COUPLING THE WASTEWATER TREATMENT PROCESS WITH AN ALGAL PHOTOBIOREACTOR FOR NUTRIENT REMOVAL AND RENEWABLE RESOURCE PRODUCTION

by

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Monica C. Rothermel, M.S.
University of Pittsburgh 2011

To address the growing need for renewable energy and high quality water, the concept of industrial symbiosis may be applied to a wastewater treatment system coupled with an algal photobioreactor (PBR). The coupled system is capable of removing nitrogen and phosphorus from wastewater while producing algal biomass containing precursors to renewable resources such as biofuels, electricity, plastics, and fertilizers.

A laboratory experiment was performed to determine the feasibility of coupling a conventional wastewater treatment system with an algal PBR for the simultaneous removal of nutrients from wastewater and production of renewable resources. An activated sludge batch reactor was set up in series with an algal PBR to feed wastewater to the algae. The nutrient concentration in the water as well as lipid content, carbohydrate content, and growth rate of the algal biomass were tested over 10 cycles to determine the capabilities of the coupled system. The study revealed complete nutrient removal in some cycles, with the average final nutrient content of 2 mg-P/L and 3 mg-N/L in effluent of the PBR. The algae biomass contained 24±3% lipids and 26±7% carbohydrates by dry weight.

A life cycle assessment of algae cultivation and harvesting revealed the highest energy demand of the coupled system occurred during harvesting of the algal mixture through

centrifugation or filtration, but the highest global warming and eutrophication impacts were due to CO_2 use and PBR construction material production, respectively. Although the use of wastewater in place of fertilizers resulted in a smaller environmental impact of an algae cultivation system, the life cycle environmental impacts could be reduced more effectively by coupling the system with waste CO_2. It is feasible for the system to treat wastewater while generating renewable resources, but the system must be optimized to reduce life cycle environmental impacts and result in a net energy gain before large-scale implementation is possible.

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ACRONYMS

APHA – American Public Health Association

ASTM – American Society for Testing and Materials

BNR – Biological nutrient removal

BOD – Biological oxygen demand

CAS – Conventional activated sludge

CFC – Chlorofluorocarbon

CH – Carbohydrate

CO_2 – Carbon dioxide

COD – Chemical oxygen demand

DEU – Direct energy use

EP – Eutrophication potential

$FeSO_4$ – Iron (II) sulfate

GHG – Greenhouse gas

GWP – Global warming potential

HRAP – High rate algal pond

HRT – Hydraulic retention time

ISO – International Organization for Standardization

KOH – Potassium hydroxide

L/ha – Liters per hectare

LCA – Life cycle assessment

LCI – Life cycle inventory

LCIA – Life cycle impact assessment

LDPE – Low-density polyethylene

LED – Light-emitting diode

M ha – Million hectares

N – Nitrogen

Na_2CO_3 – Sodium carbonate

$NaHCO_3$ – Sodium bicarbonate

O_2 – Oxygen

OD – Optical density

P – Phosphorus

PBR – Photobioreactor

PHA – Polyhydroxyalkanoate

RAS – Return activated sludge

SRT – Solids retention time

TOC – Total organic carbon

TRACI – Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts

TSS – Total suspended solids

UTEX – University of Texas

WW – Wastewater

WWTP – Wastewater treatment plant

1.0 INTRODUCTION

The wastewater treatment process currently demands large amounts of energy and material inputs. As a result of the negative impacts wastewater effluents high in nutrient content may have on receiving waterways, nutrient removal requirements have become more stringent in recent years. In general, the amount of energy and chemicals needed for nutrient removal increases as water quality is treated to higher standards. Large amounts of waste are also produced during treatment; return activated sludge (RAS) from the treatment plant is dumped in large volumes into landfills and treated water is discharged back into the environment.

In order for wastewater treatment to be more sustainable, the wastewater treatment process can be coupled with microalgal photobioreactors (PBRs) (Mallick 2002; Behzadi and Farid 2007; Campbell 2008; Johnson and Wen 2010). Microalgae is gaining specific interest as a source of biofuel because it is rapidly renewable (Behzadi and Farid 2007; Johnson and Wen 2010) and, unlike first generation biofuels, does not compete with food supplies (Chisti 2008; Dismukes, Carrieri et al. 2008; Pienkos and Darzins 2009). In the coupled system, nutrients such as nitrogen (N) and phosphorus (P) from the wastewater effluent can be fed to the microalgae, eliminating the need for synthetic nutrients (Mallick 2002; Aresta, Dibenedetto et al. 2005) which have a high environmental impact when commercially produced. As the microalgae consume the N and P, the wastewater can be treated with the potential for reuse in appropriate applications (Mallick 2002; Johnson and Wen 2010).

By coupling the wastewater treatment system with algae cultivation and harvesting for useful products, fewer chemicals, fertilizers, water, and energy are needed for an expanded system. There is a possibility that life cycle environmental impacts can be reduced when system expansion is considered due to the replacement of conventional processing techniques for products such as electricity, fuels, plastics, etc. When considering the coupled system scenario in Figure 1.1, the environmental impacts from conventional product manufacturing can be considered as avoided impacts and can be subtracted from the environmental impacts of the coupled system. Through industrial symbiosis and the reuse of products that are usually wasted, the coupled system may provide a sustainable option for the advanced treatment of wastewater and production of renewable products including biofuels, electricity, and other value-added products.

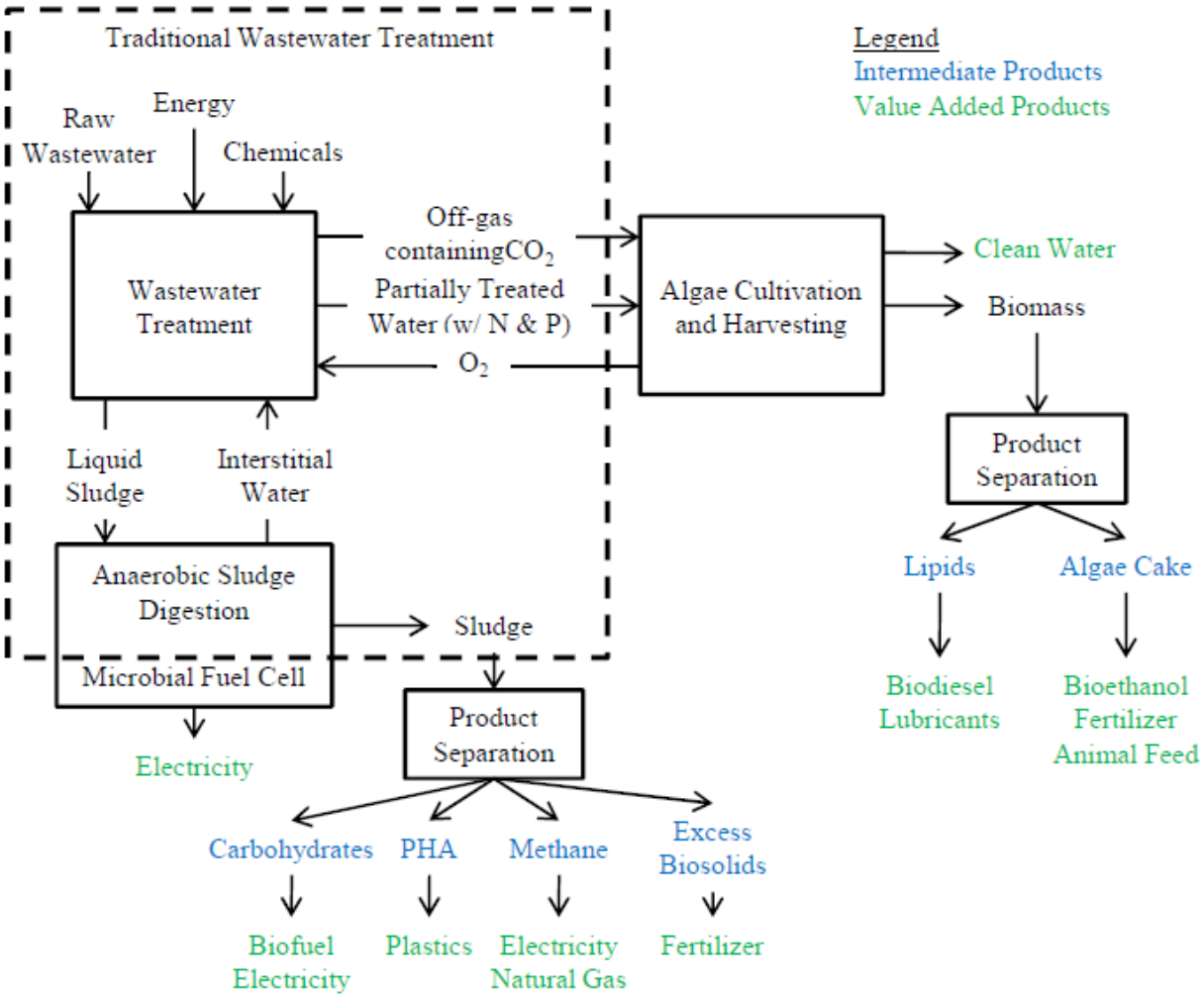


Figure 1.1. Coupled system scenario

Research pertaining to the use of algae for wastewater treatment began in the 1950's (Oswald, Gotaas et al. 1957). Interest in biofuel production from microalgae has been growing in recent years due to the unsustainable nature of fossil fuels and negative impacts found to be associated with first generation biofuels. There is little research focusing on coupling the systems for simultaneous wastewater treatment and renewable product recovery, but research efforts are growing as a result of the positive outlook of a coupled system. This work aims to

advance the knowledge of simultaneous algae cultivation and wastewater treatment in a PBR as well as the environmental life cycle impacts of algae cultivation and harvesting in a coupled system.

The focus of this research is on a portion of the expanded system (Figure 1.2) including the wastewater treatment potential of the system and the potential to create renewable resources from the algae cultivation and harvesting stages of the coupled system. While the laboratory portion of the study only involved the cultivation of algae in a PBR, algae harvesting was considered as a necessary stage to include in the study so that water leaving the coupled system would be at a comparable quality level of that undergoing nutrient removal treatment in a conventional wastewater treatment plant (WWTP). Existing studies were consulted to determine the most likely harvesting methods, and operational data from these methods was included in the current study.

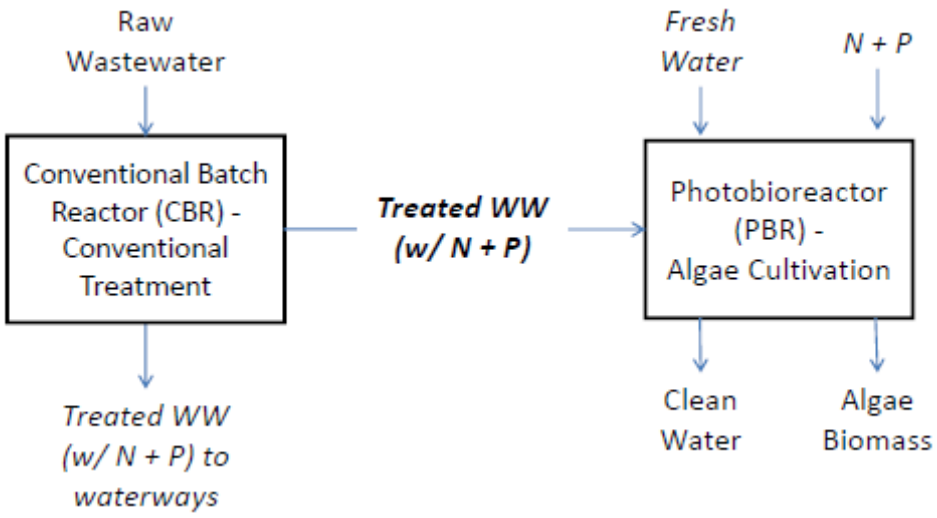


Figure 1.2. Coupling scenario focused on in this research.

In this study, a Conventional Batch Reactor (CBR) was coupled with an algal photobioreactor (PBR) through the use of partially treated wastewater (WW) effluent. WW effluent has undergone primary activated sludge treatment but still contains some nitrogen (N) and phosphorus (P). *Treated WW (w/N+P) to waterways, Fresh Water, and N + P* are avoided in this coupling scenario with the replacement of ***Treated WW (w/ N + P)*** being taken from the CBR and fed to the PBR.

Currently, no other studies could be found which combine the use of algae to treat wastewater and produce useful products in a laboratory setting while also addressing the environmental impacts of the coupled system. This study aims to assess both the feasibility and environmental sustainability of coupling the wastewater treatment system with algae cultivation.

