study, but rather the approach to evaluate the environmental impacts of microalgal biodiesel production and related policies, such as the RFS2. More details of the model are provided in Appendix A.

2.4 ORGANIZATION OF THESIS

Chapter 3 addresses Objective 1, which is to quantify life cycle impacts from microalgal biomass production. Twenty different scenarios of microalgal cultivation that utilize nutrients and CO₂ from synthetic sources and waste streams as well as the materials used to construct a PBR were examined. This work was published in the journal *Bioresource Technology* (Soratana & Landis, 2011).

Chapter 4 addresses Objectives 2 and 3 where a process LCA model was used to quantify life-cycle impacts from microalgal biodiesel and to identify the method of production that contributed minimal environmental impacts for further comparison with other types of diesel. A comparative LCA of four microalgal biodiesel production conditions was conducted using the aforementioned LCA model with MCA for uncertainty analysis. The four production scenarios included the two different production efficiencies, high and low, and two different sources of resources, waste and synthetic. The potential to meet the RFS's GHG requirement and other environmental impacts that are not regulated by the RFS was addressed. This chapter will be submitted in its entirety for publication in a peer-reviewed journal.

Chapter 5 addresses Objective 3, which is to evaluate environmental tradeoffs among different types of diesel, such as microalgal, soybean, canola and petroleum diesels. The life-cycle impacts of microalgal biodiesel from Chapter 4 were further analyzed and compared with the impacts of petroleum diesel, soybean diesel, and canola diesel from existing studies. The current RFS2 was assessed and approaches to set thresholds for EP and PSP were proposed to avoid unintended environmental consequences from the production and the utilization of biodiesels. This chapter will be submitted in its entirety for publication in a peer-reviewed journal.

Chapter 6 addresses Objective 4, which conducts an attributional LCA with system expansion to evaluate the potential of microalgal ethanol, a co-product of microalgal biodiesel production, as a product to improve life-cycle environmental footprints of microalgal biodiesel. This chapter will be submitted in its entirety for publication in a peer-reviewed journal. Conclusions of the overall results of this dissertation and recommendation and future work are discussed in Chapter 7.

3.0 EVALUATING INDUSTRIAL SYMBIOSIS AND ALGAE CULTIVATION FROM A LIFE CYCLE PERSPECTIVE

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Evaluating industrial symbiosis and algae cultivation from a life cycle perspective

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ABSTRACT

A comparative life cycle assessment (LCA) was conducted on 20 scenarios of microalgae cultivation. These scenarios examined the utilization of nutrients and CO₂ from synthetic sources and waste streams as well as the materials used to construct a photobioreactor (PBR). A 0.2-m³ closed PBR of *Chlorella vulgaris* at 30%-oil content by weight with the productivity of 25 g/m² × day was used as a case study. Results of the study show that the utilization of resources from waste streams mainly avoided global warming potential (GWP) and eutrophication impacts. Impacts from the production of material used to construct the PBR dominate total impacts in acidification and ozone depletion categories, even over longer PBR lifetimes; thus, the choice of PBR construction materials is important.

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1. Introduction

The Annual Energy Review 2009 by US Energy Information Administration reported that the world's transportation fuel demand was 30 EJ/year (2.8×10^{13} MJ/year) (US Energy Information Administration, 2010). With the significant amount of fuel demand and the depletion of petroleum diesel, other sources of transportation fuel, such as microalgae, are gaining recent attention. Microalgae is considered an ideal biodiesel feedstock due to its high growth rate, productivity, and photosynthetic efficiency while its production consumes less energy compared with feedstock of first- and second-generation biofuels (Bruce, 2008; Lehr and Posten, 2009). Consequently, the production of microalgal mass as a biodiesel feedstock have been extensively investigated by many researchers in the past several years, as can be seen in the review by Mata et al. (2010).

Microalgal diesel production consists of six main processes. The processes include strain selection and cultivation of the microalgal mass, harvesting of the microalgal slurry, dewatering to obtain the microalgal cake, drying of the dried microalgae, extraction of the microalgal oil, and conversion to the microalgal diesel (Mata et al., 2010; Uduman et al., 2010). The first two processes, strain selection and cultivation are the focus of this study because they have been shown to contribute significantly to the life cycle environmental impacts of algae fuel production and since there are significant strategies for reducing these impacts, such as utilizing waste resources as nutrients required for growth. For example, the study by Stephenson et al. (2010) on air-lift tubular PBR indicated that the algal cultivation was the most energy intensive pro-

cess and contributed the most GWP compared to other downstream processes. The algal cultivation to produce one ton of biodiesel required up to 231 GJ, while producing 13,600 kg CO₂ eq.

The microalgal cultivation depends on several parameters such as the design of cultivation system, nutrients, carbon dioxide (CO₂), water, light, temperature and pH (Carvalho et al., 2006). Some of the parameters such as nutrients, CO₂ and water can be utilized from other industries' waste streams in an industrial symbiotic relationship; nutrients, nitrogen (N) and phosphorus (P), and water can be utilized from wastewater and CO₂ can be utilized from flue gas (Greer, 2009; Kadam, 2001; Kong et al., 2010; Mallick, 2002; Mata et al., 2010).

Microalgal cultivation systems can be divided into two main categories: open ponds including circular and raceway designs and various types of closed photobioreactors (PBRs), e.g. tubular (vertical, horizontal and helical), flat plate and fermenter (Chisti, 2008). Compared to open ponds, PBRs have a better control of cultivation conditions such as mass transfer and contamination with eight times less water loss than raceway ponds (Jorquer et al., 2010; Li et al., 2008; Posten, 2009). In addition, both systems can be coupled; one example of the successful coupling system is the cultivation of *Haematococcus pluvialis* and *Chlorella* sp. in a PBR coupled with 0.02-km² seawater open pond system. The productivity of the coupled system exceeded the estimation of other oil crop productivities (Li et al., 2008).

When coupled with CO₂ producing industries, such as coal-fired power plants, microalgal cultivation has the potential to beneficially use the waste CO₂ produced from the overall system (Avagyan, 2008; Carvalho et al., 2006; Kunjapur and Eldridge, 2010; Mata et al., 2010). Since microalgae can tolerate high-CO₂ concentrations, their biomass production can utilize CO₂ from flue

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gas of power plants (Hossain et al., 2008; Li et al., 2008; Reising, 2007). Some microalgal strains can tolerate up to 5–15% of CO₂ concentration in feeding air streams from flue gases and flaring gases, while other oil crops normally utilize the typical level of CO₂ content in the atmosphere about 0.03–0.06% (Hossain et al., 2008).

Microalgal cultivation can also be combined with wastewater treatment systems to remove N and P, thus potentially reducing both energy and chemical demands of the coupled systems (Li et al., 2008; Mallick, 2002). Kong et al. (2010) showed that algae wastewater treatment system is a potentially environmentally friendly, economical and more efficient option to remove nutrients and metals from wastewater before conventional tertiary treatment. Several studies on the potential for using wastewater to grow microalgae as biofuel feedstock are discussed in the review by Pittman et al. (2011).

Life cycle assessment (LCA) is a tool to quantify the resource consumption and the environmental impacts of any product or service over its life cycle, from raw material acquisition through production, transportation, use and ultimately the products' end-of-life – i.e. from cradle to grave. LCA has four main steps: (1) goal and scope definition, (2) life cycle inventory analysis (LCI), (3) life cycle impact assessment (LCIA) and (4) improvement and interpretation. Process LCAs are defined by the ISO 14040 series and the UNEP/SETAC Life Cycle Initiative (International Organization for Standardization, 2006; Udo de Haes and van Rooijen, 2005). LCA can be used to compare different products, such as algal diesel and ultra low sulfur diesel. LCA can also be used to design and improve upon a system by analyzing the environmental impacts of alternate manufacturing processes for one product. This study presents a cradle-to-gate LCA of microalgae production, where the use and end-of-life phases are omitted.

There have been few LCA studies that investigated the industrial symbiosis of microalgal cultivation with other industries. Examples of waste resources utilized are CO₂ from flue gas of power plant and water and nutrients from municipal, industrial or agricultural wastewater of wastewater treatment plant or sewage (Greer, 2009; Kadam, 2001; Kong et al., 2010; Mallick, 2002; Mata et al., 2010; Pittman et al., 2011). In this study, municipal wastewater from secondary wastewater treatment system is considered since N and P have not been removed (Pittman et al., 2011). A comparative LCA on the production of electricity from coal firing and coal/algae co-firing found tradeoffs between environmental impacts (Kadam, 2002). Kadam found that lower greenhouse gas emissions and acidification were generated in the coal/algae co-firing electricity production, whereas natural resource depletion and eutrophication were higher. Another related study by Lardon et al. (2009) on a comparative LCA of microalgal cultivation and industrial symbiosis was conducted on the production of microalgae and other biofuel feedstocks. Another comparative LCA was conducted on the production of algae, canola and ultra-low sulfur (ULS) diesel (Campbell et al., 2011). Microalgae cultivation was conducted in raceway ponds on non-arable and arid land located close to the seawater and CO₂ waste sources in Australia. However, there is no integrated evaluation of environmental impacts from the utilization of both carbon and nutrient waste streams for microalgal cultivation from an LCA perspective.

Some LCA studies on microalgal biofuels did not focus on the utilization of CO₂ and/or nutrients from waste streams, but synthetic fertilizers or no additional fertilizers. For instance, a cradle-to-grave LCA on microalgal methane, microalgal diesel and first-generation biodiesels (rapeseed and palm) productions by Collet et al., found that the algal methane production was the highest contribution in abiotic depletion, ionizing radiation, human toxicity, GWP and land use compared to the other three biodiesels (Collet et al., 2011). Microalgae (*Chlorella vulgaris*) was cultivated

in 1000 ponds of 1000-m² open raceways supplemented by chemical fertilizers. Another study investigated the potential for biogas production from invasive aquatic plant, *Hydrilla verticillata* (Evans and Wilkie, 2010). No additional CO₂ or nutrients were required to grow this biofuel feedstock since hydrilla is in natural water. The results showed that the net energy balance ratio of some production scenarios were comparable to those for corn ethanol, soybean biodiesel and biogas productions.

Other studies that use LCA to investigate the impacts of algae-based fuel production did not include the construction of the cultivation system, but rather used LCA to evaluate the energy production of various extraction techniques such as supercritical CO₂, organic solvents and pyrolysis (Aresta et al., 2005). Another study conducted by Sander et al. investigated the energy required and greenhouse gas emissions of algae diesel production, focusing on the biomass drying process including solar drying, filter press and centrifugation (Sander and Murthy, 2010).

A comparative LCA of flat-plate and horizontal tubular PBRs and raceway open pond for marine algae was studied mainly for energy consumption (Jorquera et al., 2010). Other impacts were not investigated. Even though the materials of cultivation systems were included, only one type of PBR construction material, polyethylene, was examined for a cost estimation purpose.

The objective of this study is to evaluate the potential utilization of both CO₂ and nutrients from industrial wastes during microalgal cultivation. Moreover, as suggested by the Algae Biomass Organization (ABO), LCA of microalgal diesel production should include the utilization of N and P from wastewater as nutrients and flue gas from the smokestack as a CO₂ source for microalgal cultivation (ABO Technical Standards Committee, 2009). Therefore, environmental impacts from the production of resources consumed when utilizing nutrients and CO₂ from different sources, synthetic substances and industrial wastes, are included in this analysis. For this study, a comparative LCA of microalgal cultivation was conducted over three different assumed useful lifetimes of a PBR: 5, 10 and 20 years. Impacts from the selection of PBR construction material are also considered. Five different PBR construction materials – glass, polyvinyl chloride (PVC), polycarbonate (PC), polymethyl methacrylate (PMMA) and high-density polyethylene (HDPE) were examined.

2. Methods

A comparative LCA of different microalgal cultivation scenarios was conducted. Twenty different cultivation scenarios were evaluated for three main microalgal cultivation parameters: PBR construction materials, source of nutrients and source of CO₂. Each of these 20 scenarios was also investigated over three different lifetimes: 5, 10, and 20 years. Life cycle inventory data was collected from peer-reviewed literature and life cycle inventory databases. Inventories from the literature consisted of quantities of resources consumed during the microalgal cultivation. Inventories obtained from databases were quantities of resources consumed for the production of those resources consumed during the cultivation. The Tool for the Reduction and Assessment of Chemical and other Environmental Impacts (TRACI) version 3.01 was employed for the LCIA (Bare et al., 2003). Nine of the TRACI impact categories were evaluated: global warming potential (GWP), acidification, carcinogenics, non-carcinogenics, respiratory effects, eutrophication, ozone depletion, ecotoxicity and smog.

2.1. System boundaries

The boundaries of microalgal cultivation under various conditions are presented in Fig. 1. Twenty scenarios of microalgal culti-

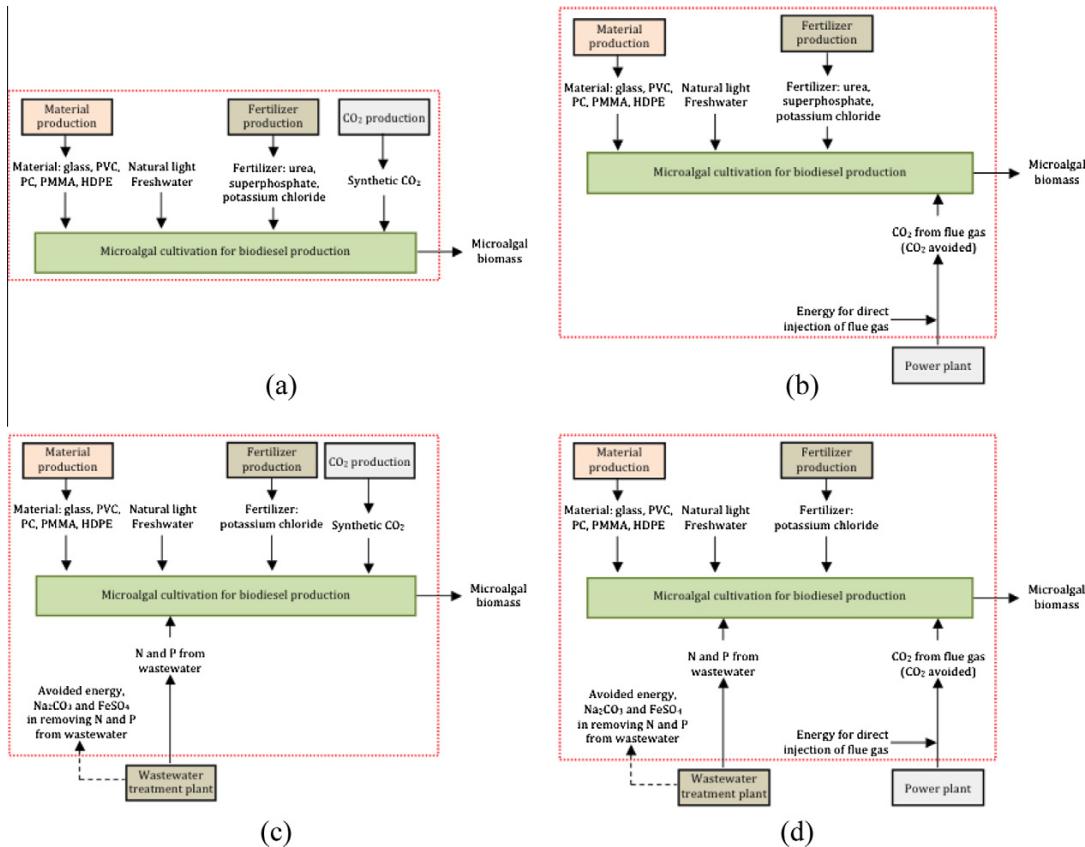


Fig. 1. System boundaries of microalgal cultivation scenarios for different nutrient and CO₂ conditions. (a) Synthetic fertilizers and synthetic CO₂, (b) synthetic fertilizers and CO₂ from flue gas, (c) N and P from wastewater and synthetic CO₂ and (d) N and P from wastewater and CO₂ from flue gas.

vation were evaluated; these scenarios were developed from combinations of five different PBR construction materials (glass, PVC, PC, PMMA and HDPE), two different sources of nutrients (synthetic fertilizers and municipal wastewaters), and two different sources of CO₂ (synthetic CO₂ and CO₂ from flue gas), as described in Table 1. The cultivation conditions investigated in this study were divided into 20 scenarios. The three main resources considered in each scenario were PBR construction materials and sources of nutrients and CO₂. Energy consumed and avoided involved with the utilizations of nutrients and CO₂ from waste streams was also considered.

The functional unit for comparison of environmental impacts amongst the 20 scenarios is the microalgal mass produced from one cultivation system operated for 20 years, which is 3650 kg of microalgae. Based on the productivity of the designed cultivation system, approximately 182.5 kg of microalgae can be produced from one PBR in one year. Therefore, approximately 3650 kg of microalgae can be produced from one PBR with a 20-year lifetime. To achieve the production of one functional unit and evaluate the impact of different PBR lifetimes, 4, 2 and 1 cultivation systems were required to be operated for 5, 10 and 20 years, respectively.

Energy and resources consumed for construction and maintenance activities of the cultivation system were assumed the same for every type of PBR construction materials and excluded from the system boundaries. Other resources for the cultivation such as water and land were not included in the analysis. One drawback of LCA is that water balances are not considered as environmental impact since they do not contribute impact to any traditional impact categories. However, there is a recent study on water footprint

from the production of microalgal diesel by Yang et al. (2011). For land usage, there is not an agreed upon methodology for calculating direct and indirect land use impacts (Klooverpris et al., 2008; Lapola et al., 2010). Moreover, compared to other biofuel feedstocks, microalgae requires significantly less land use. Microalgae with 30% oil content by biomass weight required 0.2 m² year/kg biodiesel, which is 90 times less than that of sunflower with 18% oil content by biomass weight (Mata et al., 2010).

In addition, many microalgae strains can be cultivated in high nutrient concentration wastewater (Pittman et al., 2011). This study also assumed that the selected microalgae strain could tolerate concentration of nutrients in the wastewater use and consumed significant quantities of N and P available in wastewater, therefore, water reuse was not considered and no additional pre- and post-wastewater treatments were required.

2.2. Inventories

Inventories obtained from peer-reviewed literature included the quantity of resources, PBR materials, nutrients, CO₂ and energy consumed and avoided during the microalgal cultivation, as listed in Table 2. The quantities of nutrients required for the cultivation were collected from the study on LCA of biodiesel production from microalgae by Lardon et al. (2009). Even though the study was conducted on an open pond while this study used a PBR, both studies used the same microalgal strain, *C. vulgaris*, therefore, the quantities of nutrients for microalgal cultivation were assumed to be the same between systems.

Table 1
Microalgal cultivation scenarios.

Scenario	Material	Nutrient			CO ₂ source	Energy
			Source	Fertilizers/chemicals		
S1	Glass	Fertilizers	N, P, and K		Synthetic CO ₂	–
S2	Glass	Fertilizers	N, P, and K		CO ₂ from flue gas	Energy for flue gas CO ₂
S3	Glass	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ avoided for N and P removal		Synthetic CO ₂	Energy avoided for N and P removal
S4	Glass	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ saved for N and P removal		CO ₂ from flue gas	Energy for flue gas CO ₂ , Energy avoided for N and P removal
S5	PVC	Fertilizers	N, P, and K		Synthetic CO ₂	–
S6	PVC	Fertilizers	N, P, and K		CO ₂ from flue gas	Energy for flue gas CO ₂
S7	PVC	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ avoided for N and P removal		Synthetic CO ₂	Energy avoided for N and P removal
S8	PVC	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ saved for N and P removal		CO ₂ from flue gas	Energy for flue gas CO ₂ , Energy avoided for N and P removal
S9	PC	Fertilizers	N, P, and K		Synthetic CO ₂	–
S10	PC	Fertilizers	N, P, and K		CO ₂ from flue gas	Energy for flue gas CO ₂
S11	PC	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ avoided for N and P removal		Synthetic CO ₂	Energy avoided for N and P removal
S12	PC	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ avoided for N and P removal		CO ₂ from flue gas	Energy for flue gas CO ₂ , Energy avoided for N and P removal
S13	PMMA	Fertilizers	N, P, and K		Synthetic CO ₂	–
S14	PMMA	Fertilizers	N, P, and K		CO ₂ from flue gas	Energy for flue gas CO ₂
S15	PMMA	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ avoided for N and P removal		Synthetic CO ₂	Energy avoided for N and P removal
S16	PMMA	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ avoided for N and P removal		CO ₂ from flue gas	Energy for flue gas CO ₂ , Energy avoided for N and P removal
S17	HDPE	Fertilizers	N, P, and K		Synthetic CO ₂	–
S18	HDPE	Fertilizers	N, P, and K		CO ₂ from flue gas	Energy for flue gas CO ₂
S19	HDPE	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ avoided for N and P removal		Synthetic CO ₂	Energy avoided for N and P removal
S20	HDPE	Wastewater	K, and Na ₂ CO ₃ and FeSO ₄ avoided for N and P removal		CO ₂ from flue gas	Energy for flue gas CO ₂ , Energy avoided for N and P removal

Table 2
Inventories of 0.2-m³ PBR of microalgal cultivation.

Parameters	Inventories
Microalgal strain	<i>Chlorella vulgaris</i> ^a
Microalgal areal productivity rate	25 g/m ² × day ^b
Oil content	30% by dry weight ^{b,c}
Microalgal oil heating value	41 MJ/kg ^d
Volume of PBR	0.2 m ³ ^e
PBR thickness	0.005 m ^f
Surface area to volume ratio of PBR	100 m ² /m ³ ^e
Urea (N fertilizer)	0.023 kg/kg microalgae (low N condition) ^g
Superphosphate (P fertilizer)	0.009 kg/kg microalgae ^a
Potassium chloride (K fertilizer)	0.004 kg/kg microalgae ^a
Sodium carbonate (Na ₂ CO ₃)	7.5 kg/kg N-removed ^f
Ferrous sulfate (FeSO ₄)	1.8 kg/kg P-precipitated ^f
Synthetic CO ₂ (industrial grade CO ₂)	1.85 kg CO ₂ /kg microalgae ^e
Flue gas content	14% of CO ₂ ^g
Energy avoided from N removal	14 MJ/kg N ^h
Energy avoided from P removal	24 MJ/kg P ^h
Energy for injection of CO ₂ from flue gas	0.08 MJ/kg CO ₂ ^g

^a Lardon et al. (2009).

^b Avagyan (2008).

^c Lehr and Posten (2009).

^d Huang et al. (2010).

^e Posten (2009).

^f Metcalf et al. (2003).

^g Kadam (2001).

^h Maurer et al. (2003).

The environmental impacts from the production of N and P fertilizers and the chemicals used for N and P removal from wastewater were avoided for scenarios utilizing nutrients from wastewater during cultivation. Thus, these avoided impacts are subtracting from the total impacts of microalgal cultivation. Within wastewater treatment, the N- and P-removal chemicals include Na₂CO₃, an alkalinity source of nitrification process, and FeSO₄, a chemical used in P-precipitation process (Maurer et al., 2003; Metcalf et al., 2003). Approximately 7.5 kg of Na₂CO₃ and 1.8 kg of FeSO₄ are required to remove one kg of nitrogen and phosphorus in wastewater, respectively (Metcalf et al., 2003). In order to cultivate microalgae without the use of wastewaters, synthetic N and P fertilizers are required; 0.023 kg of urea and 0.009 kg of superphosphate are required to produce one kg of microalgae. Thus, 630 kg of NaCO₃ and 60 kg of FeSO₄ can be avoided per functional unit. In addition, the environmental impacts from the production of energy consumed in N and P removal processes of wastewater treatment system, 14 and 24 MJ/kg of nutrients removed, respectively, were also avoided (Maurer et al., 2003).

For the cultivation with synthetic CO₂, impacts from the production of synthetic CO₂ were included. Energy for the direct injection of synthetic CO₂ was insignificant (0.007 MJ/kg CO₂) compared to the energy for direct injection of CO₂ from flue gas which also included other operations such as compression and dehydration (0.08 MJ/kg of CO₂); therefore it was not included (Campbell et al., 2009; Kadam, 2002). The direct injection of CO₂ from flue gas without a cleaning process was evaluated because contaminants in flue gas were assumed to have no impact on microalgae growth as presented by Kunjapur and Eldridge (2010). Therefore,

when utilizing CO₂ from flue gas, the impacts from the production of energy for direct injection of CO₂ from flue gas were considered, while the impacts of the CO₂ from flue gas were considered as the avoided impact. The assumed CO₂ content in flue gas was 14%, which was the CO₂ concentration used in the study by Kadam (2002).

Inventories of the production of PBR construction materials, fertilizers, chemicals, CO₂ and energy were extracted from ecoinvent, BUWAL and IdeMat databases (Deft University of Technology, 2001; Frischknecht et al., 2007; Spiersma, 2004) as presented in Table 3.

3. Results and discussion

Normalized impacts from the production of the materials used to construct one PBR, fertilizers and N- and P-removal chemicals are depicted in Figs. 2 and 3, respectively. TRACI impacts from 20 different scenarios of microalgal cultivation over three PBR lifetimes are also discussed to present the scenario, generally, with the least impact and its tradeoffs.

3.1. Impacts of materials for constructing a PBR

Five of materials were evaluated for constructing a PBR. The production of HDPE exhibits the lowest environmental impacts in 8 out of 9 TRACI categories, with the exception of its GWP. The production of HDPE contributes slightly higher GWP than flat glass, which contributes the lowest GWP, by 27% or contributes approximately 40 kg CO₂ eq per one 20-year lifetime HDPE PBR. Apart from the lowest contribution in GWP, the production of flat glass contributes generally low non-carcinogenic, carcinogenic and eutrophication potentials, with its tradeoffs in ozone depletion potential. Ozone depletion from the production of flat glass is the highest compared to other PBR construction materials by 99.9% or approximately 2.3×10^{-5} kg CFC-11 eq per one 20-year lifetime glass PBR. The tradeoff of flat glass results from the release of 1,1,1-trichloroethane (TCA), a cleaning and degreasing solvent, to the atmosphere.

The production of PMMA exhibits the highest environmental impact in 8 out of 9 TRACI categories with the exception of its considerably low ozone depletion compared to flat glass. PMMA contributes ozone depletion approximately less than flat glass by 99.5%. However, PMMA was the second highest ozone depletion contributor, which contributes 1.1×10^{-7} kg CFC-11 eq per one 20-year lifetime PMMA PBR. PMMA's highest impacts result from the following: CO₂ from fossil fuel combustion to GWP, sulfur diox-

ides (SO₂) to acidification and respiratory effect, dioxins to carcinogenic, ammonium ion (NH₄⁺) to eutrophication and nitrogen oxides to smog formation potentials from the production of PMMA at plant, while the high non-carcinogenic, ozone depletion and eco-toxicity potentials result from the disposal of wastes of the PMMA production.

The production of PVC contributes the second lowest impact in 6 out of 9 TRACI categories with its exception in GWP, carcinogenic and non-carcinogenic potentials. On the other hand, the production of PC contributes the second highest impact in 7 out of 9 TRACI categories with the exception of its carcinogenics and ozone depletion potentials.

3.2. Impacts of nutrients for microalgal cultivation

Fig. 3 depicts the normalized impacts during microalgal cultivation from two nutrient sources: synthetic and waste. The synthetic nutrient source includes urea, superphosphate and potassium chloride, the sources of N, P and K, respectively. Impacts from the production of urea are lower than that of superphosphate, with the exception of its GWP and ozone depletion potential due to CO₂ from fossil fuel combustion (3.37 kg CO₂ eq/kg urea) and bromochlorodifluoromethane (3.65×10^{-7} kg CFC-11 eq/kg urea) from the production of urea. According to the impacts from the production of synthetic fertilizers, the production of superphosphate, the source of P, contributes the highest impacts in 7 out of 9 TRACI categories with the exception of its GWP and ozone depletion potential, while the production of potassium chloride contributes the least impact in all of the categories. Less than 2.5% of impacts were contributed from the production of potassium chloride compared to the impacts from the production of other fertilizers. For instance, the production of urea to produce one functional unit contributes 284 kg CO₂ eq and 0.14 kg N eq, while the production of potassium chloride contributes only 8 kg CO₂ eq and 0.004 kg N eq to GWP and eutrophication, respectively.

Nutrients from wastewater include N and P in wastewater while K was obtained from a synthetic fertilizer. With this mix of nutrients, the impacts from the production of chemicals for N and P removal from wastewater are avoided by directly utilizing N and P from wastewater for the cultivation. For instance, the utilization of N and P from wastewater instead of the synthetic fertilizers can avoid approximately 100% of acidification and respiratory effects. The highest avoided impact of nutrients from waste is eutrophication potential. Comparing the cultivation with nutrients from wastewater to the cultivation with synthetic fertilizers, approximately 400% of the impact or 2.23 kg N eq to eutrophication per functional unit can be avoided mainly by utilizing N from wastewater. The production of Na₂CO₃, a chemical commonly used to remove N from wastewater, contributes significant amount of impacts compared to the production of FeSO₄, a P-removal chemical. Therefore, the utilization of N from wastewater can significantly avoid impacts when compared to the utilization of P from wastewater and nutrients from fertilizers. For instance, based on one kg of chemicals, Na₂CO₃ contributes 1.18 kg CO₂ eq and 3.53×10^{-3} kg N eq, while FeSO₄ contributes 0.12 kg CO₂ eq and 1.53×10^{-5} kg N eq to GWP and eutrophication, respectively.

3.3. Impacts from different microalgal cultivation scenarios

Microalgal cultivations were operated in PBR constructed from five different materials under four different conditions resulting in 20 cultivation scenarios (from S1 to S20), as described in Table 1. Each cultivation scenario was operated over three PBR lifetimes, 5, 10 and 20 years. In this study, due to the high uncertainty incorporated in TRACI human health related impacts (Bare, 2006), only five non-health related impacts are discussed, as presented in

Table 3
Microalgal cultivation parameters and associated inventories utilized within this study.

Process flow	Resource
PBR construction materials	Flat glass ^a (260 kg/PBR) PVC ^a (140 kg/PBR) PC ^a (120 kg/PBR) PMMA ^a (119 kg/PBR) HDPE ^a (95 kg/PBR)
Fertilizers (nutrients)	Urea (source of nitrogen) ^a Superphosphate (source of phosphorus) ^a Potassium chloride (source of potassium) ^a
Chemicals for N and P removal	Na ₂ CO ₃ (for nitrogen removal) ^b FeSO ₄ (for phosphorus precipitation) ^a
CO ₂	Synthetic CO ₂ ^c
Energy	Energy US ^d

^a ecoinvent data v2.0 (Europe).

^b ecoinvent data v2.0 (Global).

^c BUWAL 250.

^d IdeMat 2001.

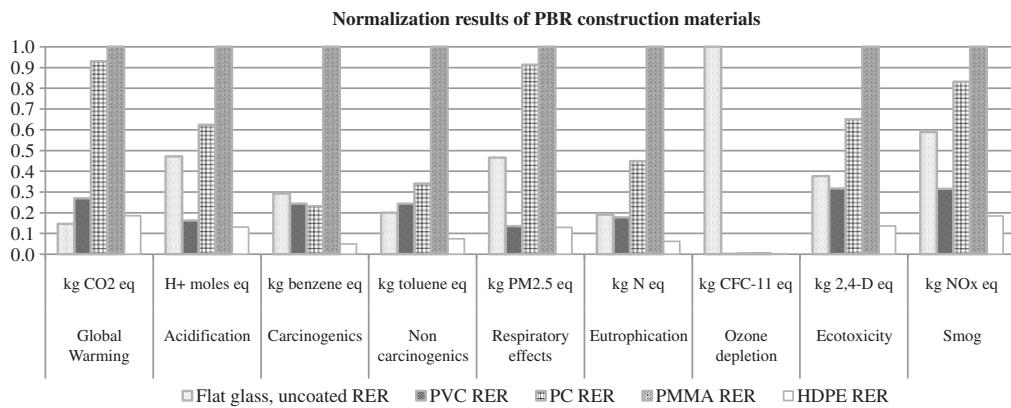


Fig. 2. Normalized TRACI impacts of one 0.2-m³ PBR. In each impact category, impacts from the production of PBR-plastic construction materials are normalized to those of the material with the highest contribution. For example, ozone depletion is normalized to that from the production of flat glass.

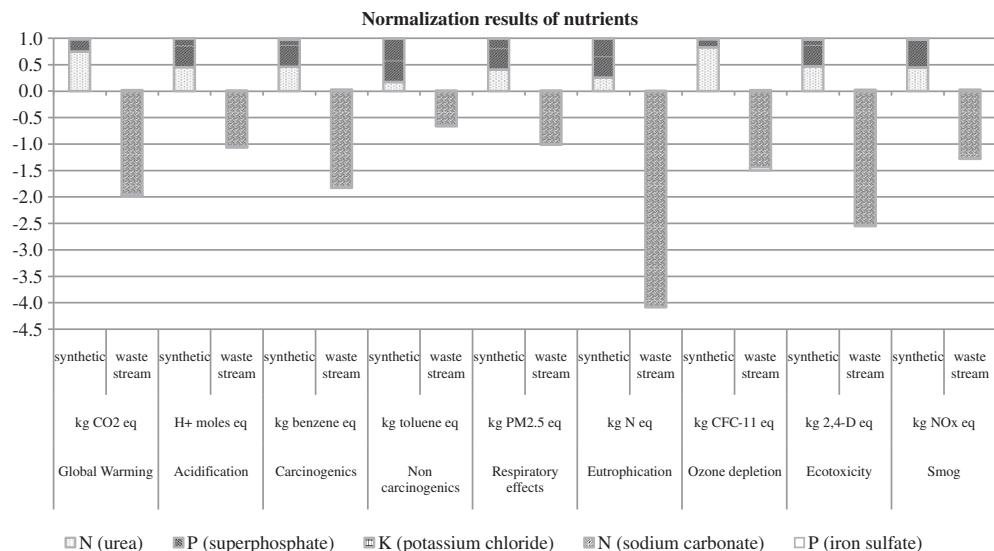


Fig. 3. The normalized impacts of nutrients obtained from synthetic versus waste resources. The impacts of 'synthetic' includes N, P and K from synthetic fertilizers, while the 'waste stream' includes avoided impacts from not using Na₂CO₃ during wastewater treatment as a N-removal chemical and FeSO₄ as a P-removal chemical, respectively.

Fig. 4. (a) GWP, (b) acidification, (c) eutrophication, (d) ozone depletion and (e) smog formation, such that 3650 kg of microalgae mass or one functional unit is obtained. For instance, in scenario 1 (S1), four PBRs were operated for 5 years each, two PBRs for 10 years and one PBR for 20 years. In order for the comparison to be equal: a total operating time of 20 years was assessed: thus, four systems with a 5-year lifetime and two systems with a 10-year lifetime were analyzed for each scenario.

3.4. Global warming potential (GWP)

Fig. 4(a) presents the GWP of 20 microalgal cultivation scenarios. For scenarios with the cultivation utilizing synthetic CO₂, the cultivation operated over a longer lifetime contributes lower GWP than those that operated over a shorter lifetime. The total GWP results are influenced by GWP from the production of synthetic CO₂ ranges from 27% to 76% of the total GWP results. However, this is not the case for S9, S11, S13 and S15 with PBR lifetime of 5 and 10 years where PC and PMMA are used as PBR construc-

tion material. Even though these scenarios also relied on synthetic CO₂, the production of the two PBR construction materials contributes considerably higher GWP than the production of synthetic CO₂. However, the percent of the GWP from the production of synthetic CO₂ increases as the system lifetime increases. The GWP results of PBRs with 5-year and 20-year lifetimes range from 27% to 65% and from 50% to 77% of the total GWP, respectively. Not only did the percent of GWP of the production of synthetic CO₂ increase as the PBR's lifetime increased, but percent of GWP of the production of fertilizers also increased. It is important to note that the production of fertilizers contributes significantly less GWP than the production of PBR construction materials. However, GWP from the production of the PBR construction material can be offset when CO₂ from flue gas is utilized. For the scenarios with the utilization of CO₂ from flue gas, approximately 6800 kg of CO₂ eq can be avoided per 3650 kg of microalgae, while the percent avoided GWP ranges from 59% to 92% of the total GWP results.

The operation of a PBR made out of PMMA utilizing fertilizers and synthetic CO₂ at a 5 year lifetime in S13 contributes the most

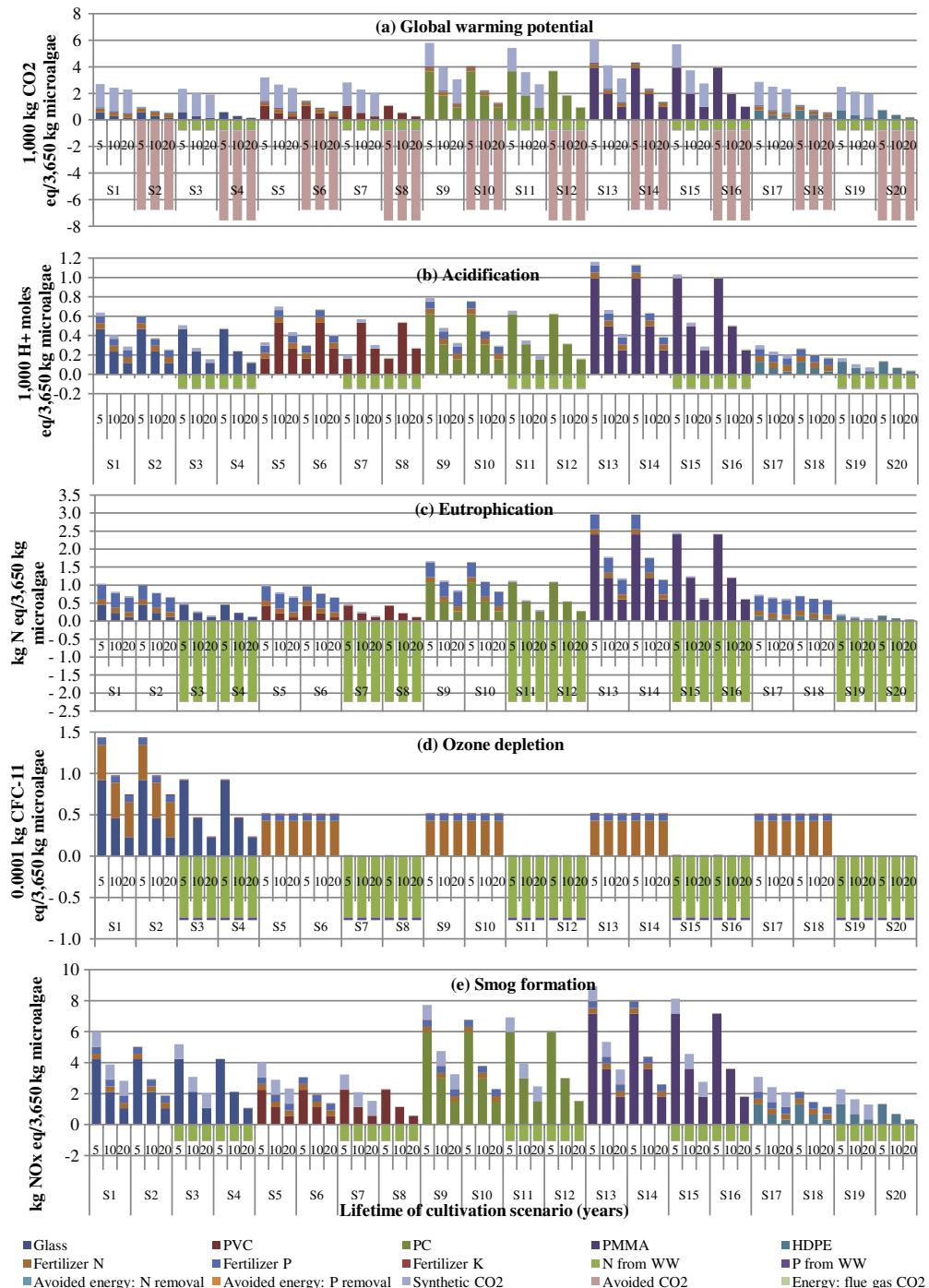


Fig. 4. Environmental impacts from producing 3650 kg of microalgae under different cultivation conditions. The different cultivation conditions are – fertilizers and synthetic CO₂, fertilizers and flue gas, wastewater and synthetic CO₂ and wastewater and flue gas. Each cultivation condition was operated at 5, 10 and 20 year lifetimes.

GWP of all scenarios, whereas the operation of glass PBR utilizing N and P from wastewater and CO₂ from flue gas at a 20 year lifetime in S4 contributes the least GWP. This is due to fossil CO₂ emission from the PMMA production, which contributes significantly to GWP.

The negative GWP in Fig. 4(a) results from two impact avoidances. The first avoidance is from the reduction of the production of energy and chemicals for the removal of N and P from wastewater since they were taken up by the microalgae. The PBR operated over longer lifetime with the utilization of nutrients from

wastewater can avoid GWP through the reduction of the production of N- and P-removal chemicals. For different nutrient scenarios, as a PBR's lifetime increases, the percentage of GWP from the production of fertilizers also increases. The GWP resulting from fertilizer production at systems operating at 5-year and 20-year PBR lifetime ranges from 3% to 13% and from 5% to 17% of the total GWP, respectively. The second avoidance was from the utilization of CO₂ from flue gas. The production of energy for direct injection of CO₂ from flue gas to the cultivation system also contributes GWP. The cultivation with CO₂ from flue gas avoided only GWP of the cultivation, not other impacts, which can be observed from S2, S6, S10, S14 and S18 in the other categories. Notably, the utilization of CO₂ from flue gas can avoid GWP more than the utilization of N and P from wastewater.

In comparison to open pond cultivation, the GWP of S19 is compared to the GWP of microalgae open pond with effluents from conventional activated sludge wastewater treatment plant and industrial grade CO₂ by Clarens et al. (2010). The PBR in S19 was constructed from generally low-impact material, HDPE, and was operated utilizing nutrients from wastewater and synthetic CO₂, which is similar to the operating conditions of the open pond study. Scenario 19 resulted in 0.02–0.09 kg CO₂ eq/kg microalgae × year or 0.001–0.008 kg CO₂ eq/MJ × year, which is approximately six times lower than the results of the study by Clarens et al., 0.02–0.05 kg CO₂ eq/MJ × year. This is due to the differences of system boundaries between the two studies. The GWP from the energy for system operation, harvesting and dewatering were included in the study by Clarens et al., while they are excluded from this study. The GWP from the production of PBR materials is included in this study, but excluded from the Clarens's study. In addition, Clarens only reports 'direct,' 'upstream,' and total GHGs, water usage, energy, and eutrophication; therefore it is impossible to determine the impacts for each process in Clarens's study for comparison to this work.

3.4.1. Acidification

Acidification potentials of 20 microalgae cultivation scenarios are presented in Fig. 4(b). The acidification potential decreases as the system lifetime increases, as expected. The production of PBR construction materials dominates the total acidification impacts in every scenario, except for the scenarios with an HDPE PBR (S17 through S20) where the impacts from the production of synthetic fertilizers and avoided impact from the utilization of wastewater are higher. Therefore, the utilization of CO₂, N and P from waste streams does result in the reduction of acidification but does not always result in impact avoidance.