1.1 RESEARCH GOALS AND OBJECTIVES

The goal of this research is to develop a PBR in order to determine the feasibility of a coupled wastewater-algae cultivation system for wastewater treatment and product recovery and to assess

the sustainability of the system in a large-scale scenario through the life cycle assessment (LCA) of algae cultivation and harvesting. Specific objectives of the research are as follows:

Objective 1 - Develop a functional PBR and determine the best practices for operating the PBR in a laboratory

Objective 1a - Assess the feasibility of the system for nutrient removal and production of algal biomass containing precursors to useful products

Objective 1b - Assess the productivity of the system in terms of nutrient removal, lipid production, carbohydrate production, and algal biomass production

Objective 2 - Determine the Global Warming Potential (GWP) Eutrophication potential (EP), and Direct Energy Use (DEU) of a theoretical, large-scale coupled algae cultivation and harvesting system

Objective 2a - Determine areas with high impacts in the life cycle and identify potential improvements

Objective 2b - Compare results with other studies to validate results

The LCA focused solely on environmental impacts in terms of GWP and EP because the function of the coupled system is to mitigate global warming, eutrophication, and energy use by providing a passive method of nutrient removal from wastewater, decreasing fertilizer use for biofuels, and producing a renewable, carbon-neutral fuel source. While the aim of sustainable system development is to reduce impacts in all categories, the tradeoffs between GWP and EP are well known (Miller, Landis et al. 2007) and are of particular interest to this study. Because inventory in the system boundary of the study primarily includes energy use and methods of

nutrient reduction, no significant areas of impact or interesting results are expected from additional impact assessment categories.

Research goals and objectives were completed as a part of a group comprised of graduate students, undergraduate students, and faculty at the University of Pittsburgh in the Department of Civil and Environmental Engineering's Sustainability and Green Design Group. Contributions from group members as a part of the overall Algae and Wastewater Treatment Project are described in Table 1.1.

Table 1.1. Contribution to Algae and Wastewater Treatment Project at the University of Pittsburgh.

Task	Contributors
Photobioreactor (PBR) design and development	Monica Rothermel
System testing protocol development	Monica Rothermel
Daily PBR maintenance	Monica Rothermel
Conventional Bioreactor (CBR) maintenance	William Barr
Water Quality and Biomass Growth Testing	Monica Rothermel, Kayla Reddington, Matthew Weschler, Grace Witter
Carbohydrate Testing	Monica Rothermel, Kayla Reddington, Matthew Weschler
Lipid Testing	Monica Rothermel
Data Analysis	Monica Rothermel
Life Cycle Assessment	Monica Rothermel, Grace Witter, Kullapa Soratana

1.2 WASTEWATER TREATMENT AND PRODUCT RECOVERY FROM MICROALGAE

1.2.1 The Need for Nutrient Removal from Wastewater

As the demand for clean water grows around the world, the need for treatment processes capable of producing high-quality water becomes evident. One aspect of wastewater quality that has gained attention by the wastewater treatment industry in recent years is the removal of nutrients from wastewaters before they are discharged from WWTPs as effluent and flow back to receiving waterways. Wastewater effluents containing nutrients such as nitrogen and phosphorus are the source of undesirable consequences related to algae growth in receiving waters, known as eutrophication (Burdick, Refling et al. 1982; de-Bashan and Bashan 2004). As a result, nutrient removal requirements have become increasingly stringent in municipalities concerned with the effects of eutrophication in receiving waterways (Foley, de Haas et al. 2010).

In order to meet these stringent wastewater effluent nutrient removal limitations, municipalities have invested in energy intensive treatment plants capable of removing nutrients to low-level concentrations (Burdick, Refling et al. 1982). A variety of nutrient removal technologies with varying energy and chemical requirements exist (Burdick, Refling et al. 1982); however, in general, energy and resource consumption at these WWTPs increases as the final nutrient level in the wastewater effluent decreases. Specifically, energy, chemical, and infrastructure requirements increase as the final nitrogen level in wastewater effluent decreases, and chemical and infrastructure requirements increase as the final phosphorus level in wastewater effluent decreases (Foley, de Haas et al. 2010). The increase in energy consumption for nitrogen removal is caused by the increase in aeration needed to supply oxygen to the plant in

a conventional nitrification reaction. Aeration is the largest source of energy use in a wastewater treatment plant (Rosso, Larson et al. 2008).

In some scenarios, the negative environmental impacts caused by advanced wastewater treatment are greater than the environmental impacts mitigated by the advanced treatment (Wenzel, Larsen et al. 2008). Therefore, methods capable of treating wastewater to high levels while limiting energy and chemical demands are desired.

1.2.1.1 Algae for wastewater treatment. One potential method for removing nutrients from wastewater is through the use of microalgae (Oswald, Gotaas et al. 1957; Craggs, McAuley et al. 1997; Mallick 2002; Kim, Lingaraju et al. 2010). Algae has been used for wastewater treatment in high-rate algal ponds (HARPs) since the 1950's (Oswald, Gotaas et al. 1957). In addition to treatment in algal ponds, wastewater can also be treated by algae in a PBR (Tamer, Amin et al. 2006).

Nitrogen is removed from wastewater in algal ponds due to the assimilation of nitrogen by the algae, desorption of ammonia into the atmosphere, and natural nitrification-denitrification in the pond (Bich, Yaziz et al. 1999). In addition to nutrient removal, chemical oxygen demand (COD) (Bich, Yaziz et al. 1999), total inorganic carbon (TOC) (Kim, Lingaraju et al. 2010), and heavy metals can also be removed from wastewater through microalgal treatment (Mallick 2002).

The main drawback to the treatment of wastewater in high rate algal ponds (HRAPs) is the inability to effectively harvest the algae from the wastewater. Without efficient harvesting technologies, wastewater effluent from HRAPs does not meet effluent discharge requirements (Sheehan, Dunahay et al. 1998). The problems and limitations associated with harvesting algae from the treated wastewater will be discussed further in Section 1.2.3.2.

1.2.2 Environmental and Energy Concerns

Concerns over the continued use of fossil fuels have arisen due to the depletion of oil reserves, the increasing demand for energy, and problems associated with energy security. Additionally, there has been a heightened awareness of environmental issues associated with fossil fuel use; primarily, the use of fossil fuels has caused an increase in global warming due to accumulation of CO_2 in the atmosphere. Because of these issues, the continued use of fossil fuels is unsustainable.

The most feasible method of replacing petroleum fuels while continuing to meet projected energy demands is through the production and use of biofuels (Demirbas 2007). First generation biofuels made from terrestrial crops were developed as a replacement for fossil fuels, but first generation biofuels may also be unsustainable due to the competition with food crops, the contribution of agricultural practices to world water shortages, the amount of land required, and the eutrophication potential (EP) caused by increased fertilizer usage for growth (Miller, Landis et al. 2007; Patil, Tran et al. 2008; Schenk, Thomas-Hall et al. 2008; Brennan and Owende 2010). In addition, first generation biofuels are unable to satisfy the existing demand for fuels (Chisti 2007).

1.2.3 Microalgal biofuels

Microalgae are a promising replacement for first generation biofuels (Li, Horsman et al. 2008; Patil, Tran et al. 2008; Griffiths and Harrison 2009) and a promising option for the contribution of renewable fuels to the existing fuel infrastructure (Rosenberg, Oyler et al. 2008; Schenk, Thomas-Hall et al. 2008; Pienkos and Darzins 2009; Pittman, Dean et al. 2011). Microalgal

biomass may be used to produce biofuels without the negative environmental and agricultural impacts associated with first generation, land-based biofuels (Dismukes, Carrieri et al. 2008).

Microalgal biomass is a source of renewable energy (Aresta, Dibenedetto et al. 2005; Amin 2009) which can be converted into useful energy sources such as biofuel oil and gas (Amin 2009). Algal biodiesel could provide a greater amount of energy than any other oilseed crop (Sheehan, Dunahay et al. 1998; Patil, Tran et al. 2008) and is theoretically capable of meeting the existing energy demand (Chisti 2007). Microalgal biofuels have great potential to progressively replace fossil fuels (Brennan and Owende 2010), and will become even more competitive as petroleum supplies decrease and associated costs of fuel increase (Campbell 2008).

1.2.3.1 Advantages of Microalgae for Biofuels. The advantages of using microalgae for biofuel production are numerous. Microalgae contain an efficient biological system for harvesting solar energy (Vonshak 1990; Aresta, Dibenedetto et al. 2005; Schenk, Thomas-Hall et al. 2008), making microalgae more efficient harvesters of solar energy than terrestrial crops (Dismukes, Carrieri et al. 2008). Microalgae have simple reproductive organs with a simple cell division cycle (Vonshak 1990; Li, Horsman et al. 2008), they have a high growth rate (Behzadi and Farid 2007; Li, Horsman et al. 2008) and there is the potential to produce a high volume of biomass through algae cultivation (Campbell 2008). Algal cells generally contain a high lipid content (Pienkos and Darzins 2009), but algal species can also be engineered to produce large concentrations of carbohydrates, lipids, proteins, or pigments (Vonshak 1990) depending on the intended use of the algal biomass.

The minimal land requirements for algae cultivation also make microalgal biofuels advantageous over terrestrial crops. The cultivation of microalgae for biofuel production would use a smaller amount of land than terrestrial biofuels (Dismukes, Carrieri et al. 2008), and

marginal lands could be used for cultivation (Campbell 2008). According to Chisti (2007), biofuels from microalgae are the only realistic option capable of replacing petroleum fuels based on land use alone (Chisti 2007). Table 1.2 shows the land area needed and the percent of existing US cropping area that would be needed to replace 50% of all transport fuel needs in the US.

Table 1.2. Comparison of some sources of biodiesel.

Taken from Chisti (2007).

Crop	*Oil yield (L/ha)*	*Land area needed (M ha)*[a]	*Percent of existing US cropping area*[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	136,900	2	1.1
Microalgae[c]	58,700	4.5	2.5

a. For meeting 50% of all transport fuel needs of the United States.
b. 70% oil (by wt) in biomass.
c. 30% oil (by wt) in biomass.

Microalgae are widely adaptable to a variety of environmental conditions. Microalgae can be grown using seawater, brackish water, and wastewater (Vonshak 1990; Aresta, Dibenedetto et al. 2005; Li, Horsman et al. 2008; Schenk, Thomas-Hall et al. 2008; Pittman, Dean et al. 2011), reducing the demand on freshwater use. Although microalgae grow with the highest productivity under specific conditions, microalgae have the capability of growing in a variety of conditions, including in water with variable temperature and pH levels (Aresta, Dibenedetto et al. 2005; Behzadi and Farid 2007).

Among the greatest advantages of using microalgae for biofuels is the potential for CO_2 fixation (Aresta, Dibenedetto et al. 2005; Behzadi and Farid 2007; Schenk, Thomas-Hall et al. 2008; Wang, Li et al. 2008; Amin 2009). The ability for microalgae to consume CO_2 for growth results in an overall process that is carbon neutral (Aresta, Dibenedetto et al. 2005; Campbell 2008) because CO_2 is released back into the atmosphere when the biofuel is combusted. It is important to note that CO_2 is also produced at other points in the life cycle of the system, so the global warming potential (GWP) is positive over the entire life cycle.

Finally, there is potential for the cost-effective production of microalgal biofuels (Campbell 2008).

1.2.3.2 Conversion of Algae to Useful Products. Microalgae can be converted to useful products through the processes shown in Figure 1.3. The processes include algae cultivation, harvesting and dewatering, extraction, and conversion to useful products.

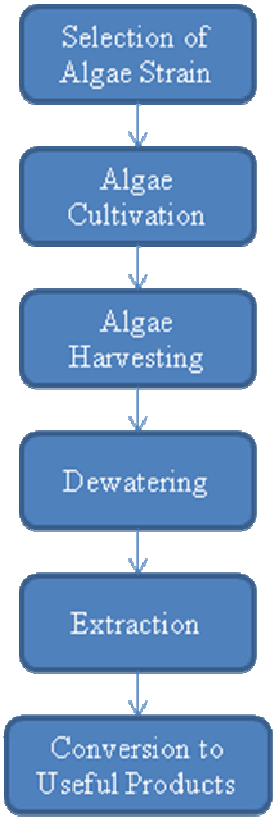


Figure 1.3. Process for resource production from microalgae.

Selection of Algae Strain. There is a wide variety of algae species available for cultivation and resource recovery. The strain is selected based on availability and species characteristics such as growth rate and biological composition. The species can be chosen based solely on the end product desired.

Cultivation. During the cultivation stage, algae are placed under natural or engineered conditions and undergo growth and multiplication of cells. Cultivation of algae can occur in a PBR or open pond (Amin 2009). Open raceway ponds, shown in Figure 1.4, are the

primary commercial production systems for outdoor systems (Carvalho, Meireles et al. 2006).

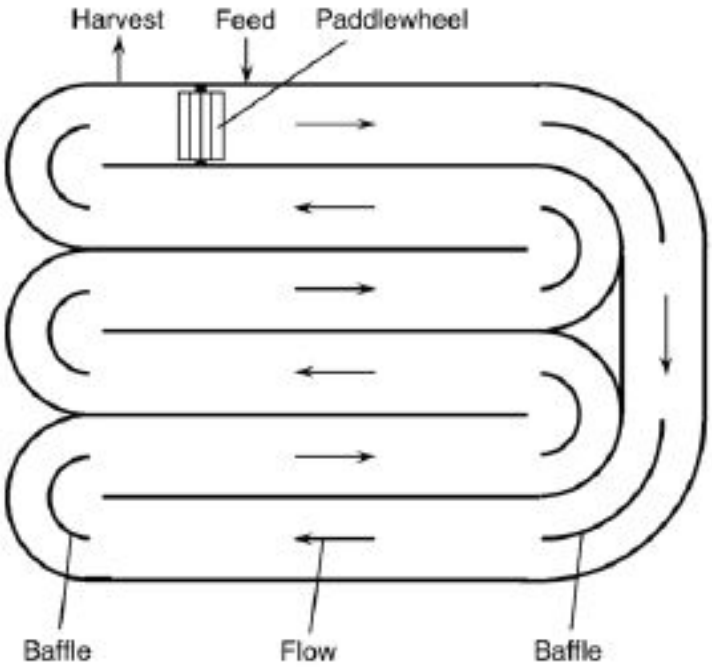


Figure 1.4. Arial view of a raceway pond.

Taken from Chisti (2007).

Because open raceway ponds cannot be controlled, engineered and controlled PBRs have been developed (Carvalho, Meireles et al. 2006). The advantage of a closed system over the open ponds is the enhanced control available to the system and the resulting increase in productivity (Carvalho, Meireles et al. 2006); however, the productivity must be improved in PBRs to the point that they are profitable and competitive (Carvalho, Meireles et al. 2006). A comparison of PBR and raceway production methods is shown in Table 1.3.

Table 1.3. Comparison of photobioreactor and raceway production methods.

Taken from Chisti (2007).

Variable	Photobioreactor facility	Raceway ponds
Annual biomass production (kg)	100,000	100,000
Volumetric productivity (kg/m^3/d)	1.535	0.117
Areal productivity (kg/m^2/d)	0.048[a] 0.072[c]	0.035[b]
Biomass concentration in broth (kg/m^3)	4.00	0.14
Dilution rate (d^{-1})	0.384	0.250
Area needed (m^2)	5681	7828
Oil yield (m^3/ha)	136.9[d] 58.7[e]	99.4[d] 42.6[e]
Annual CO_2 consumption (kg)	183,333	183,333
System geometry	132 parallel tubes/unit; 80 m long tubes; 0.06 m tube diameter	978 m^2/pond; 12 m wide, 82 m long, 0.30 m deep
Number of units	6	8

a. Based on facility area.
b. Based on actual pond area.
c. Based on projected area of photobioreactor tubes.
d. Based on 70% by wt. oil in biomass.
e. Based on 30% by wt. oil in biomass.

When designing a PBR for algae cultivation, "the best reactor system," or the one system with maximum productivity for the lowest cost, does not exist (Carvalho, Meireles et al. 2006). Rather, the best system design is dependent on the goals of the system, the type of algae being cultivated, and the intended purpose for production (Carvalho, Meireles et al. 2006; Kunjapur and Eldridge 2010).

A number of PBR configurations have been designed (Javanmardian and Palsson 1992; Sánchez Mirón, García Camacho et al. 2000; Ugwu 2002; Posten 2009; Kunjapur and Eldridge 2010). The most common PBR designs include tubular reactors (Figure 1.5), flat plate reactors (Figure 1.6), and fermenter-type reactors (Figure 1.7). Gas transfer, mixing, lighting, nutrient

level, temperature, and pH control all must be engineered in a PBR (Carvalho, Meireles et al. 2006; Kunjapur and Eldridge 2010). Additionally, high illuminated surface to volume ratio is a key parameter in reactor design (Javanmardian and Palsson 1992).

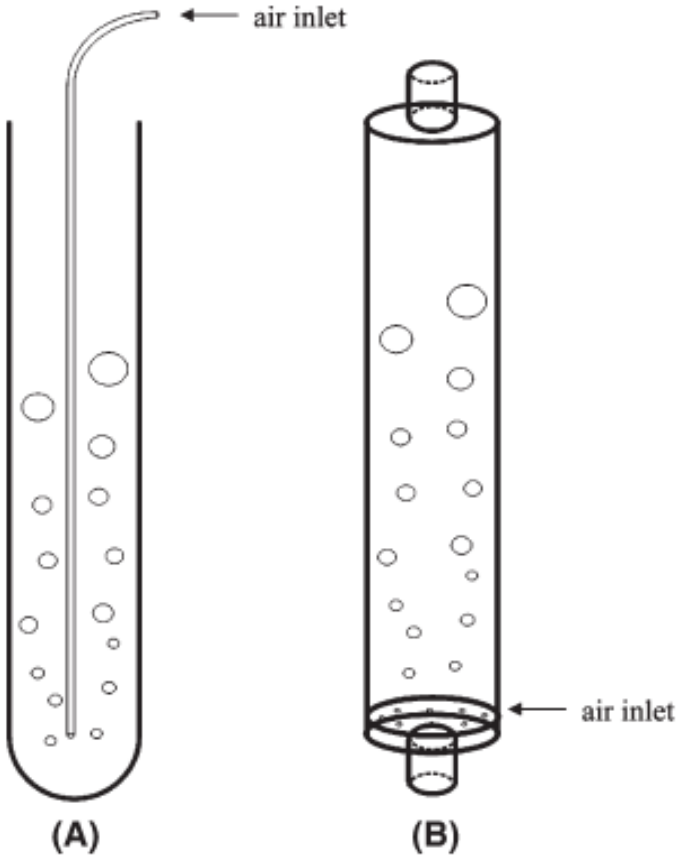


Figure 1.5. Schematic representation of airlift (A) and bubble column (B) tubular reactors. Taken from Carvalho, Meireles et al. (2006).

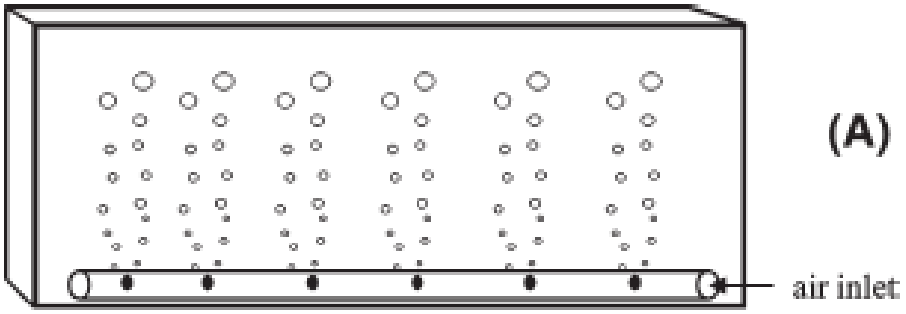


Figure 1.6. Schematic representation of flat panel reactor; flat panel bubbled on the bottom.

Taken from Carvalho, Meireles et al. (2006).

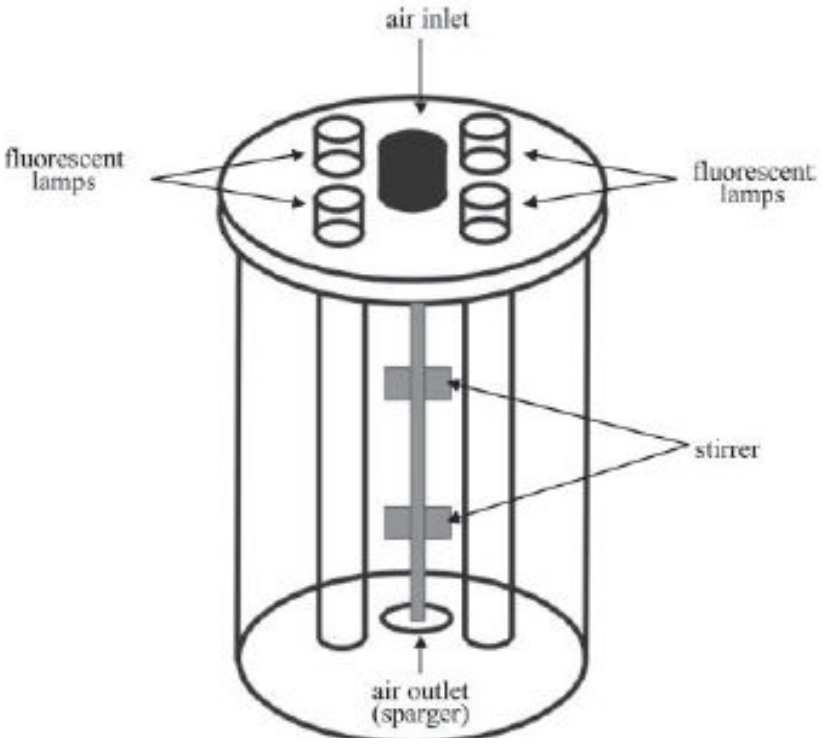


Figure 1.7. Schematic representation of fermenter-type reactor.

Taken from Carvalho, Meireles et al. (2006).

The advantages and limitations of open ponds and various PBRs were compared by Brennan and Owende (2010) in Table 1.4, and additional advantages and disadvantages of three types of PBRs are compared by Kunjapur and Eldridge (2010) in Table 1.5.

Table 1.4. Advantages and limitations of open ponds and photobioreactors.

Taken from Brennan and Owende 2010.

Production System	Advantages	Limitations
Raceway pond	Relatively cheap Easy to clean Utilizes non-agricultural land Low energy inputs Easy maintenance	Poor biomass productivity Large area of land required Limited to a few strains of algae Poor mixing, light, and CO_2 utilization Cultures are easily contaminated
Tubular photobioreactor	Large illumination surface area Suitable for outdoor cultures Relatively cheap Good biomass productivities	Some degree of wall growth Fouling Requires large land space Gradients of pH, dissolved oxygen and CO_2 along the tubes
Flat plate photobioreactor	High biomass productivities Easy to sterilize Low oxygen build-up Readily tempered Good light path Large illumination surface area Suitable for outdoor cultures	Difficult scale-up Difficult temperature control Small degree of hydrodynamic stress Some degree of wall growth
Column photobioreactor	Compact High mass transfer Low energy consumption Good mixing with low shear stress Easy to sterilize Reduced photoinhibition and photo-oxidation	Small illumination area Expensive compared to open ponds Shear stress Sophisticated construction

Table 1.5. Typical advantages and disadvantages of the three main types of closed reactors.

Taken from Kunjapur and Eldridge (2010).

Reactor Type	Typical Advantages	Typical Disadvantages
Flat plate Photobioreactor	Shortest oxygen path Low power consumption	Low photosynthetic efficiency Shear damage from aeration
Tubular Photobioreactor	High volumetric biomass density	Oxygen accumulation Photoinhibition Most land use
Vertical Photobioreactor	Greatest gas exchange Best exposure to light/dark cycles Least land use High photosynthetic efficiency	Support costs Scalability

It has been predicted that photobioreactors are likely to produce most of the biomass used for biodiesel in the future due to their high productivity levels (Chisti 2007), but, currently, most controlled PBRs cannot offer the productivity necessary to offset the high costs associated with their construction and operation (Kunjapur and Eldridge 2010).

Harvesting and Dewatering. Following algae cultivation, the microalgae must be concentrated and separated from the water through harvesting. Traditional harvesting methods include micro-screening and filtration, centrifugation, flocculation, and autoflocculation in which an interruption in the carbon dioxide supply causes algae to flocculate on its own (Amin 2009). Although there is no standard harvesting method (Brennan and Owende 2010), flocculation is often used as the first stage in which metal salts such as alum are used to aggregate algal cells to increase the effectiveness of subsequent centrifugation, filtration, or sedimentation (Pittman, Dean et al. 2011).

Centrifugation is the preferred method for the harvesting of algal cells due to its high recovery efficiency and compatibility with many species of algae, but it is also the most energy intensive method (Pittman, Dean et al. 2011). In general, harvesting accounts for highest energy input for production of biofuels from microalgae (Brennan and Owende 2010). Alternative methods with high recovery efficiency and low energy and chemical use are being investigated; many of these involve the immobilization of algal cells during cultivation so that the harvesting process downstream is more efficient (Pittman, Dean et al. 2011). Microalgae must then be dried, or dewatered, to further concentrate the algae for the subsequent extraction phase.

Oil Extraction and Energy Production. Following harvesting and dewatering, oil is extracted through a variety of methods and is then converted to useful energy through a variety of processes including transesterification, gasification, liquefaction, pyrolysis, hydrogenation, and fermentation (Amin 2009). The products which can be produced as a result of these processes are shown in Figure 1.8.