3.4.2. Eutrophication

Fig. 4(c) illustrates the results of eutrophication impacts. For the cultivation scenarios with the utilization of synthetic fertilizers, the impact from the production of N and mainly from the production of P fertilizers outweighs the impact from the production of glass PBR construction materials in S1 and S2 with lifetimes of 10 and 20 years and the production of PVC and HDPE in S5, S6, S17 and S18 over the three PBR lifetimes. The production of N fertilizer contributes approximately 0.14 kg N eq to overall eutrophication potential, while the production of P fertilizer contributes 0.40 kg N eq per functional unit.

Furthermore every PBR scenario utilizing nutrients and CO₂ from waste streams results in avoided eutrophication over the three PBR lifetimes, with one exception – the PMMA PPBR with a lifetime of 5 years does not result in net avoided eutrophication potential. The utilization of nutrients from wastewater is the major eutrophication avoidance, where the productions of chemicals and energy for N and P removal processes, 2.23 kg N eq per functional unit, can be avoided.

An LCA on microalgal diesel production that also reported results regarding eutrophication was the study by Clarens et al. (2010). Clarens et al. evaluated an open raceway pond for the production of microalgae mass as biofuel feedstock. The eutrophication of the evaluated cultivation system utilizing nutrients in effluents of conventional activated sludge ranged from 1.7×10^{-5} to 3×10^{-5} kg N eq/MJ × year. The results of the direct biomass production and upstream processes did not indicate avoided eutrophication, unlike the results from this study. The eutrophication potential of the HDPE PBR in S19 with the utilization of N from wastewater ranges from -9.1×10^{-6} to -2.4×10^{-6} kg N eq/MJ × year due to the avoidance of the impact from the production of N- and P-removal chemicals, Na₂CO₃ and FeSO₄ which are examined in this study.

3.4.3. Ozone depletion

Ozone depletion potential in every scenario decreases as the PBR's lifetime increases, as expected. The production of glass contributes the most ozone depletion potential compared to the production of other resources, as presented in Fig. 4(d). The major contribution to ozone depletion is from the release of TCA to the atmosphere, resulting in 1.8×10^{-5} kg CFC-11 eq, from the production of glass. The production of one glass PBR (260 kg of glass) contributes 2.3×10^{-5} kg CFC-11 eq, which is higher than other PBR construction materials approximately by 100%. Therefore, if ozone depletion is the impact of concern, the use of glass as the PBR construction material should be avoided. The second highest ozone depletion potential among the four PBR construction materials results from the production of PMMA, and the third, fourth and fifth are PC, PVC and HDPE, respectively. The production of one PMMA PBR (119 kg of PMMA) contributes 1.1×10^{-7} kg CFC-11 eq from releasing trichlorofluoromethane, while the production of one HDPE PBR (95 kg of HDPE) contributes 1.7×10^{-8} kg CFC-11 eq from releasing the bromotrifluoromethane.

Due to significantly low ozone depletion from the production of PVC, PC, PMMA and HDPE, the results of the scenarios with those PBR construction materials represent primarily the impacts from the production of N, P and K fertilizers, which is 5.2×10^{-5} kg CFC-11 eq per functional unit, and the avoided impacts from the utilization of N and P from wastewater, which is 7.8×10^{-5} kg CFC-11 eq per functional unit.

3.4.4. Smog formation

Similar to the acidification results in Fig. 4(b), the production of PBR construction materials generally exhibits the highest smog potential in scenarios with the utilization of synthetic fertilizers, except in S5, S6, S17 and S18 where PBR is constructed from PVC and HDPE with 10 and 20 years lifetimes. Scenarios with the utilization of nutrients solely from wastewater do not result in smog formation potential avoidance since the production of synthetic CO₂ contributes smog formation potential (0.95 kg NO_x eq per functional unit) nearly equal to the avoided impact (-1.04 kg NO_x eq per functional unit). Additionally, the utilization of CO₂ from waste streams does not result in smog formation potential avoidance in any scenario, as can be seen in Fig. 4(e). The impact avoidance occurs in S8 and S20 where nutrients and CO₂ are obtained from waste streams within the PVC PBR operating at 20 year lifetimes and for the HDPE PBR operating at 10 and 20 year lifetimes, respectively. Among different PBR material productions, PMMA contributes the highest smog potential. The result is mainly due to the contribution of nitrogen oxides released to the atmosphere during the N₂ gas blow drying process of producing PMMA.

3.5. Overview of the results

According to the life cycle assessment results, the selection of PBR construction materials is important, particularly for

acidification, eutrophication and smog formation potentials. For instance, PMMA contributes up to 65% of total GWP and 80% of total eutrophication potential in S13 with a PBR lifetime of 5 years. Moreover, over longer PBR lifetimes, impacts from the production of the PBR construction material dominate more than half of the total impacts, as can be seen from the results of acidification and smog formation potentials.

Generally, impacts from the production of PBR construction material dominate the total impacts and decrease over longer PBR lifetime. Most of the scenarios of the PBR operated over 20 years with the utilization of resources from waste streams present higher avoided impact potential than other lifetimes. In scenarios with the utilization of synthetic resources, the impacts from the production of PBR materials were suppressed by the impacts from the production of other cultivation resources such as synthetic N fertilizer or CO₂. The GWP results of the cultivation with the utilization of synthetic CO₂ are a good example of this case; the GWP slightly decreases over longer PBR lifetimes. This occurs because the total GWP results in each scenario are dominated by GWP from the production of synthetic CO₂. Therefore, after the PBR construction material with the lowest impact contribution is selected, the next step in minimizing environmental impacts should be to reduce the demand for CO₂ and nutrients to reduce the overall impacts during microalgal cultivation.

Impacts from the consumption of synthetic fertilizers and CO₂ emitted during the cultivation become more important as the lifetime of the PBR increased. For instance, by examining GWP results, the utilization of synthetic CO₂ became the major impact contributor as the PBR's lifetime increased. The utilization of wastes as resources of microalgae mass production has the potential to avoid those impacts. Referring to Fig. 4(a and c), the utilization of nutrients and CO₂ from waste streams mainly affects GWP and eutrophication potential, which is not surprising since CO₂ and N, which are the GWP and eutrophication potential indicators, are utilized by the microalgae. By combining multiple waste streams, the avoided GWP and eutrophication potential can reach 6800 kg CO₂ eq per functional unit and 2.2 kg N eq per functional unit, respectively. Even though not every scenario with nutrients and/or CO₂ from waste streams resulted in impact avoidance, the total impacts were less than scenarios not utilizing waste streams as resources. For example, in S5 the acidification of the PVC PBR operated on synthetic fertilizers and CO₂ with a 20 year lifetime resulted in approximately 4.4×10^2 H⁺ moles eq/functional unit, while the same system in S8 operated on nutrients and CO₂ from waste streams with a 20 year of lifetime contributed approximately 1.2×10^2 H⁺ moles eq/functional unit. As a result, acidification can be minimized by approximately 72% with the use of waste streams as resources. Moreover, the utilization of nutrients and CO₂ from waste streams can significantly reduce GWP. In S17 of GWP of HDPE PBR operated on synthetic CO₂ and fertilizers with a 20 year lifetime contributed approximately 2.3×10^3 kg CO₂ eq/functional unit, while the same system in S20 operated on CO₂ and nutrients from waste streams with a 20 year lifetime can avoid approximately -7.3×10^3 kg CO₂ eq/functional unit. By switching the cultivation from S17 to S20, which switches from synthetic resources to waste resources for an HDPE PBR, GWP can be reduced by 9.7×10^3 kg CO₂ eq/functional unit or approximately 420%.

When evaluating carbon credits for microalgal biofuels, the system boundary for the carbon cycle should be considered over the entire lifetime of the fuel, from cultivation to combustion. Because the scope of this paper is a cradle-to-gate LCA of microalgal cultivation for microalgal diesel production, impacts from the combustion of microalgal diesel were not included. Thus, microalgal diesel cannot be considered a carbon sink at this stage. Referring to Fig. 4(a), though GWP in most of the scenarios with the utilization of nutrients and CO₂ from waste streams can be avoided, they were

only for the production of microalgae mass production and not for microalgal diesel production. Further study on environmental impacts of harvesting, dewatering, drying, extraction, conversion and combustion of microalgal diesel should be conducted and combined with the results of this study. If the total impact from pond (or PBR) to wheel yields a negative value of GWP result, then microalgal diesel production can be considered to have carbon mitigation potential.

Impacts related to water consumption and water quality such as acidification and eutrophication should be considered. According to Fig. 4(b), there is no acidification impact avoidance when nutrients and CO₂ were from waste streams, except for glass PBR with a 20 years of lifetime and HDPE PBRs with 10 and 20 years of lifetime. Moreover, impacts from other stages of microalgal diesel production should be further investigated. For example, researchers at Colorado State University conducted a research on use phase of algae-based biodiesels (Magill, 2010). They found that the combustion of algae-based biodiesel emitted less NO_x from the tailpipe when compared to other biofuels. The results can be combined with this study and other impacts from other stages of the production in order to provide a comprehensive comparative LCA of microalgal diesel.

4. Conclusions

Environmental impacts such as GWP and eutrophication can be avoided by utilizing nutrients and CO₂ from waste streams to cultivate microalgae. The utilization of CO₂ from flue gas avoids only GWP, while the utilization of N and P from wastewater mainly avoids eutrophication. However, the avoided impact potential was often outweighed by impacts from the production of the materials used to construct the PBR. The importance of material selection is evident in acidification and smog formation results.

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References

- ABO Technical Standards Committee. 2009. ABO Recommends Lifecycle Analysis of Algal Production Systems, Vol. 2010, Algal Biomass Organization.
- Aresta, M., Dibenedetto, A., Barberio, G., 2005. Utilization of macro-algae for enhanced CO₂ fixation and biofuels production: development of a computing software for an LCA study. Fuel Processing Technology 86 (14–15), 1679–1693.
- Avagyan, A., 2008. A contribution to global sustainable development: inclusion of microalgae and their biomass in production and bio cycles. Clean Technologies and Environmental Policy 10 (4), 313–317.
- Bare, J.C., 2006. Risk assessment and Life-Cycle Impact Assessment (LCIA) for human health cancerous and noncancerous emissions: integrated and complementary with consistency within the USEPA. Human and Ecological Risk Assessment: An International Journal 12 (3), 493–509.
- Bare, J.C., Norris, G.A., Pennington, D.W., McKone, T., 2003. TRACI: the tool for the reduction and assessment of chemical and other environmental impacts. Journal of Industrial Ecology 6 (3–4), 49–78.
- Bruce, E.R. 2008. Opportunities for Renewable Bioenergy Using Microorganisms. 100, 203–212.
- Campbell, P.K., Beer, T., Batten, D. 2009. Greenhouse Gas Sequestration by Algae – Energy and Greenhouse Gas Life Cycle Studies. The 6th Australian Conference on Life Cycle Assessment.
- Campbell, P.K., Beer, T., Batten, D., 2011. Life cycle assessment of biodiesel production from microalgae in ponds. Bioresource Technology 102 (1), 50–56.
- Carvalho, A.P., Meireles, L.A., Xavier Malcata, F., 2006. Microalgal reactors: a review of enclosed system designs and performances. Biotechnology Progress 22 (6), 1490–1506.
- Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. Trends in Biotechnology 26 (3), 126–131.
- Clarens, A.F., Resurreccion, E.P., White, M.A., Colosi, L.M., 2011. Environmental life cycle comparison of algae to other bioenergy feedstocks. Environmental Science & Technology 44 (5), 1813–1819.

- Collet, P., HÈlias, 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.
- Delft University of Technology. 2001. IdeMat Online.
- Evans, J.M., Wilkie, A.C., 2010. Life cycle assessment of nutrient remediation and bioenergy production potential from the harvest of hydrilla (*Hydrilla verticillata*). *Journal of Environmental Management* 91 (12), 2626–2631.
- Frischknecht, R., Jungbluth, N., Althaus, H.-J.R., Bauer, C., Doka, G., Dones, R., Hischier, R., Nemecek, T., Primas, A., Wernet, G. 2007. ecoinvent Report No. 2, Code of Practice Data v2.0. Swiss Federal Institute of Technology Zürich (ETHZ).
- Greer, D., 2009. Algae to energy: cultivating algae in wastewater for biofuel. *Biocycle Energy* 36, 39.
- Hossain, A.B.M.S., Salleh, A., Boyce, A.N., Chowdhury, P., Naqiuuddin, M. 2008. Biodiesel fuel production from algae as renewable energy. *American Journal of Biochemistry and Biotechnology*, 4(3), 250–205.
- Huang, G., Chen, F., Wei, D., Zhang, X., Chen, G., 2010. Biodiesel production by microalgal biotechnology. *Applied Energy* 87 (1), 38–46.
- International Organization for Standardization. 2006. ISO 14040 Environmental management – Life cycle assessment – Principles and framework. Switzerland.
- Jorquera, O., Kiperstok, A., Sales, E.A., EmbirùAu, M., Ghirardi, M.L., 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresource Technology* 101 (4), 1406–1413.
- Kadam, K.L. 2001. Microalgae production from power plant flue gas: environmental implications on life cycle basis. National Renewable Energy Laboratory. NREL/TP-510-29417.
- Kadam, K.L. 2002. Environmental implications of power generation via coal-microalgae cofiring. *Energy* 27 (10), 905–922.
- Klöverpris, J., Wenzel, H., Banse, M., Milà i Canals, L., Reenberg, A., 2008. Conference and workshop on modelling global land use implications in the environmental assessment of biofuels. *The International Journal of Life Cycle Assessment* 13 (3), 178–183.
- Kong, Q.-x., Li, L., Martinez, B., Chen, P., Ruan, R., 2010. Culture of microalgae *Chlamydomonas reinhardtii* in wastewater for biomass feedstock production. *Applied Biochemistry and Biotechnology* 160 (1), 9–18.
- Kunjapur, A.M., Eldridge, R.B. 2010. Photobioreactor design for commercial biofuel production from microalgae. *Industrial & Engineering Chemistry Research*.
- Lapola, D.M., Schaldach, R., Alcamo, J., Bondeau, A., Koch, J., Koelking, C., Priess, J.A. 2010. Indirect land-use changes can overcome carbon savings from biofuels in Brazil. *Proceedings of the National Academy of Sciences*.
- Lardon, L., Helias, A., Sialve, B., Steyer, J.-P., Bernard, O. 2009. Life-cycle assessment of biodiesel production from microalgae. *Environmental Science & Technology*, 7.
- Lehr, F., Posten, C., 2009. Closed photo-bioreactors as tools for biofuel production. *Current Opinion in Biotechnology* 20 (3), 280–285.
- Li, Y., Horsman, M., Wu, N., Lan, C.Q., Dubois-Calero, N., 2008. Articles: biocatalysts and bioreactor design, biofuels from microalgae. *Biotechnology Progress* 24, 815–820.
- Magill, B. 2010. CSU researchers test emissions from burning biofuel, Checkbiotech.
- Mallick, N., 2002. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *BioMetals* 15 (4), 377–390.
- Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. *Renewable & Sustainable Energy Rev.* 14 (1), 217–232.
- Maurer, M., Schwegler, P., Larsen, A.T., 2003. Nutrients in urine: energetic aspects of removal and recovery. IWA Publishing, London, ROYAUME-UNI.
- Metcalf, Eddy, Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. *Wastewater engineering: treatment and reuse*. McGraw-Hill, Boston.
- Pittman, J.K., Dean, A.P., Osundeko, O., 2011. The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology* 102 (1), 17–25.
- Posten, C. 2009. Design principles of photo-bioreactors for cultivation of microalgae. 9, 165–177.
- Reising, T.F. 2007. Cultivating algae for liquid fuel production. *Pharmaculture Activist*.
- Sander, K., Murthy, G., 2010. Life cycle analysis of algae biodiesel. *The International Journal of Life Cycle Assessment* 15 (7), 704–714.
- Spiersma, R. 2004. SimaPro Database Manual, The BUWAL 250 Library version 2.0. PRÉ Consultants.
- 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.
- US Energy Information Administration. 2010. Annual Energy Review 2009. (DOE/EIA-0384(2009)).
- Udo de Haes, H., van Rooijen, M. 2005. Life cycle approaches, the road from analysis to practice. UNEP/SETC Life Cycle Initiative.
- Uduman, N., Qi, Y., Danquah, K.M., Forde, M.G., Hoadley, A., 2010. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of Renewable Sustainable Energy* 2 (012701), 15.
- Yang, J., Xu, M., Zhang, X., Hu, Q., Sommerfeld, M., Chen, Y., 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. *Bioresource Technology* 102 (1), 159–165.

4.0 MICROALGAL BIODIESEL AND THE RENEWABLE FUEL STANDARD'S GREENHOUSE GAS REQUIREMENT

The following chapter is a reproduction of an article under review in the journal *Energy Policy* with the citation:

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4.1 ABSTRACT

The Renewable Fuel Standard (RFS2) under the Energy Independence and Security Act (EISA) of 2007 requires 15.2 billion gallons of domestic alternative fuels per year by 2012, of which 2 billion gallons must be from advanced biofuel and emit 50% less life-cycle greenhouse gas (GHG) emissions than petroleum-based transportation fuels. Microalgal biodiesel, one of advanced biofuels, has the qualities and potential to meet the RFS's requirement. A comparative life cycle assessment (LCA) of four microalgal biodiesel production conditions was investigated using a process LCA model with Monte Carlo simulation to assess global warming potential (GWP), eutrophication, ozone depletion and ecotoxicity potentials. The four conditions represent different production efficiencies – high and low efficiencies, and different sources of carbon dioxide and nutrient resources – synthetic and waste resources. The GWP results of the four microalgal biodiesel production conditions showed that none of the assumed production conditions meet the RFS's advanced biofuel requirement. The GWP results are sensitive to energy consumption in harvesting process. Other impacts, eutrophication, ozone depletion and ecotoxicity potentials, are sensitive to percent lipid content of microalgae, service lifetime of PBRs and quantity of hexane in extraction process, respectively. Net energy ratio and other emissions should be included in future RFS for a more sustainable fuel.

4.2 INTRODUCTION

The Renewable Fuel Standard (RFS) under the Energy Independence and Security Act (EISA) of 2007 requires domestic alternative fuels to meet 15.2 billion gallons by 2012, of which 2 billion

gallons must be from advanced biofuels. Advanced biofuels, which include cellulosic biofuel, biomass-based diesel and other advanced biofuel, are the renewable fuels other than corn ethanol (U.S. Environmental Protection Agency, 2010b). In addition, life-cycle greenhouse gas (GHG) emissions from advanced biofuels must be at least 50% less than GHG emissions from petroleum-based transportation fuels distributed in 2005 (Office of Transportation and Air Quality, 2010a; Office of Transportation and Air Quality, 2010b). Microalgal biodiesel, an advanced biofuel, has the potential to support the U.S. transportation fuel and meet the RFS's advanced biofuels requirement (Ferrell & Sarisky-Reed, 2010).

Microalgae has been investigated for the production of a number of different biofuels e.g. methane, ethanol, electricity and biodiesel (Batan et al., 2010a; Li et al., 2008a; Sander & Murthy, 2010; Stephenson et al., 2010). The applications of microalgae suggested that its characteristics comply with the needs for the biomass industry established by the Roadmap for Bioenergy and Biobased Products in the U.S. which are that it is easy to grow, exhibits high yields, and provides good quality fuel (Avagyan, 2008; Biomass Research and Development Technical Advisory Committee and Biomass Research and Development Initiative, 2007). The quality of microalgal biodiesel meets American Society for Testing and Materials (ASTM) Biodiesel Standard D6751, thus can substitute for petroleum diesel (Bruce, 2008; Chisti, 2007).

Microalgae as biodiesel feedstock have a high growth rate, high productivity, and high photosynthetic efficiency (Avagyan, 2008; Bruce, 2008; Ferrell & Sarisky-Reed, 2010; Lehr & Posten, 2009; Li et al., 2008b). Moreover, microalgal cultivation has been shown to consume limited land and less water resources than terrestrial biofuel crops. The study by Chisti in 2007 suggested that the land for microalgal cultivation requires only 1-3% of the total agricultural area in the U.S. for the same oil-crop diesel yield (Chisti, 2007).

Microalgal cultivation considered in this study was assumed to occur in a closed photobioreactor (PBR). Compared to open ponds, the PBR has a better control of cultivation conditions such as mass transfer, water loss by evaporation, and contamination (Li et al., 2008b; Posten, 2009). The PBR system is suitable for sensitive strains since contamination can be controlled more easily than in an open pond. The cell mass productivity of PBRs is about three times higher than the productivity of open ponds; hence harvesting costs can be significantly reduced. Although the volume of industrial PBR is 5 to 10 m³, some designs can be scaled to larger volume of 10 to 100 m³, and the most practical method to increase the PBR volume is by adding more PBR units (Carvalho et al., 2006a; Janssen et al., 2003). While the closed PBR is a viable alternative for large scale production of microalgae biomass, their operation is still more costly than open ponds (Carvalho et al., 2006a; Posten, 2009).

Although various advantages support the potential of using microalgae as biodiesel feedstock, due to certain limitations, not many applications have reached the industrial scale (Carvalho et al., 2006a). The limitations of cultivation techniques include the low yield from open ponds and the high cost of PBRs (Lehr & Posten, 2009). High harvesting costs have been observed due to the lighting limitations of the cultivating systems and due to the low concentration of biomass in the systems, which result from the relatively small cell-size of microalgae. Drying is also an energy-consuming process due to the large water content of the harvested biomass. In addition, microalgal cultivation facilities require higher capital cost and more operation and maintenance compared to conventional agricultural activities. However, the development of new technologies is expected to overcome these limitations (Li et al., 2008b).

Life cycle assessment (LCA) is a tool that can be used to examine the resource consumption and potential impacts of any product or service (International Organization for

Standardization, 2006; Udo de Haes & van Rooijen, 2005). This tool consists of four main steps: (1) goal and scope definition, (2) life-cycle inventory (LCI), (3) life cycle impact assessment (LCIA) and (4) interpretation. LCA is applied to this study to quantify resource consumption and environmental and human health impacts from pond to wheel or from microalgal cultivation to microalgal biodiesel consumption.

The objective of this study was to conduct a comparative LCA on four conditions of microalgal biodiesel productions and then identify processes that are responsible for the major contribution to total impacts using a process LCA model. A common perception is that high-efficiency production with *synthetic* resources (condition HS) might consume more energy with better system control, while the production with *natural* and *waste* resources (i.e. conditions HW and LW) might consume more energy in preparing and cleaning resources from waste streams with unpromising yield. LCA enables researchers and policy makers to quantify the impacts of these systems and investigate tradeoffs. In addition, we use LCA results from this study to evaluate policies such as the RFS and to improve upon the production of microalgal biodiesel.

4.3 METHODS

4.3.1 Environmental Impact Assessment Using LCA

Four microalgal biodiesel production conditions were defined based on the combination of two different efficiencies of productions and two different sources of resources used during cultivation. The two efficiencies of productions examined were high-efficiency production (H) and low-efficiency production (L), described in more detail in section 4.3.1.1 and 4.3.1.2. The

two sources of resources examined were synthetic resources (S), such as synthetic CO₂ and synthetic fertilizers and natural and waste resources (W), such as sunlight, CO₂ from power plant flue gas and nutrients from municipal wastewater. Thus, four conditions for producing microalgal biodiesel are evaluated: HS, HW, LS, and LW.

The four microalgal biodiesel production conditions were examined and compared for their resource consumption and environmental impacts using LCA as an approach; the boundaries and parameters for each of the four conditions are presented in Figure 5. The input parameters for each of the conditions were evaluated for different design production conditions using Monte Carlo simulations. Monte Carlo simulation is a mathematical method by randomly pulled samples from input contributions to construct a distribution of output parameters (Soratana & Marriott, 2010). Sensitivity analysis was conducted using Tornado correlation diagrams, and the major factors influencing global warming (GWP), eutrophication, ozone depletion and ecotoxicity potentials were identified.

4.3.1.1 The High-Efficiency Production

In this study, the high-efficiency production (H) was defined as the production that cultivated genetically modified microalgae with 70% lipid content. This production method employed high-efficiency technology, which provided 90% harvesting, 98% extraction, and 87% conversion efficiencies (Lardon et al., 2009; Mata et al., 2010; Vyas et al., 2010). High-efficiency production was combined with synthetic resources (HS); synthetic resource parameters included utilization of freshwater and all synthetic resources such as urea, superphosphate and potassium chloride fertilizers, synthetic CO₂, and light provided by compact fluorescent bulbs. High-efficiency production was also combined with the utilization of waste resources (HW) to

evaluate the second production condition. Waste resources (W) included natural and waste resources such as natural light, nitrogen (N) and phosphorus (P) waste streams from municipal wastewater and CO₂ from flue gas of power plant (HW). The system boundaries for the different production conditions are shown in Figure 5, while the associated data is presented in Electronic Annex 1.

4.3.1.2 The Low-Efficiency Production

The low-efficiency production (L) was defined as the production that cultivated indigenous microalgae strains with 50% lipid content; employed harvesting, extraction and conversion which were assumed to have low efficiency than the high-efficiency production by 20%, or with 72%, 78% and 70% efficiencies, respectively. The resultant values corresponded with the possible ranges given by several other studies (Lardon et al., 2009; Mata et al., 2010; Vyas et al., 2010). Low-efficiency production was combined with synthetic resources (LS) and waste resources (LW) whose definitions were identical to those presented for combination with the high-efficiency production conditions (Section 4.3.1.1).

4.3.1.3 System Boundary

The boundaries for the LCA of microalgal biodiesel in this study are presented in Figure 5. Energy consumption and environmental and human health impacts from the four microalgal biodiesel production conditions were compared on the same functional unit basis which was 8.94 × 10¹⁰ MJ/year of biodiesel or 0.67 billion gallon of biodiesel/year which equals to one billion gallon of ethanol/year or 50% of the EISA 2007 renewable fuel volume requirement from the

advanced biofuel by 2012 (Office of Transportation and Air Quality, 2010a). The RFS sets energy requirement for biofuels in terms of bioethanol. Since this study focused on biodiesel, the functional unit was chosen to represent the basis of the RFS, which is MJ of bioethanol. Due to the lower productivity of the low-efficiency productions, LS and LW, they required more cultivation units (15 million units of 10-m³ PBR for each L condition) in order to provide the same functional unit as the high-efficiency production conditions, HS and HW (3.15 million units of 10-m³ PBR for each H condition).

This study did not include the construction of biodiesel refining facilities since the properties of microalgal oil were assumed to be compatible with the existing technology of other biodiesels. Transportation between facilities was omitted based on the assumption that each facility was established on the same location. Furthermore, no valuable by-products e.g. fertilizer, biodegradable plastic and reusable non-potable water or wastes of the production were included because of uncertainties related to yield and quality (Anderson & Dawes, 1990; Avagyan, 2008; Braunegg et al., 1998; Singh et al., 2011). Biological activity is highly dynamic (Daigger & Grady Jr, 1982), and there is a need to understand the temporal variation in the yield and quality of these products. There is also a need to better understand the trade-offs that are introduced when these additional products are collected. More long-term data is needed before we can properly incorporate these products into an LCA of this type.

A process LCA model of microalgal biodiesel production and combustion was developed. This model quantified environmental and human health impacts from different conditions of the production from pond to wheel. Emissions from combustion of microalgal biodiesel were 6.89×10^9 kg CO₂ eq, 1.21×10^5 kg N eq and 1.89×10^5 kg NO_x eq per functional unit, the same value as soybean diesel since properties of both microalgal and soybean biodiesels

are similar (Demirbas & Fatih Demirbas, 2011). The emission values are from soybean biodiesel combustion in GREET 1.8d (Argonne National Laboratory, 2010). More details are presented in Electronic Annexes 1 and 2.

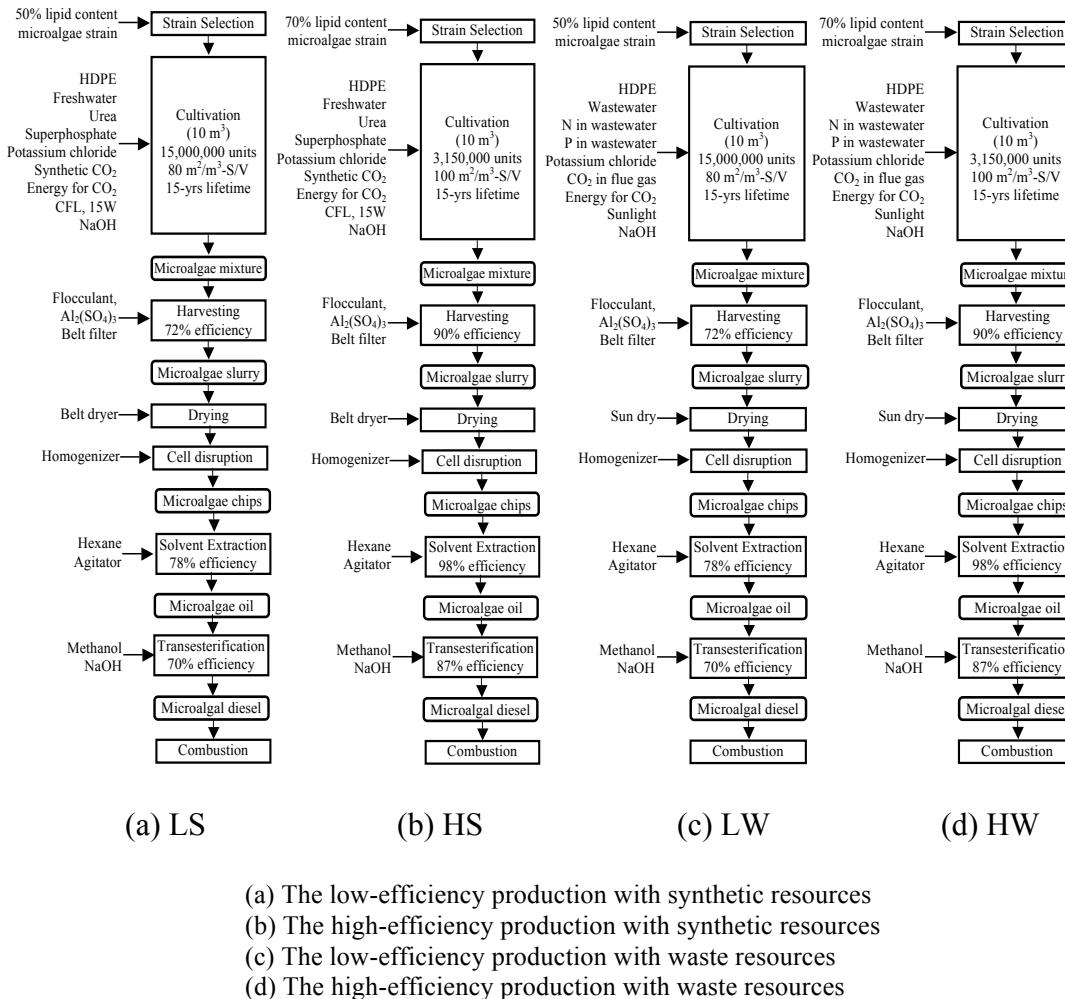


Figure 5 System boundaries of microalgal biodiesel production conditions.

4.3.1.4 Life Cycle Inventory (LCI)

Inventories of each process, from strain selection, cultivation, harvesting/dewatering, drying, cell disruption, extraction and conversion to combustion, were primarily collected from peer-reviewed literature and LCI databases, as presented in Electronic Annex 1 and Annex 2,

respectively. Inventories collected from peer-reviewed literature included the resource quantities required to produce one functional unit (Electronic Annex 1). Some inputs such as number of PBR unit and quantity of flocculant required to produce one functional unit were calculated separately.

Another set of inventories included impacts from the acquisition and production of resources, e.g. PBR construction material, fertilizers, chemicals and energy, required to produce one functional unit. The inventories were extracted from ETH-ESU, ecoinvent version 2.0, BUWAL250 and IDEMAT (Delft University of Technology, 2001; Frischknecht & Jungbluth, 2004; Frischknecht et al., 2007; Spriensma, 2004), which are all European databases, as presented in Electronic Annex 2. U.S. databases are not available for every variable considered in this work; therefore, to avoid inconsistencies, European databases are employed. Another approach is to use a mix of databases to construct the LCI; however, this approach has drawbacks in that there would be many inconsistencies related to temporal, spatial and system boundaries.

4.3.1.5 Life Cycle Impact Assessment (LCIA)

LCIA is the third step in an LCA where environmental impacts are calculated from the LCI. In this study, the U.S.-based Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts (TRACI) and IMPACT 2002+ methods were used (Bare et al., 2003; Jolliet et al., 2003). TRACI is a midpoint-oriented method developed by the U.S. Environmental Protection Agency (U.S. EPA). Nine TRACI impact categories were evaluated in this study: GWP, acidification, carcinogenics, non-carcinogenics, respiratory effects, eutrophication, ozone

depletion, ecotoxicity and smog. Non-renewable energy use (NREU) was calculated using IMPACT 2002+.

4.3.2 Sensitivity Analysis Using Monte Carlo Analysis

A sensitivity analysis was performed using Monte Carlo simulations for four TRACI impact categories: GWP, eutrophication, ozone depletion and ecotoxicity potentials. The sensitivity analysis focused on: 1) examining the uncertainty of the results and 2) investigating how sensitive the process with the highest impact is to the total impact of the microalgal biodiesel production. Input variables were assigned triangular distributions, described in Electronic Annex 1 in the online version of this article. Monte Carlo simulations were conducted for 10,000 iterations using @Risk, a risk analysis software (Palisade Corporation, 2010). Probability distributions for the outputs were determined by the Chi-squared goodness of fit test. From this data, the minimum and maximum values and the 95% confidence interval of the four impacts were obtained. Tornado diagrams were created from the Monte Carlo simulations to determine the parameters of the processes that contributed the highest impact in each category. The Tornado diagrams show how much the results will change given changes to the input parameters. For each impact category, the three activities with the highest correlation coefficient were reported and selected for further sensitivity analysis.

4.4 RESULTS

4.4.1 Environmental Impacts from the Four Microalgal Biodiesel Production Conditions

Normalized impacts from four different microalgal biodiesel production conditions are depicted in Figure 6. More details of the impact results by process are available in Electronic Annex 3. The HW production condition contributed the lowest impacts while the LS condition contributed the highest impacts in all impact categories. On the other hand, the LW condition contributes higher impacts than the HS condition, with the exception of it's carcinogenic potential due to the avoided impact from the utilization of nutrients in wastewater. As can be seen from the GWP results, HW, HS and LW conditions contribute approximately 6.9×10^{10} , 8.5×10^{10} and 1.2×10^{11} kg CO₂ eq per functional unit, or 40, 48 and 69% of GWP of LS condition, respectively.

The filtration and screening in the harvesting process contribute significantly to the impacts in half of the categories examined: GWP, acidification, respiratory effects, eutrophication potentials, and NREU. The GWP mainly results from the release of CO₂ to the atmosphere, acidification from SO_x, while respiratory effects and eutrophication potentials result from the release of NO₂ from the production of energy used during harvesting process. For instance, energy consumption of these portions of the harvesting process contributes 9.4×10^{10} and 5.4×10^{10} kg CO₂ eq per functional unit, which were up to 54 and 79% of the total GWP of the LS and LW conditions, respectively. The utilization of CO₂ and nutrients from industrial wastes in LW and HW conditions do not offset those impacts contributed from the energy intensive harvesting process.

Despite an assumed lifetime of 15 years, the HDPE materials used to construct the PBR contribute predominantly to the total carcinogenic potential of the LW and HW conditions, non-

carcinogenic potential of the LS, LW and HW conditions and ecotoxicity and smog formation potentials of all four conditions. The production of PBR construction material of the LS condition contributes 5.2×10^6 kg benzene eq per functional unit to carcinogenic potential, which accounted for 20% of the total carcinogenic potential of the LS condition, whereas the production of the PBR construction material of the HW condition contributes 1.4×10^6 kg benzene eq per functional unit or 68% of the total carcinogenic potential of the HW condition, which contributes the lowest carcinogenic potential among the four production conditions. HDPE's highest impacts result from the following: the contamination of lead in water to carcinogenic and non-carcinogenic potentials, the contribution of aluminum in water to ecotoxicity and the release of NO_x to the atmosphere to smog formation potential from the production of HDPE.

The consumption of hexane in the extraction process is the main contributor to ozone depletion, which contributes approximately 44 and 70% of the total impact in the HS and LW conditions, respectively. Even though the production of hexane for the LS condition equals to that for the LW condition (7.4×10^2 kg CFC-11 eq per functional unit) and the HS condition equals to that for the HW condition (2.7×10^2 kg CFC-11 eq per functional unit), the production of hexane contributes higher impact to the total carcinogenic of the LW condition (70%) than that of the LS condition (45%). Also, the production of hexane contributes higher impact to the HW condition (65%) than that of the HS condition (44%). The ozone depletion results from the release of bromotrifluoromethane from the production of hexane.

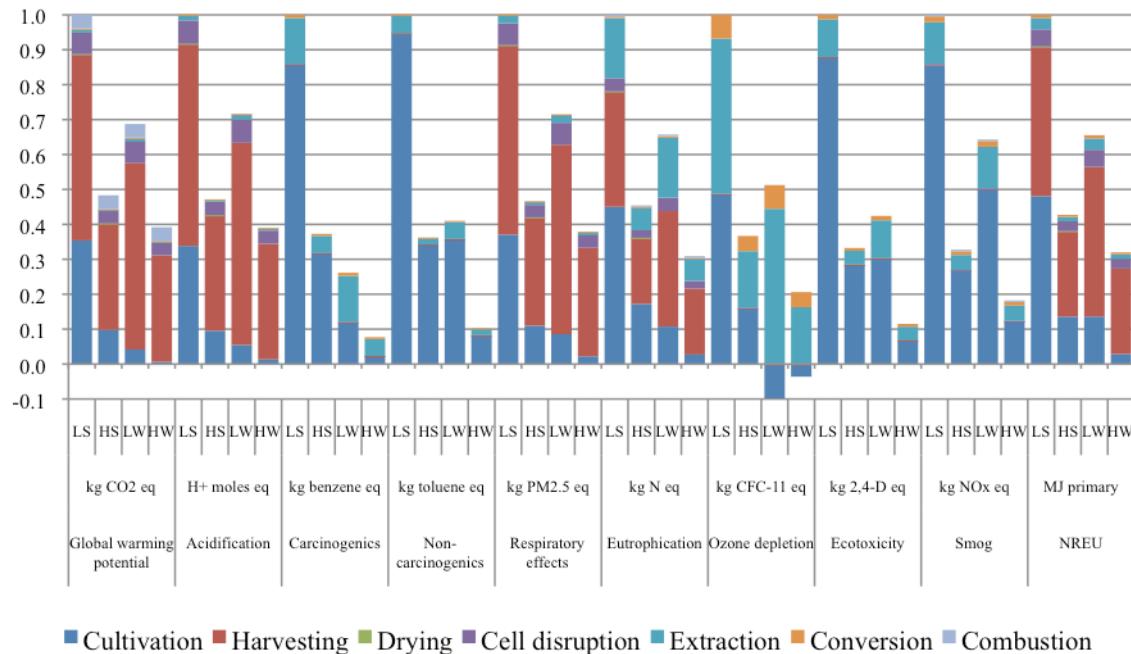


Figure 6. LCIA results for the four production conditions normalized to the highest impact in each category.

LCIA categories were calculated using TRACI with the exception of the NREU category from IMPACT 2002+.

4.4.2 Sensitivity Analysis of Impacts from Microalgal Biodiesel

The sensitivity of GWP, eutrophication, ozone depletion, and ecotoxicity potentials of the four production conditions are obtained from the Monte Carlo simulation. The sensitivity results show the effect of the input parameters on the impact results.

4.4.2.1 Tornado Correlation Coefficient Results

The GWP and eutrophication potential of the LS condition are presented in Figure 7 (a) and (b). The results indicate that both environmental impacts would change significantly given changes to the lipid content of microalgae and energy consumption of microstrainer and belt filter. In this case, energy consumptions of microstrainer and belt filter are more sensitive to the GWP results

than the lipid content. The lipid content has an inverse effect on both GWP and eutrophication potentials and is more sensitive to the eutrophication potential than the energy consumption of the microstrainer and belt filter. The lipid content of microalgae is also the most sensitive parameter with an inverse effect on ozone depletion and ecotoxicity potentials of the LS condition. Quantity of hexane consumed in extraction process and quantity of urea or N fertilizer consumed in the cultivation process are also sensitive to the ozone depletion, as presented in Figure 7 (c). Another two input parameters, which are number and service lifetime of PBR, are also sensitive to ecotoxicity, as presented in Figure 7 (d). The Tornado correlation coefficients of the LS condition and another three microalgal biodiesel production conditions are presented in Electronic Annexes 6 and 7.

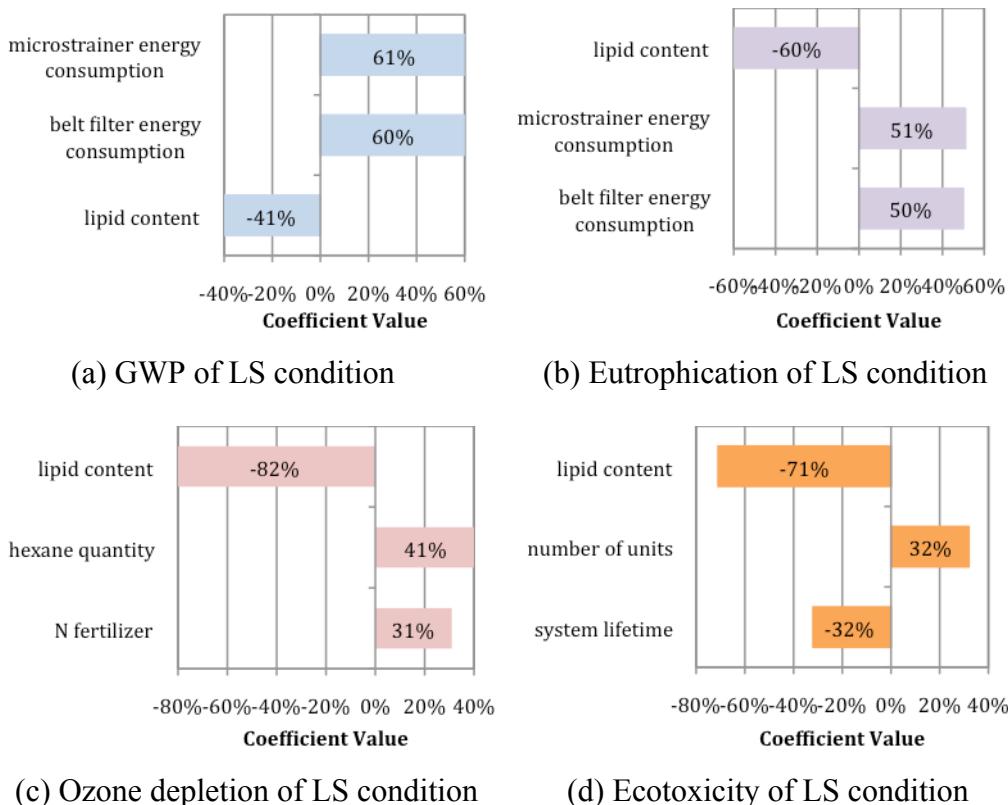


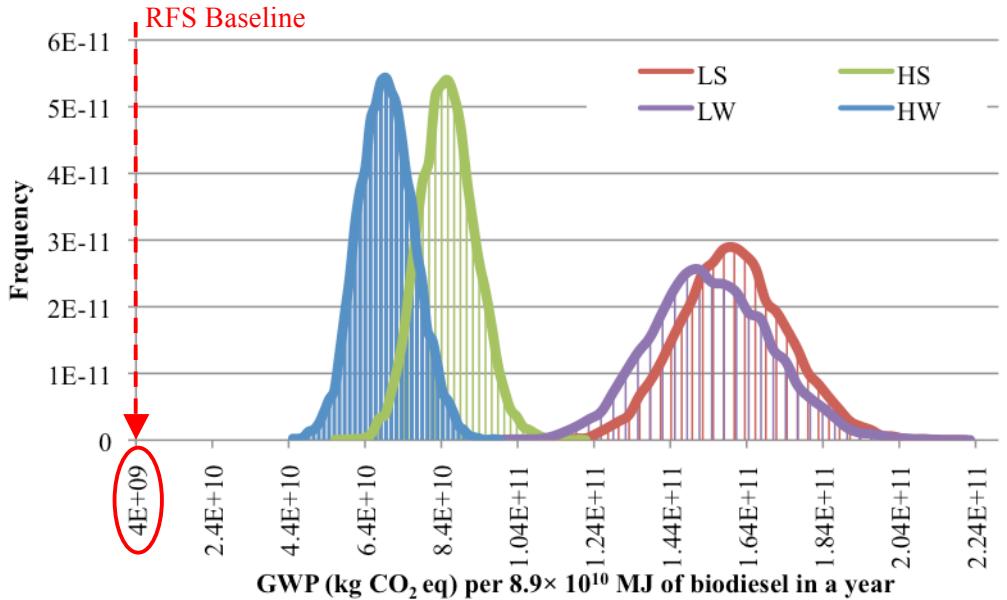
Figure 7 Tornado correlation coefficient of impacts from the LS condition.