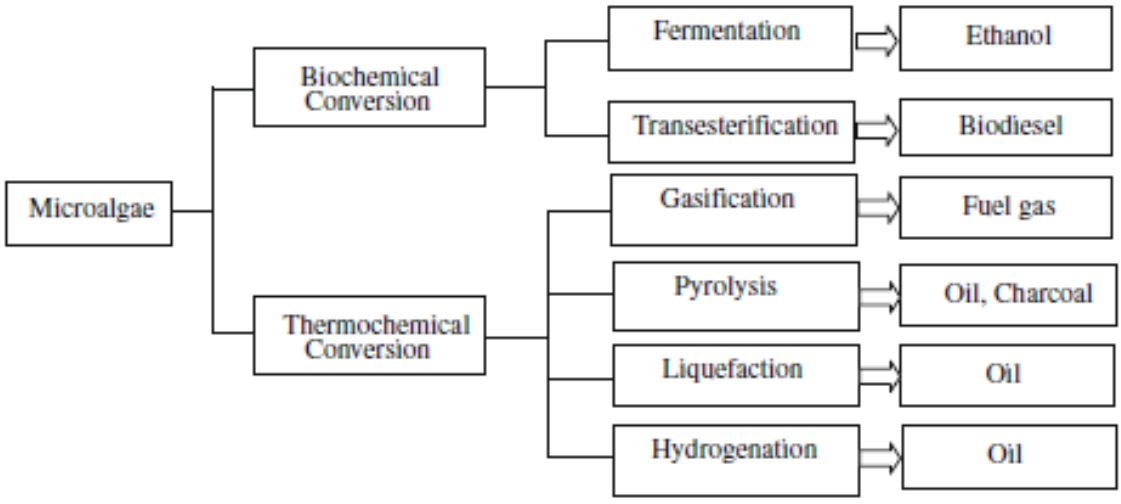


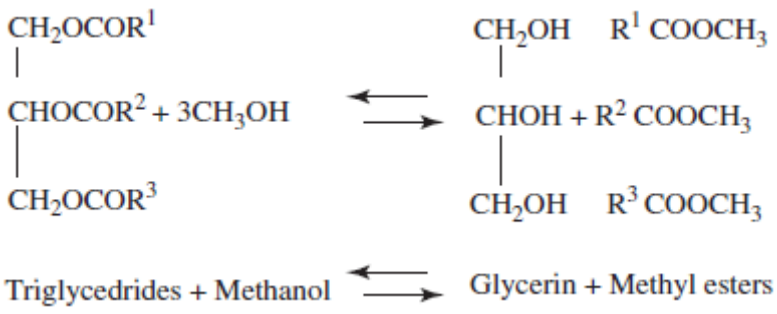
Figure 1.8. Energy conversion processes from microalgae.

Taken from Amin (2009).

Biodiesel, a substitute for petroleum diesel, is produced through the transesterification process, Equation 1, while ethanol, a substitute for gasoline, is produced through the fermentation process (Amin 2009). The biodiesel production process is shown in Figure 1.9.

Equation 1. Transesterification reaction.

Taken from Behzadi and Farid (2007).



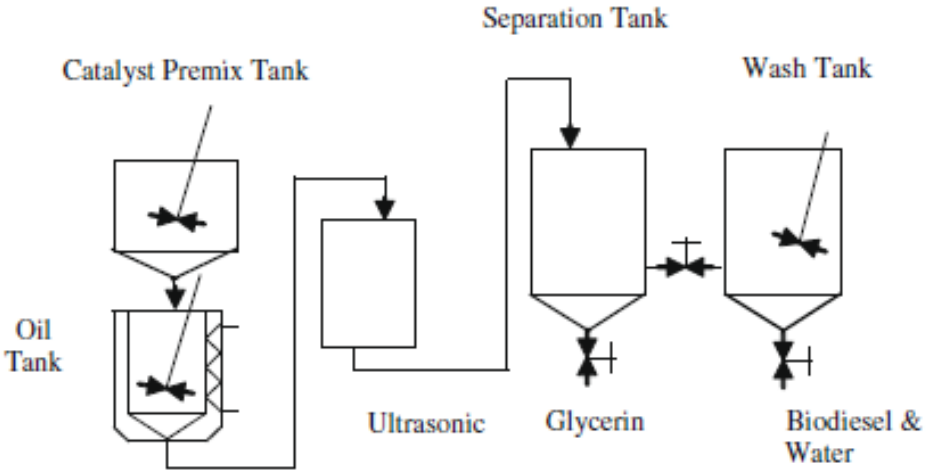


Figure 1.9. Schematic process of biodiesel production.

Taken from Amin (2009).

1.2.3.3 Oil Production Potential. The lipid content of microalgal cells is one of the primary factors that needs to be considered when choosing the algae species best for a system (Griffiths and Harrison 2009). Studies have reported a wide range of possible lipid concentrations in algal cells. The lipid content has been found to range between 5-64% dry weight (Griffiths and Harrison 2009) with an average oil content between 15-40% dry weight (Amin 2009) and up to 80% of the dry weight of algae (Chisti 2007; Patil, Tran et al. 2008). A summary of the oil content from a variety of microalga species is shown in Table 1.6.

Table 1.6. Oil content of some microalgae.

Taken from Chisti (2007).

Microalga	*Oil content (% dry weight)*
Botryococcus braunii	25-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Monallanthus salina	>20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
Phaedactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis sueica	15-23

1.2.3.4 Properties of Algal Biodiesel vs. Diesel Fuel. Algal biodiesel has properties similar to petroleum diesel (Table 1.7), and may be blended with petroleum diesel (Campbell 2008; Amin 2009) and used in engines, turbines, and for refinery feedstock (Amin 2009). Therefore, algal biodiesel can be easily incorporated into existing infrastructure. Additionally, ethanol can be used as 100% alcohol fuel or gasohol (Amin 2009).

Table 1.7. Comparison of properties of biodiesel, diesel fuel, and ASTM standard

Taken from Amin (2009).

Properties	Biodiesel from microalgae oil	Diesel fuel[a]	ASTM biodiesel standard
Density (kg/l)	0.864	0.838	0.86-0.90
Viscosity (mm^2/s, cSt at 40°C)	5.2	1.9-4.1	3.5-5.0
Flash point (°C)	115	75	Min 100
Solidifying point (°C)	-12	-50 to 10	-
Cold filter plugging point (°C)	-11	-3.0 (max -6.7)	Summer max 0 Winter max <-15
Acid value (mg KOH/g)	0.374	Max 0.5	Max 0.5
Heating value (MJ/kg)	41	40-45	-
H/C ratio	1.81	1.81	-

a. The data about diesel fuel was taken from published literature as indicated in the text.

1.2.4 Industrial Symbiosis

Industrial symbiosis is a growing portion of industrial ecology in which traditionally separate industries are combined through the use of the physical exchange of materials, energy, water, and co-products. The concept of industrial symbiosis stresses that industrial systems are not

viewed individually, but rather as a whole with other local systems in order to maximize output from all of these systems while minimizing inputs and wastes to and from the system. The greatest potential for industrial symbiosis lies at the local level, where the proximity to other industries allows transportation impacts to be minimized (Chertow 2000). Industrial symbiosis allows otherwise unrelated industries to obtain a collective benefit greater than the sum of the industries' individual benefits (Chertow 2000). There is a great potential for environmental improvement through industrial symbiosis: energy efficiency may be improved through cogeneration and by-product reuse, gray water can be recycled, and solvents and residue streams containing useful products can be reused rather than wasted in the system (Chertow 2000).

1.2.5 Coupling Wastewater Treatment with an Algal PBR

Algae can be used in the existing wastewater treatment process (Sheehan, Dunahay et al. 1998; Behzadi and Farid 2007; Li, Horsman et al. 2008; Pittman, Dean et al. 2011) to simultaneously remove nutrients from wastewater while producing algal biomass to be used for the production of biofuels and other useful products. By coupling the systems, algae farm construction can be minimized, waste from treatment plants can be reused as feed material for algae, and biodiesel costs can be reduced because of the raw material inputs and operating costs saved by coupling the system. The coupled system is shown in Figure 1.10.

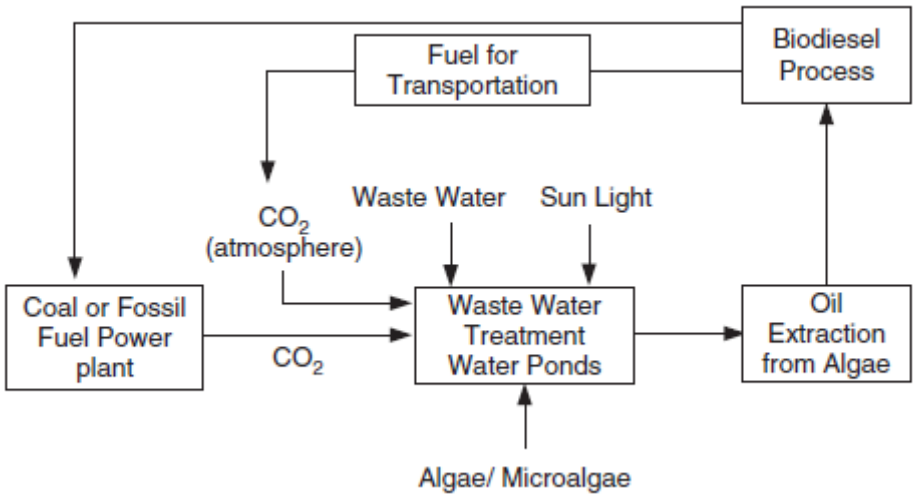


Figure 1.10. Microalgae in biodiesel process.

Taken from Behzadi and Farid (2007).

A system which couples biofuel production with wastewater treatment has the highest potential for commercial application and economic viability in the short term because of the simultaneous water treatment with biomass for biofuel production (Muñoz and Guieysse 2006; Pittman, Dean et al. 2011). Also, biodiesel productivity can be increased by using waste sources in place of fertilizers (Campbell 2008).

In addition to coupling microalgae cultivation with wastewater treatment, microalgal cultivation can be coupled with flue gas CO_2 bio-mitigation (Li, Horsman et al. 2008). Coupling the system with CO_2 recycling from power plants and other industrial processes may also help to mitigate environmental impacts and reduce costs (Muñoz and Guieysse 2006).

1.2.6 Other Renewable Resource Options for Expanded System

Multiple useful products and co-products may be produced using microalgal biomass, making the coupled system economically feasible (Muñoz and Guieysse 2006). Fuel and energy products which can be produced from the microalgae include methane gas (Sheehan, Dunahay et al. 1998), ethanol (Sheehan, Dunahay et al. 1998), biodiesel (Sheehan, Dunahay et al. 1998; Pienkos and Darzins 2009), steam or electricity from direct combustion (Sheehan, Dunahay et al. 1998), green diesel, green jet fuel, green gasoline (Pienkos and Darzins 2009), bio-oil, bio-syngas, and bio-hydrogen (Li, Horsman et al. 2008).

Other valuable products can be obtained from the microalgae. After lipids and carbohydrates are extracted from the biomass, leftover protein could be used for animal feed (McGarry and Tongkasame 1971; Dismukes, Carrieri et al. 2008). Nutritional supplements, cosmetics, dyes, and pharmaceuticals can also be produced from the microalgae (Rosenberg, Oyler et al. 2008).

The availability of fertilizers in many parts of the world has been found to be the limiting factor in crop growth for food production, particularly in developing countries (Benemann 1979). Additionally, chemical fertilizers have a high pollution potential (Benemann 1979). Phosphorus and nitrogen recovered from algal biomass can be used as fertilizer; these organic forms of fertilizer could replace chemical fertilizers and reduce the environmental impact of chemical fertilizers (Benemann 1979; Wilkie and Mulbry 2002; de-Bashan and Bashan 2004; Foley, de Haas et al. 2010). Although the economic feasibility of using algae to produce organic fertilizers is presently unknown, the demand for fertilizers and the increased realization of the negative impacts of chemical fertilizers is motivation for the continued economic development of a system which would produce fertilizers from algae (Benemann 1979). Additionally, heavy

metals and high-value chemicals in wastewater influent can be assimilated into the algal biomass and recovered for reuse (Mallick 2002; Li, Horsman et al. 2008).

Another opportunity for resource recovery in the system lies in the sludge coming from wastewater treatment: electricity can be produced through sludge digestion (Björklund, Geber et al. 2001) or from a bacterial fuel cell operating on carbohydrates (Niessen, Schröder et al. 2004) found in wastewater sludge and algal biomass. Ideally, the energy needed to produce microalgal biomass would be supplied from the energy created in the system (Chisti 2007), resulting in a self-sustaining system.

Finally, clean water can be considered as a beneficial product from the system if the wastewater is treated to the point where it could be reused as drinking water. A system capable of treating water to drinking water standards could reduce water shortages (McGarry and Tongkasame 1971) and eliminate the environmental impacts associated with further treatment of the water to potable standards.

1.3 LIFE CYCLE ASSESSMENT

LCA is a tool which is used to determine all of the environmental impacts from the cradle to grave of a product or process. The impacts from all stages of the product or processes' life cycle including raw material extraction, processing and manufacturing, use, end of life, and the transportation during and between phases are included in the analysis (Figure 1.11). It is important that an LCA is completed for all emerging products and processes, as concepts that may appear to be more sustainable in one aspect may have a higher impact when looking at all stages. It is useful to perform an LCA before important decisions are made so that time,

resources, and money are not invested in unsustainable alternatives (Baumann and Tillman 2004).

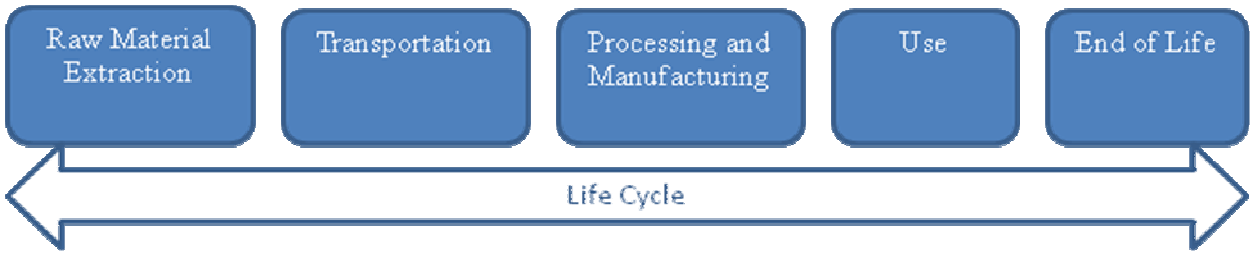


Figure 1.11. Life cycle stages.

The LCA method has been standardized by the International Organization for Standardization (ISO) according to the ISO 14040 (2006) document (ISO 2006). A process LCA includes all of the physical inputs to the system and outputs to the environment from the system over the life cycle.

1.3.1 LCA Method

An LCA is completed using the following four step process, defined by the ISO 14040 (2006) document and shown in Figure 1.12:

Goal and Scope Definition: Study boundaries and the purpose of the study are decided on, the application of the study and intentions of the results are stated

Inventory Analysis (LCI): Includes construction of the flow model, data collection for all the activities, and calculation of the system loads

Impact Assessment (LCIA): The environmental impacts of the system loads calculated in the LCI are quantified

Interpretation and Improvement Analysis: The study is revisited after completion to determine areas of improvement for future studies

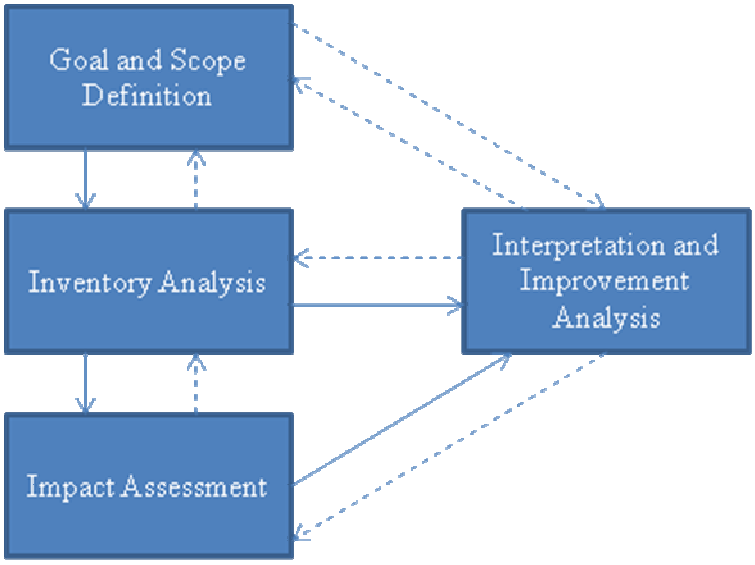


Figure 1.12. The life cycle assessment (LCA) procedure.

1.3.1.1 Goal and Scope Definition. During the goal and scope definition, the purpose of the study is defined. Boundaries of the system are defined in terms of what processes will be included in the study, what stages of the life cycle will be included, the timeline of the study, and the geographical limitations. All assumptions and limitations of the study are defined, and the impact categories that will be assessed and interpreted, as well as the impact assessment method,

are decided upon. A common unit in which all aspects of the life cycle can be compared equally, the functional unit, is defined.

The type of LCA which is being performed is also decided upon. A comparative LCA or improvement LCA may be performed. A comparative LCA is a type of LCA in which environmental impacts from alternate products or processes with the same function and functional unit are compared. An improvement LCA can be conducted to determine portions of the life cycle which account for a large part of the environmental impacts. Once these high-impact areas are identified, suggestions for improvement in the process or system can be made.

1.3.1.2 Inventory Analysis. During the inventory analysis, a flowchart showing the system boundaries and system processes is constructed. Inventory data is collected for all activities within the system boundary, and the environmental loads per functional unit are calculated. Additionally, the method of allocation is decided upon for products or processes which produce by-products within the system boundaries. Allocation impacts may be made physically in terms of economical value, product mass, or size, or through the use of system expansion. When system expansion is used, products or processes in which the production of by-products replaces may be included in an expanded system. The impacts of the traditional process can then be considered as avoided through the production of by-products.

1.3.1.3 Impact Assessment. During impact assessment, the environmental consequences of environmental loads from the inventory analysis are calculated. A variety of tools are available to calculate the environmental consequences based on characterization factors of the loads. For impacts assessed in the United States, the Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts (TRACI) may be used.

The environmental consequences of calculated loads are described by a number of impact categories, including global warming potential (GWP), acidification, carcinogenics, non-carcinogenics, respiratory effects, eutrophication potential (EP), ozone depletion, ecotoxicity, and smog.

GWP is calculated by the combination of carbon dioxide (CO_2), methane, chlorofluorocarbons (CFCs), nitrous oxide, and trace gases, also known as greenhouse gases (GHGs) which are emitted during the life cycle of the product or process. GWP is measured in terms of equivalent CO_2 emissions from all global warming causing processes.

The EP describes the impacts caused by high nutrient levels on waterways. Eutrophication is the result of increased biological productivity, often in the form of algae blooms, which them lead to oxygen consumption and results in dead zones due to lack of oxygen in the water. EP is measured in terms of equivalent N which are discharged into the environment as a result of a product or process.

1.3.1.4 Interpretation and Improvement Analysis. Following impact assessment, the results are interpreted to identify significant issues in the product or process's life cycle and to evaluate the method used for confidence in results. Conclusions and recommendations are made from the study; these can be used for product development, process improvement, and policy recommendations. The LCA methodology may also be assessed and modified as a part of the improvement analysis.

1.4 LITERATURE REVIEW

A review of the literature revealed many hypothetical scenarios involving the use of microalgal cultivation for simultaneous nutrient removal and renewable resource recovery. Still, very few studies tested this concept. All previous studies found were laboratory-scale studies and the potential for scale-up of the system is still uncertain. Only a few LCAs of the use of microalgae for biofuels were found. Some of these considered the use of wastewater as a nutrient supply to the reactor; however, only one study (Clarens, Resurreccion et al. 2010) considered the avoided impacts from wastewater treatment in the analysis.

1.4.1 Coupled Systems in the Laboratory

Very few studies involving the use of microalgae to produce renewable products while simultaneously removing nutrients from wastewater have been completed. In general, the studies which have been completed have used open ponds or PBRs with varying design parameters.

Two studies were found involving the use of open pond systems. Johnson and Wen (2010) developed an attached microalgal growth system supplied with wastewater with a goal of exploring the feasibility of the system in terms of productivity, nutrient removal, and harvesting efficiency supplied by the attached growth mechanism. The study resulted in a high potential for biofuels production with an acid methyl esters yield of 2.59 g/m^2, a productivity of 0.26g/m^2/day, and 61-79% total nitrogen and 62-93% total phosphorus removal from the wastewater. Harvested biomass had a water content of 93.75% which indicates potential of the attached microalgal growth system to eliminate or reduce primary harvesting phases (Johnson and Wen 2010).

Woertz et al. (2009) studied microalgae grown on a variety of wastewater media sources including municipal and agricultural wastes in an open pond system. Woertz et al. found peak lipid productivities ranging from 14-29% dry weight of algae with areal productivity of 2.8 g/m^2/day. Nutrients were effectively removed from wastewater; 96% ammonium removal and orthophosphate removal of greater than 99% was observed in the system (Woertz, Feffer et al. 2009).

A study by Kong et al. (2010) was conducted using Erlenmeyer flasks and PBRs for the cultivation of algae. In the study, *Chlamydomonas reinhardtii* was cultivated in artificial media and wastewater for the production of biofuels and simultaneous removal of nutrients from the

wastewater. Kong et al. found a relationship between nutrient level in the wastewater and algae growth; high nutrient concentration seemed to inhibit algae growth at the beginning of the 10 day period but then sustained high growth rates. CO_2 had a positive correlation with algae growth until a certain point at which the pH became too low in the reactors and growth declined. Dry algal biomass reached a yield of 2.0 g/L/day, while the oil content reached 25.25% (*w/w*) dry biomass weight. The system showed potential large amounts of nutrients to removed from the wastewater; 55.8 mg nitrogen/L/day and 17.4 mg phosphorus/L/day were removed from the wastewater during the 10 day period (Kong 2010).

Comparison of the results on a large scale is difficult due to the vast differences in design and operation of the systems, but all of the studies reviewed indicate the potential feasibility and productivity of a coupled system. Further testing of pilot-scale systems should be completed and the sustainability of a coupled system should be assessed before this system is implemented on a large-scale.

1.4.2 Life Cycle Assessments of Microalgae for Biofuels and Coupled Systems

A handful of LCAs have been completed to determine the environmental sustainability of using microalgae as a feedstock for biofuel production. Only a few of these studies consider the use of wastewater for algae cultivation, but all reveal important findings that can be used to optimize the process of microalgae use for biofuel production improve the sustainability of the system.

An LCA conducted by Stephenson et al. (2010) compared open pond cultivation with cultivation in a PBR. This study revealed that, if productivity goals could be met, the open pond would be more environmentally sustainable (Stephenson, Kazamia et al. 2010). When compared to the production of petroleum diesel, the GWP of an open pond system could be reduced by

approximately 80%, while the GWP would increase compared to petroleum diesel when cultivating algae in a PBR (Stephenson, Kazamia et al. 2010).

Lardon et al (2009) conducted a comparative LCA of a virtual biofuel production facility. Lardon et al. found there is potential success for biofuels from microalgae when compared with first generation biodiesel and oil diesel, but that the energy and fertilizer consumption for production must be decreased for this to be a viable option. Currently, under varying conditions, between 1.66 and 5.29 MJ of energy input was needed to produce 1 MJ biodiesel. Most of the required energy inputs came from heat needed to dry the biomass and fertilizer production (Lardon, Hélias et al. 2009). Overall, 90% of the energy consumed is related to lipid extraction; therefore, the extraction process must be improved and the process of wet extraction must be optimized to decrease energy consumption during the extraction stage. The control of nitrogen stress is also offered as a solution to decrease fertilizer use (Lardon, Hélias et al. 2009).

A study by Sander and Murthy (2010) also found that the need to efficiently process algae into its useful components is a major limitation of a current system. Results showed that thermal dewatering accounts for the majority of the energy usage for processing microalgae into its useful products, and is therefore an area of improvement for the system. Again, the authors concluded that technological improvements must be made before the use of microalgae for biofuel production can be feasible, energy efficient, and sustainable (Sander and Murthy 2010).

A study by Aresta et al. (2010) focused on different methods of fixation of CO_2 to algal biomass during the cultivation stage. This LCA revealed a potential energy benefit of recycling CO_2 for fixation to algal biomass when using wastewater effluent to supply nutrients to the algae. A potential net energy production of 11,000 $MJ/t_{dryalgae}$ was found, indicating the potential for energy production from a system utilizing waste sources (Aresta, Dibenedetto et al. 2005).

Clarens et al. (2010) was the sole study currently available which assessed the use of wastewater for nutrient supply to the microalgae and also considered the impacts of wastewater treatment which would be avoided by treating wastewater during algae cultivation. The consideration of wastewater use was motivated by the impacts of fertilizer production: fertilizer production accounts for 50% of energy use and GHG emissions during algae cultivation. The study revealed that 50-70% of the total offsets from coupling the two systems are from avoiding nutrient removal at the WWTP while 30-50% of the offsets are from avoiding fertilizer production, indicating that WWTPs may also have a vested interest in the technology in order to reduce impacts and costs at the WWTP (Clarens, Resurreccion et al. 2010). Coupling microalgal cultivation with wastewater treatment could reduce the life cycle burdens of using freshwater during algae cultivation by reducing fertilizer use and energy used during WWTr (Clarens, Resurreccion et al. 2010).

In a unique life cycle study, Yang et al. (2011) focused their LCA on the water and nutrient balance of biodiesel production from microalgae. Results of this study confirm the necessity of recycling wastewater and using seawater or wastewater as a water source for cultivation. Recycling water from the system after the algae is harvested reduces water consumption by 84% and nutrient consumption by 55%, while using wastewater reduces water consumption by 90% and eliminates the need for nutrients except for phosphate (Yang, Xu et al. 2011).

Due to the limited number of studies available, additional, more comprehensive LCAs are needed to assess the sustainability of a coupled system for the production of renewable resources from microalgae and the nutrient removal from wastewater through algal treatment. Clarens et al. (2010) is the sole study which fully addresses this topic. Lardon et al. (2009) considers the use

of wastewater as the nutrient supply for microalgae, but an LCA of a system using wastewater as the growth medium as well should be conducted (Pittman, Dean et al. 2011).

1.4.3 Problems Associated with Algae for Biofuels

Although promising results have been found, the development of a system using microalgae to produce biofuels remains in its infancy (Pienkos and Darzins 2009). Research has shown there are enough water, land, and CO_2 resources to support microalgal growth, but technology must improve before it can proceed on a large scale (Sheehan, Dunahay et al. 1998; Chisti 2007).

High costs do not allow microalgal biodiesel to be competitive with petroleum diesel (Sheehan, Dunahay et al. 1998; Chisti 2007; Campbell 2008; Johnson and Wen 2010). Also, because growth of microalgal biomass requires light, CO_2, temperature control, nutrients, and water, biofuel production from microalgae is usually more expensive than growing conventional crops (Chisti 2007). The development of cultivation and down-stream processes is needed to improve the cost-effectiveness of the system and for the system to be feasible (Li, Horsman et al. 2008; Brennan and Owende 2010; Pittman, Dean et al. 2011).