Input parameters are shown on the y-axis while the coefficient value on the x-axis represents the percent change to the impact category when the input parameter is changed.

4.4.2.2 Probability Distribution of Impacts from Microalgal Biodiesel

Figure 8 shows the resultant probability distributions for GWP. The Chi-square method was used to determine best fit distributions: LS and HW were best fit to the inverse Gaussian distribution, while the HS and LW were best fit to the log normal distribution. The RFS Baseline is the RFS's requirement in order for a biofuel to be classified as biomass-based diesel or advanced biofuel, defined as 50% of the life cycle GHGs from petroleum fuels distributed in 2005 (Office of Transportation and Air Quality, 2010b; Skone & Gerdes, 2008). The remainder of the results can be found in Electronic Annex 4. Results of the other three impact potentials, eutrophication, ozone depletion and ecotoxicity, from the four microalgal biodiesel production conditions are presented in Electronic Annex 5.

The range of GWP results of the LS and LW conditions were similar. However, the result of the LS production was best fit to the Inverse Gaussian distribution with slightly higher maximum probability density, while the result of the LW condition was best fit to the log normal distribution. On the other hand, the range of GWP of the HS and HW productions were similar. The results of the HS and HW productions were best fit to log normal and Inverse Gaussian distributions, respectively. The mean value of the HW production was lower than that of the HS production approximately by 1.7×10^{10} kg CO₂ eq per functional unit with similar maximum probability density. Yet, even under the condition with the lowest GWP, HW condition, approximately a 94% reduction in GHGs is necessary to meet the RFS Baseline.



Production conditions	Mean (kg CO ₂ eq/8.9×10 ¹⁰ MJ)	Minimum (kg CO ₂ eq/8.9×10 ¹⁰ MJ)	Maximum (kg CO ₂ eq/8.9×10 ¹⁰ MJ)
LS	1.7 × 10 ¹¹	1.1 × 10 ¹¹	2.2 × 10 ¹¹
HS	9.2 × 10 ¹⁰	6.6 × 10 ¹⁰	1.2 × 10 ¹¹
LW	1.6 × 10 ¹¹	1.0 × 10 ¹¹	2.3 × 10 ¹¹
HW	7.6 × 10 ¹⁰	4.9 × 10 ¹⁰	1.1 × 10 ¹¹

Figure 8 The probability distribution of GWP from the four microalgal biodiesel production conditions.

4.4.2.3 Comparison of the Four Conditions

The minimum, mean and maximum values of the GWP, eutrophication, ozone depletion and ecotoxicity potentials from the LS, HS, LW and HW microalgal biodiesel production conditions in Annex 5 are reported with a 95% confidence interval, as illustrated in Figure 9, and are discussed in more detail in the following paragraphs. The RFS sets a baseline only for life-cycle greenhouse gas emissions, and as such no baseline comparisons were made for other impacts.

GWP. The LS and LW conditions contribute higher GWP than the HS and HW conditions. The LS condition contributes approximately 1.7×10^{11} kg CO₂ eq per functional unit, which is about two times higher than that of the HS condition. The different results were from the lower efficiencies of harvesting, extraction and conversion processes in the LS and LW

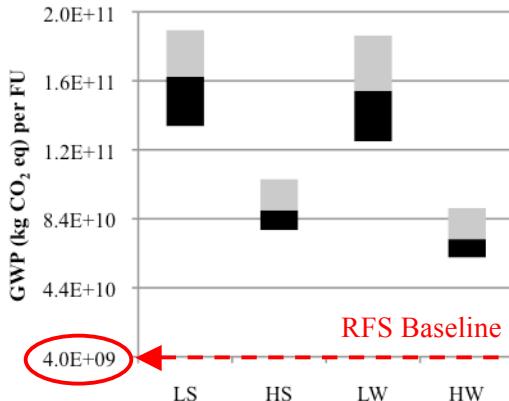
conditions, which was assumed to be 20% lower than the HS and HW conditions, respectively, as shown in Electronic Annex 1. Based on the same synthetic resources, by switching from low-efficiency production to the high-efficiency production, or from LS condition to HS condition, GWP can be reduced by 9.3×10^{10} kg CO₂ eq per functional unit or approximately 54% of the LS condition. The impact reductions are mainly from consuming less energy during lighting and harvesting processes. For the utilization of waste resources, switching from LW condition to HW condition, GWP can be reduced by 5.5×10^{10} kg CO₂ eq per functional unit or GWP from the HW condition was less than that of the LW condition by 46% from consuming less energy during harvesting process. On the other hand, the utilization of synthetic resources instead of natural and waste resources can reduce the GWP of the LS condition by 32%, and can reduce the GWP of the HS condition by 20%. The GWP results of the LS, HS, LW and HW conditions with 95% confidence interval can be seen in Figure 9 (a).

Eutrophication Potential. The eutrophication potential results of the four microalgal biodiesel production conditions follow the same trend as the GWP results, since both impacts are sensitive to the same set of parameters which are energy consumption of microstrainer and belt filter in harvesting process and lipid content of microalgae, as illustrated in Figure 9 (b). The LS condition contributes the highest eutrophication potential compared to other productions. It contributes approximately 2.6×10^7 kg N eq per functional unit. By changing from the LS condition to the LW, HS and HW conditions, eutrophication potential can be reduced by 20%, 52% and 67%, respectively.

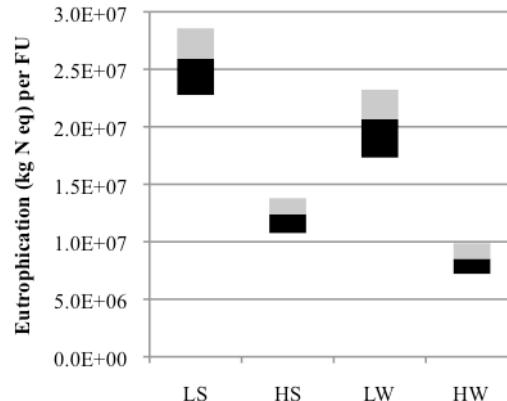
Ozone Depletion Potential. Figure 9 (c) depicts ozone depletion potentials contribute from the four microalgal biodiesel production conditions. According to Figure 7 (c), the results suggested that the lipid content of microalgae and quantity of hexane consumed in extraction

process are the two most sensitive parameters to ozone depletion potential. The LS condition, which has the largest ozone depletion potential, contributes approximately 1.5×10^3 kg CFC-11 eq per functional unit, while the HW condition, which has the smallest ozone depletion potential, contributes approximately 3.3×10^2 kg CFC-11 eq per functional unit. Although, ranges of the impact from the HS and LW conditions are overlapping, the impact from the HS condition is slightly lower. The HS and LW conditions contribute approximately 6.7×10^2 and 7.7×10^2 kg CFC-11 eq per functional unit, respectively. For the same source of resources, by switching from low-efficiency to higher-efficiency production, from LS condition to HS condition and from LW condition to HW condition, ozone depletion potential of the low-efficiency production can be offset by 54 and 57%, respectively. In addition, for the same production efficiency, the utilization of natural and waste resources instead of synthetic resources can offset ozone depletion potentials of LS and HS conditions by 47 and 50% respectively.

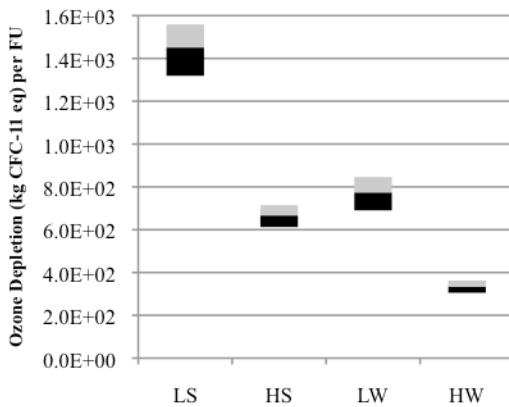
Ecotoxicity Potential. As can be seen in Figure 9 (d), the LS condition contributes approximately 1.1×10^{10} kg 2,4-D eq per functional unit to ecotoxicity potential, which is higher than the HS condition by 63%. The ecotoxicity potential of the LW condition is 5.3×10^9 kg 2,4-D eq per functional unit, which is higher than that of the HW condition by 73%. The explanation for this result is that the ecotoxicity potential of the conditions depends on the lipid content of microalgae, as shown in Figure 7 (d), and also on the number of PBRs. The lower efficiency of LS and LW conditions, as presented in Electronic Annex 1, requires more PBR units, therefore more HDPE is needed to construct PBRs in the LS and LW conditions than the HS and HW conditions in order to produce enough microalgae for one functional unit.



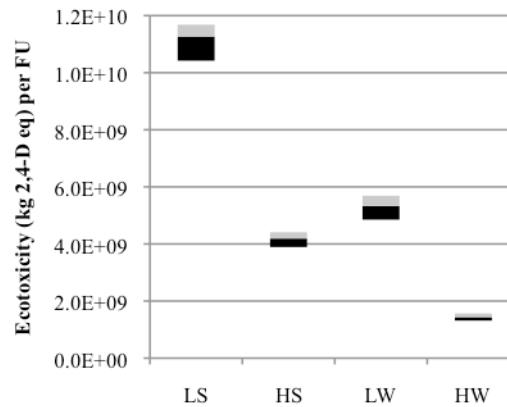
(a) GWP



(b) Eutrophication



(c) Ozone depletion



(d) Ecotoxicity

Figure 9. Total impacts with a 95% confidence interval from the four microalgal biodiesel production conditions.

Means of impacts from the four productions are represented by the line, while the edge of the bar represents the 95% confidence interval value. The RFS Baseline is the Renewable Fuel Standard requirement in order for a biofuel to be classified as biomass-based diesel or advanced biofuel, defined as 50% of the life cycle GHGs from petroleum fuels distributed in 2005 (Office of Transportation and Air Quality, 2010b; Skone & Gerdes, 2008).

4.4.2.4 Sensitivity Analysis of the Parameters with the Highest Environmental Impact

Four parameters were found to have the most impact on the system (see results from the Tornado correlation coefficients presented in Electronic Annex 7). A sensitivity analysis of these four parameters is conducted; they include: (1) lipid content of microalgae, (2) service lifetime of

PBR, (3) energy consumptions during the harvesting process, which includes energy used by both the microstrainer and belt filter and (4) quantity of hexane consumed in the extraction process. These parameters primarily affect four environmental impacts: GWP, eutrophication, ozone depletion and ecotoxicity. The GWP was evaluated in order to compare the result with the RFS's requirement. The three latter environmental impacts were selected and investigated since each impact was influenced by different parameters. The sensitivity analysis (SA) was conducted by increasing and decreasing the values of the four parameters by 50% of the base case condition, as described in Table 4.

Table 4. Eight sensitivity analysis scenarios.

SA Scenario	Parameters ^a	Description of scenarios	Environmental impact evaluated	Base case (BC) ^b
SA1	Energy consumptions of microstrainer and belt filter	Parameter reduced by 50% from the base cases	GWP Eutrophication	LS, HS, LW and HW LS and HW
SA2	Energy consumptions of microstrainer and belt filter	Parameter increased by 50% from the base cases	GWP Eutrophication	LS, HS, LW and HW LS and HW
SA3	Lipid content of microalgae	Parameter reduced by 50% from the base cases	Eutrophication Ozone depletion Ecotoxicity	LS and HS LS and HS LS and HS
SA4	Lipid content of microalgae	Parameter increased by 50% from the base cases	Eutrophication Ozone depletion Ecotoxicity	LS and HS LS and HS LS and HS
SA5	Quantity of hexane used during extraction	Parameter reduced by 50% from the base cases	Ozone depletion	LW and HW
SA6	Quantity of hexane used during extraction	Parameter increased by 50% from the base cases	Ozone depletion	LW and HW
SA7	Service lifetime of PBR	Parameter reduced by 50% from the base cases	Ecotoxicity	LW and HW
SA8	Service lifetime of PBR	Parameter increased by 50% from the base cases	Ecotoxicity	LW and HW

^a Parameters evaluated are those with the highest Tornado correlation coefficient shown in Electronic Annex 7.

^b Input values of BCs can be seen in Electronic Annex 1.

SA – Sensitivity analysis scenarios, BC – Base case (the four microalgal diesel production conditions) to compare with other SA scenarios, LS - low-efficiency production with synthetic resources, HS – high-efficiency production with synthetic resources, LW - low-efficiency production with natural and waste resources, HW - high-efficiency production with natural and waste resources, and ‘Description of scenarios’ column describes how the SA differs from the BC.

The sensitivity analyses of LS and HS production conditions and LW and HW production conditions are presented in Figure 10 and Figure 11, respectively. The GWP, eutrophication,

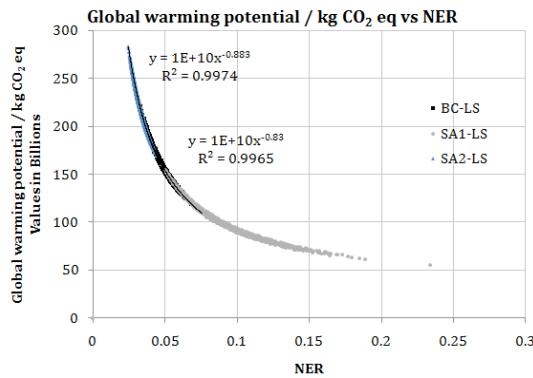
ozone depletion and ecotoxicity potential results were plotted against the net energy ratio (NER), which is a ratio of energy produced to energy consumed, to illustrate the change of the impacts as the energy consumption changes (Jorquera et al., 2010; Sander & Murthy, 2010). The results of the SA were compared to the original results from the BC scenarios. Percent of impacts and energy required of the eight microalgal diesel production scenarios compared to the four base cases are listed in Table 5. The sensitivity analysis shows how the four different input parameters affect the resultant impacts from the LS and HS base case conditions (BC-LS and BC-HS). GWP, eutrophication, ozone depletion and ecotoxicity potentials of the BC-LS and BC-HS are presented in Figure 10. When the LS and HS conditions are changed according to SA1 and SA2 (changing energy consumption of the microstrainer and belt filter), the resultant GWP overlaps with the base case (Figure 10 a.1 and Figure 10 a.2) which indicates that changing the energy consumption of microstrainer and belt filter in SA1 or SA2 does not always change the resultant GWP compared to BC-LS and BC-HS.

By changing energy consumption of microstrainer and belt filter of the BC-LS by 50%, the total GWP result of SA1-LS increases and SA2-LS decreases. Likewise, the energy required to produce one functional unit of SA1-LS increases and SA2-LS decreases. Therefore, decreasing the energy consumption for the microstrainer and belt filter shown in SA1-LS is one way to significantly reduce the overall GWP. The plots of SA1, SA2 and BC of the HS are similar to those of the LS. However, by decreasing the energy consumption of the microstrainer and belt filter of the BC-HS by 50% (SA1-HS), the total GWP result and energy consumed to produce one functional unit significantly decrease more than the decreases of the SA1-LS. In addition, the BC-HS requires less energy to produce one functional unit; therefore, it has higher potential to achieve the RFS requirement compared to the BC-LS.

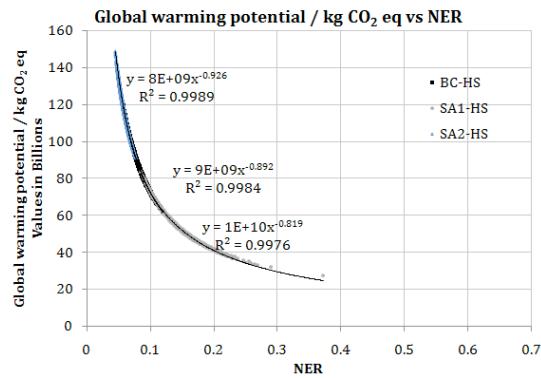
Sensitivity of the input parameter affecting lipid content (SA3 and SA4) for the LS and HS conditions were evaluated for their effect on the eutrophication potential and the NER (Figure 10 b.1 and b.2). When lipid content is reduced by 50% (SA3) in both the HS and LS conditions, NER decreases significantly and eutrophication increases significantly from the base case, with little overlap. Decreasing the lipid content of the BC-LS and BC-HS conditions by 50% (SA3) increases their eutrophication potential and energy required to produce one functional unit. However, when lipid content is increased by 50% (SA4) in both the HS and LS conditions, NER only slightly increases while eutrophication slightly decreases. These results suggest that microalgae with high lipid content is more desirable to achieve a higher NER and slight decreases in eutrophication potential. However, with lower lipid content, there is a significant impact on eutrophication potential and NER.

Ozone depletion potentials of the LS and HS conditions (BC-LS and BC-HS) are sensitive to lipid content of microalgae. The trends of ozone depletion potential are similar to the trends of eutrophication potential. Although, when the lipid content of the BCs are decreased by 50% (SA3), the ozone depletion potentials are more clustered than the eutrophication potentials, as shown in Figure 10 c.1 and c.2. Decreasing the lipid content of the BC by 50% (SA3) drastically increases ozone depletion potentials of SA3-LS and SA3-HS. Conversely, increasing the lipid content of the BC by 50% (SA4) decreases ozone depletion potential of SA4-LS and SA4-HS. The partial overlapping of the NER of SA4 and the base cases suggests that the increasing of lipid content from the base case by 50% (SA4) decreases ozone depletion potential but does not necessarily decrease the energy required to produce one functional unit. NER can be reduced to reduce life-cycle GHG emissions, thus achieve the RFS requirement, however microalgal biodiesel production still contributes to significant ozone depletion potential.

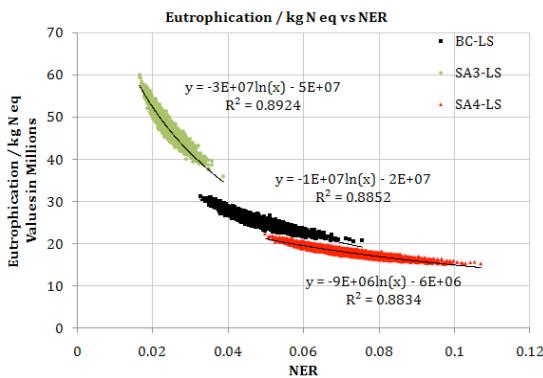
Similar to the ozone depletion potential, the ecotoxicity potential is influenced by the lipid content of microalgae (Figure 10 d.1 and d.2). The trends of the ecotoxicity potential also follow the trends of the ozone depletion potential; the results from decreasing the lipid content of the base case by 50% (SA3) does not overlap with other plots, while the results from increasing the lipid content of the base case by 50% (SA4) does partly overlap with the base case. By increasing the lipid content of the LS and HS conditions by 50% (SA3-LS and SA3-HS), the ecotoxicity potential increases, while decreasing the lipid content of the same conditions by 50% (SA4-LS and SA4-HS), the ecotoxicity potential decreases. From these results, it can be implied that increasing lipid content of microalgae potentially decreases energy required to produce one functional unit of microalgal biodiesel without any change to its ecotoxicity potential.



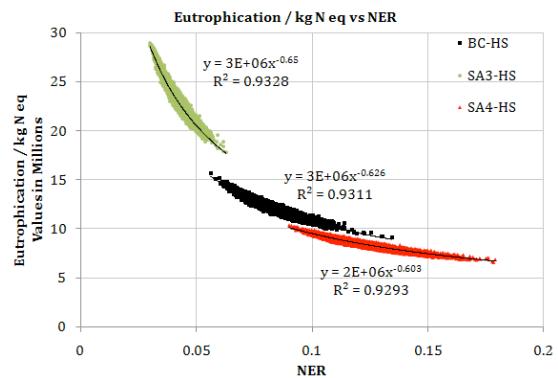
(a.1)



(a.2)



(b.1)



(b.2)

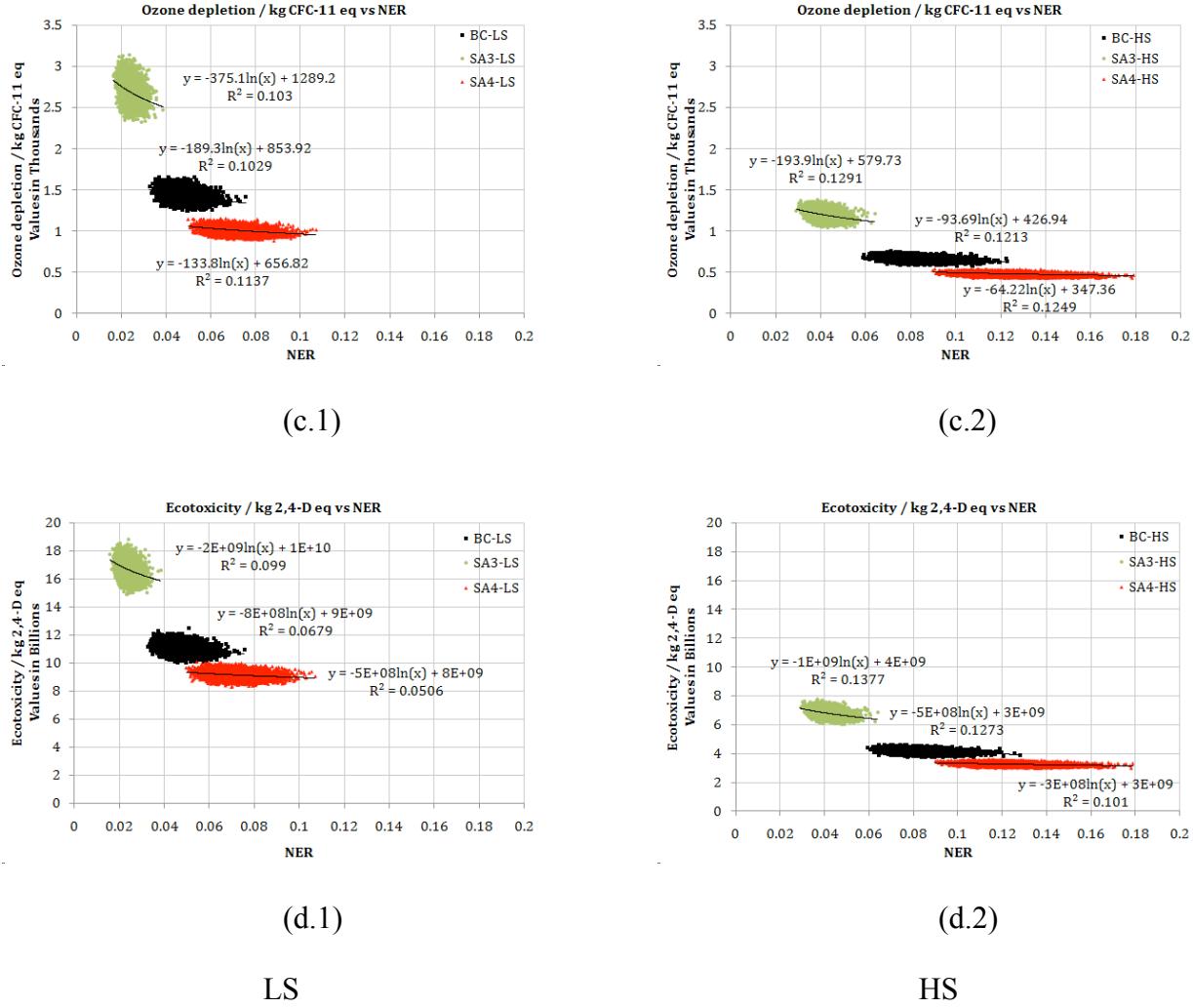


Figure 10 Sensitivity analysis scenarios for the low-efficiency production and high-efficiency production conditions with synthetic resources (LS and HS).

Sensitivity analysis was conducted for four input parameters: (a) the energy consumption of microstrainer and belt filter on the GWP, and (b, c, d) the lipid content of microalgae on the eutrophication, ozone depletion and ecotoxicity potentials, respectively. For SA1 and SA2, energy consumptions of microstrainer and belt filter are less and more than the base case (BC) by 50%, respectively. For SA3 and SA4, lipid content of microalgae was less and more than the BC by 50%, respectively. The results of SA1 through SA4-LS are compared to the result of BC-LS, while the results of SA1 through SA4-HS are compared to the results of BC-HS.

The four resultant impacts from the LW and HW base case conditions (BC-LW and BC-HW) are influenced by three different parameters, as presented in Figure 11. Energy consumed during the harvesting process by both microstrainer and belt filter (SA1 and SA2) affects GWP and eutrophication potential; quantity of hexane consumed during extraction process (SA 5 and

SA6) affects ozone depletion potential; service lifetime of PBR (SA 7 and SA8) affects ecotoxicity. The partial overlapping of GWP from the base case and the scenario with lower and higher energy consumptions during the harvesting process than the BC condition by 50% (SA1 and SA2) in Figure 11 a.1 and a.2 indicate that changing the energy consumption during harvesting process does not always change the resultant GWP compared to the base case conditions. By decreasing energy consumption during the harvesting process of the BC by 50% (SA1), the total GWP results of SA1-LW and SA1-HW decrease by 34 - 63% and 35 - 69%, respectively. These results indicate that SA1-HW has a higher potential to achieve the RFS requirement, compared to the SA1-LW. Approximately a 71-93% reduction in GHG emissions of SA1-HW is necessary to meet the RFS requirement.

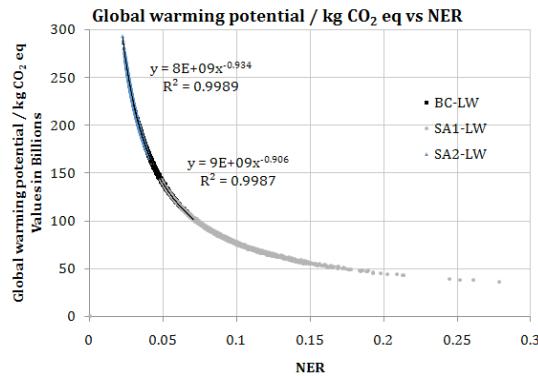
Similarly, when changing the energy consumption during the harvesting process (SA1 & SA2), the resultant eutrophication potentials of the LW and HW conditions overlap with the sensitivity analysis scenarios as can be seen in Figure 11 b.1 and b.2. The eutrophication potential results suggest that changing the energy consumption during the harvesting process does not always change the resultant eutrophication potential. Compared to the base cases, BC-LW and BC-HW, the total eutrophication of SA1-LW decreases by 26 – 39% and SA1-HW decreases by 26 – 42%, respectively. On the other hand, the total eutrophication potential of SA2-LW increases by 22 – 37%, and SA2-HW increases by 29 – 46% compared to the base case conditions. Based on these results, decreasing the energy consumption during harvesting process by 50%, the 50% reduction of life cycle GHG emissions of the BC-HW can be achieved, while the maximum reduction of eutrophication potential is only 46%. Therefore, even the microalgal biodiesel production that can reduce GHG emissions to meet the RFS's requirement still contributes significant impact on eutrophication potential.

The total ozone depletion potential is sensitive to the quantity of hexane and the ecotoxicity potential is sensitive to the service lifetime of the PBR for both the LW and HW conditions. Decreasing the quantity of hexane consumed during the extraction process of the LW condition by 50% (SA5) results in a decrease in the total ozone depletion potential, while increasing the quantity of hexane of the LW condition by 50% (SA6) increases the total ozone depletion potential, as can be seen from the plots in Figure 11 (c.1) and (c.2). A similar trend is also observed in SA5, SA6 and the BC-HW conditions. The NER of SA5 and SA6 (changing the quantity of hexane during extraction process) overlaps with the base cases, LW and HW conditions. The results showed that changing of hexane quantity during extraction process only changes the total ozone depletion potential, but has little impact on the NER.

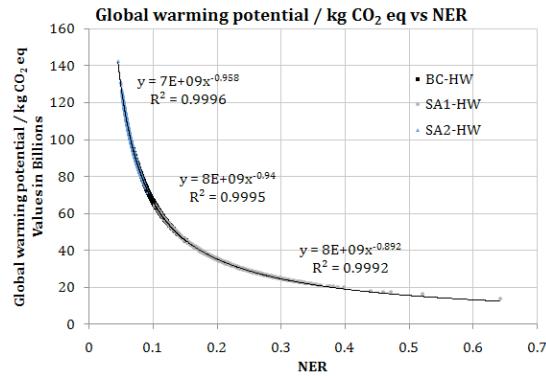
Changing the service lifetime of PBR (SA7 and SA8) affects the total ecotoxicity potential, whereas there is no effect on the NER as shown in Figure 11 (d.1) and (d.2). The microalgal biodiesel production using PBR with system lifetime longer than the BC-LW by 50% (SA8-LW) decreases the total ecotoxicity potential, while the production using PBR with shorter lifetime than the BC-LW by 50% (SA7-LW) increases the total ecotoxicity potential. Likewise, increasing a PBR's lifetime by 50% of the HW condition (SA8-HW) decreases the total ecotoxicity potential, while decreasing the PBR's lifetime by 50% of the HW condition significantly increases ecotoxicity potential. Hence, PBR's lifetime should be considered when designing the microalgal biodiesel production system or evaluating life cycle impacts of the microalgal biodiesel production.

The reduction of energy required to produce one functional unit in some cases can reduce GHG emissions and meet the RFS requirement, however other environmental impacts, such as

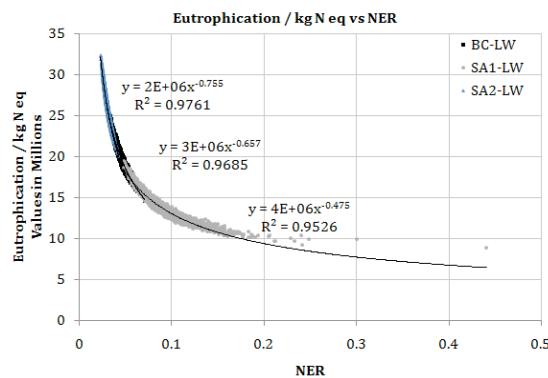
ozone depletion and ecotoxicity potentials, are also important and RFS should also take them into consideration.



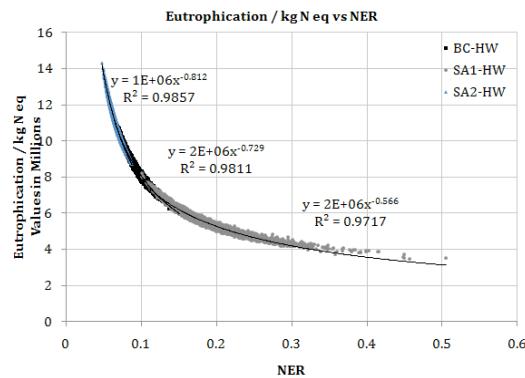
(a.1)



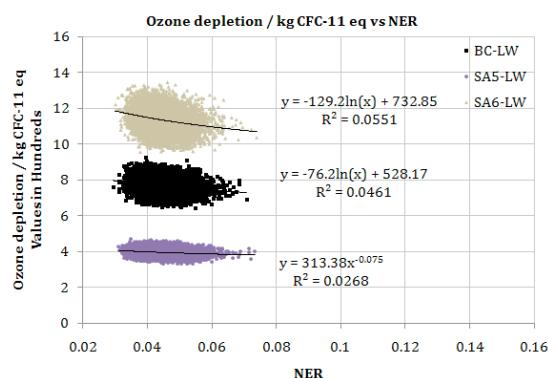
(a.2)



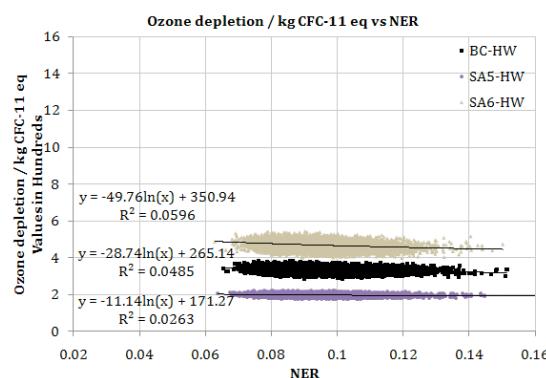
(b.1)



(b.2)



(c.1)



(c.2)

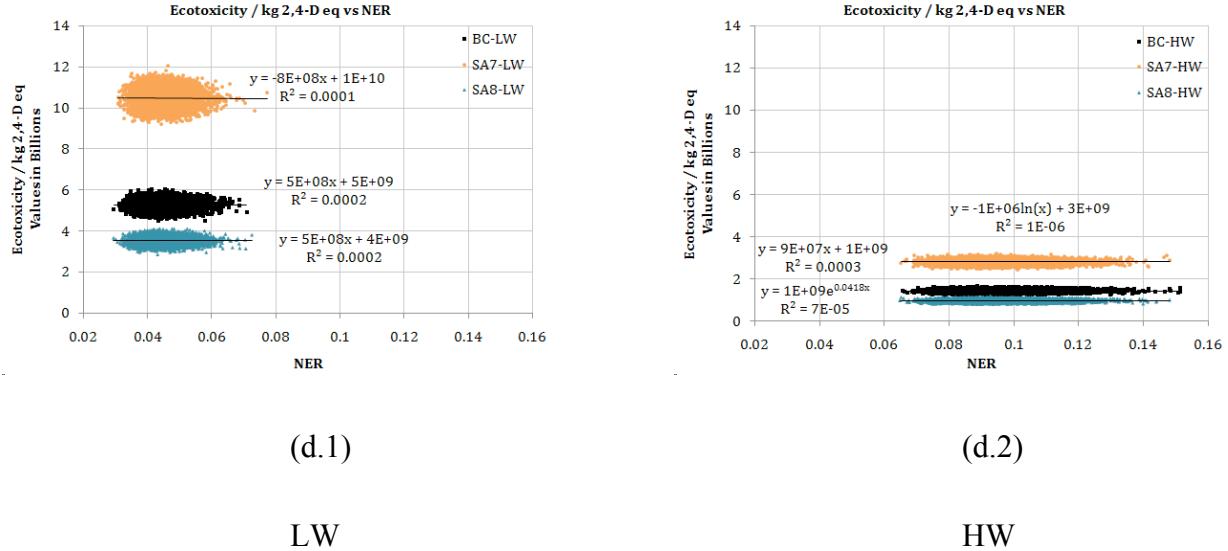


Figure 11 Environmental impact potentials from the low-efficiency production and high-efficiency production with natural and waste resources (LW and HW) sensitivity analysis scenarios.

The parameters considered are energy consumption of microstrainer and belt filter on GWP and eutrophication potential in (a) and (b), respectively, quantity of hexane in extraction process on ozone depletion potential in (c) and service lifetime of PBR on ecotoxicity potential in (d). For SA1 and SA2, energy consumptions of microstrainer and belt filter were less and more than the base case (BC) by 50%, respectively. For SA5 and SA6, quantity of hexane was less and more than the BC by 50%, respectively. For SA7 and SA8, service lifetime of PBR was shorter and longer than the BC by 50%, respectively. The results of SA1, SA2 and SA5 through SA8-LW were compared to the result of BC-LW, while the results of SA1, SA2 and SA5 through SA8-HW were compared to the result of BC-HW.

Table 5 The percent of impacts and energy required of the eight microalgal biodiesel production scenarios compared to the four base cases.

Scenario	GWP % of BC	Eutrophication % of BC	Ozone depletion % of BC	Ecotoxicity % of BC	Energy Required % of BC
SA1-LS	> BC by 28 - 45%	-	-	-	< BC by 32 - 60%
SA2-LS	< BC by 28 - 45%	-	-	-	> BC by 33 - 61%
SA3-LS	-	> BC by 76 - 92%	< BC by 87 - 89%	> BC by 49 - 50%	> BC by 95 - 98%
SA4-LS	-	< BC by 27 - 29%	> BC by 29 - 30%	< BC by 17 - 20%	< BC by 29 - 35%
SA1-HS	> BC by 31 - 56%	-	-	-	< BC by 33 - 68%
SA2-HS	< BC by 24 - 48%	-	-	-	> BC by 27 - 56%
SA3-HS	-	> BC by 85 - 95%	< BC by 81 - 83%	> BC by 61 - 68%	> BC by 92 - 102%
SA4-HS	-	< BC by 28 - 34%	> BC by 25 - 28%	< BC by 21 - 22%	< BC by 25 - 38%
SA1-LW	< BC by 34 - 63%	< BC by 26 - 39%	-	-	< BC by 37 - 73%
SA2-LW	> BC by 30 - 59%	> BC by 22 - 37%	-	-	> BC by 31 - 67%
SA5-LW	-	-	< BC by 48 - 49%	-	< BC by 3 - 5%
SA6-LW	-	-	> BC by 46 - 49%	-	< BC by 2 - 4%
SA7-LW	-	-	-	> BC by 100 - 105%	< BC by 4 - 8%
SA8-LW	-	-	-	< BC by 32 - 37%	< BC by 2%, > BC by 0.4%
SA1-HW	< BC by 35 - 69%	< BC by 26 - 42%	-	-	< BC by 37 - 76%
SA2-HW	> BC by 44 - 58%	> BC by 29 - 46%	-	-	> BC by 45 - 64%
SA5-HW	-	-	< BC by 39 - 41%	-	< BC by 16%, > BC by 3%
SA6-HW	-	-	> BC by 40 - 42%	-	> BC by 1 - 4%
SA7-HW	-	-	-	> BC by 92 - 105%	> BC by 1 - 2%
SA8-HW	-	-	-	< BC by 28 - 33%	> BC by 1 - 2%

4.5 DISCUSSION

GWP results of the four production conditions from the pond to pump LCA suggested that GWP of the HW and LS production conditions are 6.2×10^{10} and 1.7×10^{11} kg CO₂ eq per functional unit or 0.69 to 1.88 kg CO₂ eq per MJ, respectively, while the pond-to-wheel GWP from the two production conditions are 0.71 and 1.93 kg CO₂ eq per MJ, respectively. According to the Argonne National Laboratory's Greenhouse gases, Regulated Emissions, and Energy use in Transportation (GREET) Model version 1.8d.1, the well-to-wheel GWP is approximately 0.09 - 0.10 kg CO₂ eq per MJ (Argonne National Laboratory, 2010; Huang et al., 2010; Independent Statistics and Analysis, 2011; Research and Innovative Technology Administration, 2010). The GWP from the HW condition, which is the condition with the lowest impact among the four production conditions, is approximately 8 times higher than the GWP from the conventional diesel production. This does not meet the RFS's requirement for advanced biofuels, which requires 50% fewer life-cycle GHG emissions than that from the petroleum-based transportation fuels distributed in 2005 (Office of Transportation and Air Quality, 2010a; Office of Transportation and Air Quality, 2010b). Other environmental impacts from conventional diesel and biodiesels can be compared to results from other studies and databases such as ecoinvent (Batan et al., 2010a; Clarens et al., 2011; Frischknecht et al., 2007; Sheehan, 1998; Stephenson et al., 2010; Wang et al., 2008). Allocation can be conducted on various types of co-products, such as bioethanol, algae meal, methane, electricity, glycerol and potassium sulfate. Previous research shows that environmental impacts such as GWP can be allocated from more than 100% to very little depending on quantity and quality of the co-products as well as the specific conditions and system boundaries of the systems that microalgal biodiesel production is compared to (Sander & Murthy, 2010; Stephenson et al., 2010). Due to the uncertainty and variation among possible co-

products and by-products and due to their respective lack of data availability, allocation was not implemented in this study.

Sensitivity analysis was conducted on the parameters that were determined to have the most influence on the resulting environmental impacts. The GWP and eutrophication potential of the base case conditions, energy consumptions of the microstrainer and belt filter reduced by 50% from the base case condition (SA1) and energy consumptions of the microstrainer and belt filter increased by 50% from the base case condition (SA2) resulted in a power function curve. The results suggest that the effect of the improvement of the energy consumption of the microstrainer and belt filter on GWP and eutrophication potential has a diminishing return rate. The eutrophication, ozone depletion and ecotoxicity potentials of the base case conditions productions and the two SA scenarios evaluating the change in lipid content (SA3 and SA4), resulted in natural logarithm curves, and a diminishing return rate also occurs. For the ozone depletion of SA5 and 6, by switching from the scenario consuming more hexane during the extraction process than the base case condition by 50% (SA6) to the BC-LW or from the BC-LW to the scenario consuming less hexane during the extraction process than the base case condition by 50% (SA5), ozone depletion potential can be decreased, however, NER remains the same. The trends of the ecotoxicity potentials of SA7 and 8 are similar to the trends of the ozone depletion of SA5 and 6. By switching from the scenario with shorter service lifetime of PBR than the base case condition by 50% (SA7) to the BC-LW or from the BC-LW to the scenario with longer service lifetime of PBR by 50% (SA8), ecotoxicity potential can be decreased, while NER remains the same.

The NER results (shown in Figure 6, Figure 10 and Figure 11, and presented in more detail in Annex 11-13) of microalgal biodiesel are very low compared to conventional fuels and

other first-generation biofuels such as corn ethanol (Argonne National Laboratory, 2010). Microalgal biodiesel production modeled in this study based on current technologies and without allocation to co-products does not meet the RFS's GHG requirement for the US's energy security purpose at present. The NER of microalgal biodiesel can be increased through the improvement of production technologies and the use of industrial symbiosis approaches, which are the two strategies investigated in this study. Reducing energy consumption during the harvesting process by 50% (SA1) alone can increase NER of the BC-HS and BC-HW by 3 to 6 and 4 to 11 times, respectively. Under the SA1 condition, changing from synthetic to natural and waste resources can reduce the energy required to produce microalgal biodiesel in half. Another strategy to increase the NER is to develop valuable co-products, such as microalgal bioethanol, methane or electricity, which may improve the yield and the potential of microalgal biodiesel production to achieve the RFS's GHG emission reduction requirement. Therefore, when developing policy for renewable fuels, and microalgal biodiesel in particular, other factors, such as NER (Shaw et al., 2010), eutrophication potential and industrial symbiosis should be considered.

The GWP results from the four microalgal biodiesel production conditions indicate that none of the assumed conditions in this study meet the RFS's requirement for advanced biofuel. However, the results from this study suggest potential for improvement of the production. The GWP and eutrophication potential results of the process LCA model indicate that the harvesting process is an energy intensive process. Based on the results from Tornado correlation coefficients, energy consumption of the microstrainer and belt filter, which are the methods used in harvesting process of this study, are one of the main contributors to GWP in the four production conditions and are also the main contributor to eutrophication potential in the LW and HW production conditions. Another parameter of importance is the lipid content of microalgae;

it inversely influences eutrophication, ozone depletion and ecotoxicity potentials of the LS and HS production conditions. In addition, the quantity of hexane consumed during the extraction process and the service lifetime of PBRs are the major contributor to ozone depletion and ecotoxicity potentials, respectively, for both the LW and HW production conditions. Therefore, decreasing the energy consumption of the microstrainer and belt filter during the harvesting process, the recycling of hexane from the extraction process, and/or increasing of the lipid content of microalgae can result in an overall decrease in the life-cycle environmental impact of microalgal biodiesel production and can help move them toward fuels that meet the RFS2.

4.6 CONCLUSION

Microalgal biodiesel was evaluated from four different production conditions: LS, HS, LW and HW. None of the four different conditions investigated in this study meet the RFS's requirement, which requires that advanced biofuels exhibit 50% less life-cycle GHG emissions than of that from the petroleum-based transportation fuels distributed in 2005. Monte Carlo analysis was conducted to calculate probability distributions for the environmental impacts of microalgal biodiesel production. The Tornado correlation coefficient was used to identify the parameters with high contribution to the total impacts. The four parameters that had the largest impact on the results were lipid content of microalgae, service lifetime of PBRs, energy consumption of the microstrainer and belt filter in the harvesting process and quantity of hexane consumed in the extraction process. Improving these parameters can reduce GWP, eutrophication, ozone depletion and ecotoxicity potentials of microalgal biodiesel production. In order to meet the RFS, the high-efficiency production with the utilization of waste resources under the condition where

energy consumption of the microstrainer and belt filter in harvesting process is reduced by 50% of the HW condition (SA1-HW) must reduce GHG emissions by an additional 71-93% to achieve the RFS baseline. The policies for renewable fuels should also determine other factors, such as NER and other emissions, in addition to GHG emission.

4.7 ACKNOWLEDGEMENT

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5.0 RE-ENVISIONING THE RENEWABLE FUEL STANDARD TO MINIMIZE THE UNINTENDED CONSEQUENCES

The following chapter is a reproduction of an article under review in the journal *Environmental Science & Technology* with the citation:

Soratana, K., V. Khanna, and A.E. Landis, “Re-envisioning the Renewable Fuel Standard to Minimize Unintended Consequences.” Environmental Science & Technology, 2012: Under review.

The article appears as submitted following the first peer-review, journal of *Environmental Science & Technology*. Supporting Information submitted with the journal of *Environmental Science & Technology* appears in Appendix C.

5.1 ABSTRACT

The Renewable Fuel Standard 2 (RFS2) program under the Energy Independence and Security Act of 2007 set a life-cycle emission reduction threshold to only greenhouse gas (GHG) emissions; this type of single-dimensional threshold could lead to the unintended trading of one environmental problem for another. Many of the environmental impacts resulting over the life cycle of biodiesel fuels manifest in the agricultural phase of production in the form of water quality degradation. This study investigated the extent to which different biofuels meet the RFS GHG requirement, and presents alternative strategies for minimizing unintended consequences. In addition to life-cycle GWP, the eutrophication potential and photochemical smog formation potential from microalgal diesel is compared to the impacts resulting from petroleum-based diesel, soybean diesel and canola diesel. The results showed tradeoffs between GWP and eutrophication potential when microalgal diesel is compared to soybean diesel. Future RFS criteria should include eutrophication potential and photochemical smog formation potential metrics, however establishing thresholds like the GHG management approach may not be appropriate for these other impacts. Two possible strategies to setting life-cycle eutrophication standards are to establish a threshold based on first generation biofuels, as opposed to petro-fuels or to set maximum levels of eutrophication potential loads for major watersheds or coastal areas. To decrease photochemical smog formation potential, apart from existing standards for tailpipe emissions, future RFSSs should include the well-to-pump emissions from biofuels and petroleum fuels accounting for temporal and seasonal variations.

5.2 INTRODUCTION

The Renewable Fuel Standard (RFS2) under the Energy Independence and Security Act of 2007 (EISA 2007) established an annual biofuel production mandate and the first life-cycle greenhouse gas (GHG) emissions reduction threshold for four different types of biofuel: conventional, cellulosic, advanced biofuels, and biomass-based diesel. Conventional biofuels are required to have at least a 20% reduction in lifecycle GHG emissions relative to baseline petroleum-derived transportation fuels produced and distributed in 2005, cellulosic biofuels at least a 60% reduction and advanced biofuels and biomass-based diesel (e.g. biofuels produced from microalgae) a 50% reduction (Office of Transportation and Air Quality, 2010a; Office of Transportation and Air Quality, 2010b). The life-cycle GHG emissions reduction threshold was analyzed and set by the U.S. Environmental Protection Agency (EPA) based on various factors from several models such as the Greenhouse gases, Regulated Emissions, and Energy use in Transportation Model (GREET), the Forest and Agricultural Sector Optimization Model (FASOM) and the integrated Food and Agricultural Policy and Research Institute (FAPRI) international model (Assessment and Standard Division Office of Transportation and Air Quality, 2010).

The EPA also evaluated other emissions such as nitrogen oxides (NO_x), volatile organic carbon (VOC), carbon monoxide (CO), and particulate matter 10 and $2.5 \mu\text{m}$ in diameter (PM10 and PM2.5), which contribute to additional environmental impacts, e.g. eutrophication potential (EP) and photochemical smog formation potential (PSP), but not from a life-cycle perspective. RFS2 does not set thresholds for any other emission or environmental impact resulting from biofuels. Environmental impact such as EP are directly related to the discharge of ammonia

(NH_3), nitrate (NO_3^-) and phosphate (PO_4^{3-}) from fertilizer use during agricultural production of the biofuel feedstock. PSP is influenced by the release of NO_x from the combustion of biofuels (Clarens et al., 2010; Environmental Protection Authority, 2004; Hadj Amor et al., 2008; Miller et al., 2007). Neither EP nor PSP threshold impacts were included in the Renewable Fuel Standard program (RFS2) under EISA. Such single metric approaches without attention to other environmental impacts could lead to the unintended trading of one environmental problem for another.

Due to environmental impacts of conventional biofuels and unsuccessful commercial-scale production of cellulosic biofuels (Miller et al., 2007; Schnoor, 2011), advanced biofuels have emerged as a sustainable biofuel solution. Microalgal diesel, which is categorized as an advanced biofuel, shows higher potential as a biodiesel feedstock due to its high productivity and minimal land use and freshwater requirements, compared to first- and second-generation biodiesel feedstocks. Microalgae can be cultivated on non-arable land with water deemed unsuitable for other oil crops (e.g. wastewater and/or brackish water). It can also utilize carbon dioxide (CO_2) in flue gas from power plants in its photosynthesis process (Avagyan, 2008; Chisti, 2007; Pittman et al., 2011; Soratana & Landis, 2011). However, the microalgal harvesting and drying processes can be energy intensive (Uduman et al., 2010). Therefore, multiple environmental impacts of biofuels over their entire life-cycle must be evaluated to determine which biofuels are actually more sustainable than conventional fuels.

The objectives of this study were (1) to assess the suitability of different biomass to meet the existing 50% life-cycle GHG emissions reduction threshold set by the U.S. EPA (Office of Transportation and Air Quality, 2010b), (2) to evaluate environmental tradeoffs among different

types of diesel from a life cycle perspective and (3) to propose an approach for establishing life cycle EP and PSP threshold for future RFSs.

5.3 METHODS

Life cycle assessment (LCA) was used in this study to evaluate GHG, EP and PSP impacts over the fuels' lifecycle following a process approach similar to International Organization for Standardization 14040 (ISO 14040) (International Organization for Standardization, 2006; Udo de Haes & van Rooijen, 2005).

The GWP, EP and PSP of microalgal diesel were evaluated from well to wheel using a process LCA model combined with Monte Carlo uncertainty analysis. The results were analyzed and compared to the results from conventional diesel, low-sulfur diesel (LSD), soybean and canola derived biodiesel, and microalgae feedstock using existing databases including: GREET (the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation) 1.8d model, ecoinvent data v2.0, IDEMAT, and peer-reviewed literature. Even though conventional diesel has been replaced by ultra low-sulfur diesel (ULSD), the existing LCA models and databases (i.e. GREET and ecoinvent) still provide inventories for conventional diesel and LSD; therefore, conventional diesel was chosen for investigation in this study. The impacts from different diesels produced under different conditions were compared on the same functional unit basis, 8.94×10^{10} MJ of biodiesel/year or 0.67 billion gallon of biodiesel/year (BGY), which equals one BGY of bioethanol or 50% of the RFS's volume required from advanced biofuels in 2012 (Office of Transportation and Air Quality, 2010a; Office of Transportation and Air Quality, 2010b). The

RFS referred to the energy requirement in term of bioethanol, while this study focused on biodiesel, therefore the functional unit was chosen.

5.3.1 LCA of Microalgal Diesel

The system boundaries of microalgal diesel LCA model included the following processes: strain selection, cultivation, harvesting, drying, extraction, conversion, and combustion. The microalgal diesel was produced from microalgae with 70% lipid content in 3.15 million units of a 10-m³ photobioreactor (PBR) with a 15-year service lifetime. Although, 70% lipid content is high, it is achievable and represents future optimistic scenario; the lipid content used in other studies may vary from 20 to 50% (Batan et al., 2010a; Chisti, 2007; Gouveia & Oliveira, 2009; Mata et al., 2010). The microalgal diesel production is assumed to employ high-efficiency technologies that provided 90% harvesting, 98% extraction and 87% conversion efficiencies. The cultivation system utilized natural and waste resources such as natural light, nitrogen (N) and phosphorus (P) from municipal wastewater and CO₂ from flue gas of power plants, as presented in Figure 12.

The use phase of microalgal diesel accounted for combustion emissions of CO₂, CH₄, N₂O and NO_x (Argonne National Laboratory, 2010; Hermann, 2010). Environmental impacts were determined using the Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts (TRACI) (Bare et al., 2003).

The model did not include the construction of biodiesel production facilities since microalgal oil properties were assumed to be compatible with the existing refinery technology for producing biodiesel from other feedstocks. Transportation between facilities was omitted and the facilities were assumed close to one another, and no by-product or waste allocations were included. However, we discuss the impact of mass allocation to evaluate the yield of biodiesel

from microalgal biomass and bioethanol from de-oiled microalgal biomass. Life cycle inventories (LCIs) of resources utilized within the microalgal diesel production, for example, PBR construction material, fertilizers, chemicals for nutrient removal from wastewater, energy etc., from ecoinvent data v2.0, ETH-ESU 96, BUWAL 250 and IDEMAT were used (Delft University of Technology, 2001; Frischknecht & Jungbluth, 2004; Frischknecht et al., 2007; Spriensma, 2004). More details on LCIs and Monte Carlo simulation model input variables are available in the Supporting Information (SI).

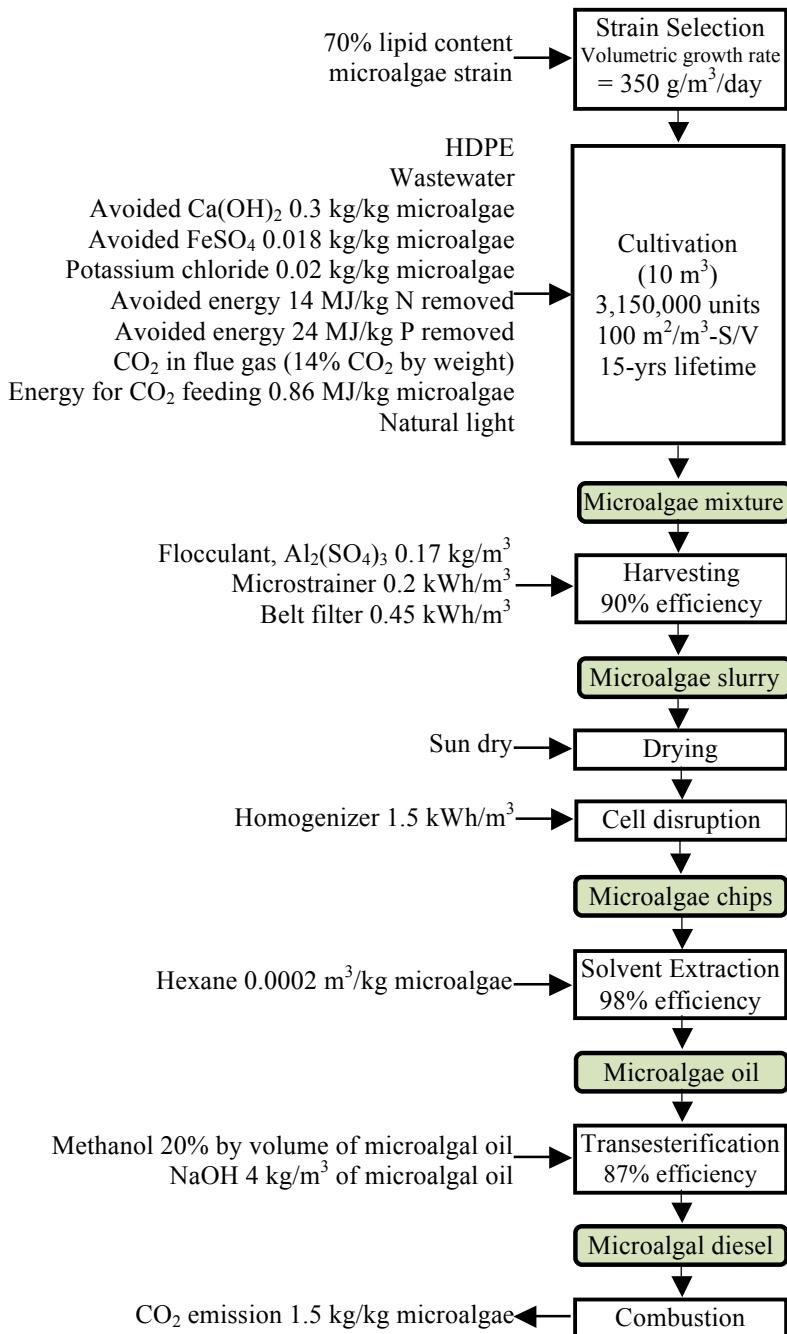


Figure 12 System boundaries and LCI of microalgal diesel LCA

(Collet et al., 2011; Greenwell et al., 2010; Hermann, 2010; Janssen, 2002; Kadam, 2002; Kadam, 2001; Lardon et al., 2009; Mata et al., 2010; Maurer et al., 2003; Posten, 2009; Sander & Murthy, 2010; Schenk et al., 2008; Shelef et al., 1984; Soratana & Landis, 2011; Tchobanoglous et al., 2003; Tickell & Tickell, 2003; Vijayaraghavan & Hemanathan, 2009; Vyas et al., 2010; Zebib, 2008).

5.3.2 Environmental Impact Results from Existing Biodiesel Studies

GWP, EP and PSP from databases and other studies were collected for soybean and canola biodiesels in order to compare these biofuels to the microalgal diesel LCA presented herein; results from the studies are summarized in Table 6. The original GWP, EP and PSP values collected from other studies were converted to the functional unit used in this study (8.94×10^{10} MJ of biodiesel/year). System boundaries of microalgal diesel from other LCA studies are summarized in Table 7.

Table 6 Environmental impacts over the life cycle of petroleum diesel and biodiesels.

Three environmental impacts, global warming potential (GWP), eutrophication potential (EP) and photochemical smog formation potential (PSP) of conventional diesel (CD), low-sulfur diesel (LSD), soybean diesel, canola diesel and microalgal diesel, were quantified using GREET (the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation) model, IDEMAT, ecoinvent databases and peer-reviewed literature. Functional unit (FU) was 8.94×10^{10} MJ of biodiesel/year.

Types of diesel	GWP (kg CO ₂ eq)		EP (kg N eq)		PSP (kg NO _x eq)		References
	Original no.	per FU	Original no.	per FU	Original no.	per FU	
CD	$4 \times 10^{-1}/\text{mile}$	7.35×10^9	$1.17 \times 10^{-3}/\text{MJ}$	1.05×10^5	$3.46 \times 10^{-2}/\text{MJ}$	3.10×10^6	GREET (Argonne National Laboratory, 2010)
	$4.60 \times 10^2/\text{m}^3$	1.07×10^9	$2.16 \times 10^{-1}/\text{m}^3$	5.01×10^5	$3.12/\text{m}^3$	7.22×10^6	IDEMAT (Delft University of Technology, 2001)
	$9.31 \times 10^{-2}/\text{MJ}$	8.32×10^9	n/a	n/a	n/a	n/a	(Batan et al., 2010a)
	$8.6 \times 10^{-2}/\text{MJ}$	7.69×10^9	n/a	n/a	n/a	n/a	(Stephenson et al., 2010)
	$10^2/\text{mmBtu}$	8.47×10^9	n/a	n/a	n/a	n/a	(Wang et al., 2008)
LSD	$6.23 \times 10^{-1}/\text{kg}$	1.22×10^9	$2.91 \times 10^{-4}/\text{kg}$	5.70×10^5	$2.14 \times 10^{-3}/\text{kg}$	4.19×10^6	CH ^(a) (Frischknecht et al., 2007)
	$4.95 \times 10^{-1}/\text{kg}$	9.70×10^8	$1.64 \times 10^{-3}/\text{kg}$	3.21×10^6	$1.78 \times 10^{-3}/\text{kg}$	3.50×10^6	RER ^(b) (Frischknecht et al., 2007)
Soybean diesel	$5/\text{mmBtu}$	4.24×10^8	n/a	n/a	n/a	n/a	(Wang et al., 2008)
	$3.2 \times 10/\text{mmBtu}$	2.71×10^9	n/a	n/a	n/a	n/a	(Wang et al., 2008)
	$9.6 \times 10^{-2}/\text{mile}$	1.76×10^9	n/a	n/a	$3.46 \times 10^{-2}/\text{MJ}$	3.10×10^6	GREET (Argonne National Laboratory, 2010)
-esterification plant	-1.36/kg	-3.26×10^9	$2.79 \times 10^{-2}/\text{kg}$	6.72×10^7	$3.97 \times 10^{-3}/\text{kg}$	9.55×10^6	US ^(c) (Frischknecht et al., 2007)
-service station	-1.18/kg	-2.84×10^9	$2.86 \times 10^{-2}/\text{kg}$	6.88×10^7	$5.27 \times 10^{-3}/\text{kg}$	1.27×10^7	CH ^(a) (Frischknecht et al., 2007)
Canola diesel	$5.01 \times 10^{-3}/\text{MJ}$	4.48×10^8	n/a	n/a	n/a	n/a	(Batan et al., 2010a)
	$3/\text{km}$	6.64×10^{10}	n/a	n/a	n/a	n/a	(Clarens et al., 2011)
Microalgal diesel	$1.3/\text{km}$	1.17×10^8	n/a	n/a	n/a	n/a	(Clarens et al., 2011)
	$1.19 \times 10/\text{kg}$	2.86×10^{10}	n/a	n/a	n/a	n/a	(Stephenson et al., 2010)
	$5.6 \times 10^{-2}/\text{MJ}$	5.02×10^9	n/a	n/a	n/a	n/a	(Sander & Murthy, 2010)
Microalgal mass (dry)	$2.13 \times 10^{-1}/\text{MJ}$	1.90×10^{10}	n/a	n/a	n/a	n/a	(Sander & Murthy, 2010)
	$2 \times 10^{-2}/\text{MJ}$	8.68×10^9	$1.7 \times 10^{-5}/\text{MJ}$	1.52×10^6	n/a	n/a	(Clarens et al., 2010)
	$5 \times 10^{-2}/\text{MJ}$	1.14×10^{10}	$3.0 \times 10^{-2}/\text{MJ}$	2.68×10^6	n/a	n/a	(Clarens et al., 2010)

Table 6 (continued)

Types of diesel	GWP (kg CO ₂ eq) Original no.		EP (kg N eq) Original no.		PSP (kg NO _x eq) Original no.		References
	per FU	per FU	per FU	per FU	per FU	per FU	
Microalgal mass (wet)	1.0×10 ⁻³ /MJ	6.98×10 ⁹	-9.1×10 ⁻⁶ /MJ	-8.14×10 ⁵	n/a	n/a	(Soratana & Landis, 2011)
	8.0×10 ⁻³ /MJ	7.61×10 ⁹	-2.4×10 ⁻⁶ /MJ	-2.15×10 ⁵	n/a	n/a	(Soratana & Landis, 2011)

(a) ecoinvent CH - Switzerland (Frischknecht et al., 2007)

(b) ecoinvent RER - Europe (Frischknecht et al., 2007)

(c) ecoinvent US - The United States (Frischknecht et al., 2007)

Conversion factors: CD passenger car = 29.77 mile per gallon (MPG)
(Argonne National Laboratory, 2010)
Biodiesel (BD) passenger car = 25.89 MPG (from calculation)
Low heating value (LHV) of CD = 137,380 Btu/gallon
(Argonne National Laboratory, 2010)
LHV of BD = 119,550 Btu/gallon (Argonne National Laboratory, 2010)

LHVs and MPG of CD and BD were used to calculate kg of emission per MJ of diesel fuels.

Table 7 System boundaries of the other microalgal diesel LCAs considered in this study.

Author	Process							Resources	Products		
	Cultivation		Harvesting	Dewatering Drying	Extraction	Conversion	Combustion				
	Open pond	PBR									
This study	✓	✓	✓	✓	✓	✓	✓	synthetic fertilizer/ flue gas CO ₂	biodiesel		
	✓	-	✓	✓	-	-	-	commercial fertilizer/ pure CO ₂	dry biomass		
	✓	-	✓	✓	✓	✓	-	commercial fertilizer/ wastewater effluent	biodiesel/ bioelectricity		
	✓	-	✓	✓	✓	✓	-	nutrients/ flue gas CO ₂	biodiesel/ ethanol		
	✓	✓	✓	-	✓	✓	-	wastewater after secondary treatment/ flue gas CO ₂	biodiesel/ methane		
	✓	✓	✓	-	✓	✓	✓	synthetic fertilizer/ flue gas CO ₂	biodiesel/ methane		
	-	✓	-	-	-	-	-	municipal wastewater/ flue gas CO ₂	wet biomass		

5.4 RESULTS AND DISCUSSION

5.4.1 Global Warming Potential

GWP results from petroleum diesels and biodiesels (soybean, canola and microalgae) are presented in Figure 13. The life-cycle GWP of microalgal diesel in this study is 60%-100% higher than in other microalgal diesel studies due to the differences in system boundaries and inventories, as described in Table 7. The microalgal GWP is also higher than other studies' on conventional diesel and soybean diesel results by approximately 90% and 98%, respectively.

Based on the results of this study, soybean diesel, as a conventional biofuel, meets the RFS2 life-cycle GHG emissions reduction threshold, which equals a 50% reduction relative to the life-cycle GHG (90 g CO₂ eq/ MJ baseline) emissions from the petroleum diesel produced and distributed in 2005, as mandated by EISA (Assessment and Standard Division Office of Transportation and Air Quality, 2010). Canola diesel, a qualified biomass-based diesel and advanced diesel as specified in RFS2, contributes a GWP slightly lower (~8%) than the mean value of the GWP of microalgal diesel of this study. However, all of the results from potential biomass-based diesels – microalgal and canola diesels – exceed the life-cycle GHG emissions reduction threshold.

Soybean diesel, a conventional biodiesel, contributes less GWP than conventional diesel and LSD since the production and combustion of soybean diesel emits less GHG emissions (Sheehan, 1998). Soybean diesel contributes even less GWP than canola and microalgal diesels, both of which are advanced biofuels. The main factor influencing this difference is that LCAs of soybean diesel included several co-products (i.e. soybean meal and glycerin) compared to canola

diesel. Moreover, the production of soybean diesel does not require energy intensive processes such as harvesting, dewatering and drying as microalgae diesel (Uduman et al., 2010).

The harvesting, dewatering, and drying processes are the primary areas where the greatest environmental improvements to microalgal diesel production can be realized. Approximately 80% of the life-cycle GWP of microalgal diesel in this study resulted from the energy consumed in microstrainer and belt filter harvesting processes (more details on the life-cycle GWP are presented in Supporting Information). The selection of harvesting process should be appropriate to the size and properties of the microalgal cell, downstream processes and the final product from microalgal biomass (microalgal diesel in this study) (Mata et al., 2010; Singh et al., 2011; Uduman et al., 2010; Xu et al., 2011). The four primary harvesting methods for microalgal biomass are microstraining, belt filtering, flotation and sedimentation (Weissman, 1987). A flotation process is suitable for the harvesting of microalgae with high oil content since the microalgal cells tend to float. In addition, the flotation process is not as time consuming as a simple sedimentation, and requires lower operation cost (Brennan & Owende, 2010; Singh et al., 2011; Uduman et al., 2010). The drying process can be implemented by natural air or sun drying, however this method is time and area consuming and can potentially lose some bioreactive products (Li et al., 2008b; Vijayaraghavan & Hemanathan, 2009). Other common drying methods are drum-drying, freeze-drying and spray-drying, however, spray-drying is not economically feasible for low value product e.g. biofuels (Mata et al., 2010). There have also been efforts to avoid the drying processes, but it was found not to be cost-effective (Xu et al., 2011). The authors suggested a co-location of the drying process with the equipment that provides a controlled source of heat or airflow, e.g. vents or condenser units in industrial

facilities, to lower energy consumption for the drying process since it is one of the major contributions to GWP from microalgal diesel production (Uduman et al., 2010; Xu et al., 2011).

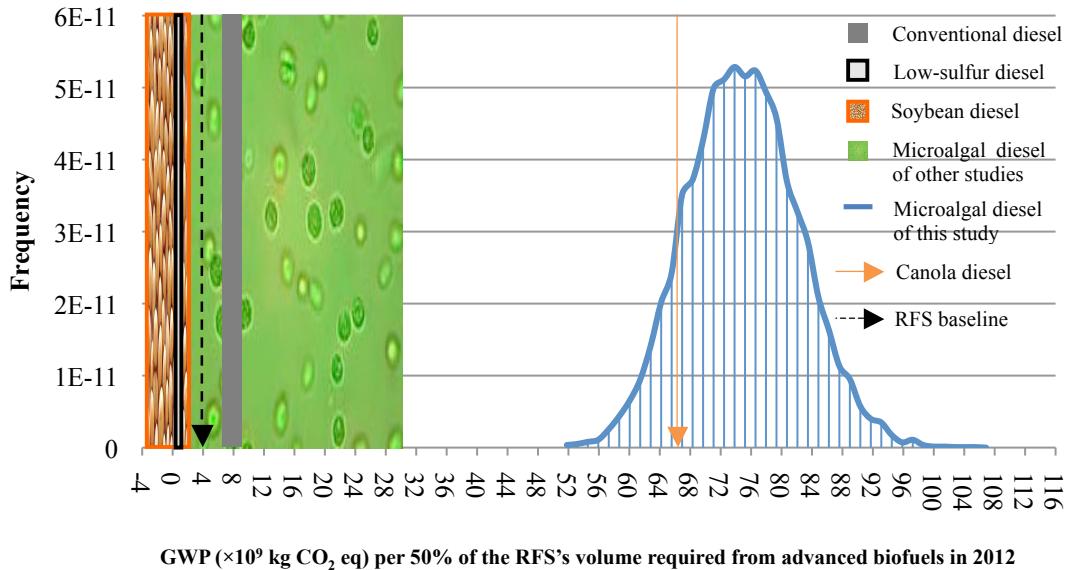


Figure 13 GWP of Biodiesels.

The probability distribution for microalgal diesel investigated in this study is compared to ranges from other studies. Original data from other studies is summarized in **Table 6**. 80% of the life-cycle GWP of microalgal diesel investigated in this study results from the energy consumed in microstrainer and belt filter harvesting processes.

5.4.2 Eutrophication Potential

The life-cycle EP of conventional diesels are already so low (1.31×10^{-11} g N eq/MJ), that biofuels will never achieve a reduction like the RFS2 GHG threshold. The EP impact from conventional biofuels can be lessened by utilizing agricultural management strategies such as reducing tillage, optimizing fertilizer application, constructing wetland buffers, cover cropping or planting perennials to reduce runoff (Committee on Environmental and Natural Resources, 2010; Miller et al., 2007).

In contrast to other studies, the environmental impacts of microalgal diesel in this study were higher since they were evaluated from well to wheel. A high EP from microalgal diesel is

mainly a result of the energy intensive harvesting process. Other comparative studies do not have similar system boundaries, for example the study by Clarens *et al.* (Clarens et al., 2010) on a comparative LCA of dried microalgal biomass and other bioenergy feedstocks did not include the biomass upgrading into fuels and coproducts. Another study by Soratana and Landis (Soratana & Landis, 2011) conducted a comparative LCA only for microalgal biomass production in PBRs using synthetic and waste resources for CO₂ and nutrients; harvesting was not included.

In order to produce sustainable biofuels, RFSs should include water quality criteria to prevent water pollution from biofuel production such as eutrophication and hypoxia. Hypoxia results when the concentration of dissolved oxygen in water is less than 2-3 mg/L (Committee on Environmental and Natural Resources, 2010; Miller et al., 2007). The contribution of nitrogen compounds (NH₃, NO₃⁻ and NO₂⁻) and a phosphorus compound (PO₄³⁻) on EP are subject to seasonal, spatial and temporal variations. For example, in the life-cycle aquatic EP results of a coastal lagoon in Tunisia, NH₃ and PO₄³⁻ were the major cause of EP in summer, while NO₃⁻ was the major contribution in winter, and both N and P compounds were higher in summer than in winter (Committee on Environmental and Natural Resources, 2010; Hadj Amor et al., 2008). Consequently, the seasonal variation of EP impact should be taken into consideration when regulating life-cycle EP for biofuels.

Several different approaches to setting life-cycle EP baseline for biofuels could be considered. A possible strategy would be to set conventional biofuels as the baseline for EP. However, based on the existing RFS2 program and the EP results presented in Figure 14, EPs from soybean diesel might be a better baseline for advanced biofuels since soybean diesel has the highest contribution to EP impact among currently available fuels. In this policy scenario

utilizing baselines or thresholds, none of the life-cycle EP from biofuels should exceed the life-cycle EP of soybean diesel or conventional biofuel. Another strategy might set maximum contaminant loads for each watershed or coastal area, similar to the Total Maximum Daily Load (TMDL). This strategy would utilize water quality standards as a life-cycle EP threshold for biofuels. For example, the TDML developed by the U.S. EPA to reduce N, P and sediment in Chesapeake Bay covers several states, including Delaware, Maryland, New York, Pennsylvania, Virginia, West Virginia and the District of Columbia. The annual emissions contributing to life-cycle EPs of biofuels in the specified areas should not exceed these limits: 84.3 million kg of N and 5.7 million kg of P or 25% reduction in N and 24% reduction in P of the annual emissions (U.S. Environmental Protection Agency, 2010a). These limits are set in terms of nutrient emissions, thus, the quantity of nutrients in effluent of the production facilities and from agricultural operations would have to be estimated or monitored and reported along with the life-cycle EP impacts.

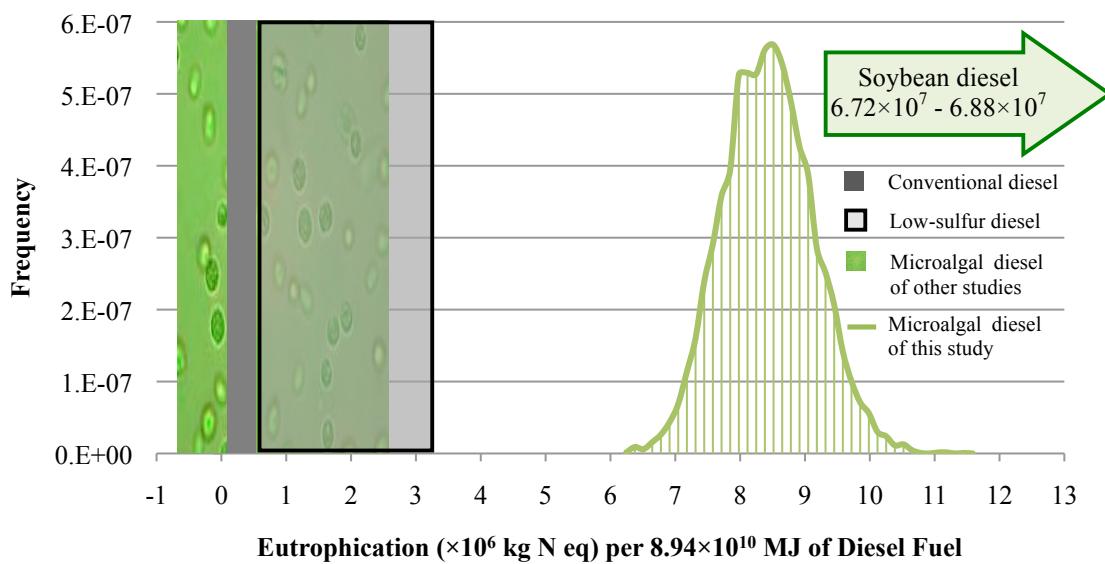


Figure 14 Eutrophication of Biodiesels.

The probability distribution for microalgal diesel investigated in this study is compared to ranges from other studies' fuels. Data from other studies summarized in **Table 6**.

5.4.3 Photochemical Smog Formation Potential

Another environmental impact of importance for transportation fuels is PSP, which is caused by tailpipe emissions such as nitrogen oxides (NO_x) and volatile organic compounds (VOCs). Photochemical reactions of ozone formation precursors such as NO_x and VOCs contribute to a formation of photochemical oxidants, namely tropospheric ozone (O_3), peroxyacetyl nitrate (PAN) and hydrogen peroxide (H_2O_2). Anthropogenic NO_x and VOCs, such as benzene and formaldehyde, are emitted primarily from typical engine combustion processes (Shah & Ries, 2009; U.S. Environmental Protection Agency, 1994a). Because liquid fuel formulations are efficient, the emission levels depend mainly on the car model. For example, results from a particular study on tailpipe emissions from algal fatty acid methyl esters (FAME) by Fisher *et al.* showed that NO_x emissions from algal FAME are less than those from soybean and canola methyl esters due to the decreased premixed combustion of algal FAME in the ignition zone (Fisher, 2010). To reduce the tailpipe emissions, VOCs and NO_x , automobile manufacturers attempt to improve engine design and install advanced emission control equipment (U.S. Environmental Protection Agency, 1994a).

The 1990 Clean Air Act (CAA) includes more stringent tailpipe standards expected to reduce more than 40% of the automobile-related emissions by a provision through controlling O_3 or urban smog, NO_x and particulate matters from diesel engines (U.S. Environmental Protection Agency, 2000; U.S. Environmental Protection Agency, 1994b). Most of the CAA programs such as Clean Fleets, Refueling Control and Inspection and Maintenance programs and Heavy-Duty Engine and Vehicle Standards and Highway Diesel Fuel Sulfur Control Requirements focus on fuel quality and vehicle operation to reduce emissions such as PM and NO_x and to meet the

CAA's tailpipe emission standards. The more stringent tailpipe standards phased in since 2007 limit non-methane hydrocarbon emissions to 0.14 gram per brake-horsepower-hour (g/bhp-hr) and limits NO_x to 0.2 g/bhp-hr (U.S. Environmental Protection Agency, 2000). However, tailpipe emissions are only part of the total PSP of biofuels, other emissions result throughout the life cycle (U.S. Environmental Protection Agency, 1994b).

In addition to tailpipe emissions, biofuel production and other upstream processes should be considered when calculating and legislating PSP. Most published LCA studies focused on biofuels generally do not include PSP impact, thus data from GREET and the life-cycle databases ecoinvent and IDEMAT (Delft University of Technology, 2001; Frischknecht et al., 2007) were used to compare other biofuels with PSP from microalgal diesel in this study. The life-cycle PSP results in Figure 15 indicate that microalgal diesel generally contributes lower PSP than soybean diesel. Up to 85% of the life-cycle PSP from microalgal diesel or 6.89×10^6 kg NO_x eq per functional unit results from HDPE used in the construction of PBR (Soratana et al., 2012a). The impact is related to the amount of HDPE used and service lifetime of the PBR, and mainly arises from ethylene released to the environment during the polymerization process of HDPE production. Even though impacts from the production of HDPE are not of concern with other biodiesels or petroleum diesels, PSP might be generated from other related upstream processes and not only from the tailpipe emissions. It has been shown previously that the PSP impacts will increase if different types of PBR construction material such as glass, polyvinyl chloride (PVC), polycarbonate (PC) and polymethyl methacrylate (PMMA) are used (Soratana & Landis, 2011).

Strictly because clean fuels meet tailpipe standards does not necessarily imply that their production processes are also clean. Various combustion processes and other activities emit NO_x

and VOCs over the life cycle of biofuels as well as petroleum fuels. Since concentrations of O₃ and its anthropogenic precursors are location dependent (Shah & Ries, 2009) and biofuels production facilities are located in different areas, criteria for PSP from upstream processes should be spatially specific and be considered in addition to tailpipe emissions. The impact of NO_x and VOC emissions on PSP are also temporally dependent. NO_x is more sensitive to temporal variability than to spatial variability, while VOCs are less sensitive to temporal variability compared to NO_x (Shah & Ries, 2009). Consequently, in addition to decreasing these PSP precursors – NO_x and VOCs – from tailpipe emissions, there should also be standards to control well-to-pump PSP emissions from biofuels and petroleum fuels accounting for temporal and seasonal variations.

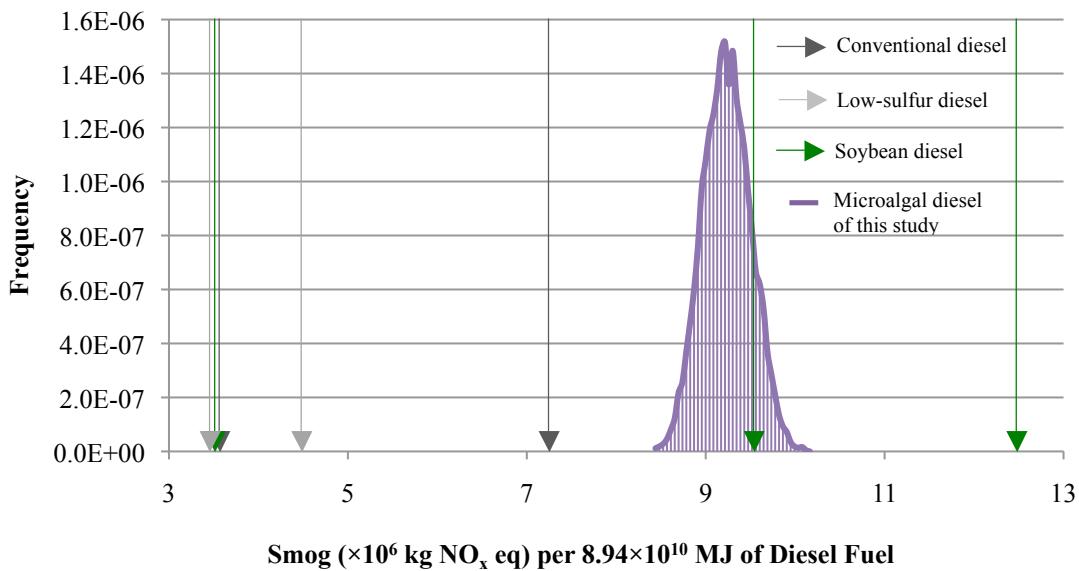


Figure 15 Smog of Biodiesels.

The probability distribution for microalgal diesel investigated in this study is compared to ranges from other studies fuels. Data from other studies summarized in **Table 6**.

5.4.4 Tradeoffs Between GWP and EP

Studies by several researchers have shown that there are tradeoffs between GWP and EP of bioproducts (Miller, 2007). Likewise, according to the GWP and EP results of biodiesels presented in **Figure 16**, life-cycle EP from microalgal diesel are lower than lifecycle EP from soybean diesel, while algal life-cycle GWP impacts are higher. Similar tradeoffs are also present between LSD and conventional diesel; GWP is higher for conventional diesel than LSD, but its EP is lower. Switching from petroleum diesel to soybean diesel results in trading one environmental problem for another; the GHG profile is improved, but the nitrogen cycle and associated water quality are affected.

The RFS2 based solely on life-cycle GWP impacts risks increasing these and other unintended consequences. If the RFS2 were based on the percent reduction of emissions contribution to EP, such as N emissions, microalgal diesel or even petroleum diesels may prove to be environmentally preferable to soybean diesel.

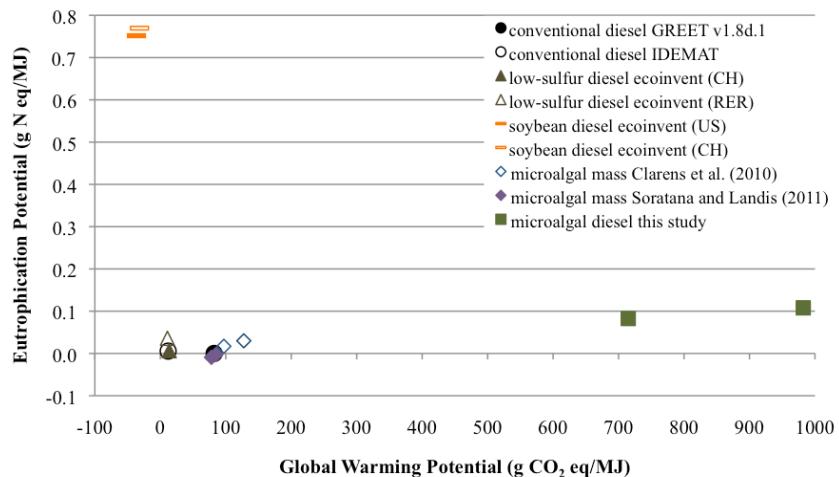


Figure 16 EP vs. GWP for Diesel Fuels.

Tradeoffs among different types of petroleum-based diesels and biodiesels (soybean diesel and microalgal diesel) from GREET, databases and literature. The life-cycle GWP and EP of microalgal diesel investigated in this study are presented with a 90% confidence interval.