Development related to algae productivity and lipid accumulation is also needed. Lipid accumulation is greater with nitrogen deficiency, but the growth rate is slower under nitrogen-deficient conditions. A species which can maintain high productivity under nitrogen-deficient conditions must be found (Lardon, Hélias et al. 2009). Also, high productivity has not been seen in the less energy intensive open ponds (Pittman, Dean et al. 2011).

In general, large scale implementation of the coupled system is limited by the harvesting stage (Schenk, Thomas-Hall et al. 2008). When nutrients are assimilated by algae, organic suspended solids form in the treated wastewater effluent and must be removed in order for

effluents to meet standards for reuse or discharge into receiving waterways (Bich, Yaziz et al. 1999). Separation and harvesting techniques have been developed, but are costly and complicated due to high energy and chemical demands (Bich, Yaziz et al. 1999; Pittman, Dean et al. 2011). Harvesting is limited to the lowest-cost option due to economic realities; however, the lowest-cost option might not be the most effective option for harvesting (Sheehan, Dunahay et al. 1998).

There are also issues associated with the scale up of working laboratory systems to full-scale systems. Many systems that work in the lab do not end up working at a larger scale; specifically, oxygen degassing is possible at a laboratory scale but is difficult at a large scale (Carvalho, Meireles et al. 2006). The method of scaling up PBRs so that they can benefit from economies of scale and produce meaningful quantities of algal biomass must also be determined (Pienkos and Darzins 2009; Kunjapur and Eldridge 2010).

Finally, issues related to social sustainability must be addressed. Cultivation will most likely take place in previously undeveloped areas; therefore, ecological impacts, public perception, and permitting issues will have to be resolved before a large-scale system is implemented (Pienkos and Darzins 2009).

1.4.4 Critique of Literature

Because the development of microalgae for biofuels is still in its infancy, there are still many conflicting results and knowledge gaps related to a coupled wastewater treatment and algae cultivation system. Additional lab scale testing is needed to determine the most efficient reactor design and operation methods when using wastewater as a water and nutrient source for algae cultivation. Issues related to high energy use and cost in a PBR and low productivity in open

ponds must be resolved in order to determine which system is the most effective as this technology develops.

In order to effectively couple the algae cultivation system with the wastewater treatment process, the level of pretreatment needed at the wastewater treatment plant must be determined. Although nutrients entering wastewater treatment systems from raw influents are present in the form of ammonium and orthophosphate (Craggs, McAuley et al. 1997), the removal efficiency of other nitrogen species which may form during pretreatment of the influent must be determined. The levels of treatment needed in the WWTP combined with the nutrient removal and biomass productivity during algae cultivation must be optimized in order to develop a coupled system which produces the greatest amount of products and removes the greatest amounts of nutrients while using the lowest amounts of energy and chemicals.

Microalgae have been described as a sustainable energy resource by multiples sources (Li, Horsman et al. 2008; Amin 2009; Sander and Murthy 2010), but these claims have not been supported. A few assessments of environmental sustainability have been completed with conflicting results, but the economic and social aspects of sustainability must be addressed in order to label microalgae for biofuel production as sustainable.

As shown in Figure 1.13, the concept of using microalgae to produce products and treat wastewater in individual and coupled systems has been covered to a large extent in the literature. Still, laboratory studies which assess a coupled system are limited. LCAs of the coupled system are also limited, and an LCA of the system based on the productivity and nutrient removal capability of algae in a coupled scenario has not been completed. This study aims to improve upon existing studies which couple the systems in the laboratory, and also completes an LCA of

the system based on the productivity and nutrient removal capability of algae in a coupled scenario.

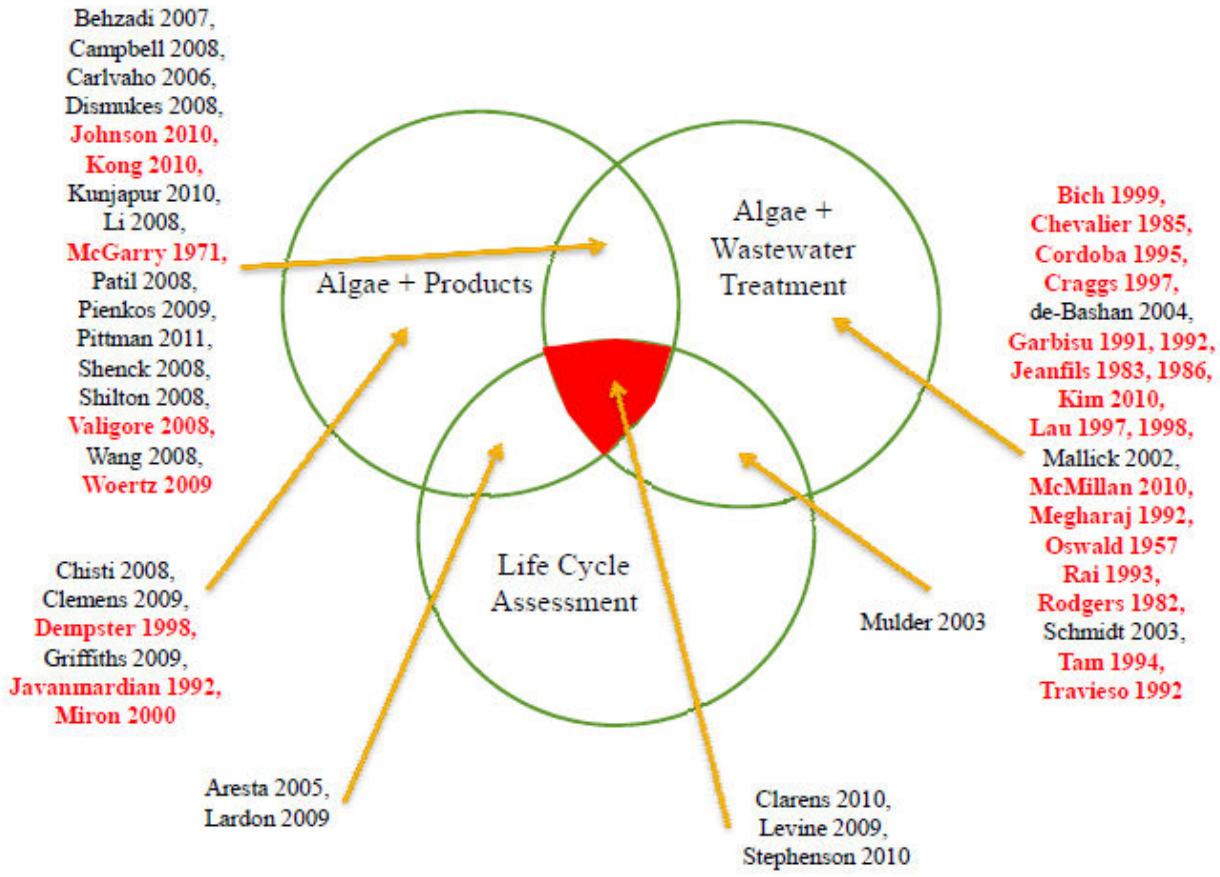


Figure 1.13. Related studies.

(Laboratory Studies). This study is the first study which has been found that combines using algae for wastewater treatment and product recovery and assesses the sustainability of the coupled system using Life Cycle Assessment based off of productivity and nutrient removal capability in the laboratory.

2.0 COUPLING THE WASTEWATER TREATMENT PROCESS WITH AN ALGAL PHOTOBIOREACTOR FOR NUTRIENT REMOVAL AND RENEWABLE RESOURCE PRODUCTION

A laboratory experiment was performed to determine the feasibility of coupling a conventional wastewater treatment system with an algal photobioreactor (PBR) for the removal of nutrients from wastewater and production of renewable resources. An activated sludge batch reactor was set up in series with an algal PBR to feed wastewater to the algae. The nutrient concentration in the water as well as lipid content, carbohydrate content, and growth rate of the algal biomass were tested over 10 cycles to determine the capabilities of the coupled system. The study revealed complete nutrient removal in some cycles, with the average final nutrient content of 2 mg-P/L and 3 mg-N/L in effluent of the PBR. The algae biomass contained 24±3% lipids and 26±7% carbohydrates by dry weight. A life cycle assessment revealed the highest energy demand occurred during harvesting of the algal mixture through centrifugation or filtration, but the highest global warming and eutrophication impacts were due to CO_2 use and PBR construction material production. It is feasible for the system to treat wastewater while generating renewable resources, but the system must be optimized to reduce life cycle environmental impacts and result in a net energy gain before large-scale implementation is possible.

2.1 INTRODUCTION

To meet growing clean water and energy demands, the concept of industrial symbiosis may be applied to a water and energy paradigm in which a wastewater treatment system is coupled with an algal photobioreactor (PBR). The paradigm has potential to reduce the environmental impacts of water and energy production by recovering resources from system wastes and using them to fuel other processes, making both water and energy production more sustainable.

The removal of nutrients from wastewater improves local water quality by decreasing eutrophication in receiving waterways (Burdick, Refling et al. 1982; Foley, de Haas et al. 2010), and may allow water to meet criteria for reuse (McGarry and Tongkasame 1971). The current solution to improving water quality through nutrient removal is to invest more energy and resources into wastewater treatment plants (WWTPs) (Burdick, Refling et al. 1982; Foley, de Haas et al. 2010). The nutrient concentration of wastewater can be reduced to low levels, but the advantages of advanced treatment are often offset by the additional consumption of energy and resources (Burdick, Refling et al. 1982; Foley, de Haas et al. 2010). The additional burden placed on WWTPs will continue to increase as discharge requirements become more stringent in the future (Landers). It is estimated that the addition of nitrification to an activated sludge wastewater treatment plant results in an increase of 60-80% of the energy consumed at the plant (Maurer, Schwegler et al. 2003), and evidence suggests that nitrous oxide emissions increase when biological nutrient removal is added to a WWTP (Kampschreur, van der Star et al. 2008).

Meanwhile, the growing demand of energy has prompted the search for a safe, sustainable, and renewable energy source. The environmental, geopolitical, and economic consequences of fossil fuel production and use are well established (Hill, Nelson et al. 2006; Brennan and Owende 2010). First generation biofuels produced from corn, soybeans, and other

food crops compete with food supplies and would require vast amounts of land to replace fossil fuels (Hill, Nelson et al. 2006; Chisti 2007; Brennan and Owende 2010; Sander and Murthy 2010; Stephenson, Kazamia et al. 2010).

Interest has been growing in third generation microalgal biofuels as an alternative to fossil fuels or first generation biofuels (Chisti 2007; Brennan and Owende 2010; Johnson and Wen 2010; Sander and Murthy 2010; Stephenson, Kazamia et al. 2010). The advantages of microalgal biofuel production are numerous: they are renewable with short harvesting periods, do not compete with food sources, do not require large amounts of land, and can be grown with wastewater sources (Chisti 2007; Brennan and Owende 2010; Clarens, Resurreccion et al. 2010; Sander and Murthy 2010; Stephenson, Kazamia et al. 2010). Despite tangible benefits of algae based biofuels, high production costs have inhibited commercial viability (Sheehan, Dunahay et al. 1998; Johnson and Wen 2010; Sander and Murthy 2010). Stephenson et al. reports the phase with the highest impact during the biofuel production process is algae cultivation (Stephenson, Kazamia et al. 2010), and is therefore an area for potential improvement. Nutrients such as nitrogen and phosphorus are necessary for algae growth (Chisti 2007; Brennan and Owende 2010; Clarens, Resurreccion et al. 2010), but industrial fertilizer production results in about 50% of the energy use and greenhouse gas emissions in algae cultivation (Clarens, Resurreccion et al. 2010).

Wastewater can be used to supply nutrients to microalgal photobioreactors (PBRs) (Brennan and Owende 2010; Stephenson, Kazamia et al. 2010), thus reducing the impacts of the algae cultivation stage (Clarens, Resurreccion et al. 2010). As algae consume nutrients during growth, wastewater quality is improved (Oswald, Gotaas et al. 1957; Craggs, McAuley et al. 1997; Bich, Yaziz et al. 1999; Shilton 2008; Brennan and Owende 2010; Clarens, Resurreccion

et al. 2010) and the energy requirement at municipal WWTPs can be reduced (Clarens, Resurreccion et al. 2010). As algae consume nutrients, microalgal biomass in the reactor grows and accumulates lipids (Chisti 2007; Johnson and Wen 2010; Sander and Murthy 2010; Stephenson, Kazamia et al. 2010) which can then be extracted and processed to produce biodiesel (Chisti 2007; Brennan and Owende 2010; Stephenson, Kazamia et al. 2010), while carbohydrates found in algal biomass can be used to produce bioethanol (Brennan and Owende 2010; Sander and Murthy 2010; Stephenson, Kazamia et al. 2010). Other useful products including biopolymers, fertilizer, and feedstock can be produced from algal biomass (Chisti 2007; Brennan and Owende 2010; Sander and Murthy 2010; Stephenson, Kazamia et al. 2010), resulting in a robust system.