5.4.5 Allocation Issues

The life-cycle environmental impact results of microalgal diesel from this study are higher than other studies mainly due to the different system boundaries and the allocation of co-products such as animal feeds and bioethanol, which are not included in this study. On the other hand, the system boundaries of this study include impacts from upstream processes such as the production of HDPE for the PBR, which contributes a significant amount of emissions to GWP, EP and PSP, while other studies did not include the construction of the PBR (Soratana & Landis, 2011). Even though microalgae can be used to generate fertilizer and a plastic precursor, no allocations of co-products from microalgal diesel production were considered in this study because of uncertainties related to yield and quality (Anderson & Dawes, 1990; Avagyan, 2008; Braunegg et al., 1998; Singh et al., 2011). More long-term data is needed before these products can be incorporated to prevent overstating the positive impact of microalgal diesel production. The results of this study are based on cultivating microalgae with 70% lipid content and only 30% biomass remains with which to produce co-products such as animal feeds or bioethanol. If the 30% biomass remains were converted to microalgal ethanol with 46% bioethanol yield from fermentable carbohydrate composition in microalgae, less than one MJ of bioethanol (~0.07 MJ) can be produced from one kg of microalgal biomass (Brennan & Owende, 2010; Singh & Dhar, 2011; Taherzadeh & Karimi, 2008; Wall et al., 2008). On the other hand, with 77% biodiesel yield, 22 MJ of biodiesel can be produced per kg of microalgal biomass (Lardon et al., 2009; Mata et al., 2010; Vyas et al., 2010). Therefore, the impacts will not be significantly different.

5.4.6 Policy Implications

The study performed by the U.S. EPA to determine the percent life-cycle GHG emissions reduction threshold for biofuels has the foundation for lessening GHG emissions impact of the transportation sector. However, the method used to set the 50% life-cycle GHG emission reduction for advanced biofuels should be more transparent so that a similar strategy for other life cycle environmental impacts such as EP and PSP can be established in future RFSs. Moreover, providing a threshold as a range or a probability distribution maybe more appropriate than a single point estimate since the upstream and downstream production processes vary greatly from one system to another. Biofuels also contribute other environmental impacts in addition to GWP; therefore, GHG emissions should not be the only emission evaluated from a life-cycle perspective. Other impacts such as EP and PSP should also be included in future RFSs. Soybean diesel, for example, presents a tradeoff in the form of decreased GWP but increased EP and PSP as compared to microalgal diesel. Similar tradeoffs exist for soybean diesel and conventional diesel and LSD. Based on the relationship between biofuels and environmental impacts, baselines of EP and PSP impacts should be included in future RFSs. Implementing LCA along with specific spatial and temporal criteria can also minimize impacts. A localized approach, where suitable plants are cultivated should be encouraged for more efficient production and minimal EP impacts (Committee on Environmental and Natural Resources, 2010). Future RFS emission reduction thresholds for EP should be set to meet local or regional EP limits, or at least should be less than the EP from conventional biodiesel such as soybean diesel. For PSP impacts, future RFS criteria should be extended to include the emissions from spatially relevant upstream processes, not only the tailpipe emissions. Since PSP is a spatially-specific impact and PSP impacts from upstream processes and tailpipe affect the environment in

different locations, an emission reduction threshold for PSP could be categorized into two stages: emissions from well-to-pump and tailpipe emissions.

5.5 ACKNOWLEDGEMENT

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6.0 CO-PRODUCTS FROM MICROALGAL BIOFUELS TO REDUCE ENVIRONMENTAL FOOTPRINTS

6.1 INTRODUCTION

The Renewable Fuel Standard finalized in 2010 (RFS2) under the Energy Independence and Security Act of 2007 (EISA 2007) is aimed at increasing biofuels production in the US. To meet the total renewable fuel requirement, the RFS2 increases volume of total advanced biofuels from 2.0 billion gallons per year (BGY) in 2012 to 5.5 BGY in 2015. The RFS2 also mandates the first reduction threshold of advanced biofuel's life-cycle greenhouse gas (GHG) emissions. For any biofuel to be classified as advanced biofuels, life-cycle GHG emissions of the biofuel must be at least 50% less than the life-cycle GHG emissions of the gasoline or diesel distributed in 2005. Petroleum diesel contributes approximately 90 g CO₂ Eq/MJ over its life cycle (Office of Transportation and Air Quality, 2010a; Office of Transportation and Air Quality, 2010b; Skone & Gerdes, 2008).

Microalgae has several characteristics as a biodiesel feedstock that may enable it to sustainable replace fossil fuels. For instance, microalgae has higher biomass productivity with shorter crop cycle compared to other oil crops, produces a suitable type of lipid for biodiesel conversion, and can be cultivated in non-arable area; therefore, microalgal cultivation does not compete with food crops (Avagyan, 2008; Das et al., 2011; Lehr & Posten, 2009). The utilization

of nutrients and CO₂ from waste streams during microalgal cultivation can avoid mainly global warming potential (GWP) and eutrophication potential (EP) (Soratana & Landis, 2011). Nutrients such as nitrogen (N) and phosphorus (P) in e.g. municipal and agricultural wastewaters and carbon dioxide (CO₂) in flue gas of power plant and smelter can be utilized during microalgal cultivation (Greer, 2009; Kadam, 2001; Kong et al., 2010; Mallick, 2002; Mata et al., 2010; Pittman et al., 2011). However, these industrial symbiosis processes might not be adequate to achieve the RFS2's requirement.

One potential strategy to offset environmental impacts and energy consumption from microalgal biofuels is to increase the amount of useful co-products from the microalgae feedstock. Valuable co-products can increase the net energy ratio (NER) (i.e. the energy produced per energy required), of microalgal biodiesel production by converting carbohydrates in de-oiled microalgal mass to microalgal bioethanol via fermentation (Jorquera et al., 2010; Ritslaid et al., 2010; Sheehan, 2000). Generally, microalgae consists of carbohydrate, lipid, protein and nucleic acid (Singh et al., 2011). After lipid is extracted from microalgal mass for microalgal biodiesel production, carbohydrates e.g. starch and cellulose in de-oiled mass are hydrolyzed and fermented for microalgal bioethanol with CO₂ as a by-product (Ritslaid et al., 2010). Unlike other cellulosic bioethanol production, microalgal bioethanol production does not require lignocellulosic pretreatment to remove lignin, which cannot be fermented, therefore energy for heating and chemicals for the treatment can be avoided (John et al., 2011; Sander & Murthy, 2010). CO₂ from the fermentation can be recovered and cleaned for carbonation of beverages and frozen into dry-ice for food industry (Ritslaid et al., 2010; Xu et al., 2010). Approximately, only 5% to 7% of the total CO₂ produced from the fermentation process are captured (U.S. Environmental Protection Agency, 2010b).

This study evaluated the life-cycle environmental impacts from 1) microalgal biodiesel and microalgal bioethanol cultivated using CO₂ from flue gas (MD+ME), 2) microalgal biodiesel and corn ethanol cultivated using CO₂ from flue gas (MD+CE), and 3) microalgal biodiesel and microalgal bioethanol cultivated using CO₂ recovery from a fermentation process (MD+ME+CO₂). The cradle-to-gate production of microalgal biodiesel consists of strain selection, photobioreactor (PBR) cultivation, harvesting, drying, extraction and conversion (Soratana & Landis, 2011). De-oiled biomass from the extraction process can be hydrolyzed and fermented to produce microalgal bioethanol (Ritslaid et al., 2010). Only GWP and EP from the co-production of microalgal biodiesel and bioethanol were quantified since GWP and EP were the main tradeoffs of concern based on the life-cycle impact results from Chapter 4 and Chapter 5 of this dissertation (Soratana et al., 2012a; Soratana et al., 2012b).

Life cycle assessment (LCA) was used to quantify the environmental impacts of the aforementioned systems. LCA is a tool commonly used to quantify resource consumption and to evaluate environmental and human health impacts over a product's or service's lifetime. LCA consists of four stages – goal and scope definition, life cycle inventory analysis (LCI), life cycle impact assessment (LCIA) and interpretation and improvement of the results (International Organization for Standardization, 2006). The objectives of this study are to examine the potential of utilizing de-oiled microalgal mass to produce bioethanol to lessen GWP and EP impacts from the production of microalgal biodiesel, and to evaluate and compare environmental impacts from the production of microalgal biodiesel with and without the recovery of CO₂ from the bioethanol production. A similar study was conducted by Sander and Murthy (Sander & Murthy, 2010), however, the recovery of CO₂ from bioethanol production for microalgal cultivation was not considered, which was included in this study.

6.2 METHODS

We conducted a comparative cradle-to-gate LCA of microalgal biodiesel and bioethanol from microalgae and corn. The three scenarios compared were Scenario 1, microalgal biodiesel and microalgal bioethanol from de-oiled microalgal mass using CO₂ from flue gas for microalgal cultivation (MD+ME), Scenario 2, microalgal biodiesel and corn ethanol using CO₂ from flue gas (MD+CE), and Scenario 3, microalgal biodiesel and microalgal bioethanol using the CO₂ recovered from fermentation process and CO₂ from flue gas for microalgal cultivation (MD+ME+CO₂). Attributional LCA with systems expansion was applied to the MD+ME scenario where the original system boundary of microalgal biodiesel was expanded to include the production of bioethanol, the co-product of microalgal biodiesel to quantify environmental impacts from life-cycle microalgal biodiesel and its subsystems. Inventories were collected from peer-reviewed literature and existing life-cycle databases. Environmental impacts, GWP and EP, of the three scenarios were quantified using the Tool for Reduction and Assessment of Chemical and other environmental Impacts (TRACI) as a LCIA tool to analyze the environmental impacts on midpoint-oriented basis. TRACI was developed particularly for the U.S. by the U.S. Environmental Protection Agency (U.S. EPA) (Bare et al., 2003).

6.2.1 System Boundary

A cradle-to-gate LCA of microalgal biodiesel and microalgal bioethanol, as a co-product, was conducted. The microalgal strain considered was *Chlorella vulgaris* with 30% lipid content and 37% starch content by weight of microalgae (Avagyan, 2008; Brennan & Owende, 2010; Lehr & Posten, 2009; Singh & Dhar, 2011). This strain was selected since it has potential to be used as a

feedstock both in biodiesel and bioethanol production (Brennan & Owende, 2010; Mata et al., 2010). Microalgae was cultivated in 10-m³ PBR utilizing N and P from municipal wastewater, synthetic K fertilizer (superphosphate) and CO₂ from flue gas of power plant, as depicted in Figure 17. The source of CO₂ in MD+ME+CO₂ scenario was from both flue gas and fermentation. The functional unit (FU) of this study is 1.32×10^{11} MJ, which is the sum of 8.94×10^{10} MJ of microalgal biodiesel per year or one BGY (which is the unit specified according to the RFS2) and 4.25×10^{10} MJ of microalgal bioethanol produced from the de-oiled microalgal mass used to produce one BGY of microalgal biodiesel.

Microalgal bioethanol from the de-oiled microalgal mass may displace bioethanol from other feedstock, therefore, LCA of corn ethanol was considered in MD+CE scenario for a fair comparison to other scenarios. Transportation between each process facility was omitted because those facilities were assumed to be closely located to one another and have similar distances among all three scenarios. Moreover, bioethanol fermentation production processes using microalgae or corn as a feedstock are the same (Balan et al., 2009; Krylova et al., 2008; Singh & Dhar, 2011; Taherzadeh & Karimi, 2008; Wall et al., 2008).

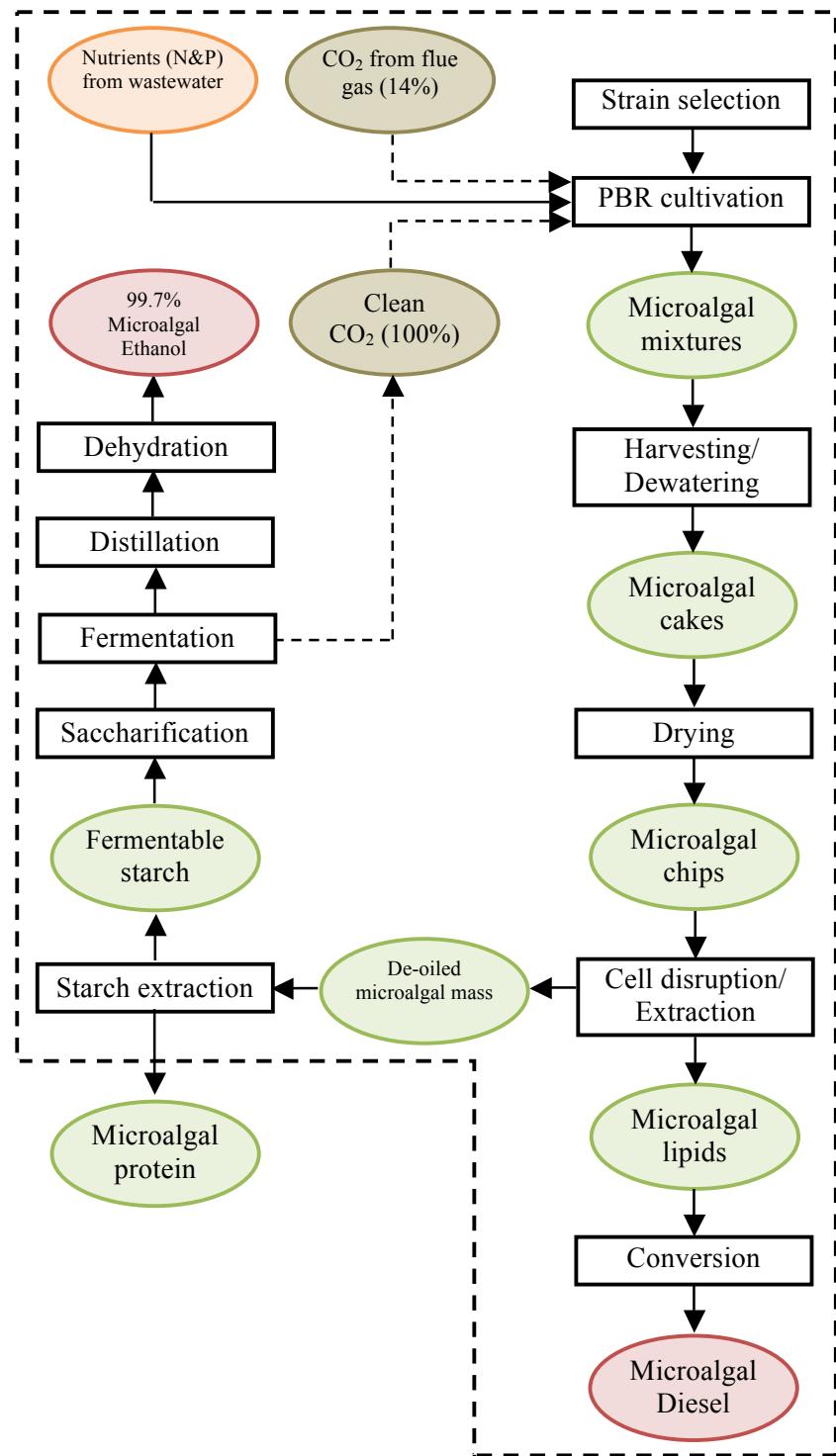


Figure 17 System boundary of the co-production of microalgal biodiesel and microalgal bioethanol.

Two sources of CO₂ for microalgal cultivation are CO₂ from flue gas and CO₂ from fermentation process are presented by the dashed arrows.

6.2.2 Life Cycle Inventories (LCI)

Inventories for the productions of microalgal biodiesel, microalgal bioethanol and corn ethanol were obtained from literature and life-cycle databases such as ecoinvent, ETH-ESU, BUWAL 250 and Franklin (Frischknecht & Jungbluth, 2004; Frischknecht et al., 2007; Norris, 2003; Spijensma, 2004), as listed in Table 8, Table 9 and Table 10, respectively. Inventories obtained from literature such as volume and thickness of PBR construction materials were calculated for the material used to construct PBRs to produce one functional unit.

The energy consumption of the microstrainer and belt filter in the harvesting process and the homogenizer in the cell disruption processes were obtained from Shelef et al. (Shelef et al., 1984), therefore, energy efficiencies of the three equipments were assumed to be increased by 50% of the original values. Moreover, based on the sensitivity analysis results from the study in Chapter 4, both GWP and EP are sensitive to the energy consumption of microstrainer and belt filter during harvesting process. To increase microalgal biodiesel's potential to achieve the RFS's life-cycle GHG emission reduction requirement, the energy consumption during harvesting process should be a primary target for energy reductions. Quantities of chemicals for N and P removal from municipal wastewater, Ca(OH)₂ and FeSO₄, were used to calculate for avoided impacts since the nutrients in wastewater were removed by microalgae. Energy consumed in N and P removal process of a wastewater treatment plant was calculated as avoided energy use. Energy consumed during the injection of CO₂ to microalgal cultivation was also included. The energy consumed by the injection of CO₂ from flue gas was higher than that of the CO₂ from fermentation process due to the lower concentration of CO₂ in flue gas (14%). For bioethanol production, either from microalgae or from corn, the processes were the same (John et al., 2011; Tan et al., 2008; Wall et al., 2008). Microalgal bioethanol yield was calculated from

the de-oiled microalgal mass left from oil extraction process, whereas quantity of corn mass was calculated based on its energy content to achieve the same energy provided by the microalgal bioethanol.

Table 8 Life-cycle data of microalgal biodiesel used within this study.

Microalgal Biodiesel Production		
Parameter	Inventory	Reference
Microalgal strain	<i>Chlorella vulgaris</i>	(Brennan & Owende, 2010; Singh & Dhar, 2011)
Microalgal volumetric productivity rate	350 g/m ³ × day	(Mata et al., 2010; Rodolfi et al., 2009; Shen et al., 2010)
Lipid content	30%	(Brennan & Owende, 2010; Singh & Dhar, 2011)
Carbohydrate (starch) content	37%	(Brennan & Owende, 2010; Singh & Dhar, 2011)
Microalgal oil heating value	41 MJ/kg	(Huang et al., 2010)
Volume of PBR	10 m ³	(Carvalho et al., 2006a)
PBR construction material	HDPE	(Soratana & Landis, 2011)
PBR thickness	0.01 m ³	(Carvalho et al., 2006a; Posten, 2009)
Surface area to volume ratio	100 m ² /m ³	(Posten, 2009)
PBR lifetime	15 years	Assumption
Light	Natural light	
N nutrient in wastewater	0.05 kg/kg microalgae	(Collet et al., 2011; Lardon et al., 2009)
P nutrient in wastewater	0.01 kg/kg microalgae	(Collet et al., 2011; Lardon et al., 2009)
Potassium chloride (K fertilizer)	0.02 kg/kg microalgae	(Collet et al., 2011; Lardon et al., 2009)
Calcium hydroxide (Ca(OH) ₂)	5.2 kg/kg N-removal	(Tchobanoglous et al., 2003)
Ferrous sulfate (FeSO ₄)	1.8 kg/kg P-removal	(Tchobanoglous et al., 2003)
Energy avoided from N removal	14 MJ/kg	(Maurer et al., 2003)
Energy avoided from P removal	24 MJ/kg	(Maurer et al., 2003)
CO ₂ consumption by microalgae	1.85 kg CO ₂ /kg microalgae	(Kadam, 2001; Mata et al., 2010; Schenk et al., 2008)
Flue gas content	14% of CO ₂	(Kadam, 2001; Mata et al., 2010; Schenk et al., 2008)
Energy for injection of CO ₂ from flue gas	0.079 MJ/ kg flue gas	(Kadam, 2001)
Energy for injection of pure CO ₂ from fermentation	0.025 MJ/ kg CO ₂	(Kadam, 2001)
Harvesting efficiency	90%	(Greenwell et al., 2010; Mata et al., 2010)
Flocculant – Al ₂ (SO ₄) ₃	0.17 kg/m ³ microalgal slurry	Calculation
Microstrainer	0.10 kWh/m ³ microalgal slurry	50% of (Shelef et al., 1984)
Belt filter	0.23 kWh/m ³ microalgal slurry	50% of (Shelef et al., 1984)
Drying	Natural air dry	(Kadam, 2002)
Homogenizer	0.75 kWh/m ³ of microalgal cake	50% of (Shelef et al., 1984)
Extraction efficiency	98%	(Mata et al., 2010; Sander & Murthy, 2010)

Table 8 (continued).

Microalgal Biodiesel Production		
Parameter	Inventory	Reference
Solvent - Hexane	0.0002 m ³ /kg microalgae	(Lardon et al., 2009; Vijayaraghavan & Hemanathan, 2009)
Conversion efficiency	87%	(Mata et al., 2010)
Methanol	20% by volume of microalgal oil	(Sander & Murthy, 2010; Tickell & Tickell, 2003)
Catalyst – NaOH	4 kg/ m ³ microalgal oil	(Vijayaraghavan & Hemanathan, 2009)

Table 9 Life-cycle data of microalgal bioethanol and corn ethanol used within this study.

Microalgal Bioethanol Production		
Parameter	Inventory	Reference
Microalgal bioethanol energy content	29.96 MJ/kg microalgal bioethanol	(Carvalho et al., 2006a; Feinberg, 1984; Ritslaid et al., 2010)
Actual yield of CO ₂ from fermentation	44.1%	(Singh & Dhar, 2011; Taherzadeh & Karimi, 2008; Wall et al., 2008)
Corn Ethanol Production		
Parameter	Inventory	Reference
Starch content	70%	(Mousdale, 2008)
Corn ethanol energy content	29.65 MJ/kg corn ethanol	(Argonne National Laboratory, 2010; Mousdale, 2008)
Actual yield of CO ₂ from fermentation	44.1%	(Singh & Dhar, 2011; Taherzadeh & Karimi, 2008; Wall et al., 2008)

Table 10 Parameters and inventories of microalgal and corn biofuels used within this study.

Process flow	Resource	Database
PBR construction material	HDPE	ecoinvent (Europe) (Frischknecht et al., 2007)
Nutrients	Ca(OH) ₂	ETH-ESU (Frischknecht & Jungbluth, 2004)
	FeSO ₄	ETH-ESU (Frischknecht & Jungbluth, 2004)
	KCl	ecoinvent (Europe) (Frischknecht et al., 2007)
Harvesting	Al ₂ (SO ₄) ₃	ecoinvent (Europe) (Frischknecht et al., 2007)
Extraction	C ₆ H ₆	ecoinvent (Europe) (Frischknecht et al., 2007)
Conversion	CH ₃ OH	ecoinvent (North America) (Frischknecht et al., 2007)
	NaOH	ecoinvent (North America) (Frischknecht et al., 2007)
CO ₂	Synthetic CO ₂	BUWAL 250 (Spriensma, 2004)
Energy	Electricity	Franklin US LCI (Norris, 2003)
Corn	Corn	ecoinvent (United States) (Frischknecht et al., 2007)
Bioethanol production	Ethanol 95%	ecoinvent (United States) (Frischknecht et al., 2007)
	Ethanol 99.7%	ecoinvent (United States) (Frischknecht et al., 2007)

6.2.3 Life Cycle Impact Assessment (LCIA)

The LCIA method employed was the TRACI version 3.01 (Bare et al., 2003). GWP and EP, two of the available nine TRACI impact categories, are discussed in this study for the three aforementioned microalgal biodiesel production scenarios. Monte Carlo Analysis (MCA) was conducted for 10,000 iterations on LCA of microalgal biodiesel and microalgal bioethanol using CO₂ from flue gas (MD+ME scenario) with 30% ± 20% of lipid content and 37% ± 20% of fermentable carbohydrate content. Relationships among the total energy produced from the two microalgal fuels, lipid content, carbohydrate content, GWP and EP were investigated.

6.3 RESULTS AND DISCUSSION

Normalized life-cycle GWP and EP impacts from microalgal biodiesel and bioethanol productions from different feedstocks, corn and microalgae, and different sources of CO₂, flue gas and fermentation, were evaluated for the major impact contributions in each scenario. These three scenarios are referred to as microalgal biodiesel and microalgal bioethanol from de-oiled microalgal mass using CO₂ from flue gas for microalgal cultivation (MD+ME scenario), microalgal biodiesel and corn ethanol using CO₂ from flue gas (MD+CE scenario), and microalgal biodiesel and microalgal bioethanol using the CO₂ recovered from fermentation process and CO₂ from flue gas for microalgal cultivation (MD+ME+CO₂ scenario), respectively. The correlations of the total energy produced from the two microalgal fuels, lipid content, carbohydrate content, GWP and EP from MD+ME scenario are also discussed.

6.3.1 Impacts from Microalgal Biodiesel and Bioethanol Productions

Normalized life-cycle GWP and EP impacts from the three scenarios of microalgal biodiesel and bioethanol productions are illustrated in **Figure 18**. Impacts from the production of microalgal biodiesel in each scenario are the same. The differences are from the production of bioethanol because of the different feedstocks (corn and microalgae) and the different sources of CO₂.

GWP of each scenario results from energy consumed by the microstrainer and belt filter during the harvesting process, which contributes approximately 67% of the total GWP of each scenario or 2.1×10^{11} kg CO₂ Eq/FU. The second highest GWP contributor is the use of flocculants, which are also consumed during microalgal harvesting process. Therefore, the harvesting process contributed up to 91% of the total GWP. The avoided GWP of MD+ME scenario is approximately -2.7%, while the avoided GWP of MD+CE scenario is approximately -4.5% of the total GWP or about -0.1 kg CO₂ Eq/MJ. The difference of GWP between MD+ME and MD+CE scenarios results from the quantity of CO₂ released from microalgal bioethanol production and the CO₂ avoided in corn mass production. The GWP of MD+ME scenario is higher than GWP of MD+CE scenario by 0.04 kg CO₂ Eq/MJ due to the releasing of CO₂ from microalgal bioethanol production, fermentation. The fermentation process produces 0.01 kg CO₂ Eq/MJ or only 0.14 kg CO₂/kg microalgal mass, which is insufficient to maintain the microalgal cultivation condition. Microalgae require 1.5 kg of CO₂ to produce one kg of microalgal mass (Posten, 2009; Rodolfi et al., 2009; Schenk et al., 2008).

The utilization of CO₂ from the fermentation process can reduce GWP footprint of the co-production of microalgal biodiesel and microalgal bioethanol. However, GWP impact from MD+ME+CO₂ scenario is still higher than GWP from MD+CE scenario by 0.6% or 14 g CO₂ Eq/MJ.

There are tradeoffs between GWP and EP in each scenario. EP from MD+CE scenario is the highest (approximately 1.5×10^{-3} kg N Eq/MJ), while EP from MD+ME scenario is the lowest. The flocculant used in harvesting process contributes the largest percentage to EP; approximately 40% of the total EP impact. For the 95% and 99.7% bioethanol productions (Table 10), which are assumed to be the same for both corn ethanol and microalgal bioethanol, each bioethanol production contributes almost 19% of the total EP in all the three scenarios.

Compared to the total EP from MD+ME scenario, the total EP from MD+CE scenario is higher by 0.7% (1×10^{-5} kg N Eq/MJ), and the EP from MD+ME+CO₂ scenario is higher than MD+ME scenario by 0.1% (1.3×10^{-6} kg N Eq/MJ) resulting from the utilization of CO₂ from flue gas and fermentation process. For MD+ME+CO₂ scenario, the utilization of CO₂ from flue gas reduces EP by -5.7×10^{-7} kg N Eq/MJ (-0.04% of the total EP), whereas the utilization of CO₂ from fermentation process reduces EP by 6.0×10^{-8} kg N Eq/MJ (-0.004% of the total EP). The recovery of CO₂ from fermentation process for microalgal cultivation in MD+ME+CO₂ scenario also avoids GWP by -90 g CO₂ Eq/MJ, whereas EP is slightly increased by 0.001 g N Eq/MJ compared to the impact results from MD+ME scenario, where only CO₂ from flue gas is fed to the cultivation system.

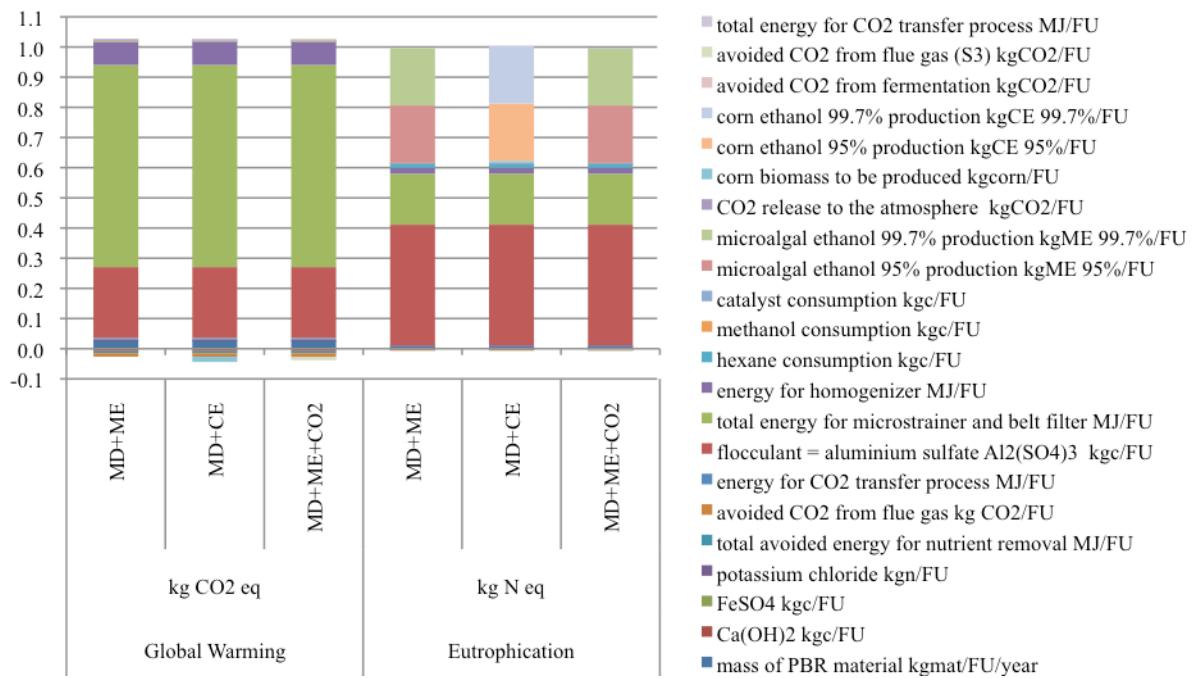


Figure 18 Normalized global warming and eutrophication potentials from microalgal biodiesel (MD) and bioethanol productions.

Feedstocks for bioethanol production are corn for corn ethanol (CE) in MD+CE scenario and microalgae for microalgal bioethanol (ME) in MD+ME and MD+ME+CO₂ scenarios. CO₂ for MD+ME and MD+CE scenarios is from flue gas, while CO₂ for MD+ME+CO₂ scenario is from both flue gas and fermentation process. The impact results are normalized to the highest impact in each category.

6.3.2 Uncertainty Analysis

Only MD+ME scenario was investigated for uncertainty analysis as a base case scenario to provide baseline for the co-production of microalgal biodiesel and microalgal bioethanol. Out of 10,000 iterations of LCA of the co-production using MCA, only 270 iterations or 50 cases with the highest and the lowest total energy produced from microalgal biodiesel and microalgal bioethanol, GWP and EP are presented in the subsequent figures. These 50 cases illustrate the boundaries of the range of values estimated by MCA, and are presented as such to enable the reader to see the boundary results of multiple factors contributing to the uncertainty of the results.

The results suggested that GWP from microalgal biodiesel and microalgal bioethanol production using only CO₂ from flue gas in Scenario 1 are strongly related to lipid content of microalgae, as presented in Figure 19. For the case with the highest total energy produced from both microalgal fuels, which is 1.4×10^{11} MJ with the GWP results ranging from 2.6×10^{11} to 3.8×10^{11} kg CO₂ Eq, the lipid content could range from 24% to 36% with approximately 44% of carbohydrate content.

The EP is influenced by carbohydrate contents as much as lipid content of microalgae, as depicted in Figure 20. At the maximum value of total energy produced from the two microalgal fuels, EP results range from 1.9×10^8 to 2.4×10^8 kg N Eq, while at the minimum value of the total energy, EP results range from 1.6×10^8 to 2.0×10^8 kg N Eq.

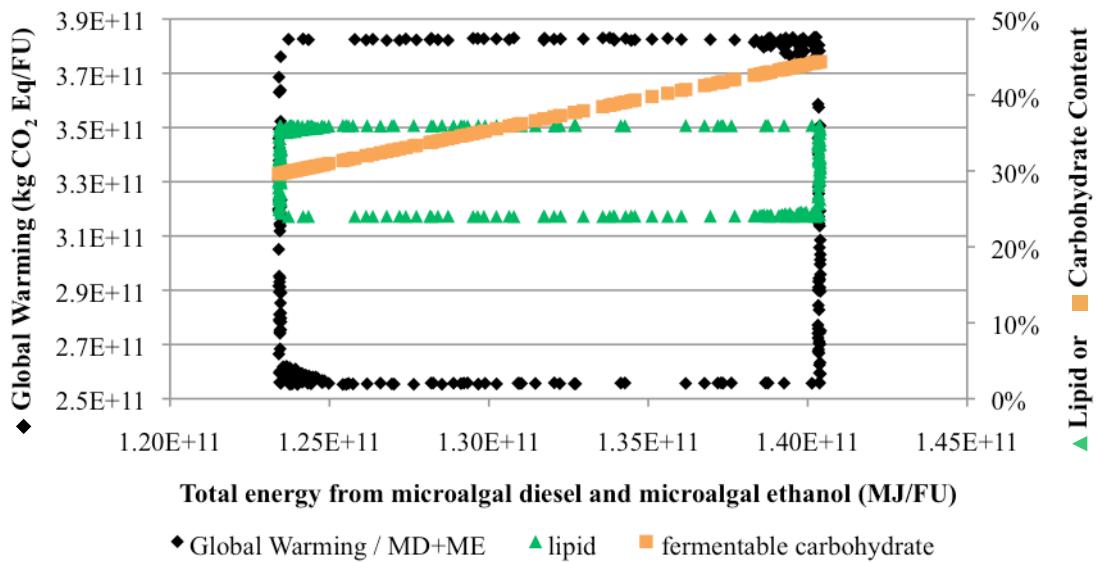


Figure 19 Relationships among the total energy produced from the two microalgal biofuels, lipid content, carbohydrate content, and global warming potential.

The GWP from the production of microalgal biodiesel and microalgal bioethanol cultivated using CO₂ from flue gas for microalgal cultivation, and lipid and carbohydrate contents in microalgae.

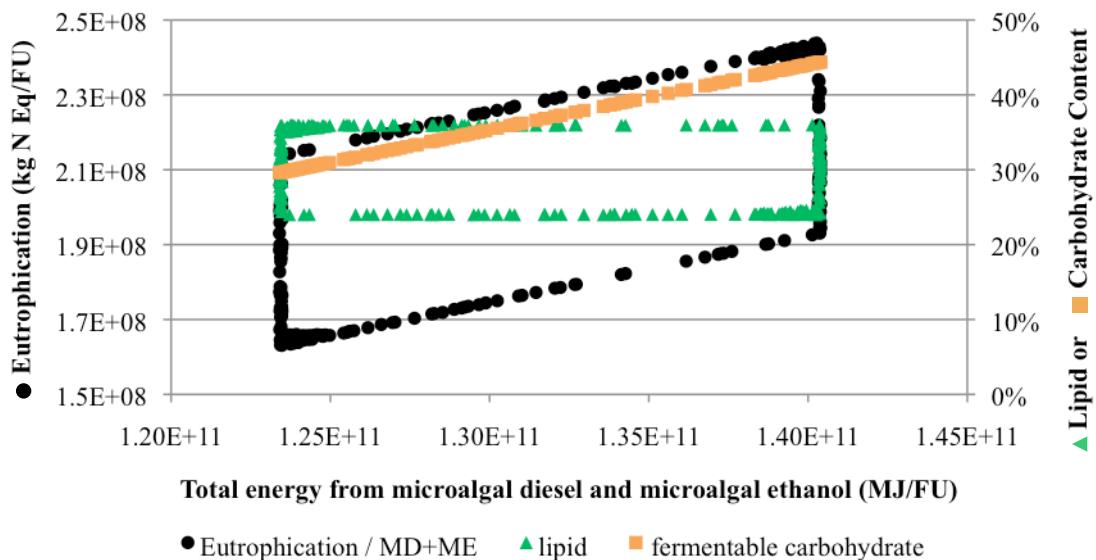


Figure 20 Relationships among the total energy produced from the two microalgal biofuels, lipid content, carbohydrate content, and eutrophication potential.

The EP from the production of microalgal biodiesel and microalgal bioethanol cultivated using CO₂ from flue gas for microalgal cultivation, and lipid and carbohydrate contents in microalgae.

The conditions we aim for in any fuels production are to maximize the yield while minimizing the environmental impacts. In this study, increasing the total energy produced by 13% increases GWP impact by 8% compared to the case with the minimum GWP, which is approximately 2.9 kg CO₂ Eq/MJ of the total energy produced. On the other hand, decreasing EP by 23% decreases the total energy produced by 14% compared to the case with minimum EP, which is approximately 1.8 kg N Eq/MJ of the total energy produced. To maximize the total energy produced from microalgal biodiesel and microalgal bioethanol and minimize GWP and EP impacts, lipid content of microalgae should be in the range of 24 and 36%, and the carbohydrate content should approximately be 44%.

The ratio of microalgal biodiesel and microalgal bioethanol from *Chlorella vulgaris* investigated in this study is approximately 1:0.48. Therefore, based on the energy content allocation, microalgal bioethanol is responsible for approximately 32% of the total GWP and the total EP. Consequently, the co-production of microalgal bioethanol contributes 0.75 kg CO₂

Eq/MJ and 0.46 kg N Eq/MJ, while the microalgal biodiesel contributes 1.57 kg CO₂ Eq/MJ and 0.96 kg N Eq/MJ. Without microalgal bioethanol as a co-product, microalgal biodiesel would be responsible for 3.41 kg CO₂ Eq/MJ and 2.01 kg N Eq/MJ. However, based on the LCA model, microalgal bioethanol is directly responsible for only 0.5% of the total GWP impact or 0.01 kg CO₂ Eq/MJ. Considering microalgal bioethanol as a co-product of microalgal biodiesel can increase the potential of microalgal biodiesel to reach the RFS's life-cycle GHG emission reduction requirement which is 0.045 kg CO₂ Eq/MJ (U.S. Environmental Protection Agency, 2010b).

6.4 CONCLUSIONS

Based on the energy content allocation of microalgal biodiesel and microalgal bioethanol, the utilization of de-oiled microalgal mass from microalgal oil extraction process to produce microalgal bioethanol has the potential to decrease GWP and EP approximately by 54% and 52%, respectively. However, the recovery of CO₂ from fermentation process for microalgal cultivation slightly increases EP impact compared to the microalgal fuels production using CO₂ only from flue gas. There are also tradeoffs between GWP and EP impacts among the three scenarios. Microalgal biodiesel and microalgal bioethanol from de-oiled microalgal mass using CO₂ from flue gas for microalgal cultivation (MD+ME scenario) contributes the highest GWP and the least EP when compared to the other two scenarios. The lipid content and carbohydrate content of microalgae should be 24-36% and 44% by weight, respectively, in order to provide the highest energy yield with minimal GWP and EP impacts.

7.0 CONCLUSIONS

7.1 SUMMARY

The goal of this study was to examine the environmental feasibility of microalgal biodiesel, as a potential replacement of other biodiesels, from a life-cycle perspective. The life-cycle impact results of microalgal biodiesel and their uncertainties were quantified using a process LCA model with MCA for sensitivity and uncertainty analysis. The LCA model could aid in green design and engineering choices for redesigning environmentally friendly microalgal biodiesel production systems. The life-cycle impact results were also used to evaluate the existing policies such as the RFS and to improve upon the production of microalgal biodiesel to avoid unintended consequences, as witnessed in first- and second-generation biofuels (Costello et al., 2009; Landis & Theis, 2008).

Microalgal cultivation has the potential to participate in industrial symbiosis with wastewater and CO₂ producing industries. The utilization of CO₂ from flue gas instead of synthetic CO₂ avoids GWP, whereas the utilization of N and P nutrients from wastewater instead of synthetic N and P fertilizers can avoid water quality impacts such as eutrophication potential. However, the material used to construct PBR should also be closely considered since the impacts from the material production often outweighed the avoided impacts from industrial symbiosis, as evident in acidification and smog formation potentials (Soratana & Landis, 2011).

A process LCA model of microalgal biodiesel with MCA was used to evaluate environmental impacts among various different methods of producing microalgal biodiesel. Each method represent different production efficiencies – high and low efficiencies, and different sources of carbon dioxide and nutrient resources – synthetic and waste resources. The environmental impact results suggested that the condition using natural and waste resources with high-efficiency production and a 50% reduction of energy consumption during the harvesting process, which contributed the lowest GWP among the four production conditions evaluated herein, required approximately a 71-93% reduction of GHG emissions in order to meet the RFS baseline. Using Tornado correlation coefficients to identify the most critical parameter to the total GWP, eutrophication, ozone depletion and ecotoxicity impacts, the following parameters were identified as targets for achieving the most improvements to the systems' life cycle impacts: lipid content of microalgae, service lifetime of PBR, energy consumption in harvesting process and hexane consumption in extraction process.

The well-to-wheel environmental impact results from the production condition with the lowest GWP was further examined and compared with other types of diesel including petroleum, soybean and canola diesels, to evaluate the environmental tradeoffs and to investigate which different biodiesels meet the RFS's GHGs requirement. To avoid unintended environmental impacts, the future RFS criteria should include eutrophication and smog formation potential metrics. This study also provided possible strategies to setting life-cycle eutrophication and smog formation potentials standards.

Valuable co-products, such as ethanol, were evaluated in order to investigate how allocation and co-products can improve upon the LCA for microalgal biodiesel. Results from the LCA case study on the co-production of microalgal biodiesel and microalgal ethanol indicated

that co-production of ethanol has the potential to minimize the environmental footprint of microalgal biodiesel and to enhance microalgal biodiesel in achieving the RFS's requirements. Based on energy content allocation, GWP and EP impacts can be reduced by 54 and 52%, respectively, compared to the production of microalgal biodiesel alone. Percent lipid content and percent carbohydrate content of microalgae in order to yield the maximum total energy with minimum GWP and EP impacts were quantified. The utilization of CO₂ from fermentation process for microalgal cultivation can reduce GWP impact, but slightly increase EP impact compared to the utilization of CO₂ only from flue gas.

7.2 RECOMMENDATIONS FOR FUTURE WORK

The production of microalgal biodiesel also produces various types of co-product such as algae meal for animal feed, algal residues for bioethanol, methane and electricity and glycerol for the pharmaceutical industry. Even though information on those co-products are currently uncertain and limited, energy and impact allocation of microalgal biodiesel and co-products should be investigated. Co-product allocation will decrease energy consumption and impacts per MJ of microalgal biodiesel and also enhance the potential of microalgal biodiesel in achieving the RFS's volume and life-cycle GHG requirement.

Other industrial ecology approaches, apart from the utilization of CO₂ and nutrients from waste streams should be explored, in order to minimize impacts from the production of microalgal biodiesel. Examples of other industrial ecology approaches are the utilization of heat or airflow from e.g. vents or condenser units in industrial facilities in drying process, and the utilization of solvents or alcohols in extraction and conversion processes from other industries.

The process LCA model of microalgal biodiesel developed in this study can be improved by including more options to each process and industrial data, which is still limited and proprietary. Life-cycle cost analysis should be introduced for economic viable of the production, which will bring the production even closer to the current industrial situation.

APPENDIX A

LIFE CYCLE ASSESSMENT MODEL FOR MICROALGAL BIODIESEL

A.1 SYSTEM BOUNDARY

System boundaries of this LCA model for microalgal biodiesel include strain selection, cultivation using a PBR, harvesting and dewatering, drying, cell disruption, extraction, conversion and consumption. Environmental impacts from each process are converted and compared on the same basis or one functional unit (MJ of microalgal biodiesel energy content), which can be defined by the user.

Microalgal strains are not provided since some strains can be genetically modified; rather the strain can be selected based on its lipid content and productivity rate. In the cultivation stage, only cultivation via a PBR is considered since PBRs have better control of cultivation conditions, such as mass transfer and contamination with less water loss than open ponds (Jorquera et al., 2010; Posten, 2009). Similar to microalgal strains, PBR types are not provided; rather the PBR is designed based on SVR and volume. Eight input parameters are considered in the computation of environmental impacts from cultivation stage. The eight parameters are PBR construction materials, sources of water, sources of nutrients, types of mixing and recirculation system, sources of CO₂, pH control, sources of light and temperature.

For the harvesting and dewatering stage, flocculation is the main harvesting process with four other additional separation techniques, which are filtration and screening, gravity sedimentation, flotation, and centrifugation. Environmental impacts from this stage are calculated from energy consumption per volume (kWh/m^3) of microalgal suspension from PBR. Likewise, environmental impacts from drying stage are calculated on kWh m^{-3} basis. The model provides three drying methods, sun drying, drum drying and conveyor drying. Four different cell disruption methods provided are homogenizer, bead mills, ultrasonic and autoclave. Cell disruption is generally required in order to improve extraction yield (Greenwell et al., 2010).

The extraction methods available in this model are solvent extraction with five different solvent options, which are acetone ($(\text{CH}_3)_2\text{CO}$), hexane (C_6H_{14}), methanol (CH_3OH), ethanol ($\text{C}_2\text{H}_5\text{OH}$), and C_6H_{14} and $\text{C}_2\text{H}_5\text{OH}$ mixture), and Soxhlet extraction. The conversion process considered in this model is transesterification in order to convert microalgal oil to microalgal biodiesel. Two main types of chemicals involved in this process are alcohol and catalyst. Therefore, environmental impacts from transesterification were calculated based on impacts from the production of chemicals used in the process e.g. CH_3OH , $\text{C}_2\text{H}_5\text{OH}$, sodium hydroxide (NaOH) and potassium hydroxide (KOH).

The construction of microalgal biodiesel production infrastructure is omitted from the boundaries since the quality of microalgal oil suggested that the production can occurred within existing systems for biodiesel production from other feedstocks (ExxonMobil Research and Engineering, 2009). No transportations and distribution systems are included in this model.

A.2 LIFE CYCLE INVENTORIES AND IMPACT ASSESSMENT

There are two sets of inventories provided in the process LCA model of microalgal biodiesel, (1) quantity of resources such as CO₂ and nutrients required in order to produce one functional unit and (2) environmental impacts such as GWP, EP and ozone depletion potential contributed from one unit of resource, as listed in Table A - 1. The first set of inventories is defined by the user. The second set is a result from the multiplication of quantity of raw materials required to produce one unit of resource consumed during the microalgal biodiesel production and the characterization factor (CF) from TRACI. The inventory and the LCIA CF are multiplied to present environmental impacts from the production of resources required to produce one functional unit of microalgal biodiesel.

It should also be noted that impacts from the production of nutrient-removal chemicals from wastewater and related energy required can be avoided if the source of water for microalgal cultivation is wastewater with sufficient nutrients. For conversion processes, generally, the molar ratio of alcohol to oil in order to complete transesterification reaction is from 6 to 56 portions of alcohol to one portion of oil (Mata et al., 2010; Vijayaraghavan & Hemanathan, 2009). Catalysts can be reused for a certain times therefore the quantity of catalyst for one kg of microalgal mass, for microalgal mass produced over the PBR system's lifetime and for one FU are measured based on quantity and number of times catalyst is reused.

Table A - 1. Inventories and formulation of parameters considered in the LCA model of microalgal biodiesel.

Variables	Definition	Suggested values	References
Energy content	Heating value of microalgal biodiesel.	41 MJ/kg	(Huang et al., 2010; Lardon et al., 2009; Sander & Murthy, 2010)
Microalgal biodiesel density	Density of microalgal biodiesel produced from microalgal oil	870 kg/m ³	(Sander & Murthy, 2010)
Lipid content	Percent lipid in dried microalgal mass.	40 to 70% dry weight of microalgal mass	(Mata et al., 2010)
Volumetric microalgal mass productivity	Microalgal mass that can be produced per volume of PBR within a day.	300 to 360 g _M /m ³ × day	(Mata et al., 2010; Rodolfi et al., 2009; Shen et al., 2010)
Harvesting efficiency	Efficiency of a harvesting process to collect microalgal mass from microalgal suspension in PBR.	80 to 100%	(Greenwell et al., 2010; Lardon et al., 2009; Mata et al., 2010)
Extraction efficiency	Efficiency of an extraction process to extract microalgal oil from dried microalgal mass.	70 and 98%	(Lardon et al., 2009; Mata et al., 2010; Sander & Murthy, 2010)
Conversion efficiency	Efficiency of a conversion process (transesterification) to convert microalgal oil to microalgal biodiesel.	98%	(Mata et al., 2010)
Surface area per volume ratio (SVR)	One of the design parameters of PBR. Most designs require high SVR for shorter length of light path and therefore can support higher concentration of biomass.	80-100 m ² /m ³	(Posten, 2009; Schenk et al., 2008)
Volume of PBR unit	Volume of one unit of PBR.	5-10 m ³	(Carvalho et al., 2006b)
Number of PBR	Number of PBR required for one functional unit.	PBR units/system	
System lifetime	Designed service lifetime of PBR.	15 Years	
Material thickness	Thickness of selected PBR construction material.	0.005 m	(Posten, 2009)
PBR construction material	Eight types of PBR construction materials are available – high-density polyethylene (HDPE), low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), polycarbonate (PC), polyvinyl chloride (PVC), polymethyl methacrylate (PMMA), flat glass and glass tube.	HDPE	(Soratana & Landis, 2011)
Sources of water	Two sources of water, freshwater and wastewater are provided without recycle.	Wastewater	(Soratana & Landis, 2011)
Volume of water	Volume of water, either freshwater or wastewater, requires during microalgal cultivation.	0.001 m ³ /kg microalgae	(Campbell et al., 2011)
Sources of nutrients	Two sources of nitrogen (N) and phosphorus (P), synthetic fertilizers (urea and superphosphate) and municipal wastewater, are provided. Only synthetic fertilizer, potassium chloride, is an option for a source of potassium (K).	Municipal wastewater	(Lardon et al., 2009; Soratana & Landis, 2011)
Quantity of synthetic fertilizers	The quantities of synthetic fertilizers required to produce one kg of microalgal mass: Urea (normal and low N conditions) Superphosphate (normal and low N conditions) Potassium chloride (normal and low N conditions)	0.02 and 0.12 kg _F /kg _M 0.009 and 0.02 kg _F /kg _M 0.004 and 0.02 kg _F /kg _M	(Lardon et al., 2009; Soratana & Landis, 2011)
Quantity of nutrients from wastewater	The quantities of N, P and K required to produce one kg of microalgal mass.	0.05 kg of N, 0.01 kg of P and 0.008 kg of K/kg _M	(Lardon et al., 2009)
Denitrification chemicals	Chemicals use to remove one kg of N from wastewater. The options provided are CaO, Ca(OH) ₂ , NaOH and Na ₂ CO ₃	4.0, 5.2, 8.1 and 7.5 kg of chemical/kg _M	(Metcalf et al., 2003)
Precipitation chemical	Chemical use to remove one kg of P from wastewater. The only option provided is FeSO ₄ .	1.8 kg of chemical/kg _M	(Metcalf et al., 2003)
Energy consumption for nutrient removal	The amount of energy consumed to remove one kg of nutrient.	14 MJ/kg of N and 24 MJ/kg of K	(Maurer et al., 2003)
Types of mixing/recirculation	Pumping options available in this model are diaphragm, centrifugal, peristaltic and airlift pumps. CO ₂ feeding can be applied instead of mixing system	CO ₂ feeding	(Carvalho et al., 2006a; Jorquera et al., 2010; Posten, 2009)
Energy consumption of selected pump	The amount of power required to operate the selected pump.	n/a (Watt)	
Pumping rate	The quantity of water and TSS pumped within an hour.	n/a (m ³ /hour)	
Duration of pumping employed	The duration of pump being used per day.	24 hours/day	

Table A – 1 (continued).

Variables	Definition	Suggested values	References
Sources of CO ₂	Two options are made available which are (1) synthetic CO ₂ and (2) CO ₂ in flue gas	CO ₂ in flue gas	(Soratana & Landis, 2011)
Quantity of CO ₂ required	The quantity of CO ₂ , either from synthetic CO ₂ and flue gas, being fed to PBR in order to produce one kg of microalgal mass	1.8 to 2.0 kg of CO ₂ /kg _M	(Posten, 2009)
CO ₂ content	The percent of CO ₂ fed to PBR: CO ₂ from flue gas Synthetic CO ₂	14 to 15% 99 to 100%	(Batan et al., 2010a; Kadam, 2002; Kadam, 2001; Mata et al., 2010)
Energy consumption for CO ₂ feeding process	The amount of energy consumed for feeding CO ₂ from: Synthetic CO ₂ CO ₂ in flue gas CO ₂ in flue gas with monoethanolamine (MEA) extraction	0.002 kWh/kg of CO ₂ 0.022 kWh/kg of CO ₂ 0.033 kWh/kg of CO ₂	(Kadam, 2002; Kadam, 2001)
pH control	There are two pH adjustment methods available, by adding CO ₂ and acid/base solution such as HCl/NaOH.	Suitable pH range for microalgal cultivation is from 7.5 to 8.1	(Rodolfi et al., 2009)
Sources of light	Sunlight, light-emitting diode (LED), compact fluorescent lamp (CFL)		(Das et al., 2011; Janssen et al., 2003)
Energy consumption of selected light	Sunlight LED CFL	0 Watt 100-150 Watts 18, 20, 32 and 40 Watts	(Janssen, 2002)
Duration of light employed	The number of hour the selected light is used.	18 hours/lamp/day	(Lanvens & Sorgeloos, 1996)
Number of lamp	The number of lamp required for PBR.		
Lifetime of lamp	The service lifetime of selected light source	9,000-26,000 hours	
Local temperature	The temperature of the place where PBR is located		User define
Optimal temperature	The temperature suitable for PBR operation	20-26 °C	(Mata et al., 2010)
Energy consumption to increase/decrease 1°C	The amount of energy required to increase or decrease 1°C	0.001 MJ/kg × °C	
Energy consumption to maintain temperature	The amount of energy required to maintain temperature of PBR under designed condition	Calculation (MJ/kg _M)	
Concentration of microalgal slurry	The concentration of microalgal mass obtained from harvesting process	1-6% TSS	(Shelef et al., 1984)
Concentration of microalgal cake	The concentration of microalgal mass obtained from dewatering process	15-25% TSS	(Shelef et al., 1984)
Type of flocculant	Three choices of flocculant are available, which are aluminium sulfate (Al ₂ (SO ₄) ₃), ferrous sulfate (FeSO ₄) and calcium oxide (CaO).		(Shelef et al., 1984)
Quantity of flocculant	The quantity of selected flocculant consumed during harvesting process.	0.08-0.25 kg of Al(SO ₄) ₃ 0.05-0.9 kg of FeSO ₄ 0.5-0.7 kg of CaO/m ³	(Shelef et al., 1984)
Additional separation techniques	Four separation techniques are available: (1) filtration and screening, (2) gravity sedimentation, (3) flotation and (4) centrifugation		(Batan et al., 2010b; Greenwell et al., 2010; Posten, 2009; Shelef et al., 1984)
Energy consumption of additional separation techniques	The amount of energy required to separate microalgal mass from microalgal slurry: Belt filter Chamber filter press Cylindrical sieve Filter Basket Filter thickener Suction filter	0.45 kWh/m ³ 0.88 kWh/m ³ 0.30 kWh/m ³ 0.20 kWh/m ³ 1.60 kWh/m ³ 0.10 kWh/m ³	(Batan et al., 2010a; Greenwell et al., 2010; Posten, 2009; Shelef et al., 1984; Singh et al., 2011)
Drying process	A process to remove moisture content in microalgal slurry to 12-15% by weight. The methods available in this model are: Sun drying Rotary or drum drying Conveyor drying		(Lardon et al., 2009; Shelef et al., 1984)
Cell disruption	A process to aid in oil extraction efficiency. The equipments provided are: homogenizer, bead mills, ultrasonic and autoclave.	Homogenizer: 1.5 kWh	(Greenwell et al., 2010)

Table A – 1 (continued).

Variables	Definition	Suggested values	References
Extraction process	A process to extract metabolite such as oil from microalgae. The process options provided are: solvent and Soxhlet extractions		(Batan et al., 2010a; Demirbas, 2009b; Lardon et al., 2009; Mulbry et al., 2009; Stephenson et al., 2010; Vijayaraghavan & Hemanathan, 2009)
Solvents for extraction process	Acetone Hexane Methanol Ethanol Hexane:ethanol mixture Chloroform:methanol	0.002 kg/m ³ 0.0002 kg/m ³ 0.004 kg/m ³	(Batan et al., 2010a; Demirbas, 2009b; Lardon et al., 2009; Mulbry et al., 2009; Stephenson et al., 2010; Vijayaraghavan & Hemanathan, 2009)
Energy consumption of additional separation techniques	The amount of energy required to separate microalgal mass from microalgal slurry: Belt filter Chamber filter press Cylindrical sieve Filter Basket Filter thickener Suction filter	0.45 kWh/m ³ 0.88 kWh/m ³ 0.30 kWh/m ³ 0.20 kWh/m ³ 1.60 kWh/m ³ 0.10 kWh/m ³	(Batan et al., 2010a; Greenwell et al., 2010; Posten, 2009; Shelef et al., 1984; Singh et al., 2011)
Conversion process	Transesterification is the conversion process used in this model to convert microalgal oil to microalgal biodiesel. Transesterification is a reaction between TAG and alcohol with the present of catalyst		(Brennan & Owende, 2010; Vijayaraghavan & Hemanathan, 2009; Vyas et al., 2010)
Types of alcohol for conversion process	Methanol Ethanol	20% v/v of microalgal oil 22% v/v of microalgal oil	(Greenwell et al., 2010; Mata et al., 2010; Sander & Murthy, 2010; Tickell & Tickell, 2003; Vijayaraghavan & Hemanathan, 2009)
Types of catalyst	Only basic catalyst is available which are: NaOH KOH NaOCH ₃	4 kg/m ³ of microalgal oil 6 kg/m ³ of microalgal oil	(Greenwell et al., 2010; Reijnders & Huijbregts, 2009; Vijayaraghavan & Hemanathan, 2009; Zebib, 2008)
Combustion	Combustion of microalgal biodiesel in typical engine	1.5 kg CO ₂ / kg microalgal mass (or same as the quantity fo CO ₂ intake)	(Fisher, 2010; Hermann, 2010)

APPENDIX B

ANNEXES OF THE STUDY MICROALGAL BIODIESEL AND THE RENEWABLE FUEL STANDARD'S GREENHOUSE GAS REQUIREMENT

B.1 ANNEX 1 INPUTS AND INVENTORIES FOR THE LCA MODEL OF MICROALGAL BIODIESEL PRODUCTION

Process		LS	HS	LW	HW
Cultivation	Strain selection	Energy content ^a 41 MJ kg ⁻¹ of biodiesel	41 MJ kg ⁻¹ of biodiesel	41 MJ kg ⁻¹ of biodiesel	41 MJ kg ⁻¹ of biodiesel
	Lipid content ^b 50%		70%	50%	70%
	Volumetric productivity ^{c, d, e} 200 g m ⁻² day ⁻¹	200 g m ⁻² day ⁻¹	350 g m ⁻² day ⁻¹	200 g m ⁻² day ⁻¹	350 g m ⁻² day ⁻¹
	Surface area: volume ratio ^f	80 m ⁻¹	100 m ⁻¹	80 m ⁻¹	100 m ⁻¹
	Unit volume ^g	10 m ³	10 m ³	10 m ³	10 m ³
	Number of unit [*]	15,000,000 units system ⁻¹	3,150,000 units system ⁻¹	15,000,000 units system ⁻¹	3,150,000 units system ⁻¹
	System lifetime ^{**}	15 years	15 years	15 years	15 years
	Material ^{g, h}	High-density polyethalene	High-density polyethalene	High-density polyethalene	High-density polyethalene
	Water	Freshwater	Freshwater	Wastewater	Wastewater
	Nutrient: Nitrogen ^{c, i, j, k}	Urea (0.12 kg kg ⁻¹ biomass)	Urea (0.12 kg kg ⁻¹ biomass)	Wastewater (avoided Ca(OH) ₂) 0.3 kg kg ⁻¹ biomass)	Wastewater (avoided Ca(OH) ₂) 0.3 kg kg ⁻¹ biomass)
	Nutrient: Phosphorus ^{c, i, k}	Superphosphate (0.02 kg kg ⁻¹ biomass)	Superphosphate (0.02 kg kg ⁻¹ biomass)	Wastewater (avoided FeSO ₄ 0.018 kg kg ⁻¹ biomass)	Wastewater (avoided FeSO ₄ 0.018 kg kg ⁻¹ biomass)

B.1 (continued)

Process	LS	HS	LW	HW
Nutrient: Potassium ^{c, i}	Potassium chloride (0.02 kg kg ⁻¹ biomass)	Potassium chloride (0.02 kg kg ⁻¹ biomass)	Potassium chloride (0.02 kg kg ⁻¹ biomass)	Potassium chloride (0.02 kg kg ⁻¹ biomass)
Energy for N removal ^l	-	-	14 MJ kg ⁻¹ nutrient removed	14 MJ kg ⁻¹ nutrient removed
Energy for P removal ^l	-	-	24 MJ kg ⁻¹ nutrient removed	24 MJ kg ⁻¹ nutrient removed
CO ₂ ^{b, d, f, j, m, n}	Synthetic CO ₂ (1.5 kg CO ₂ kg ⁻¹ biomass)	Synthetic CO ₂ (1.5 kg CO ₂ kg ⁻¹ biomass)	CO ₂ from flue gas (14% of CO ₂ by wt.)	CO ₂ from flue gas (14% of CO ₂ by wt.)
Energy for CO ₂ feeding ^{c, m, o, **}	0.04 MJ kg ⁻¹ biomass	0.04 MJ kg ⁻¹ biomass	0.86 MJ kg ⁻¹ biomass	0.86 MJ kg ⁻¹ biomass
Light ^{p, q}	397,622,951 15-W CFLs (equiv to 60 W)	397,622,951 15-W CFLs (equiv to 60 W)	Natural light	Natural light
Harvesting/ Dewatering	Efficiency ^c	72%	90%	72%
	Harvesting method	Flocculation	Flocculation	Flocculation
	Flocculant	Al ₂ (SO ₄) ₃ (0.17 kg m ⁻³)	Al ₂ (SO ₄) ₃ (0.17 kg m ⁻³)	Al ₂ (SO ₄) ₃ (0.17 kg m ⁻³)
Drying	Microstrainer/ belt filter ^r	0.2/ 0.45 kWh m ⁻³	0.2/ 0.45 kWh m ⁻³	0.2/ 0.45 kWh m ⁻³
	Drying method ^c	Conveyor dryer, 4kW kg ⁻¹ biomass	Conveyor dryer, 4kW kg ⁻¹ biomass	Sun dry
Cell Disruption	Homogenizer ^r	1.5 kWh m ⁻³	1.5 kWh m ⁻³	1.5 kWh m ⁻³
Extraction	Efficiency ^b	78%	98%	78%
	Extraction method ^{c, s}	Solvent extraction	Solvent extraction	Solvent extraction
	Solvent ^{c, t}	Hexane	Hexane	Hexane
	Solvent quantity ^{c, t}	0.0002 m ³ kg ⁻¹ microalgae	0.0002 m ³ kg ⁻¹ microalgae	0.0002 m ³ kg ⁻¹ microalgae
Conversion	Efficiency ^u	70%	87%	70%
	Conversion method ^u	Trans- esterification	Trans- esterification	Trans- esterification
	Alcohol ^{b, j, t, v}	Methanol	Methanol	Methanol
	Alcohol quantity ^w	20% by vol of microalgal oil	20% by vol of microalgal oil	20% by vol of microalgal oil
	Catalyst ^{j, x, y}	NaOH	NaOH	NaOH
	Catalyst:oil molar ratio ^t	4 kg m ⁻³	4 kg m ⁻³	4 kg m ⁻³
Combustion	CO ₂ emission ^z	0.08 kg CO ₂ MJ ⁻¹	0.08 kg CO ₂ MJ ⁻¹	0.08 kg CO ₂ MJ ⁻¹
	N emission ^z	1.4×10 ⁻⁶ kg N MJ ⁻¹	1.4×10 ⁻⁶ kg N MJ ⁻¹	1.4×10 ⁻⁶ kg N MJ ⁻¹
	NO _x emission ^z	2.1×10 ⁻⁶ kg NO _x MJ ⁻¹	2.1×10 ⁻⁶ kg NO _x MJ ⁻¹	2.1×10 ⁻⁶ kg NO _x MJ ⁻¹

** The energy for CO₂ feeding for LW and HW conditions are higher than that for LS and HS conditions because of the CO₂ content (14%) in flue gas (Kadam, 2002; Mata et al., 2010).

Quantity of CO₂ required for microalgal cultivation = 1.5 kg CO₂/kg microalgae

CO₂ content in flue gas = 14 % by weight (Kadam, 2002; Mata et al., 2010)

Energy for direct injection of CO₂ from flue gas = 0.0222 kWh/kg flue gas (Kadam, 2002; Mata et al., 2010)

Conversion factor: 1 kWh = 3.6 MJ

Therefore, $(1.5/14\%) \times 0.0222 \times 3.6 = 0.856 \text{ MJ/kg microalgae}$

^a (Huang et al., 2010).

^b (Mata et al., 2010).

^c (Lardon et al., 2009).

^d (Rodolfi et al., 2009).

^e (Shen et al., 2010).

^f (Posten, 2009).

^g (Carvalho et al., 2006a).

^h (Soratana & Landis, 2011).

ⁱ (Collet et al., 2011).

^j (Greenwell et al., 2010).

^k (Tchobanoglous et al., 2003).

^l (Maurer et al., 2003).

^m (Kadam, 2001).

ⁿ (Schenk et al., 2008).

^o (Kadam, 2002).

^p (Das et al., 2011).

^q (Janssen, 2002).

^r (Shelef et al., 1984).

^s (Mulbry et al., 2009).

^t (Vijayaraghavan & Hemanathan, 2009).

^u (Vyas et al., 2010).

^v (Sander & Murthy, 2010).

^w (Tickell & Tickell, 2003).

^x (Reijnders & Huijbregts, 2009).

^y (Zebib, 2008).

^z (Argonne National Laboratory, 2010).

**B.2 ANNEX 2 DATABASES OF INVENTORIES OF RESOURCES UTILIZED
WITHIN THE MICROALGAL BIODIESEL PRODUCTION**

Process	Data	Resource	Database
Cultivation	PBR material	HDPE	ecoinvent data v2.0 (Europe) ^a
	Fertilizers	Urea	ecoinvent data v2.0 (Global) ^a
		Superphosphate	ecoinvent data v2.0 (Europe) ^a
		Potassium chloride	ecoinvent data v2.0 (Europe) ^a
	Chemicals for nutrient removal from wastewater	Ca(OH) ₂	ETH-ESU 96 ^b
		FeSO ₄	ecoinvent v2.0 data (Europe) ^a
	CO ₂	Synthetic CO ₂	BUWAL 250 ^c
Harvesting	Flocculant	Al ₂ (SO ₄) ₃	ecoinvent data v2.0 (Europe) ^a
Extraction	Chemical	Hexane	ecoinvent data v2.0 (Europe) ^a
Conversion	Alcohol	Methanol	ecoinvent data v2.0 (Global) ^a
	Catalyst	NaOH	ecoinvent data v2.0 (North America) ^a
All processes	Energy	Energy US	IDE-MAT ^d

^a (Frischknecht et al., 2007).

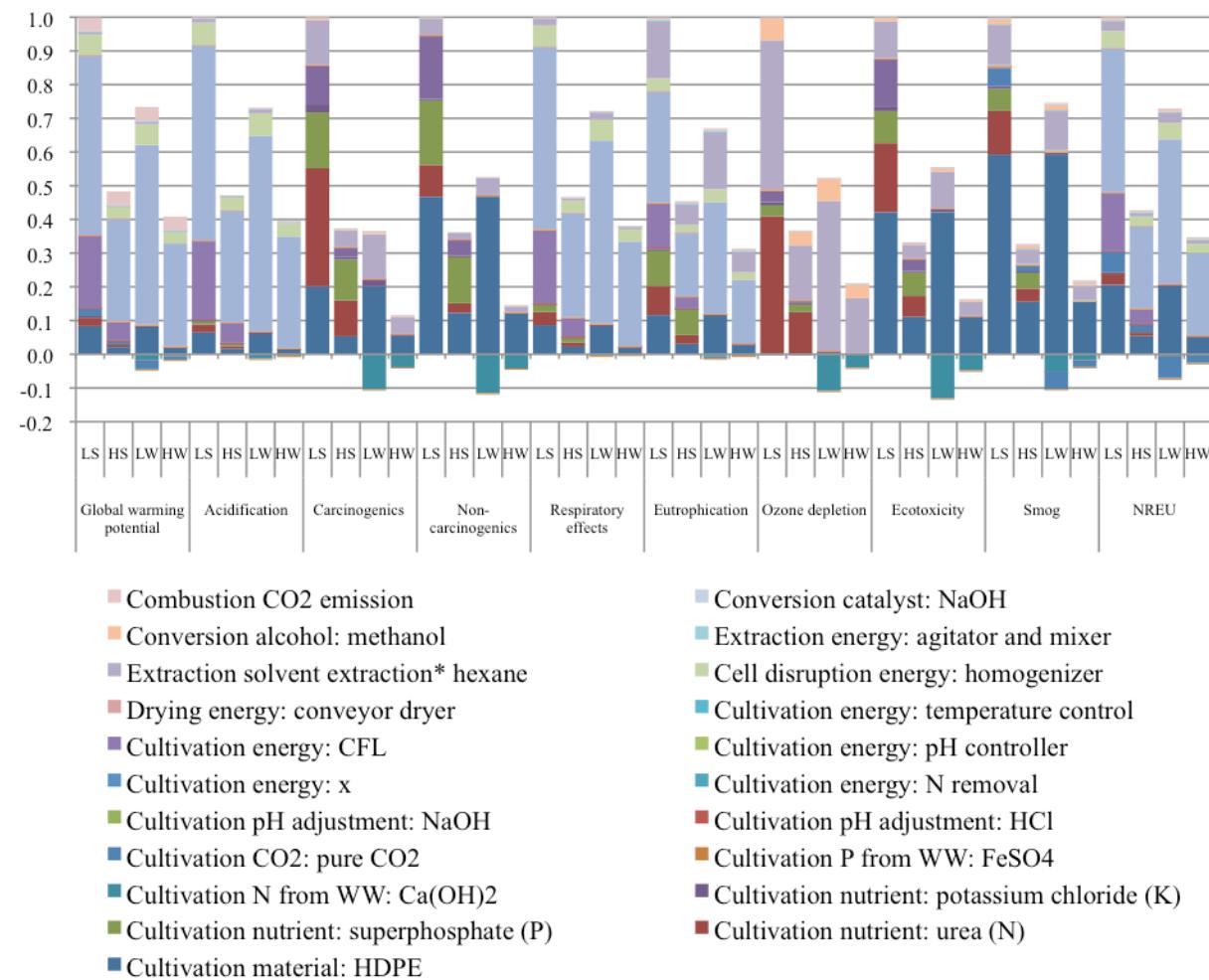
^b (Frischknecht & Jungbluth, 2004).

^c (Spriensma, 2004).

^d (Delft University of Technology, 2001).

B.3 ANNEX 3 LCIA RESULTS FOR THE FOUR PRODUCTION CONDITIONS NORMALIZED TO THE HIGHEST IMPACT IN EACH CATEGORY

LCIA categories were calculated using TRACI with the exception of the NREU category from IMPACT 2002+. LS condition is the low-efficiency production with synthetic resources, HS condition is the high-efficiency production with synthetic resources, LW condition is the low-efficiency production with waste resources and HW condition is the high-efficiency production with waste resources.



B.4

ANNEX 4 LIFE-CYCLE ENVIRONMENTAL RESULTS FROM FOUR SCENARIOS

(LS, HS, LW AND HW) OF MICROALGAL BIODIESEL.

Process	Resources	GWP (kg CO ₂ eq/FU)				Acidification (kg H+ moles eq/FU)				Carcinogenes (kg benzene eq/FU)			
		LS	HS	LW	HW	LS	HS	LW	HW	LS	HS	LW	HW
Cultivation	HDPE	1.46×10 ¹⁰	3.83×10 ⁹	1.46×10 ¹⁰	3.83×10 ⁹	2.58×10 ⁹	6.78×10 ⁸	2.58×10 ⁹	6.78×10 ⁸	5.19×10 ⁶	1.36×10 ⁶	5.19×10 ⁶	1.36×10 ⁶
Urea	4.48×10 ⁹	1.37×10 ⁹	-	-	9.51×10 ⁸	2.90×10 ⁸	-	-	9.01×10 ⁶	2.75×10 ⁶	-	-	
Superphosphate	5.99×10 ⁸	4.38×10 ⁸	-	-	4.79×10 ⁸	3.51×10 ⁸	-	-	4.27×10 ⁸	3.13×10 ⁶	-	-	
KCl	1.18×10 ⁸	3.45×10 ⁷	1.18×10 ⁸	4.31×10 ⁷	2.42×10 ⁷	7.10×10 ⁶	2.42×10 ⁷	8.87×10 ⁶	5.87×10 ⁶	1.72×10 ⁵	5.87×10 ⁵	2.15×10 ⁵	-
Ca(OH) ₂	-	-	-	-	-	-	-	-	-	-	-	2.67×10 ⁶	-9.78×10 ⁷
FeSO ₄	-	-	-	-	-	-	-	-	-	-	-	-3.77×10 ²	-1.38×10 ²
CO ₂	4.32×10 ⁹	1.58×10 ⁹	-4.32×10 ⁹	-1.58×10 ⁹	9.13×10 ⁷	3.34×10 ⁷	-9.13×10 ⁷	-3.34×10 ⁷	-	-	-	-	-
Lighting	9.94×10 ⁷	2.60×10 ⁷	-	-	3.37×10 ⁷	8.80×10 ⁶	-	-	3.05×10 ⁶	7.97×10 ³	-	-	
Energy for N removal	-	-	-5.40×10 ⁸	-1.98×10 ⁸	-	-	-	-	-1.33×10 ⁸	-4.88×10 ⁷	-	-	
Energy for P removal	-	-	-1.85×10 ⁸	-6.77×10 ⁷	-	-	-	-	-4.57×10 ⁷	-1.67×10 ⁷	-	-	
CO ₂ transfer	2.91×10 ⁷	1.07×10 ⁷	6.60×10 ⁸	2.42×10 ⁸	7.20×10 ⁶	2.64×10 ⁶	1.63×10 ⁸	5.97×10 ⁷	-	-	-	-	-
Energy for lighting	3.78×10 ¹⁰	9.89×10 ⁹	-	-	9.35×10 ⁹	2.44×10 ⁹	-	-	-	-	-	-	-
Harvesting	Al ₂ (SO ₄) ₃	9.39×10 ³	3.44×10 ³	9.39×10 ³	3.44×10 ³	8.60×10 ³	3.15×10 ³	8.60×10 ³	3.15×10 ³	3.80×10 ³	1.39×10 ³	3.80×10 ³	1.39×10 ³
Energy for filtration and screening	9.35×10 ¹⁰	5.35×10 ¹⁰	9.35×10 ¹⁰	5.35×10 ¹⁰	2.31×10 ¹⁰	1.32×10 ¹⁰	2.31×10 ¹⁰	1.32×10 ¹⁰	-	-	-	-	-
Drying	Energy for conveyor dryer	1.21×10 ⁷	5.52×10 ⁶	-	-	2.98×10 ⁶	1.37×10 ⁶	-	-	-	-	-	-
Cell disruption	Energy for homogenizer	6.17×10 ⁹	1.08×10 ¹⁰	6.17×10 ⁹	2.67×10 ⁹	1.52×10 ⁹	2.67×10 ⁹	1.52×10 ⁹	-	-	-	-	-
Extraction	Hexane	1.33×10 ⁹	4.87×10 ⁸	1.33×10 ⁹	4.87×10 ⁸	5.33×10 ⁸	1.95×10 ⁸	5.33×10 ⁸	1.95×10 ⁸	5.39×10 ⁷	1.24×10 ⁶	3.39×10 ⁶	1.24×10 ⁶
Conversion	Energy for agitator	2.18×10 ⁸	2.18×10 ⁸	2.18×10 ⁸	2.18×10 ⁸	5.39×10 ⁷	5.39×10 ⁷	5.39×10 ⁷	5.39×10 ⁷	-	-	-	-
Methanol	5.01×10 ⁸	3.21×10 ⁸	5.01×10 ⁸	3.21×10 ⁸	4.73×10 ⁷	3.03×10 ⁷	4.73×10 ⁷	3.03×10 ⁷	2.28×10 ³	1.46×10 ³	2.28×10 ³	1.46×10 ³	
NaOH	8.89×10 ⁶	5.69×10 ⁶	8.89×10 ⁶	5.69×10 ⁶	3.84×10 ⁶	2.46×10 ⁶	3.84×10 ⁶	2.46×10 ⁶	5.20×10 ³	3.33×10 ³	5.20×10 ³	3.33×10 ³	
Combustion		6.89×10 ⁹	6.89×10 ⁹	6.89×10 ⁹	6.89×10 ⁹	-	-	-	-	-	-	-	-

B.4 (continued)

Process	Resources	Non-carcinogenics (kg toluene eq/FU)				Respiratory Effects (kg PM _{2.5} eq/FU)				Eutrophication (kg N eq/FU)				
		LS	HS	LW	HW	LS	HS	LW	HW	LS	HS	LW	HW	
Cultivation	HDIPE	1.49×10 ¹¹	3.92×10 ¹⁰	1.49×10 ¹¹	3.92×10 ¹⁰	1.07×10 ⁷	2.80×10 ⁶	1.07×10 ⁷	2.80×10 ⁶	2.96×10 ⁶	7.77×10 ³	2.96×10 ⁶	7.77×10 ³	
Urea	3.01×10 ¹⁰	9.17×10 ⁹	-	-	-	4.79×10 ⁶	1.46×10 ⁶	-	-	2.19×10 ⁶	6.69×10 ³	-	-	
Superphosphate	6.10×10 ¹⁰	4.46×10 ⁹	-	-	-	2.91×10 ⁶	2.13×10 ⁶	-	-	2.72×10 ⁶	1.99×10 ⁶	-	-	
KCl	1.79×10 ⁹	5.23×10 ⁸	1.79×10 ⁹	6.54×10 ⁸	1.29×10 ⁹	3.78×10 ⁴	1.29×10 ⁷	4.73×10 ⁷	5.39×10 ⁷	1.58×10 ⁴	5.39×10 ⁷	1.97×10 ⁴	-	
Ca(OH) ₂	-	-	3.69×10 ¹⁰	1.35×10 ¹⁰	-	-	-8.79×10 ⁴	-3.22×10 ⁴	-	-	-1.78×10 ⁵	-6.53×10 ⁴	-	
FeSO ₄	-	-	-6.61×10 ⁶	-2.42×10 ⁶	-	-	-1.59×10 ¹⁰	-5.82	-	-	-3.05×10 ¹⁰	-1.12×10 ¹⁰	-	
CO ₂	-	-	-	-	9.08×10 ⁴	3.32×10 ⁴	-9.08×10 ⁴	-3.32×10 ⁴	9.69×10 ⁴	3.55×10 ⁴	-9.69×10 ⁴	-3.55×10 ⁴	-	
Lighting	6.05×10 ¹⁰	1.58×10 ¹⁰	-	-	8.62×10 ³	2.25×10 ³	-	-	1.74×10 ³	4.55×10 ³	-	-	-	
Energy for N removal	-	-	-	-	-	-	-3.86×10 ³	-1.41×10 ³	-	-	-4.85×10 ⁴	-1.77×10 ⁷	-	
Energy for P removal	-	-	-	-	-	-	-1.32×10 ³	-4.85×10 ⁴	-	-	-1.66×10 ⁴	-6.08×10 ¹	-	
CO ₂ transfer	-	-	-	-	-	2.09×10 ⁴	7.63×10 ³	4.73×10 ³	1.73×10 ⁵	2.62×10 ³	9.58×10 ²	5.93×10 ⁴	2.17×10 ⁴	-
Energy for lighting	-	-	-	-	-	2.71×10 ¹⁰	7.08×10 ⁶	-	-	3.40×10 ¹⁰	8.89×10 ³	-	-	-
Harvesting	Flocculant Al ₂ (SO ₄) ₃	1.59×10 ⁷	5.81×10 ⁷	1.59×10 ⁷	5.81×10 ⁷	4.91×10 ³	1.08×10 ³	4.91×10 ³	1.80×10 ³	1.04×10 ³	3.81×10 ²	1.04×10 ³	3.81×10 ²	-
Energy for filtration and screening	-	-	-	-	-	6.69×10 ⁷	3.83×10 ⁷	6.69×10 ⁷	3.83×10 ⁷	8.40×10 ⁶	4.80×10 ⁶	8.40×10 ⁶	4.80×10 ⁶	-
Energy for conveyor	-	-	-	-	-	8.64×10 ³	3.95×10 ³	-	-	1.09×10 ³	4.96×10 ²	-	-	-
Drying	Energy for dryer	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell disruption	Energy for homogenizer	-	-	-	-	7.72×10 ⁶	4.42×10 ⁶	7.72×10 ⁶	4.42×10 ⁶	9.70×10 ⁵	5.54×10 ⁵	9.70×10 ⁵	5.54×10 ⁵	-
Extraction	Hexane	1.57×10 ¹⁰	5.73×10 ⁹	1.57×10 ¹⁰	5.73×10 ⁹	2.65×10 ⁶	9.69×10 ⁵	2.65×10 ⁶	4.35×10 ⁶	1.59×10 ⁶	4.35×10 ⁶	1.59×10 ⁶	-	-
Energy for agitator	-	-	-	-	-	1.56×10 ⁵	1.56×10 ⁵	1.56×10 ⁵	1.56×10 ⁵	1.96×10 ⁴	1.96×10 ⁴	1.96×10 ⁴	1.96×10 ⁴	-
Conversion	Methanol	1.12×10 ⁸	7.14×10 ⁸	1.12×10 ⁹	7.14×10 ⁸	1.81×10 ⁷	1.16×10 ⁷	1.81×10 ⁷	1.16×10 ⁷	1.16×10 ⁷	7.56×10 ⁴	7.56×10 ⁴	-	-
NaOH	2.72×10 ⁷	1.74×10 ⁷	2.72×10 ⁷	1.74×10 ⁷	1.49×10 ³	9.51×10 ³	1.49×10 ⁴	9.51×10 ³	1.01×10 ⁵	6.48×10 ³	1.01×10 ⁵	6.48×10 ³	-	-
Combustion	-	-	-	-	-	-	-	-	-	1.21×10 ³	1.21×10 ³	1.21×10 ³	1.21×10 ³	-

B.4 (continued).