Previous studies have demonstrated the feasibility of a coupled system for the treatment of wastewater and cultivation of algae for biofuels on a small scale (Woertz, Feffer et al. 2009; Johnson and Wen 2010; Kim, Miyahara et al. 2010; Kong 2010); however, the long term feasibility and sustainability of algae production must be evaluated before production is considered on a large scale (Brennan and Owende 2010; Clarens, Resurreccion et al. 2010; Sander and Murthy 2010). Life cycle assessment (LCA) is a tool which can be used to assess environmental sustainability in terms of the cradle-to-grave environmental impacts of a process before it is fully implemented (Baumann and Tillman 2004). Previous life cycle studies of algae cultivation have relied upon theoretical yields of biomass cultivated in wastewater, and many do not consider the additional treatment of wastewater as an added benefit to biofuels production. The goal of this research is to determine the energy production and nutrient removal capabilities of an algal PBR being fed with wastewater, and the associated energy demands and environmental impacts of a coupled WWTP/PBR system.

2.2 MATERIALS AND METHODS

2.2.1 Laboratory Setup

A conventional bioreactor (CBR) consisting of primary wastewater treatment with basic activated sludge secondary treatment for the removal of organics was a 5 L liquid volume sequencing batch reactor operated with a 24 hour cycle (Figure 2.1, Figure 2.2). The biomass for the reactor came from the McKeesport, PA wastewater treatment plant. The sequencing batch system was automated as follows: 20 hour and 10 minute mixing period, 3 hour and 45 minute settling period, and 3 minute effluent discharge. The CBR was fed synthetic wastewater with acetate as the primary substrate. The biological oxygen demand (BOD) of the synthetic wastewater was 300 mg/L and nutrients were fed in the form of ammonium chloride, potassium phosphate (mono- and di-basic) in a BOD:N:P ratio of 30:3:1 along with trace metals. The solid retention time (SRT) and hydraulic retention time (HRT) of the CBR were 20 and 10 days, respectively. The CBR pH control was automated to remain between 7 and 7.5 using sodium hydroxide and hydrochloric acid. Mixing and aeration were provided using a magnetic stir rod and air pump.

Effluent from the CBR was used to supply the PBR with water and nutrients. Algae were cultivated in the PBR in seven day cycles. The PBR used in this setup was a glass cylinder, 15 cm tall and 7.5 cm in diameter with a working volume of 700 mL. The PBR was inoculated with the *Chlorella vulgaris* algae strain, cultivated at the University of Texas at Austin (UTEX 1803). Continuous lighting was provided by two 8-Watt tube fluorescent LEDs located 7 cm from either side of the PBR. The PBR was mixed using a stir plate and magnetic stir rod. Mixing allowed

nutrients to be dispersed throughout the reactor and provided algae with beneficial light and dark cycles conducive to growth in a concentrated system (Qiang and Richmond 1996).

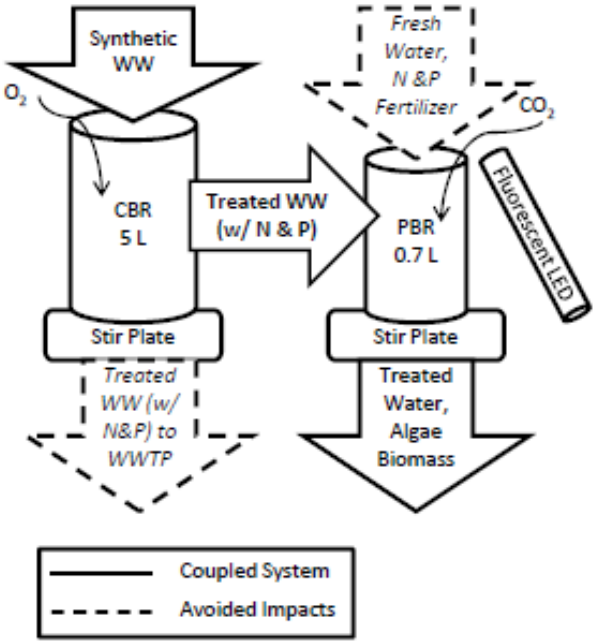


Figure 2.1. Laboratory setup of coupled system.

The coupled system consisted of a 5 L conventional bioreactor (CBR) and a 0.7 L photobioreactor (PBR). Both reactors were mixed using a stir rod and stir plate. Oxygen was added to the CBR for aeration, while carbon dioxide was added to the PBR to stimulate algae growth. Lighting for the PBR was provided continuously by a fluorescent LED tube light. The lightweight solid line represents the coupled system used in the experiment, while the dashed line indicates water and nutrient flows which can be avoided by coupling the system.

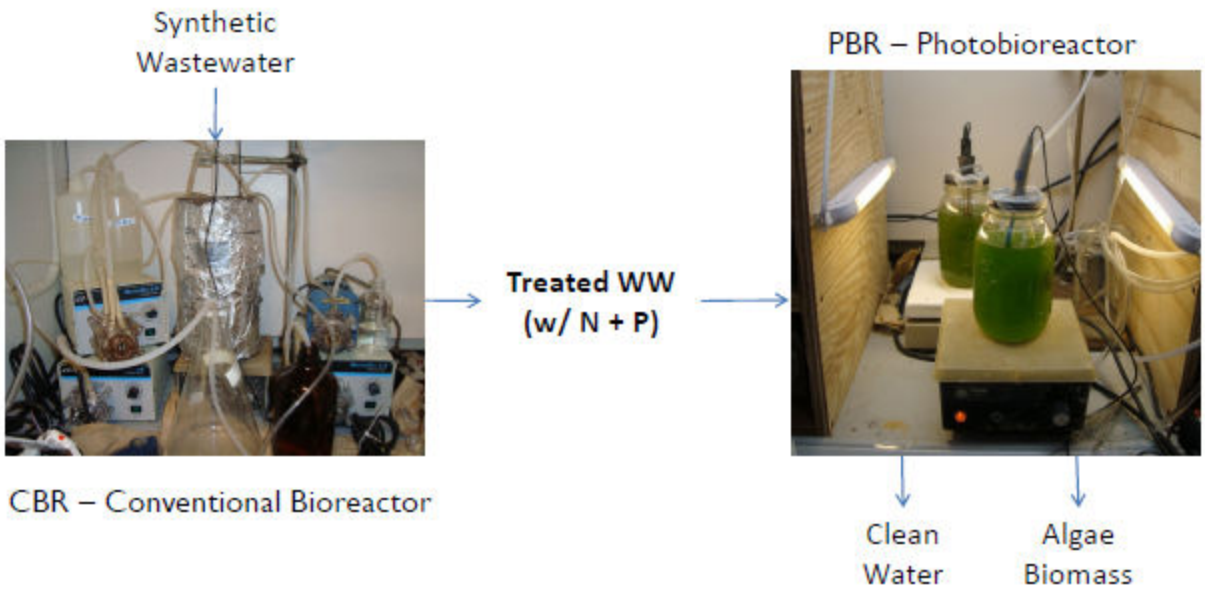


Figure 2.2. Coupled CBR and PBR in the laboratory.

Carbon dioxide CO_2 was supplied to the PBR from a k-size tank via stone aerators. Because of the great impact a continuous CO_2 feed would have on the pH of the PBR, a discontinuous feed of CO_2 was supplied at a rate of about 5 g CO_2/day. The pH in the PBR was monitored by a pH probe. Because of the rapid decrease in the pH of the water when CO_2 was added to the PBR, $NaHCO_3$ was added as a buffer to stabilize the pH when needed. Temperature was kept constant at 20°C in the CBR and PBR. Each week, 250 mL of the algae mixture was harvested from the PBR and replaced with 250 mL of nutrient-rich effluent wastewater from the CBR.

2.2.2 Analytical Methods

Water going into the CBR (influent) was a synthetic wastewater feed with controlled nutrient levels comparable to influent at a wastewater treatment plant. Water quality was tested on water coming out of the CBR (CBR effluent), and in the PBR at the beginning and end of each cycle for 10 consecutive cycles (Cycles 1-10). Cycles were defined as the 7-day growth period following the addition of CBR effluent to the PBR. Each cycle ended with the harvesting of 250 mL of algae mixture from the PBR, which was then replaced by 250 mL CBR effluent for the next cycle. Standard Methods (APHA 1992) were used to measure ammonia-nitrogen (APHA 4500-NH_3 C), nitrite-nitrogen (APHA 4500-NO_2^- B), nitrate-nitrogen (APHA 4500-NO_3^- D), orthophosphate-phosphorus (APHA 4500-P C), and total suspended solids (APHA 2540 D). Ammonia-nitrogen, nitrite-nitrogen, and orthophosphate-phosphorus concentrations were measured using a Spectronic 20 spectrophotometer (Bausch and Lomb), while nitrate-nitrogen concentrations were measured using a nitrate electrode (Oakton Nitrate Double-junction Ion-Selective Electrode). The optical density of the samples was measured at 600nm using a Spectronic 20 spectrophotometer (Bausch and Lomb) as a non-destructive estimate of total suspended solids (TSS). The change in TSS was used as a measure of biomass growth in the PBR.

During Cycle 9, the water quality and optical density were measured daily to evaluate nutrient removal patterns in the reactor throughout the cycle. These measurements were compared to an additional low concentration PBR during Cycle 9 only; the low concentration PBR consisted of a *C. vulgaris* algae mixture diluted to 100 mg/L at the beginning of the cycle and was set up and run in the same manner as the original reactor.

Lipids were measured to determine the expected biodiesel yields, while carbohydrates were measured to determine the possible bioethanol production capabilities from the PBR. The algal biomass was analyzed using fluorescent microscopy to detect lipid droplets (Listenberger and Brown 2001). A sample containing algae cells was taken at the end of each cycle and analyzed visually using a Nikon Eclipse E800 Biorad confocal fluorescent microscope (B&B Microscopes, Ltd.) at 60 times magnification to determine the approximate lipid content of the sample. The carbohydrate content in the algae biomass at the end of each PBR cycle was measured by the anthrone method (Dreywood 1946). The absorbance of treated biomass samples was measured using a Spectronic 20 spectrophotometer (Bausch and Lomb), and these absorbance measurements were compared with those of treated glucose samples to determine the carbohydrate content of the algal biomass.

2.2.3 Life Cycle Assessment Methodology

The environmental impacts of the algae cultivation and harvesting phases in a coupled system were analyzed using a Life Cycle Assessment (LCA). The ISO 14040 method (ISO 2006) was used to conduct the LCA; the four steps of an LCA, including 1) Goal and Scope Definition, 2) Life Cycle Inventory, 3) Life Cycle Impact Assessment, and 4) Interpretation and Improvement Analysis, were completed as a part of this study.

2.2.3.1 Goal and Scope Definition. The goal of this study was to determine the life cycle environmental impacts of algae cultivation and harvesting in a system coupled with wastewater treatment in terms of global warming potential (GWP) and eutrophication potential (EP), and also to determine the direct energy use during cultivation and harvesting of microalgae for biofuels and other renewable products. Although other environmental impacts could be assessed, the focus of the study is the reduction of GWP and EP through the use of the coupled system and therefore, these impact categories were chosen as the focus of the study.

The functional unit was defined in this study as 1,000 MJ of microalgal diesel. The function of the algae cultivation system in this study was assumed to be to produce energy in the form of biodiesel from algae; therefore, the functional unit was based on a unit of energy which could be obtained from the algae. By normalizing impacts to a unit of energy, the life cycle impacts of algae cultivation and harvesting can easily be compared to sources of energy obtained through other algae cultivation and harvesting scenarios as well as to energy obtained from other sources such as fossil fuels or 1st generation biofuels.

The process LCA conducted is an improvement LCA; it is the aim of the study to show that the life cycle environmental impacts of algae cultivation for biofuels can be reduced through coupling the system, and to determine other areas or processes in the algae cultivation and harvesting process where impacts could be reduced.

The portion of the expanded water and energy paradigm (Figure 1.1) in which this study focuses on is shown in Figure 2.3. The focus of this study was the cultivation stage, but it is assumed that the algae mixture would have to go through the harvesting stage in order for water to be at an equivalent quality level to that being treated by nitrification/denitrification and chemical phosphorus removal. Therefore, both the cultivation and harvesting stages were

considered in this assessment. It is assumed that, aside from PBR construction, existing infrastructure could be used for cultivation and harvesting, and transportation would be minimized by cultivating algae close to the wastewater treatment plant.

When coupling the wastewater treatment system with an algal photobioreactor, the need for synthetic fertilizers for algae cultivation and the need for denitrification and phosphorus removal in the wastewater treatment plant is eliminated. When considering algae cultivation and harvesting for the production of biofuels only in the LCA, the ability of the algae to remove nutrients during cultivation is not directly accounted for. Therefore, the impacts of the energy and chemicals that would have been needed to produce final products equal in quality to those produced by the coupled system were considered to be avoided impacts in the system and were taken as negative values in the life cycle impact assessment.