Process	Resources	Ozone depletion (kg CFC-11 eq/FU)				Ecotoxicity (kg 2,4-D eq/FU)				Smog (kg NO _x eq/FU)			
		LS	HS	LW	HW	LS	HS	LW	HW	LS	HS	LW	HW
Cultivation													
HDPE	1.52	3.98×10 ⁻¹	1.52	3.98×10 ⁻¹	5.22×10 ⁹	1.37×10 ⁹	5.22×10 ⁹	1.37×10 ⁹	2.63×10 ⁷	6.89×10 ⁶	2.63×10 ⁷	6.89×10 ⁶	
Urea	6.76×10 ⁻²	2.06×10 ²	-	-	2.54×10 ⁹	7.74×10 ⁸	-	-	5.73×10 ⁶	1.75×10 ⁶	-	-	
Superphosphate	5.35×10	3.92×10	-	-	1.18×10 ⁹	8.66×10 ⁸	-	-	2.89×10 ⁶	2.11×10 ⁶	-	-	
KCl	1.56×10	4.57	1.56×10	5.72	1.41×10 ⁸	4.12×10 ⁷	1.41×10 ⁸	5.15×10 ⁷	3.64×10 ⁵	1.07×10 ⁵	3.64×10 ⁵	1.33×10 ⁵	
Ca(OH) ₂	-	-	-1.79×10 ²	-6.56×10 ⁻¹	-	-	-1.62×10 ⁹	-5.92×10 ⁸	-	-	-2.21×10 ⁶	-8.10×10 ⁵	
FeSO ₄	-	-	-1.02×10 ⁻¹	-3.72×10 ⁻²	-	-	-2.14×10 ⁵	-7.82×10 ⁴	-	-	-3.89×10 ²	-1.42×10 ²	
CO ₂	-	-	-	-	-	-	-	-	2.34×10 ⁶	8.57×10 ⁵	-2.34×10 ⁶	-8.57×10 ⁵	
Lighting													
5.90×10	1.54×10	-	-	-	1.78×10 ⁹	4.66×10 ⁸	-	-	2.16×10 ³	5.65×10 ⁴	-	-	
Energy for N removal	-	-	-	-	-	-	-	-	-	-	-9.36×10 ²	-3.43×10 ²	
Energy for P removal	-	-	-	-	-	-	-	-	-	-	-3.21×10 ²	-1.17×10 ²	
CO ₂ transfer	-	-	-	-	-	-	-	-	-	-	5.06×10 ¹⁰	1.15×10 ³	
Energy for lighting	-	-	-	-	-	-	-	-	6.57×10 ⁴	1.72×10 ⁴	-	-	
Flocculant Al₂(SO₄)₃	7.43×10 ⁻²	2.72×10 ²	7.43×10 ²	2.72×10 ⁻²	1.21×10 ⁷	4.41×10 ⁶	1.21×10 ⁷	4.41×10 ⁶	2.69×10 ³	9.86×10 ²	2.69×10 ³	9.86×10 ²	
Energy for filtration and screening	-	-	-	-	-	-	-	-	-	-	1.62×10 ⁵	9.28×10 ⁴	
Energy for conveyor dryer	-	-	-	-	-	-	-	-	2.10×10	9.58	-	-	
Drying													
Energy for homogenizer	-	-	-	-	-	-	-	-	1.87×10 ⁴	1.07×10 ⁴	1.87×10 ⁴	1.07×10 ⁴	
Extraction													
Hexane	7.35×10 ²	2.69×10 ²	2.69×10 ²	1.35×10 ⁹	4.93×10 ⁸	1.35×10 ⁹	4.93×10 ⁸	5.30×10 ⁶	1.94×10 ⁶	5.30×10 ⁶	1.94×10 ⁶		
Energy for agitator	-	-	-	-	-	-	-	-	3.79×10 ²	3.79×10 ²	3.79×10 ²	3.79×10 ²	
Conversion													
Methanol	1.11×10 ²	7.12×10	7.12×10	1.56×10 ⁸	9.96×10 ⁷	1.56×10 ⁸	9.96×10 ⁷	7.25×10 ⁵	4.64×10 ⁵	7.25×10 ⁵	4.64×10 ⁵		
NaOH	1.01	6.48×10 ⁻¹	6.48×10 ⁻¹	4.60×10 ⁵	2.94×10 ⁵	4.60×10 ⁵	2.94×10 ⁵	-	-	1.52×10 ⁴	2.37×10 ⁴	1.52×10 ⁴	
Combustion	-	-	-	-	-	-	-	-	1.89×10 ⁵	1.89×10 ⁵	1.89×10 ⁵	1.89×10 ⁵	

B.4 (continued)

Process	Resources	Non-renewable energy use (MJ primary/FU)			
		LS	HS	LW	HW
Cultivation					
HDPE	5.91×10 ¹¹	1.55×10 ¹¹	5.91×10 ¹¹	1.55×10 ¹¹	
Urea	9.19×10 ¹⁰	2.80×10 ¹⁰	-	-	
Superphosphate	1.08×10 ¹⁰	7.90×10 ⁸	-	-	
KCl	2.14×10 ⁹	6.25×10 ⁸	2.14×10 ⁹	7.82×10 ⁸	
Ca(OH) ₂	-	-	1.47×10 ¹⁰	-5.37×10 ⁹	
FeSO ₄	-	-	-5.25×10 ⁶	-1.92×10 ⁶	
CO ₂	1.86×10 ¹¹	6.82×10 ¹⁰	-	-	
Lighting	1.53×10 ⁹	3.99×10 ⁸	-	-	
Energy for N removal	-	-	-7.10×10 ⁹	-2.60×10 ⁹	
Energy for P removal	-	-	-2.43×10 ⁹	-8.91×10 ⁸	
CO ₂ transfer	3.83×10 ⁸	1.40×10 ⁸	8.69×10 ⁹	3.18×10 ⁹	
Energy for lighting	4.98×10 ¹¹	1.30×10 ¹¹	-	-	
Flocculant Al ₂ (SO ₄) ₃	1.71×10 ⁷	6.28×10 ⁶	1.71×10 ⁷	6.28×10 ⁶	
Harvesting					
Energy for filtration and screening	1.23×10 ¹²	7.04×10 ¹¹	1.23×10 ¹²	7.04×10 ¹¹	
Drying					
dryer	1.59×10 ⁸	7.27×10 ⁷	-	-	
Cell disruption					
Energy for homogenizer	1.42×10 ¹¹	8.12×10 ¹⁰	1.42×10 ¹¹	8.12×10 ¹⁰	
Extraction					
Hexane	8.94×10 ¹⁰	3.27×10 ¹⁰	8.94×10 ¹⁰	3.27×10 ¹⁰	
Energy for agitator	2.87×10 ⁹	2.87×10 ⁹	2.87×10 ⁹	2.87×10 ⁹	
Conversion					
Methanol	2.67×10 ¹⁰	1.71×10 ¹⁰	2.67×10 ¹⁰	1.71×10 ¹⁰	
NaOH	1.35×10 ⁸	8.66×10 ⁷	1.35×10 ⁸	8.66×10 ⁷	
Combustion	-	-	-	-	

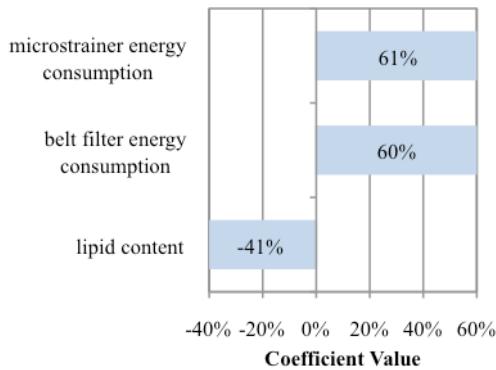
B.5 ANNEX 5 LIST OF MONTE CARLO SIMULATION MODEL INPUT VARIABLES.

Most of the input variables were fitted to Triangular Distribution with 90% significance, while some were fitted to Normal Distribution with 90% significance (in *italic*).

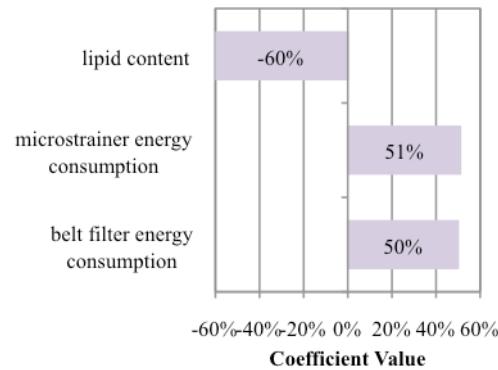
Variables	Values
Lipid Content	Average value \pm 10%
Harvesting Efficiency	Average value \pm 1%
Drying Efficiency	Average value \pm 1%
Conversion Efficiency	Average value \pm 1%
Number of units	Average value \pm 5%
System Lifetime	Average value \pm 5%
Material Thickness	Average value \pm 5%
Quantity of Freshwater/ Wastewater	Average value \pm 10%
Quantity of N, P and K	Average value \pm 10%
Quantity of N Removal Chemical (Ca(OH)_2)	Average value \pm 10%
Quantity of P precipitation Chemical ($\text{Fe(SO}_4\text{)}_3$)	Average value \pm 10%
<i>Energy Consumption for N/ P Removal</i>	Average value \pm 5%
Quantity of synthetic CO_2 / CO_2 from Flue gas	Average value \pm 10%
CO_2 Content in Flue gas	Average value \pm 5%
Energy Consumption for the Direct Injection of synthetic CO_2 / CO_2 from Flue Gas	Average value \pm 10%
Power of Compact Fluorescent Lamp (CFL)	Average value \pm 5%
Number of CFLs	Average value \pm 5%
Lifetime of CFLs	Average value \pm 10%
Algal Slurry Concentration	5% \pm 3%
(%Total Suspended Solid, %TSS)	
Algal Cake Concentration (%TSS)	20% \pm 5%
Quantity of Flocculant ($\text{Al}_2(\text{SO}_4)_3$)	Average value \pm 10%
<i>Energy Consumption of Microstrainer</i>	Average value \pm 5%
<i>Energy Consumption of Belt Filter</i>	Average value \pm 5%
Energy Consumption of Conveyor Dryer	Average value \pm 10%
Quantity of Solvent in Extraction Process (C_6H_{14})	Average value \pm 10%
Quantity of Alcohol in Conversion Process (CH_3OH)	Average value \pm 2%
Quantity of Catalyst in Conversion Process (NaOH)	Average value \pm 5%
Cycle of Catalyst Recycled	3 Cycles \pm 10%

B.6 ANNEX 6 THE TORNADO CORRELATION COEFFICIENTS OF THE LS, HS, LW AND HW MICROALGAL BIODIESEL PRODUCTION CONDITIONS.

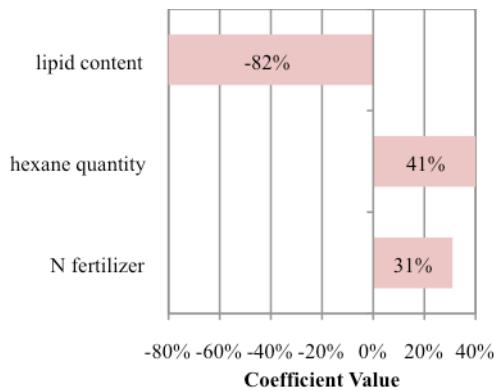
The four conditions are a low-efficiency production with synthetic resources (LS), high-efficiency production with synthetic resources (HS), low-efficiency production with natural and waste resources (LW) and high-efficiency production with natural and waste resources (HW).



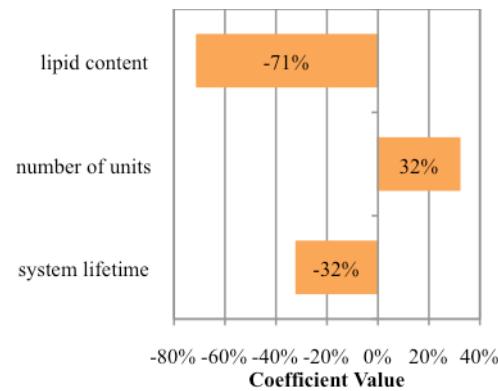
(a) GWP of LS condition



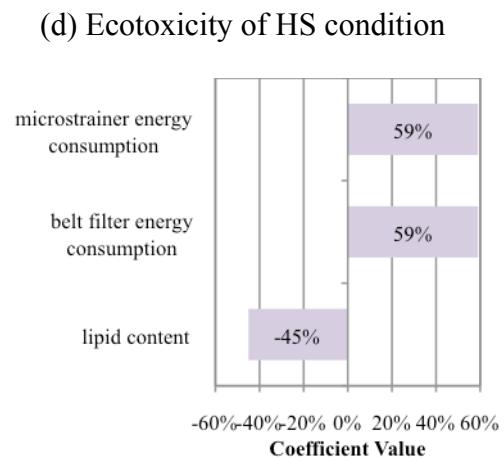
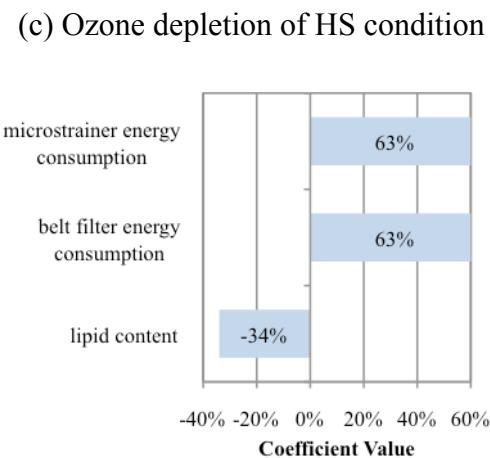
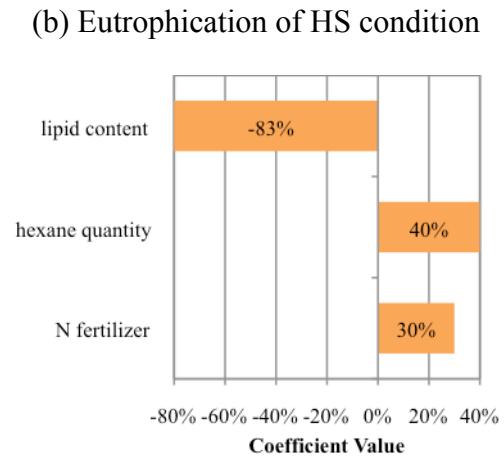
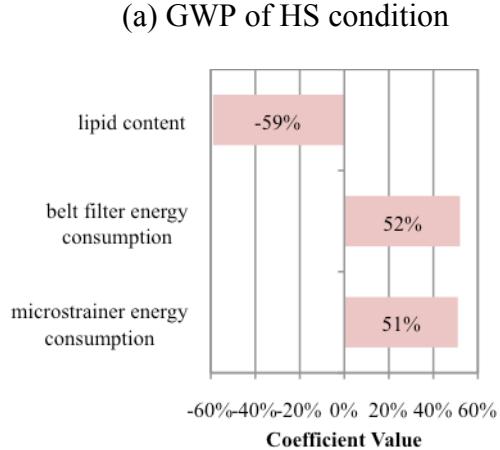
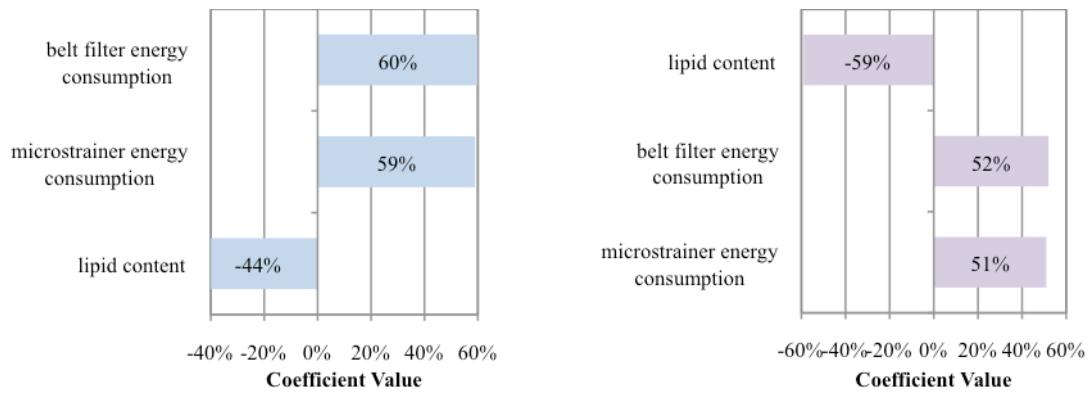
(b) Eutrophication of LS condition



(c) Ozone depletion of LS condition

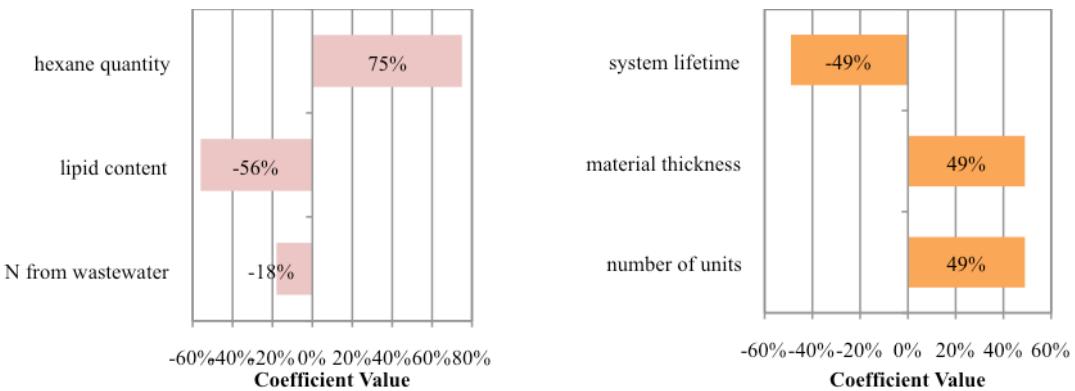


(d) Ecotoxicity of LS condition

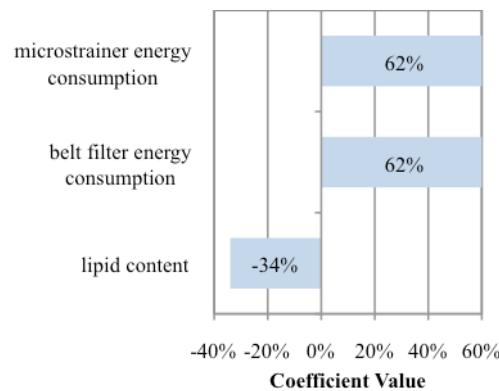


(a) GWP of LW condition

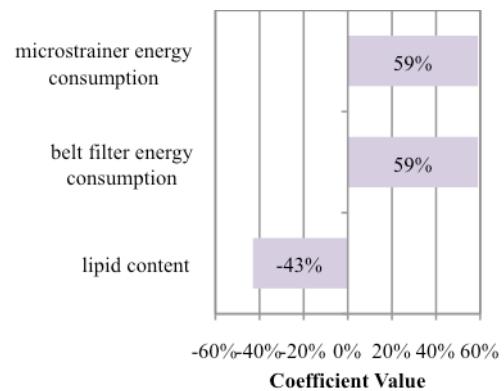
(b) Eutrophication of LW condition



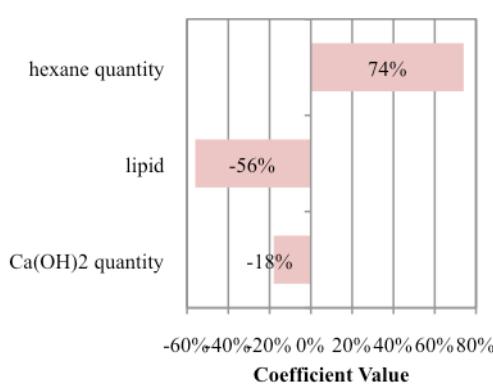
(c) Ozone depletion of LW condition



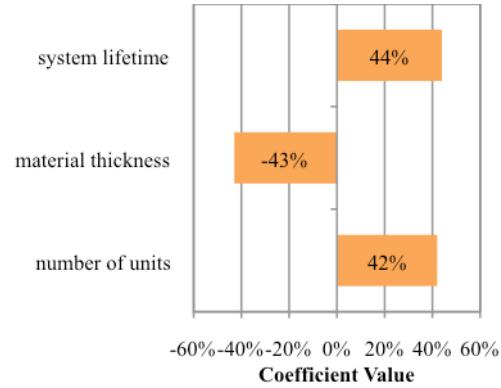
(d) Ecotoxicity of LW condition



(a) GWP of HW condition



(b) Eutrophication of HW condition



(c) Ozone depletion of HW condition

(d) Ecotoxicity of HW condition

B.7 ANNEX 7 TORNADO CORRELATION COEFFICIENT OF THE FOUR MICROALGAL BIODIESEL PRODUCTION CONDITIONS.

The four conditions are a low-efficiency production with synthetic resources (LS), high-efficiency production with synthetic resources (HS), low-efficiency production with natural and waste resources (LW) and high-efficiency production with natural and waste resources (HW).

Impact category	Production condition	Process	Parameter	Tornado correlation coefficient
Global warming potential	LS	Cultivation, Harvesting	Microstrainer energy consumption	61%
			Belt filter energy consumption	60%
			Lipid content	-41%
	HS	Cultivation, Harvesting	Belt filter energy consumption	60%
			Microstrainer energy consumption	59%
			Lipid content	-43%
	LW	Cultivation, Drying	Microstrainer energy consumption	64%
			Belt filter energy consumption	51%
			Lipid content	-36%
	HW	Cultivation, Drying	Microstrainer energy consumption	63%
			Belt filter energy consumption	62%
			Lipid content	-37%
Eutrophication	LS	Cultivation, Harvesting	Lipid content	-80%
			Microstrainer energy consumption	51%
			Belt filter energy consumption	50%
	HS	Cultivation, Harvesting	Lipid content	-58%
			Microstrainer energy consumption	52%
			Belt filter energy consumption	52%
	LW	Cultivation, Drying	Microstrainer energy consumption	60%
			Belt filter energy consumption	59%
			Lipid content	-44%
	HW	Cultivation, Drying	Microstrainer energy consumption	60%
			Belt filter energy consumption	59%
			Lipid content	-45%
Ozone depletion	LS	Cultivation, Extraction	Lipid content	-82%
			Hexane quantity	41%
			N fertilizer quantity	31%
	HS	Cultivation, Extraction	Lipid content	-83%
			Hexane quantity	40%
			N fertilizer quantity	30%
	LW	Cultivation, Extraction	Hexane quantity	75%
			Lipid content	-58%
			N quantity from wastewater	-18%
	HW	Cultivation, Extraction	Hexane quantity	74%
			Lipid content	-56%
			Ca(OH) ₂ quantity (N-removal chemical)	-18%
Ecotoxicity	LS	Cultivation	Lipid content	-71%
			Number of units	32%
			System lifetime	-32%
	HS	Cultivation	Lipid content	-81%
			P fertilizer quantity	25%
			N fertilizer quantity	23%
	LW	Cultivation	System lifetime	-49%
			Material thickness	48%
			Number of units	48%
	HW	Cultivation	Number of units	44%
			Material thickness	44%
			System lifetime	-43%

B.7 (continued)

Impact category	Production condition	Process	Parameter	Tornado correlation coefficient
Acidification	LS	Cultivation, Harvesting	Microstrainer energy consumption	61%
			Belt filter energy consumption	60%
			Lipid content	-41%
	HS	Cultivation, Harvesting	Belt filter energy consumption	60%
			Microstrainer energy consumption	59%
	LW	Cultivation, Drying	Lipid content	-43%
			Microstrainer energy consumption	62%
			Belt filter energy consumption	62%
	HW	Cultivation, Drying	Lipid content	-36%
			Microstrainer energy consumption	62%
			Belt filter energy consumption	62%
			Lipid content	-35%
Carcinogenics	LS	Cultivation, Harvesting	Belt filter energy consumption	-84%
			P fertilizer quantity	32%
			N fertilizer quantity	29%
	HS	Cultivation	Lipid content	-85%
			P fertilizer quantity	33%
			N fertilizer quantity	27%
	LW	Cultivation, Harvesting, Extraction	Hexane quantity	47%
			N from wastewater	-39%
			Ca(OH) ₂ quantity	-38%
	HW	Cultivation, Harvesting, Extraction	Hexane quantity	54%
			Ca(OH) ₂ quantity	-43%
			N from wastewater	-42%
Non-carcinogenics	LS	Cultivation	Lipid content	-67%
			P fertilizer	44%
			System lifetime	-32%
	HS	Cultivation	Material thickness	-76%
			P fertilizer quantity	46%
			N fertilizer quantity	19%
	LW	Cultivation, Extraction	Hexane quantity	75%
			Lipid content	-56%
			N quantity from wastewater	-18%
	HW	Cultivation	System lifetime	-48%
			Material thickness	48%
			Number of units	47%
Respiratory Effects	LS	Cultivation, Harvesting	Microstrainer energy consumption	61%
			Belt filter energy consumption	60%
			Lipid content	-41%
	HS	Cultivation, Harvesting	Microstrainer energy consumption	59%
			Belt filter energy consumption	59%
			Lipid content	-45%
	LW	Cultivation, Harvesting	Microstrainer energy consumption	62%
			Belt filter energy consumption	62%
			Lipid content	-35%
	HW	Cultivation, Harvesting	Microstrainer energy consumption	62%
			Belt filter energy consumption	61%
			Lipid content	-36%

B.7 (continued)

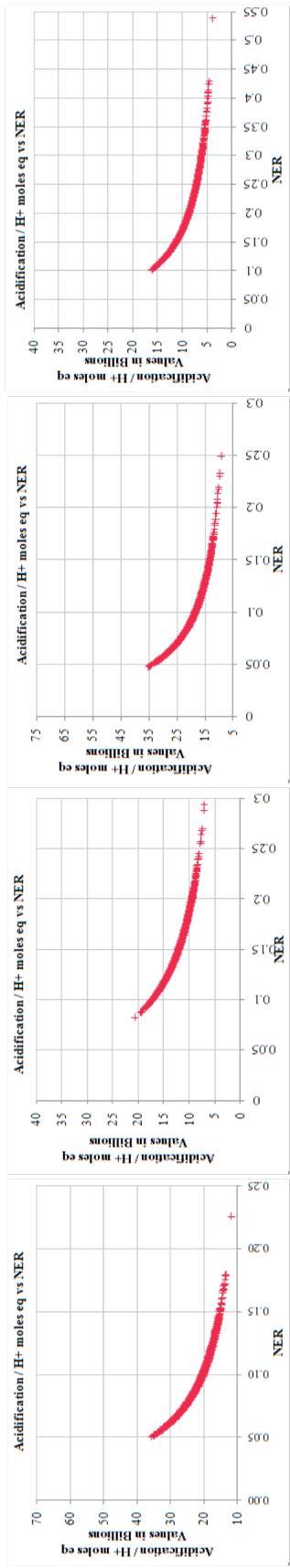
Impact category	Production condition	Process	Parameter	Tornado correlation coefficient
Smog	LS	Cultivation	Lipid content	-55%
			System lifetime	-44%
			Material thickness	44%
	HS	Cultivation	Lipid content	-70%
			Material thickness	33%
			Number of units	33%
	LW	Cultivation	System lifetime	-55%
			Material thickness	54%
			Number of units	54%
	HW	Cultivation	System lifetime	-52%
			Material thickness	52%
			Number of units	51%
Non-renewable Energy Use (NREU)	LS	Cultivation, Harvesting	Microtrainer energy consumption	59%
			Belt filter energy consumption	59%
			Lipid content	-43%
	HS	Cultivation, Harvesting	Microtrainer energy consumption	59%
			Belt filter energy consumption	58%
			Lipid content	-46%
	LW	Cultivation, Harvesting	Microtrainer energy consumption	63%
			Belt filter energy consumption	62%
			Lipid content	-34%
	HW	Cultivation, Harvesting	Microtrainer energy consumption	62%
			Belt filter energy consumption	62%
			Lipid content	-34%

B.8 ANNEX 8 LIST OF SUGGESTED SENSITIVITY ANALYSIS SCENARIOS.

Scenario (SA)	Parameters ^a	Description of scenarios	Environmental impact evaluated	Base case (BC) ^b
SA1.1	Energy consumptions of microstrainer and belt filter	Parameter reduced by 50% from the base cases	Acidification Respiratory effects NREU Carcinogenics	LS, HS, LW and HW LS, HS, LW and HW LS, HS, LW and HW LS
SA2.1	Energy consumptions of microstrainer and belt filter	Parameter increased by 50% from the base cases	Acidification Respiratory effects NREU	LS, HS, LW and HW LS, HS, LW and HW LS, HS, LW and HW
SA3.1	Lipid content of microalgae	Parameter reduced by 50% from the base cases	Carcinogenics Carcinogenics Non-carcinogenics Smog	LS HS LS LS and HS
SA4.1	Lipid content of microalgae	Parameter increased by 50% from the base cases	Carcinogenics Non-carcinogenics Smog	HS LS LS and HS
SA5.1	Quantity of hexane used during extraction	Parameter reduced by 50% from the base cases	Carcinogenics Non-carcinogenics	LW and HW LW
SA6.1	Quantity of hexane used during extraction	Parameter increased by 50% from the base cases	Carcinogenics Non-carcinogenics	LW and HW LW
SA7.1	Service lifetime of PBR	Parameter reduced by 50% from the base cases	Non-carcinogenics	HW
SA8.1	Service lifetime of PBR	Parameter increased by 50% from the base cases	Smog Non-carcinogenics	LW and HW HW
SA9	Thickness of PBR construction material	Parameter increased by 50% from the base cases	Non-carcinogenics	HS
SA10	Thickness of PBR construction material	Parameter reduced by 50% from the base cases	Non-carcinogenics	HS

B.9

ANNEX 9 EXAMPLES OF SENSITIVITY ANALYSIS OF THE SUGGESTED SCENARIOS, AS LISTED IN APPENDIX B.8

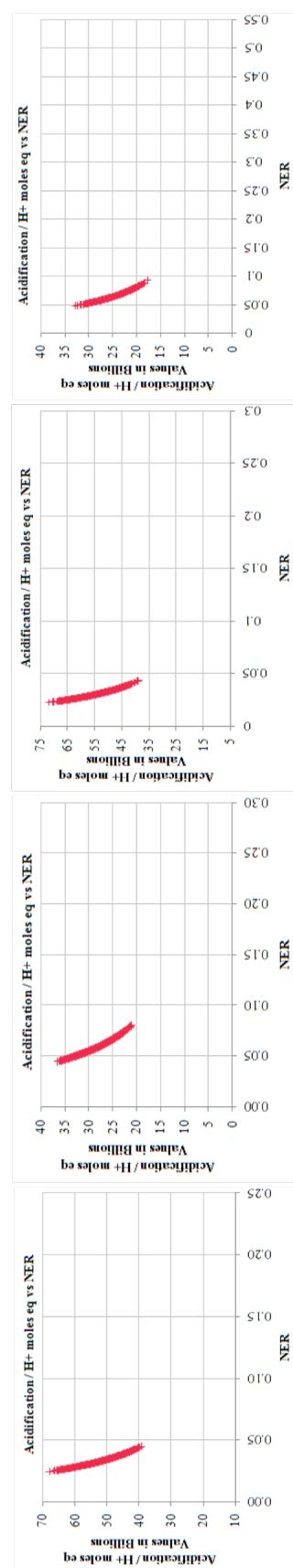


SA1.1-LS

SA1.1-HS

SA1.1-HW

SA1.1-LW

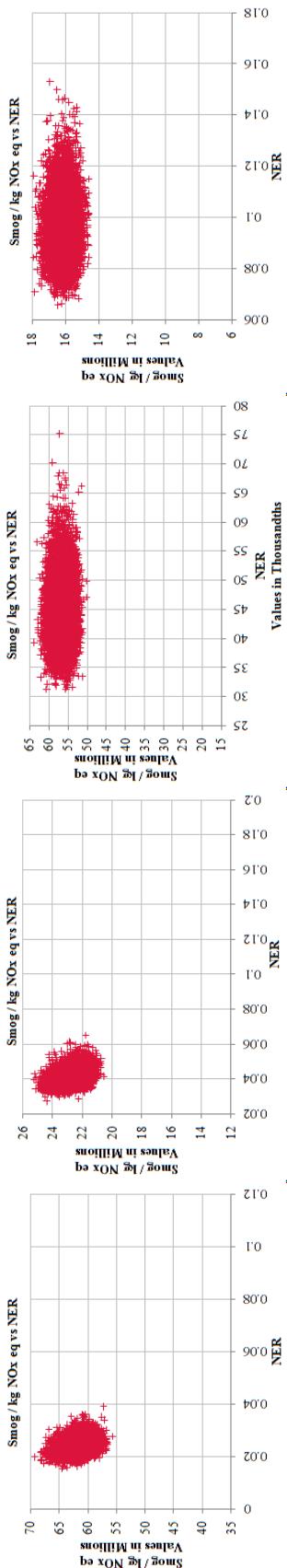


SA2.1-LS

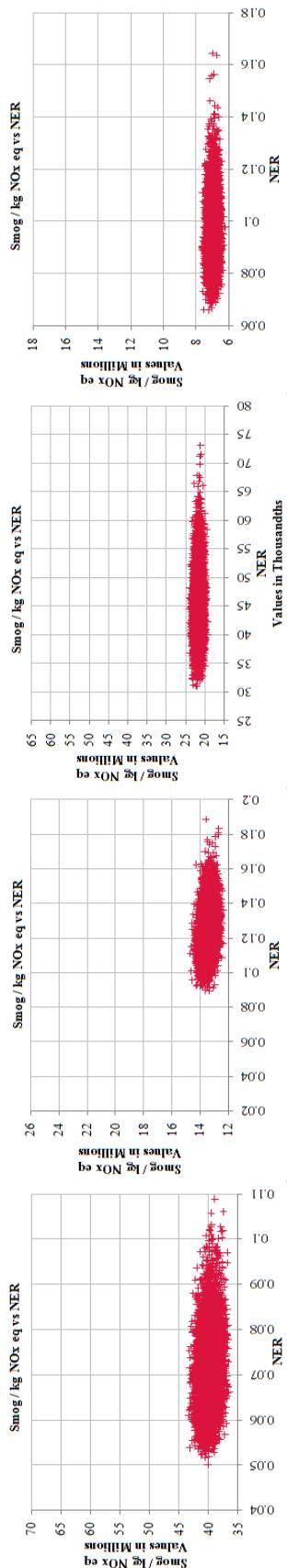
SA2.1-HS

SA2.1-HW

Acidification



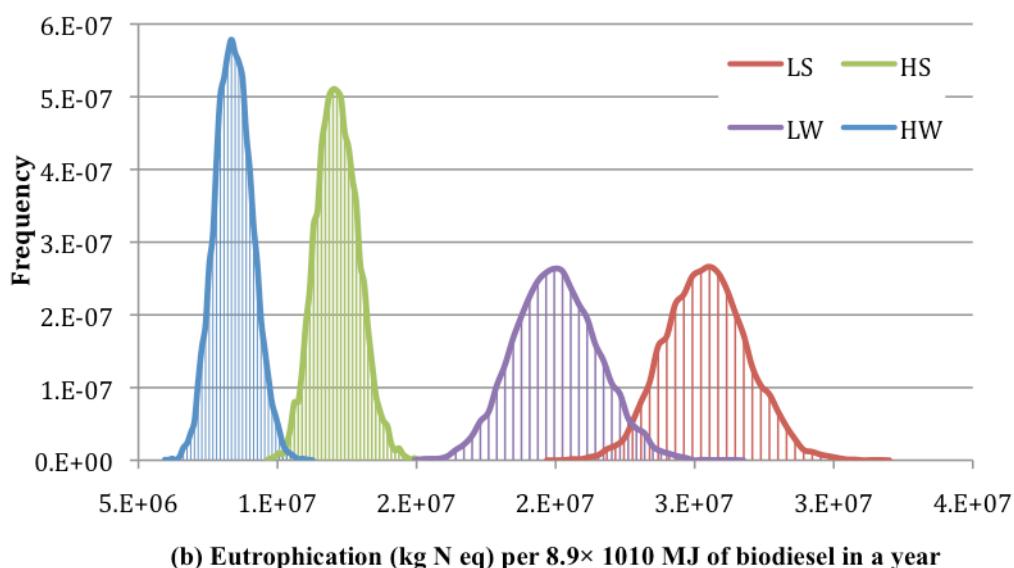
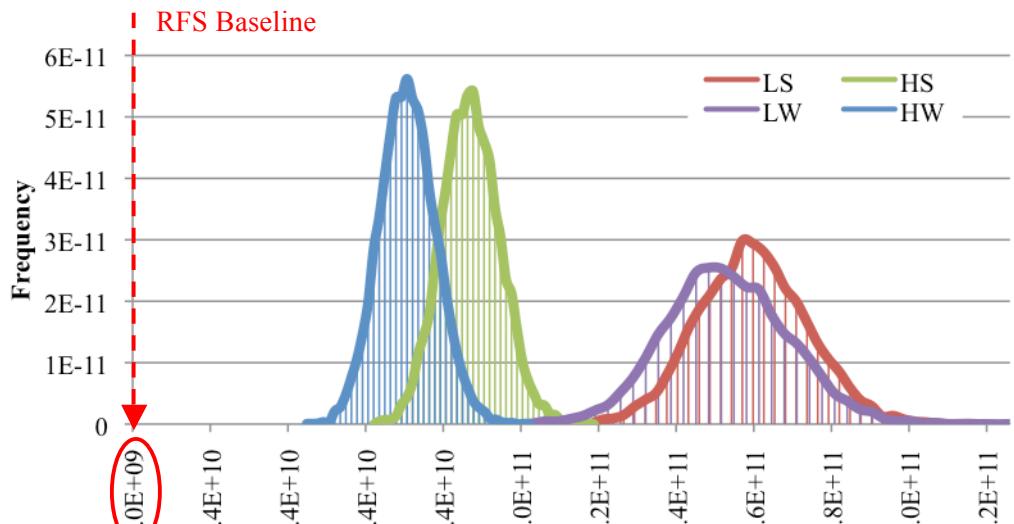
SA7.1-HW

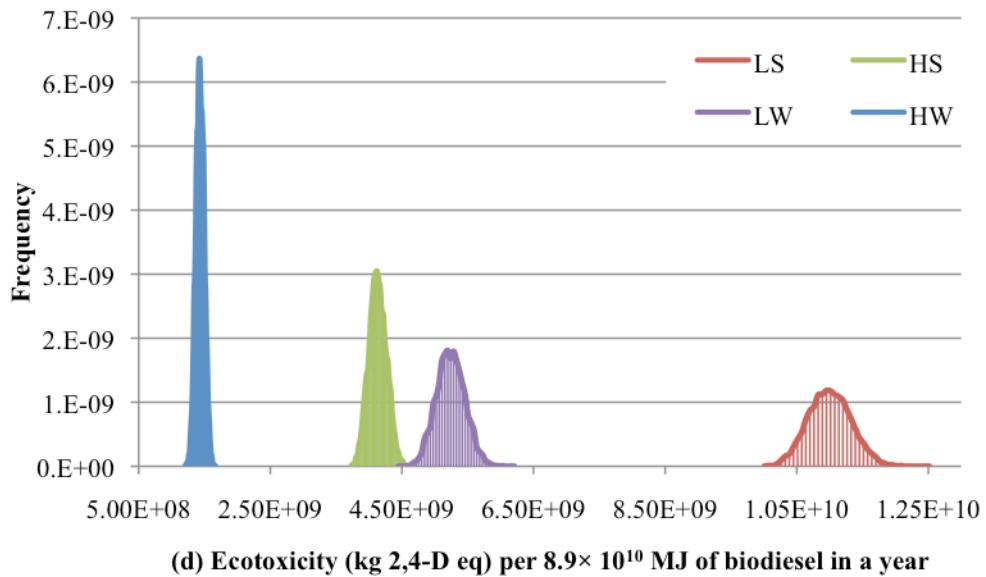
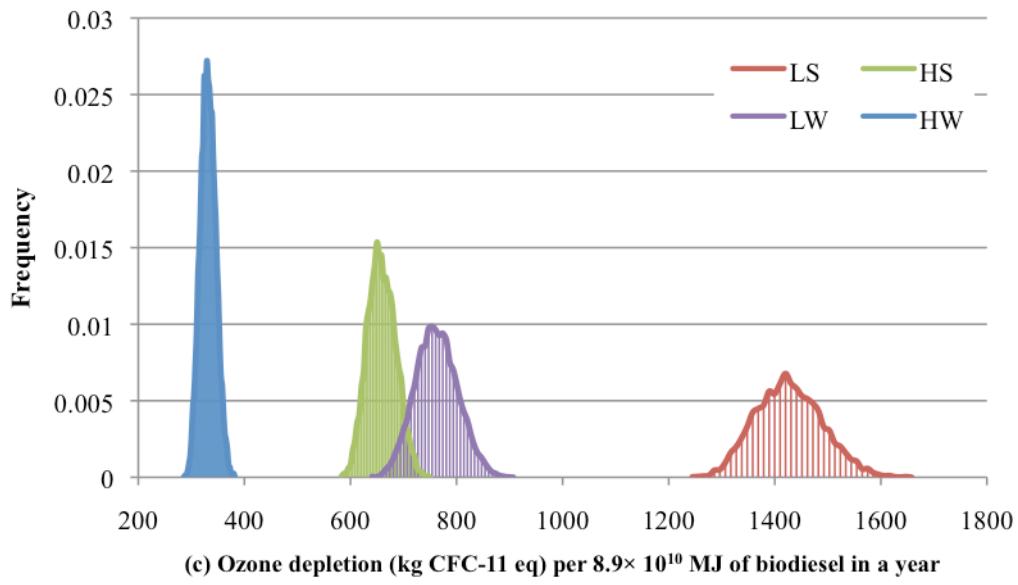


Smog

B.10 ANNEX 10 PROBABILITY DISTRIBUTIONS OF THE GWP, EUTROPHICATION, OZONE DEPLETION AND ECOTOXICITY POTENTIALS FROM THE FOUR MICROALGAL BIODIESEL PRODUCTION CONDITIONS.

The impacts are from low-efficiency production with synthetic resources (LS), high-efficiency production with synthetic resources (HS), low-efficiency production with natural and waste resources (LW) and high-efficiency production with natural and waste resources (HW). Their minimum, mean and maximum impact potentials and the best-fit distribution to the results are reported.





B.11 ANNEX 11 PROBABILITY DISTRIBUTION OF ENVIRONMENTAL IMPACTS AND NET ENERGY RATIO (NER)

The impacts are from low-efficiency production with synthetic resources (LS), high-efficiency production with synthetic resources (HS), low-efficiency production with natural and waste resources (LW) and high-efficiency production with natural and waste resources (HW). Their minimum, mean and maximum impact potentials, and the best-fit distribution to the results are reported.

Environmental Impact	Production Scenario	Mean	Minimum	Maximum	95% Confidence Interval		Probability Distribution
					Lower limit	Upper limit	
GWP (kg CO ₂ eq/ functional unit)	LS	1.65×10^{11}	1.11×10^{11}	2.22×10^{11}	1.38×10^{11}	1.93×10^{11}	Inverse Gaussian
	HS	9.18×10^{10}	6.62×10^{10}	1.23×10^{11}	7.75×10^{10}	1.07×10^{11}	Inverse Gaussian
	LW	1.59×10^{11}	1.04×10^{11}	2.34×10^{11}	1.28×10^{11}	1.90×10^{11}	Log normal
	HW	7.56×10^{10}	4.89×10^{10}	1.06×10^{11}	6.16×10^{10}	9.02×10^{10}	Log normal
Eutrophication (kg N eq/ functional unit)	LS	2.56×10^7	1.97×10^7	3.20×10^7	2.28×10^7	2.86×10^7	Log normal
	HS	1.22×10^7	9.63×10^6	1.52×10^7	1.08×10^7	1.38×10^7	Inverse Gaussian
	LW	2.02×10^7	1.49×10^7	2.67×10^7	1.73×10^7	2.32×10^7	Beta
	HW	8.50×10^6	5.95×10^6	1.13×10^7	7.21×10^6	9.88×10^6	Inverse Gaussian
Ozone depletion (kg CFC-11 eq/ functional unit)	LS	1.45×10^3	1.24×10^3	1.65×10^3	1.32×10^3	1.56×10^3	Beta
	HS	6.67×10^2	5.83×10^2	7.46×10^2	6.12×10^2	7.14×10^2	Beta
	LW	7.74×10^2	6.41×10^2	9.00×10^2	6.90×10^2	8.46×10^2	Beta
	HW	3.34×10^2	2.86×10^2	3.80×10^2	3.05×10^2	3.62×10^2	Beta
Ecotoxicity (2,4-D eq/ functional unit)	LS	1.13×10^{10}	1.00×10^{10}	1.25×10^{10}	1.04×10^{10}	1.17×10^{10}	Beta
	HS	4.19×10^9	3.74×10^9	4.62×10^9	3.89×10^9	4.41×10^9	Beta
	LW	5.33×10^9	4.54×10^9	6.17×10^9	4.85×10^9	5.69×10^9	Inverse Gaussian
	HW	1.44×10^9	1.22×10^9	1.65×10^9	1.32×10^9	1.56×10^9	Inverse Gaussian
Net Energy Ratio (NER)	LS	0.05	0.03	0.08	0.04	0.06	Log normal
	HS	0.08	0.06	0.12	0.07	0.10	Inverse Gaussian
	LW	0.05	0.03	0.07	0.04	0.06	Lognormal
	HW	0.10	0.06	0.16	0.08	0.12	Pearson

B.12 ANNEX 12 LIFE-CYCLE IMPACTS AND NET ENERGY RATIO (NER) FROM THE FOUR MICROALGAL BIODIESEL PRODUCTION

Global warming potential (GWP), eutrophication, ozone depletion and ecotoxicity potentials from the four base-case microalgal diesel production conditions, which are the production with lower-efficiency production with synthetic resources (BC-LS), high-efficiency production with synthetic resources (BC-HS), lower-efficiency production with natural and waste resources (BC-LW) and high-efficiency production with natural and waste resources (BC-HW).

Base case	GWP kg CO ₂ eq/FU	Eutrophication kg N eq/FU	Ozone depletion kg CFC-11 eq/FU	Ecotoxicity kg 2,4-D eq/FU	Energy required MJ/FU	NER
BC-LS	1.1 × 10 ¹¹ - 2.2 × 10 ¹¹	2.0 × 10 ⁷ - 3.2 × 10 ⁷	1.2 × 10 ³ - 1.7 × 10 ³	1.0 × 10 ¹⁰ - 1.3 × 10 ¹⁰	1.2 × 10 ¹² - 2.7 × 10 ¹²	0.03 - 0.08
BC-HS	6.6 × 10 ¹⁰ - 1.2 × 10 ¹¹	9.6 × 10 ⁶ - 1.5 × 10 ⁷	5.8 × 10 ² - 7.5 × 10 ²	3.7 × 10 ⁹ - 4.6 × 10 ⁹	7.5 × 10 ¹¹ - 1.6 × 10 ¹²	0.06 - 0.12
BC-LW	1.0 × 10 ¹¹ - 2.3 × 10 ¹¹	1.5 × 10 ⁷ - 2.7 × 10 ⁷	6.4 × 10 ² - 9.0 × 10 ²	4.5 × 10 ⁹ - 6.2 × 10 ⁹	1.3 × 10 ¹² - 3.0 × 10 ¹²	0.03 - 0.07
BC-HW	4.9 × 10 ¹⁰ - 1.1 × 10 ¹¹	6.0 × 10 ⁶ - 1.1 × 10 ⁷	2.9 × 10 ² - 3.8 × 10 ²	1.2 × 10 ⁹ - 1.7 × 10 ⁹	5.9 × 10 ¹² - 1.4 × 10 ¹²	0.06 - 0.16

B.13 ANNEX 13 LIFE-CYCLE IMPACTS AND NET ENERGY RATIO (NER) FROM THE EIGHT MICROALGAL BIODIESEL PRODUCTION SCENARIOS

Scenarios 1 and 2 (SA1 and SA2) consume higher and lower energy in harvesting process than the base case by 50%, respectively.

Scenarios 3 and 4 (SA3 and SA4) have higher and lower lipid content in microalgae than the base case by 50%, respectively.

Scenarios 5 and 6 (SA5 and SA6) consume higher and lower hexane in extraction process than the base case by 50%, respectively.

Scenarios 7 and 8 (SA7 and SA8) have longer and shorter system lifetime of photobioreactor than the base case by 50%, respectively.

Scenario	GWP kg CO ₂ eq/ FU	Eutrophication kg N eq/ FU	Ozone depletion kg CFC-11 eq/ FU	Ecotoxicity kg 2,4-D eq/ FU	Net Energy Ratio (NER)	Energy required MJ/ FU
SA1-LS	6.2×10^{10} - 1.6×10^{11}	-	-	-	0.05 - 0.19	4.7×10^{11} - 1.9×10^{12}
SA2-LS	1.6×10^{11} - 2.8×10^{11}	-	-	-	0.03 - 0.05	1.9×10^{12} - 3.6×10^{12}
SA3-LS	-	3.6×10^7 - 6.0×10^7	2.3×10^3 - 3.1×10^3	1.5×10^{10} - 1.9×10^{10}	0.02 - 0.04	2.3×10^{12} - 5.4×10^{12}
SA4-LS	-	1.5×10^7 - 2.2×10^7	8.8×10^2 - 1.2×10^3	8.3×10^9 - 1.0×10^{10}	0.05 - 0.11	8.4×10^{11} - 1.8×10^{12}
SA1-HS	2.7×10^{10} - 8.3×10^{10}	-	-	-	0.09 - 0.37	2.4×10^{11} - 1.1×10^{12}
SA2-HS	9.1×10^{10} - 1.5×10^{11}	-	-	-	0.05 - 0.08	1.2×10^{12} - 1.9×10^{12}
SA3-HS	-	1.8×10^7 - 2.9×10^7	1.0×10^3 - 1.4×10^3	6.0×10^9 - 7.7×10^9	0.03 - 0.06	1.4×10^{12} - 3.0×10^{12}
SA4-HS	-	6.6×10^6 - 2.2×10^7	4.3×10^2 - 5.4×10^2	3.0×10^9 - 3.6×10^9	0.09 - 0.18	5.0×10^{11} - 10.0×10^{11}
SA1-LW	3.8×10^{10} - 1.5×10^{11}	8.9×10^6 - 2.0×10^7	-	-	0.05 - 0.26	3.4×10^{11} - 1.9×10^{12}
SA2-LW	1.6×10^{11} - 3.0×10^{11}	2.0×10^7 - 3.2×10^7	-	-	0.02 - 0.04	2.1×10^{12} - 4.0×10^{12}
SA5-LW	-	-	3.3×10^2 - 4.7×10^2	-	0.03 - 0.07	1.2×10^{12} - 2.9×10^{12}
SA6-LW	-	-	9.6×10^2 - 1.4×10^3	-	0.03 - 0.07	1.2×10^{12} - 3.0×10^{12}
SA7-LW	-	-	-	9.2×10^9 - 1.2×10^{10}	0.03 - 0.08	1.2×10^{12} - 3.0×10^{12}
SA8-LW	-	-	-	2.8×10^9 - 4.1×10^9	0.03 - 0.07	1.2×10^{12} - 3.1×10^{12}
SA1-HW	1.4×10^{10} - 6.5×10^{10}	3.5×10^6 - 8.2×10^6	-	-	0.10 - 0.64	1.4×10^{11} - 8.7×10^{11}
SA2-HW	7.1×10^{10} - 1.4×10^{11}	8.7×10^6 - 1.4×10^7	-	-	0.05 - 0.09	9.7×10^{11} - 2.0×10^{12}
SA5-HW	-	-	1.8×10^2 - 2.2×10^2	-	0.06 - 0.18	5.0×10^{11} - 1.4×10^{12}
SA6-HW	-	-	4.0×10^3 - 5.4×10^2	-	0.06 - 0.15	6.0×10^{11} - 1.4×10^{12}
SA7-HW	-	-	-	2.5×10^9 - 3.2×10^9	0.07 - 0.15	6.0×10^{11} - 1.4×10^{12}
SA8-HW	-	-	-	8.1×10^8 - 1.2×10^9	0.07 - 0.15	6.0×10^{11} - 1.4×10^{12}

APPENDIX C

SUPPORTING INFORMATION FOR THE STUDY RE-ENVISIONING THE RENEWABLE FUEL STANDARD TO MINIMIZE UNINTENDED CONSEQUENCES

C.1 INPUTS FOR THE PROCESS LCA MODEL OF MICROALGAL DIESEL WITH MONTE CARLO UNCERTAINTY ANALYSIS AND THE INVENTORIES OF RESOURCES FOR MICROALGAL DIESEL PRODUCTION.

Process	Resource	Input	Reference	Database
Strain Selection	Energy content	41 MJ kg ⁻¹ of biodiesel	(Huang et al., 2010)	
	Lipid content	70%	(Chisti, 2007; Mata et al., 2010)	
	Volumetric productivity	350 g m ⁻² day ⁻¹	(Lardon et al., 2009; Rodolfi et al., 2009; Shen et al., 2010)	
Cultivation	Surface area: volume ratio	100 m ⁻¹	(Posten, 2009)	
	Unit volume	10 m ³	(Carvalho et al., 2006a)	
	Number of unit	3,150,000 units system ⁻¹	Calculation	
	System lifetime	15 years	Assumption	
	PBR material	HDPE	(Carvalho et al., 2006a; Soratana & Landis, 2011)	ecoinvent data v2.0 (Europe) (Frischknecht et al., 2007)
Water	Water	Wastewater		
	Nutrient: Nitrogen	Wastewater (avoided Ca(OH) ₂) 0.3 kg kg ⁻¹ biomass	(Collet et al., 2011; Greenwell et al., 2010; Lardon et al., 2009; Tchobanoglous et al., 2003)	ETH-ESU 96 (Frischknecht & Jungbluth, 2004)
	Nutrient: Phosphorus	Wastewater (avoided FeSO ₄ 0.018 kg kg ⁻¹ biomass)	(Collet et al., 2011; Lardon et al., 2009; Tchobanoglous et al., 2003)	ecoinvent data v2.0 (Europe) (Frischknecht et al., 2007)
	Nutrient: Potassium	Potassium chloride (0.02 kg kg ⁻¹ biomass)	(Collet et al., 2011; Lardon et al., 2009)	ecoinvent data v2.0 (Europe)(Frischknecht et al., 2007)
	Energy for N removal	14 MJ kg ⁻¹ nutrient removed	(Maurer et al., 2003)	IDEMAT (Delft University of Technology, 2001)
	Energy for P removal	24 MJ kg ⁻¹ nutrient removed	(Maurer et al., 2003)	IDEMAT (Delft University of Technology, 2001)
	CO ₂	CO ₂ from flue gas (14% of CO ₂ by wt.)	(Kadam, 2002; Mata et al., 2010; Posten, 2009; Schenk et al., 2008)	
	Energy for CO ₂ feeding	0.86 MJ kg ⁻¹ biomass	(Kadam, 2002; Kadam, 2001; Lardon et al., 2009; Mata et al., 2010; Posten, 2009; Schenk et al., 2008)	IDEMAT (Delft University of Technology, 2001)

C.1 (continued)

Process	Resource	Input	Reference	Database
	Light	Natural light	(Janssen, 2002)	
Harvesting/ Dewatering	Efficiency	90%	(Lardon et al., 2009)	
	Flocculation: Flocculant	$\text{Al}_2(\text{SO}_4)_3$ (0.17 kg m ⁻³)	Calculation	ecoinvent data v2.0 (Europe) (Frischknecht et al., 2007)
	Microstrainer/ belt filter	0.2/ 0.45 kWh m ⁻³	(Shelef et al., 1984)	IDEMAT (Delft University of Technology, 2001)
Drying	Drying method	Sun dry	(Greenwell et al., 2010; Mata et al., 2010)	
Cell Disruption	Homogenizer	1.5 kWh m ⁻³	(Shelef et al., 1984)	IDEMAT (Delft University of Technology, 2001)
Extraction	Efficiency	98%	(Mata et al., 2010)	
	Solvent extraction: Solvent	Hexane	(Lardon et al., 2009; Vijayaraghavan & Hemanathan, 2009)	ecoinvent data v2.0 (Europe) (Frischknecht et al., 2007)
	Solvent quantity	0.0002 m ³ kg ⁻¹ microalgae	(Lardon et al., 2009; Vijayaraghavan & Hemanathan, 2009)	
Conversion	Efficiency	87%	(Vyas et al., 2010)	
	Conversion method	Transesterification	(Vyas et al., 2010)	
	Alcohol	Methanol	(Greenwell et al., 2010; Mata et al., 2010; Sander & Murthy, 2010; Vijayaraghavan & Hemanathan, 2009)	ecoinvent data v2.0 (Global) (Frischknecht et al., 2007)
	Alcohol quantity	20% by vol of microalga oil	(Tickell & Tickell, 2003)	
	Catalyst	NaOH	(Greenwell et al., 2010; Reijnders & Huijbregts, 2009; Zebib, 2008)	ecoinvent data v2.0 (North America) (Frischknecht et al., 2007)
	Catalyst:oil molar ratio	4 kg m ⁻³ microalgal oil	(Vijayaraghavan & Hemanathan, 2009)	
Combustion	CO ₂ emission	0.08 kg CO ₂ MJ ⁻¹	(Hermann, 2010)	
	N emission	1.4×10^{-6} kg N MJ ⁻¹		
	NO _x emission	2.1×10^{-6} kg NO _x MJ ⁻¹		

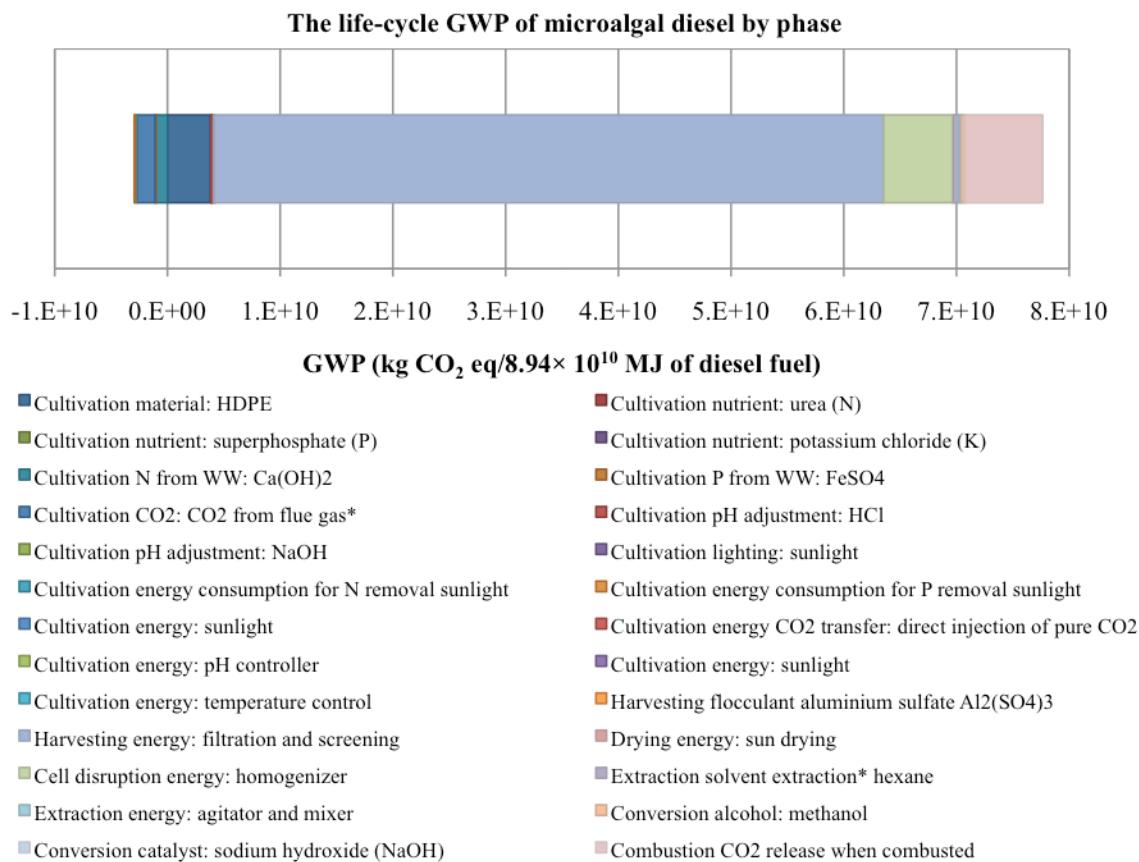
C.2 LIST OF INPUT VARIABLES OF LCA MODEL WITH MONTE CARLO UNCERTAINTY SIMULATION.

Most of the input variables were fitted to Triangular Distribution with 90% significance, while some were fitted to Normal Distribution with 90% significance (in italic).

Variables	Values
Lipid Content	Average value $\pm 10\%$
Harvesting Efficiency	Average value $\pm 1\%$
Drying Efficiency	Average value $\pm 1\%$
Conversion Efficiency	Average value $\pm 1\%$
Number of units	Average value $\pm 5\%$
System Lifetime	Average value $\pm 5\%$
Material Thickness	Average value $\pm 5\%$
Quantity of Wastewater	Average value $\pm 10\%$
Quantity of K	Average value $\pm 10\%$
Quantity of N Removal Chemical ($\text{Ca}(\text{OH})_2$)	Average value $\pm 10\%$
Quantity of P precipitation Chemical ($\text{Fe}(\text{SO}_4)_2$)	Average value $\pm 10\%$
<i>Energy Consumption for N/P Removal</i>	Average value $\pm 5\%$
Quantity of synthetic CO_2 / CO_2 from Flue gas	Average value $\pm 10\%$
CO_2 Content in Flue gas	Average value $\pm 5\%$
Energy Consumption for the Direct Injection of CO_2 from Flue Gas	Average value $\pm 10\%$
Algal Slurry Concentration (%Total Suspended Solid, %TSS)	$5\% \pm 3\%$
Algal Cake Concentration (%TSS)	$20\% \pm 5\%$
Quantity of Flocculant ($\text{Al}_2(\text{SO}_4)_3$)	Average value $\pm 10\%$
<i>Energy Consumption of Microstrainer</i>	Average value $\pm 5\%$
<i>Energy Consumption of Belt Filter</i>	Average value $\pm 5\%$
Energy Consumption of Conveyor Dryer	Average value $\pm 10\%$
Quantity of Solvent in Extraction Process (C_6H_{14})	Average value $\pm 10\%$
Quantity of Alcohol in Conversion Process (CH_3OH)	Average value $\pm 2\%$
Quantity of Catalyst in Conversion Process (NaOH)	Average value $\pm 5\%$
Cycle of Catalyst Recycled	3 Cycles $\pm 10\%$

C.3 THE GREENHOUSE GAS EMISSIONS BY LIFE-CYCLE PHASE OF MICROALGAL DIESEL INVESTIGATED IN THIS STUDY.

The energy consumed during harvesting process by filtration and screening or microstrainer and belt filter contributes up to 80% of the life-cycle GWP of microalgal diesel.



BIBLIOGRAPHY

2009. Energy Balance: Cumulative Fossil Fuel Demand and Solar Energy Conversion Efficiency of Transport Biofuels, pp. 49-74.
2007. Possible Fix For Global Warming?: Environmental Engineers Use Algae to Capture Carbon Dioxide.
- Anderson, A., Dawes, E. 1990. Occur, Metabolism, Metabolic Role, and Industrial Uses of Bacterial Polyhydroxyalkanoates. *Microbiological reviews*, **54**, 450-472.
- Aresta, M., Dibenedetto, A., Barberio, G. 2005. Utilization of Macro-algae for Enhanced CO₂ Fixation and Biofuels Production: Development of a computing software for an LCA study. *Fuel Processing Technology*, **86**(14-15), 1679-1693.
- Argonne National Laboratory. 2010. GREET Model, Vol. 1.8d.1, U.S. Department of Energy.
- Assessment and Standard Division Office of Transportation and Air Quality, U.S.E. 2010. Renewable Fuel Standard Program (RFS2) Regulatory Impact Analysis. EPA-420-R-10-006.
- Avagyan, A. 2008. A Contribution to Global Sustainable Development: Inclusion of microalgae and their biomass in production and bio cycles. *Clean Technologies and Environmental Policy*, **10**(4), 313-317.
- Balan, V., Rogers, C.A., Chundawat, S.P.S., Costa Sousa, L.d., Slininger, P.J., Gupta, R., Dale, B.E. 2009. Conversion of Extracted Oil Cake Fibers into Bioethanol Including DDGS, Canola, Sunflower, Sesame, Soy, and Peanut for Integrated Biodiesel Processing. *Journal of the American Oil Chemists' Society (JAOCS)*, **86**(2), 157-165.
- Bare, J., Gloria, T., Norris, G. 2006. Development of the Method and U.S. Normalization Database for Life Cycle Impact Assessment and Sustainability Metrics. *Environmental Science & Technology*, **40**(16), 5108-5115.
- Bare, J.C., Norris, G.A., Pennington, D.W., McKone, T. 2003. TRACI: The Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts. *Journal of Industrial Ecology*, **6**(3-4), 49-78.
- Batan, L., Quinn, J., Bradley, T., Willson, B. 2010a. Net Energy and Greenhouse Gas Emissions Evaluation of Biodiesel Derived from Microalgae. *Environmental Science & Technology*, **45**(3), 1160-1160.

Batan, L., Quinn, J., Willson, B., Bradley, T. 2010b. Net Energy and Greenhouse Gas Emission Evaluation of Biodiesel Derived from Microalgae. *Environmental Science & Technology*, null-null.

Biomass Research and Development Technical Advisory Committee and Biomass Research and Development Initiative. 2007. Roadmap for Bioenergy and Biobased Products in the United States.

Braunegg, G., Lefebvre, G., Genser, K.F. 1998. Polyhydroxyalkanoates, Biopolymers from Renewable Resources: Physiological and engineering aspects. *Journal of Biotechnology*, **65**(2-3), 127-161.

Brennan, L., Owende, P. 2010. Biofuels from Microalgae: A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, **14**(2), 557-577.

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*, null-null.

Bruce, E.R. 2008. Opportunities for Renewable Bioenergy Using Microorganisms, Vol. 100, pp. 203-212.

Campbell, P.K., Beer, T., Batten, D. 2011. Life Cycle Assessment of Biodiesel Production from Microalgae in Ponds. *Bioresource Technology*, **102**(1), 50-56.

Carnegie Mellon University Green Design Institute. 2008. Economic Input-Output Life Cycle Assessment (EIO-LCA), Vol. 2011.

Carvalho, A.P., Meireles, L.A., Malcata, F.X. 2006a. Microalgal Reactors: A review of enclosed system designs and performances. *Biotechnology Progress*, **22**(6), 1490-1506.

Carvalho, A.P., Meireles, L.A., Xavier Malcata, F. 2006b. Microalgal Reactors: A Review of Enclosed System Designs and Performances, Vol. 22, pp. 1490-1506.

Chisti, Y. 2007. Biodiesel from Microalgae. *Biotechnology Advances*, **25**, 13.

Chisti, Y. 2008. Biodiesel from Microalgae Beats Bioethanol. *Trends in Biotechnology*, **26**(3), 126-131.

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*, 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*.

- Collet, P., HÈlias, 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.
- Committee on Environmental and Natural Resources. 2010. Scientific Assessment of Hypoxia in U.S. Coastal Waters. Interagency Working Group on Harmful Algal Blooms, Hypoxia, and Human Health of the Joint Subcommittee on Ocean Science and Technology.
- Committee on Environmental and Natural Resources. 2010. Scientific Assessment of Hypoxia in U.S. Coastal Waters. Interagency Working Group on Harmful Algal Blooms, Hypoxia, and Human Health of the Joint Subcommittee on Ocean Science and Technology.
- Costello, C., Griffin, W.M., Landis, A.E., Matthews, H.S. 2009. Impact of Biofuel Crop Production on the Formation of Hypoxia in the Gulf of Mexico. *Environmental Science & Technology*, **43**(20), 7985-7991.
- Daigger, G.T., Grady Jr, C.P.L. 1982. The Dynamics of Microbial Growth on Soluble Substrates: A unifying theory. *Water Research*, **16**(4), 365-382.
- Das, P., Lei, W., Aziz, S.S., Obbard, J.P. 2011. Enhanced Algae Growth in Both pPhototrophic and Mixotrophic Culture Under Blue Light. *Bioresource Technology*, **102**(4), 3883-3887.
- Delft University of Technology. 2001. IdeMat Online.
- Demirbas, A. 2009a. Production of Biodiesel from Algae Oils, Vol. 31, Taylor & Francis, pp. 163 - 168.
- Demirbas, A. 2009b. Thermochemical Conversion Processes Biofuels, Springer London, pp. 261-304.
- Demirbas, A., Fatih Demirbas, M. 2011. Importance of Algae Oil as a Source of Biodiesel. *Energy Conversion and Management*, **52**(1), 163-170.
- Ekvall, T., B.P.Weidema. 2004. System Boundaries and Input Data in Consequential Life Cycle Inventory Analysis. *International Journal of Life Cycle Assessment*, **9**(3), 161-171.
- Environmental Protection Authority. 2004. Photochemical Smog: what it means for us. Government of South Australia.,
- Eriksen, N. 2008. The technology of microalgal culturing. *Biotechnology Letters*, **30**(9), 1525-1536.
- Evans, J.M., Wilkie, A.C. 2010. Life Cycle Assessment of Nutrient Remediation and Bioenergy Production Potential from the Harvest of Hydrilla (*Hydrilla verticillata*). *Journal of Environmental Management*, **91**(12), 2626-2631.
- ExxonMobil Research and Engineering, C. 2009. Algae Biofuels, Vol. 2009.

- Feinberg, D.A. 1984. Technical and Economic Analysis of Liquid Fuel Production from Microalgae, (Ed.) I. Solar Energy Research, Vol. 231-2608, Solar Energy Research Institute. Golden, Colo.
- Ferrell, J., Sarisky-Reed, V. 2010. National Algal Biofuels Technology Roadmap, A technology roadmap resulting from the National Algal Biofuels Workshop.
- Fisher, B.C., Marchese, A. J., Volckens, J., Lee, T. and Collett, J. 2010. Measurement of Gaseous and Particulate Emissions from Algae-Based Fatty Acid Methyl Esters. *SAE Int. J. Fuels Lubr.*, **3**, 292-321.
- Frischknecht, R., Jungbluth, N. 2004. SimaPro Database Manual, The ETH-ESU 96 Libraries version 2.1. ESU-services.
- Frischknecht, R., Jungbluth, N., Althaus, H.-J.r., Bauer, C., Doka, G., Dones, R., Hischier, R., Nemecek, T., Primas, A., Wernet, G. 2007. ecoinvent Report No. 2, Code of Practice Data v2.0. Swiss Federal Institute of Technology Zürich (ETHZ).
- Goedkoop, M., De Schryver, A., Oele, M. 2008. SimaPro 7, Intorduction to LCA. PRé Consultants.
- Gouveia, L., Oliveira, A. 2009. Microalgae as a Raw Material for Biofuels Production. *Journal of Industrial Microbiology and Biotechnology*, **36**(2), 269-274.
- Greenwell, H.C., LLaurens, L.M.L., Shields, R.J., Flynn, K.J. 2010. Placing Microalgae on the Biofuels Priority List: A review of the technological challenges. *J. R. Soc. Interface*, **7**(46), 24.
- Greer, D. 2009. Algae to Energy: Cultivating algae in wastewater for biofuel. *Biocycle Energy*, 36-39.
- Hadj Amor, R., Quaranta, G., Gueddari, F., Million, D., Clauer, N. 2008. The Life Cycle Impact Assessment Applied to a Coastal Lagoon: The Case of the Slimane lagoon (Tunisia) by the Study of Seasonal Variations of the Aquatic Eutrophication Potential. *Environmental Geology*, **54**(5), 1103-1110.
- Hermann, W. 2010. Arizona Set to Become Center for Algae-Based, Biofuel Industry, Vol. 2010, The Arizona Republic.
- Huang, G., Chen, F., Wei, D., Zhang, X., Chen, G. 2010. Biodiesel Production by Microalgal Biotechnology. *Applied Energy*, **87**(1), 38-46.
- Huntley, Mark, Redalje, Donald. 2007. CO₂ Mitigation and Renewable Oil from Photosynthetic Microbes: A New Appraisal. *Mitigation and Adaptation Strategies for Global Change*, **12**(4), 573-608.
- Independent Statistics and Analysis. 2011. Voluntary Reporting of Greenhouse Gases Program: Fuel Emission Coefficients, Vol. 2011, U.S. Energy Information Administration.,

International Organization for Standardization. 2006. ISO 14040 Environmental Management - Life cycle assessment - Principles and framework. Switzerland.

Janssen, M. 2002. Cultivation of Microalgae: Effect of light/ dark cycles on biomass yield, Vol. Ph.D., Wageningen University, pp. 184.

Janssen, M., Tramper, J., Mur, L.R., Wijffels, R.H. 2003. Enclosed Outdoor Photobioreactors: Light regime, photosynthetic efficiency, scale-up, and future prospects. *Biotechnology and Bioengineering*, **81**(2), 193-210.

Jenkins, M.B., Williams, B.R. 2006. Bioenergy - Clean Alternative?, Vol. 2010, Climate Action Team Forum. Fresno, California.

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.

Johnson, M.B., Wen, Z. 2009. Production of Biodiesel Fuel from the Microalga *Schizochytrium limacinum* by Direct Transesterification of Algal Biomass. *Energy & Fuels*, **23**(10), 5179-5183.

Jolliet, O., Margni, M., Charles, R., Humbert, S., Payet, J., Rebitzer, G., Rosenbaum, R. 2003. IMPACT 2002+: A new life cycle impact assessment methodology. *The International Journal of Life Cycle Assessment*, **8**(6), 324-330.

Jorquera, O., Kiperstok, A., Sales, E.A., EmbiruÁu, M., Ghirardi, M.L. 2010. Comparative Energy Life-cycle Analyses of Microalgal Biomass Production in Open Ponds and Photobioreactors. *Bioresource Technology*, **101**(4), 1406-1413.

Kadam, K.L. 2002. Environmental Implications of Power Generation via Coal-Microalgae Cofiring. *Energy*, **27**(10), 905-922.

Kadam, K.L. 2001. Microalgae Production from Power Plant Flue Gas: Environmental implications on life cycle basis. National Renewable Energy Laboratory. NREL/TP-510-29417.

Kaufman, A.S., Meier, P.J., Sinistore, J.C., Reinemann, D.J. 2010. Applying Life-cycle Assessment to Low Carbon Fuel Standards: How allocation choices influence carbon intensity for renewable transportation fuels. *Energy Policy*, **38**(9), 5229-5241.

Kong, Q.-x., Li, L., Martinez, B., Chen, P., Ruan, R. 2010. Culture of Microalgae *Chlamydomonas reinhardtii* in Wastewater for Biomass Feedstock Production. *Applied Biochemistry and Biotechnology*, **160**(1), 9-18.

Krylova, A., Kozyukov, E., Lapidus, A. 2008. Ethanol and Diesel Fuel from Plant Raw Materials: A review. *Solid Fuel Chemistry*, **42**(6), 358-364.

Kyndt, J. 2010. Algae-based biodiesel: Current procedures and innovations, Vol. 2010, AlgaeForBiofuels.

- Landis, A.E., Miller, S.A., Theis, T.L. 2007. Life Cycle of the Corn-Soybean Agroecosystem for Biobased Production. *Environmental Science & Technology*, **41**(4), 1457-1464.
- Landis, A.E., Theis, T.L. 2008. Comparison of life cycle impact assessment tools in the case of biofuels. *IEEE International Symposium on Electronics and the Environment*, 1-7.
- Lanvens, P., Sorgeloos, P. 1996. Manual on the production and use of live food for aquaculture, (Ed.) Food and Agriculture Organization of the United Nations, Vol. 361. Rome, pp. 295.
- Lardon, L., Helias, A., Sialve, B., Steyer, J.-P., Bernard, O. 2009. Life-Cycle Assessment of Biodiesel Production from Microalgae. *Environmental Science & Technology*, **7**.
- Lee, A., Lewis, D., Ashman, P. 2009. Microbial Flocculation, a Potentially Low-Cost Harvesting Technique for Marine Microalgae for the Production of Biodiesel. *Journal of Applied Phycology*, **21**(5), 559-567.
- Lehr, F., Posten, C. 2009. Closed Photo-bioreactors as Tools for Biofuel Production. *Current Opinion in Biotechnology*, **20**(3), 280-285.
- Li, Q., Du, W., Liu, D. 2008a. Perspectives of Microbial Oils for Biodiesel Production. *Applied Microbiology & Biotechnology*, **80**(5), 749-756.
- Li, Y., Horsman, M., Wu, N., Lan, C.Q., Dubois-Calero, N. 2008b. Articles: Biocatalysts and Bioreactor Design, Biofuels from Microalgae. *Biotechnol. Prog.*, **24**, 815-820.
- Lippiatt, B.C. 1999. Selecting Cost-Effective Green Building Products: BEES Approach. *Journal of Construction Engineering and Management*, **125**(6), 448-455.
- Lutz, K., Mühlbauer, W., Müller, J., Reisinger, G. 1987. Development of a Multi-Purpose Solar Crop Dryer for Arid Zones. *Solar & Wind Technology*, **4**(4), 417-424.
- Mallick, N. 2002. Biotechnological Potential of Immobilized Algae for Wastewater N, P and Metal Removal: A review. *BioMetals*, **15**(4), 377-390.
- Mata, T.M., Martins, A.A., Caetano, N.S. 2010. Microalgae for Biodiesel Production and Other Applications: A review. *Renewable and Sustainable Energy Reviews*, **14**(1), 217-232.
- Maurer, M., Schwegler, P., Larsen, A., T. 2003. *Nutrients in Urine: Energetic aspects of removal and recovery*. IWA Publishing, London, ROYAUME-UNI.
- McDonough, W., Braungart, M. 2002. *Cradle to Cradle: Remaking the Way We Make Things*. First ed. North Point Press.
- Metcalf, Eddy, Tchobanoglous, G., Burton, F.L., Stensel, H.D. 2003. *Wastewater engineering : treatment and reuse*. McGraw-Hill, Boston.
- Miller, S.A., et al. 2007. A Comparative Life Cycle Assessment of Petroleum and Soybean-based Lubricants. *Environmental Science & Technology*, **41**, 7.

- Miller, S.A., Landis, A.E., Theis, T.L. 2007. Feature: Environmental Trade-offs of Biobased Production. *Environmental Science & Technology*, **41**(15), 5176-5182.
- Moser, B. 2009. Biodiesel production, properties, and feedstocks. *In Vitro Cellular & Developmental Biology - Plant*, **45**(3), 229-266.
- Mousdale, D.M. 2008. *Biofuels: Biotechnology, chemistry, and sustainable development*. CRC Press, Boca Raton.
- Mulbry, W., Kondrad, S., Buyer, J., Luthria, D. 2009. Optimization of an Oil Extraction Process for Algae from the Treatment of Manure Effluent. *Journal of the American Oil Chemists' Society*, **86**(9), 909-915.
- Norris, G.A. 2003. SimaPro Database Manual, The Franklin US LCI Library version 2.0. Sylvatica.
- Office of Transportation and Air Quality. 2010a. EPA Finalizes Regulations for the National Renewable Fuel Standard Program for 2010 and Beyond. United States Environmental Protection Agency. EPA-420-F-10-007.
- Office of Transportation and Air Quality. 2010b. EPA Lifecycle Analysis of Greenhouse Gas Emissions from Renewable Fuels. United States Environmental Protection Agency. EPA-420-F-10-006.
- Office of Transportation and Air Quality. 2011. EPA Proposes 2012 Renewable Fuel Standards and 2013 Biomass-Based Diesel Volume. EPA-420-F-11-018.
- Palisade Corporation. 2010. @Risk Industrial.
- Pittman, J.K., Dean, A.P., Osundeko, O. 2011. The Potential of Sustainable Algal Biofuel Production Using Wastewater Resources. *Bioresource Technology*, **102**(1), 17-25.
- Posten, C. 2009. Design Principles of Photo-bioreactors for Cultivation of Microalgae. **9**(3), 165-177.
- Reijnders, L., Huijbregts, M. 2009. Transport Biofuels: Their Characteristics, Production and Costs. in: *Biofuels for Road Transport*, Springer London, pp. 1-48.
- Reising, T.F. 2007. Cultivating Algae for Liquid Fuel Production. *Pharmaculture Activist*.
- Research and Innovative Technology Administration. 2010. Average Fuel Efficiency of U.S. Passenger Cars and Light Trucks, Vol. 2011.
- Ritslaid, K., Küüt, A., Olt, J. 2010. State of the Art in Bioethanol Production. *Agronomy Research*, **8**(1), 236-254.
- Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R. 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass

cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, **102**(1), 100-112.

Rosenberg, J.N., Oyler, G.A., Wilkinson, L., Betenbaugh, M.J. 2008. A Green Light for Engineered Algae: Redirecting Metabolism to Fuel a Biotechnology Revolution. *Current Opinion in Biotechnology*, **19**(5), 430-436.

Sander, K., Murthy, G. 2010. Life Cycle Analysis of Algae Biodiesel. *The International Journal of Life Cycle Assessment*, **15**(7), 704-714.

Schenk, P., Thomas-Hall, S., Stephens, E., Marx, U., Mussgnug, J., Posten, C., Kruse, O., Hankamer, B. 2008. Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *BioEnergy Research*, **1**(1), 20-43.

Schnoor, J.L. 2011. Cellulosic Biofuels Disappoint. *Environmental Science & Technology*, **45**(17), 7099-7099.

Shah, V., Ries, R. 2009. A Characterization Model with Spatial and Temporal Resolution for Life Cycle Impact Assessment of Photochemical Precursors in the United States. *The International Journal of Life Cycle Assessment*, **14**(4), 313-327.

Sharif Hossain, A.B.M., Salleh, A., Boyce, A.N., Chowdhury, P., Naqiuddin, M. 2008. Biodiesel Fuel Production from Algae as Renewable Energy. *American Journal of Biochemistry and Biotechnology*, **4**(3), 250-254.

Shaw, D., Hung, M.-F., Lin, Y.-H. 2010. Using Net Energy Output as the Base to Develop Renewable Energy. *Energy Policy*, **38**(11), 7504-7507.

Sheehan, J. 2000. The Road to Bioethanol: A Strategic Perspective of the U.S. Department of Energy's National Ethanol Program. in: *Glycosyl Hydrolases for Biomass Conversion*, Vol. 769, American Chemical Society, pp. 2-25.

Sheehan, J., Camobreco, V., et al. 1998. Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus. National Renewable Energy Laboratory, U.S. Department of Energy and U.S. Department of Agricultural.

Sheehan, J., Dunahay, T., Benemann, J., Roessler, P. 1998. A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae. National Renewable Energy Laboratory. NREL/TP-580-24190.

Shelef, G., Sukenik, A., Green, M. 1984. Microalgae Harvesting and Processing: A literature review. Solar Energy Research Institute. SERI/STR-231-2396.

Shen, Y., Yuan, W., Pei, Z., Mao, E. 2010. Heterotrophic Culture of Chlorella protothecoides in Various Nitrogen Sources for Lipid Production. *Applied Biochemistry and Biotechnology*, **160**(6), 1674-1684.

- Singh, A., Nigam, P.S., Murphy, J.D. 2011. Mechanism and Challenges in Commercialisation of Algal Biofuels. *Bioresource Technology*, **102**(1), 26-34.
- Singh, N., Dhar, D. 2011. Microalgae as Second Generation Biofuel: A review. *Agronomy for Sustainable Development*, 1-25.
- Sivakumar, G., Vail, D.R., Xu, J., Burner, D.M., Lay, J.O., Ge, X., Weathers, P.J. 2010. Bioethanol and biodiesel: Alternative liquid fuels for future generations. *Engineering in Life Sciences*, **10**(1), 8-18.
- Skone, T.J., Gerdes, K. 2008. Development of Baseline Data and Analysis of Life Cycle Greenhouse Gas Emissions of Petroleum-Based Fuels. DOE/NETL-2009/1346.
- Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and , Miller, H.L. 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.
- Soratana, K., Harper Jr., W., Landis, A.E. 2012a. Microalgal Biodiesel and the Renewable Fuel Standard's Greenhouse Gases Requirement. *Energy Policy*, accepted pending revisions.
- Soratana, K., Khanna, V., Landis, A.E. 2012b. Re-envisioning the Renewable Fuel Standard to Minimize Unintended Consequences. *Environmental Science & Technology*, under review.
- Soratana, K., Landis, A.E. 2011. Evaluating Industrial Symbiosis and Algae Cultivation from a Life Cycle Perspective. *Bioresource Technology*, **102**(13), 6892-6901.
- Soratana, K., Marriott, J. 2010. Increasing Innovation in Home Energy Efficiency: Monte Carlo simulation of potential improvements. *Energy and Buildings*, **42**(6), 828-833.
- Sorgeloos, P., Persoone, G., de Winter, F., Bossuyt, E., de Pauw, N. 1977. Air-Water Pumps as Cheap and Convenient Tools for High Density Culturing of Microscopic Algae. *Proceedings of the annual meeting - World Mariculture Society*, **8**(1-4), 173-184.
- Spirinckx, C., Carolin, Ceuterick, D. 1996. Biodiesel and Fossil Diesel Fuel: Comparative Life Cycle Assessment. *The International Journal of Life Cycle Assessment*, **1**(3), 127-132.
- Spriensma, R. 2004. SimaPro Database Manual, The BUWAL 250 Library version 2.0. PRÉ Consultants.
- 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.
- Taherzadeh, M.J., Karimi, K. 2008. Bioethanol: Market and production processes. in: *Biofuels Refining and Performance*, (Ed.) A. Nag, McGraw-Hill Companies, Inc. Blacklick, OH, USA, pp. 69-105.

- Tan, K.T., Lee, K.T., Mohamed, A.R. 2008. Role of Energy Policy in Renewable Energy Accomplishment: The case of second-generation bioethanol. *Energy Policy*, **36**(9), 3360-3365.
- Tchobanoglou, G., Burton, F.L., Stensel, H.D., Metcalf, Eddy. 2003. *Wastewater Engineering: Treatment and reuse*. McGraw-Hill, Boston.
- Thomassen, M., Dalgaard, R., Heijungs, R., de Boer, I. 2008. Attributional and Consequential LCA of Milk Production. *The International Journal of Life Cycle Assessment*, **13**(4), 339-349.
- Tickell, J., Tickell, K. 2003. *From the Fryer to the Fuel Tank: The Complete Guide to Using Vegetable Oil as an Alternative Fuel*. Joshua Tickell Media Productions.
- Tsukahara, K., Sawayama, S. 2005. Liquid Fuel Production Using Microalgae. *Journal of the Japan Petroleum Institute*, **48**(5), 251-259.
- U.S. Energy Information Administration. 2011. International Energy Statistics, Official Energy Statistics from the U.S. Government.
- U.S. Environmental Protection Agency. 1994a. Automobile Emissions: An Overview. Fact Sheet OMS-5.
- U.S. Environmental Protection Agency. 2010a. Chesapeake Bay Total Maximum Daily Load for Nitrogen, Phosphorus and Sediment.
- U.S. Environmental Protection Agency. 2000. Heavy-Duty Engine and Vehicle Standards and Highway Diesel Fuel Sulfur Control Requirements. EPA420-F-00-057.
- U.S. Environmental Protection Agency. 1994b. Motor Vehicles and the 1990 Clean Air Act. Fact Sheet OMS-11.
- U.S. Environmental Protection Agency. 2010b. Renewable Fuel Standard Program (RFS2) Regulatory Impact Analysis.
- Udo de Haes, H. 2006. How to Approach Land Use in LCIA or, How to Avoid the Cinderella Effect? *The International Journal of Life Cycle Assessment*, **11**(4), 219-221.
- Udo de Haes, H., van Rooijen, M. 2005. Life Cycle Approaches: The road from analysis to practice. UNEP/SETAC Life Cycle Initiative.
- Uduman, N., Qi, Y., Danquah K., M., Forde M., G., Hoadley, A. 2010. Dewatering of Microalgal Cultures: A major bottleneck to algae-based fuels. *J. Renewable Sustainable Energy*, **2**(012701), 15.
- University of California. 2007. A Low-Carbon Fuel Standard for California, Part 1: Technical Analysis. in: *Life cycle assessment*.

- Vijayaraghavan, K., Hemanathan, K. 2009. Biodiesel Production from Freshwater Algae. *Energy & Fuels*, **23**(11), 5448-5453.
- Vyas, A.P., Verma, J.L., Subrahmanyam, N. 2010. A Review on FAME Production Processes. *Fuel*, **89**(1), 1-9.
- Wall, J.D., Harwood, C.S., Demain, A. 2008. *Bioenergy*. ASM Press, Washington, D.C.
- Wang, B., Li, Y., Wu, N., Lan, C. 2008. CO₂ Bio-mitigation Using Microalgae. *Applied Microbiology and Biotechnology*, **79**(5), 707-718.
- Weissman, J.C. 1987. Design and Analysis of Microalgal Open Pond Systems for the Purpose of Producing Fuels: A subcontract report. Solar Energy Research Institute.
- 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.
- Xu, Y., Isom, L., Hanna, M.A. 2010. Adding Value to Carbon Dioxide from Ethanol Fermentations. *Bioresource Technology*, **101**(10), 3311-3319.
- Yang, J., Xu, M., Zhang, X., Hu, Q., Sommerfeld, M., Chen, Y. 2011. Life-cycle Analysis on Biodiesel Production from Microalgae: Water footprint and nutrients balance. *Bioresource Technology*, **102**(1), 159-165.
- Yanqun, L., Mark, H., Nan, W., Christopher, Q.L., Nathalie, D.-C. 2008. Biofuels from Microalgae, Vol. 24, pp. 815-820.
- Zebib, T. 2008. Microalgae Grown in Photobioreactors for Mass Production of Biofuel. Department of Bioenvironmental Engineering, Rutgers University.