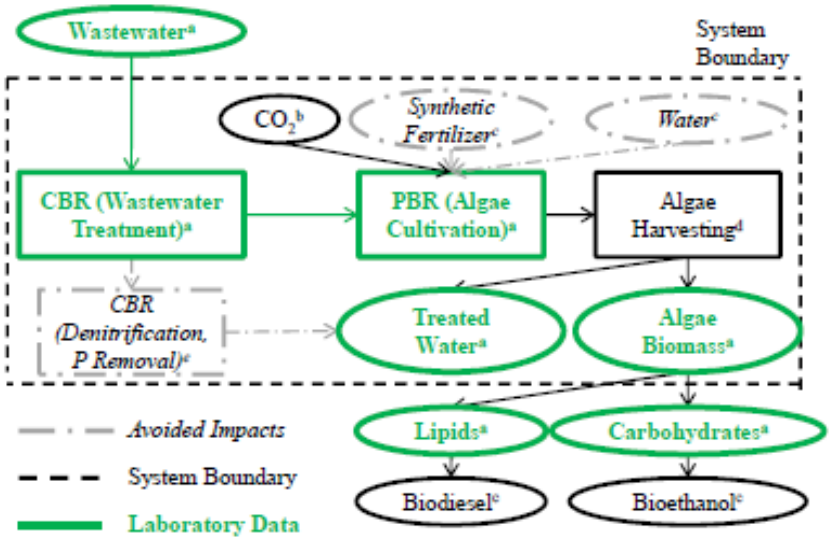


Figure 2.3. Life cycle assessment study boundaries.

The system boundaries include partial treatment in a conventional bioreactor (CBR) coupled with algae cultivation in an algal photobioreactor (PBR) and harvesting of the algae. Final products produced in the system are treated water and algae biomass. Equivalent products could be produced through the use of further treatment in the CBR consisting of denitrification and phosphorus removal, or through the use of synthetic fertilizers for algae cultivation. These products and processes are therefore considered to be avoided impacts. The life cycle inventory was calculated using data from a) laboratory results, b) laboratory results and (Kadam 2002), c) not directly considered in study, d) (Shelef, Sukenik et al. 1984; Batan and al. 2010), e) (Maurer, Schwegler et al. 2003; Metcalf, Eddy et al. 2004).

The life cycle environmental impacts of algae cultivation and harvesting were calculated assuming a large scale process would be capable of biomass yields, lipid yields, and nutrient removal efficiency from the system in the study, but that production would take place in an industrial setting in the United States. Therefore, industrial type PBR configurations, mixing, CO_2 transfer, harvesting, and conventional wastewater treatment were considered in a scaled-up

coupled system for the LCA. Because there are currently no full scale coupled systems in existence, the scale up of the laboratory system was completed based on the best available data and projections of how a full scale algae cultivation and harvesting system would likely operate based on existing industrial processes. Laboratory data as well as other published data was used in the study; the source of the data used for each process are shown in Figure 2.3 and Table 2.2.

2.2.3.2 Life Cycle Inventory. Life cycle inventory data was collected from various databases as well as lab data. The inventory and databases used are shown in Table 2.1, while calculated inputs to the system as well as the source of the inputs are shown in Table 2.2.

Table 2.1. Life cycle inventory databases.

Processes	**Portion of Coupled System**	**Database**
LDPE	PBR Material	ETH-ESU 96
Urea (N)	Avoided Synthetic Fertilizer	Ecoinvent
Superphosphate (P)	Avoided Synthetic Fertilizer	Ecoinvent
Soda, Powder (Na_2CO_3)	Avoided Nitrogen Removal Chemical	USLCI
Iron Sulfate ($FeSO_4$)	Avoided P-Precipitation Chemicals, Flocculation	ETH-ESU
CO_2 B250	Carbon Dioxide	BUWAL 250
Electricity avg. kWh USA	Nitrogen and Phosphorus Removal in WWTP, Mixing, CO_2 injection, Centrifugation, Filtration	Franklin USA 98

Table 2.2. Life cycle inventory inputs to the Excel model.

Parameter	Input	Reference
Energy Content of microalgal diesel	37.8 MJ/$kg_{microdiesel}$	(Lardon, Hélias et al. 2009)
Lipid Content	24% by dry weight	Lab data
Microalgae Productivity	0.9 g/m^2/day	Lab data
Harvesting Efficiency	100%	Assumption
Extraction Efficiency	100%	Assumption
Conversion Efficiency	100%	Assumption
Surface area/volume	400 m^2/m^3	(Schenk, Thomas-Hall et al. 2008)
Unit Volume	10 m^3	(Carvalho 2006)
System Lifetime	15 years	Assumption
PBR Material	LDPE, 1 cm thick	Assumption
Water loss	0%	Lab data
Wastewater use	0.0012 $m^3/kg_{microalgal\ mass}$	Lab data
Nitrogen Required	0.013 $kg_{Nitrogen}/kg_{microalgal\ mass}$	Lab data
Phosphorus Required	0.0069 $kg_{Phosphorus}/kg_{microalgal\ mass}$	Lab data
Denitrification (Avoidance)	7.5 kg-$Na_2CO_3/kg_{Nitrogen}$	(Metcalf, Eddy et al. 2004)
Phosphorus Precipitation (Avoidance)	1.8 kg-$FeSO_4/kg_{Phosphorus}$	(Metcalf, Eddy et al. 2004)
Nitrogen Removal Energy (Avoidance)	14.0 MJ/$kg_{Nitrogen}$	(Maurer, Schwegler et al. 2003)
Phosphorus Removal Energy (Avoidance)	24.0 MJ/$kg_{Phosphorus}$	(Maurer, Schwegler et al. 2003)
Fertilizer – urea (N) (Avoidance)	0.013 $kg_{Nitrogen}/kg_{microalgal\ mass}$	Assumption
Fertilizer – Superphosphate (P) (Avoidance)	0.0069 $kg_{Phosphorus}/kg_{microalgal\ mass}$	Assumption
Mixing	Peristaltic Pump – 300 W, 24 h/day	Assumption
Pure CO_2	150 kg-$CO_2/kg_{microalgal\ mass}$	Lab data
Energy for CO_2 transfer	0.2 MJ/$kg_{microalgal\ mass}$	(Kadam 2002)
Lighting	Natural Light	Assumption
Temperature Control	None	Lab data
Algal slurry Concentration (from harvesting process)	5% TSS	(Shelef, Sukenik et al. 1984)
Flocculent	0.07 kg-$FeSO_4/m^3$	(Shelef, Sukenik et al. 1984)
Centrifugation	1.0 kWh/m^3	(Batan and al. 2010)
Screening (Microstrainers)	0.2 kWh/m^3	(Shelef, Sukenik et al. 1984)
Filtration (Suction Filter)	0.1 kWh/m^3	(Shelef, Sukenik et al. 1984)

2.2.3.3 Life Cycle Impact Assessment. Inputs to the system were analyzed using the Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts (TRACI) (Bare 2002) to determine the life cycle environmental impacts of a hypothetical large-scale algae cultivation and harvesting system in the US. The GWP and EP of the system were looked at in detail as these are areas of concern specifically related to the study. Additionally, the direct energy use (DEU) associated with each portion of the cultivation and harvesting stages was reported (Table 2.3). Direct energy use is defined as the energy used during the cultivation and harvesting stages and does not include upstream or downstream energy use associated with these stages.

Table 2.3. Direct Energy Use (DEU), Global Warming Potential (GWP), and Eutrophication Potential (EP) of system during cultivation and harvesting stages.

Stage		DEU (MJ/1,000 MJ)	GWP (kg CO_2-eq/1000 MJ)	EP (kg N-eq/1000 MJ)
Cultivation	Nitrogen Removal (Avoided Energy)	-1.99×10^{1}	-1.38	-1.24×10^{-4}
	Phosphorus Removal (Avoided Energy)	-1.82×10^{1}	-1.27	-1.14×10^{-4}
	Nitrogen Removal (Avoided Na_2CO_3)	0	-9.87	-5.22×10^{-4}
	Phosphorus Removal (Avoided $FeSO_4$)	0	-1.11×10^{-3}	-1.44×10^{-7}
	Urea (Avoided Fertilizer)	0	-4.78	-2.34×10^{-3}
	Superphosphate (Avoided Fertilizer)	0	-2.05	-9.32×10^{-3}
	Mixing	5.31×10^{1}	4.77×10^{-3}	7.64×10^{2}
	Industrial CO_2	0	4.29×10^{3}	9.61×10^{-2}
	Injection of Industrial CO_2	4.16×10^{2}	2.89×10^{1}	2.60×10^{-3}
	Waste CO_2	0	-4.29×10^{3}	-9.61×10^{-2}
	Injection of Waste CO_2	9.42×10^{3}	6.55×10^{2}	5.88×10^{-2}
	PBR (LDPE)	0	3.35×10^{2}	3.28×10^{-1}
Harvesting	Flocculant	0	1.75×10^{-3}	7.74×10^{-7}
	Centrifugation	3.97×10^{4}	2.76×10^{3}	2.48×10^{-1}
	Filtration/Screening	1.19×10^{4}	8.28×10^{2}	7.43×10^{-2}

2.3 RESULTS AND DISCUSSION

Experimental testing of the coupled system indicates that wastewater treatment can be coupled with a microalgal PBR to remove nutrients in the wastewater while producing precursors to multiple useful, renewable products. The examination of water quality in the coupled system showed removal of soluble orthophosphate and total inorganic nitrogen as well as individual nitrogen species. The production of precursors to beneficial products was evaluated, and final beneficial product yields from the system were estimated based on product content and additional literature. Life cycle environmental impacts of the system were calculated to determine areas with great environmental impacts in the system, and total impacts were compared to existing related studies.

2.3.1 Water Quality

The quality of the effluent wastewater varied each week, which was consistent with performance variations at a full-scale treatment plant. In this study, water quality was measured by the concentration of phosphorus and nitrogen species. N and P transformations were observed through each cycle of water treatment in the CBR and algae cultivation in the PBR.

Water quality was measured on samples of CBR effluent going into the PBR for each cycle. Water quality in the CBR was monitored throughout the week for its daily cycles. The total suspended solids (TSS) concentration in water coming directly from the CBR was 11.3 mg/L, on average, while the pH was maintained at 7.2 on average with the addition of NaOH or HCl as needed. The nutrient content of the CBR effluent for each cycle is shown in Table 2.4.

Table 2.4. CBR effluent water quality.

Cycle	Phosphorus (mg-P/L)	Total Inorganic Nitrogen (mg-N/L)	Ammonia (mg-N/L)	Nitrite (mg-N/L)	Nitrate (mg-N/L)
1	--	--	--	--	--
2	28.1	19.9	3.8	0.01	16.1
3	7.8	18.8	2.0	0.07	16.7
4	2.2	5.5	1.0	0.00	4.5
5	7.8	14.7	2.0	0.00	12.7
6	9.1	9.6	1.7	0.00	7.9
7	7.8	5.9	1.8	0.00	4.0
8	10.4	12.3	1.1	0.00	11.3
9	1.7	16.1	0.0	0.03	16.0
10	1.0	11.7	0.0	0.01	11.7
Average	8.5	12.7	1.5	0.02	11.2
St. Dev.	8.2	5.2	1.2	0.02	4.8

The average nutrient concentrations at various points in the system are shown in Figure 2.4. The average reduction of soluble orthophosphate through the system was 8.2 mg-P/L (82% reduction), while the average reduction of total inorganic nitrogen was 27.3 mg-N/L (91% reduction).

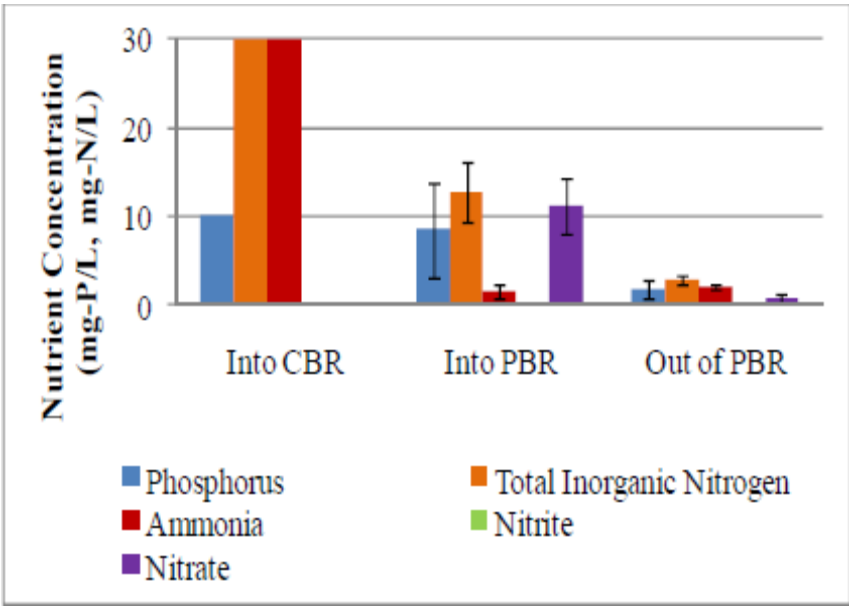


Figure 2.4. Nutrient concentration at various points in the system. Phosphorus, total inorganic nitrogen, ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen levels in water going into the conventional bioreactor (CBR), coming out of the CBR and going into the photobioreactor (PBR), and coming out of the PBR. Total inorganic nitrogen is the sum of ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen. 95% confidence intervals are shown for nutrient concentration levels. A synthetic feed containing 10 mg/L phosphorus and 30 mg/L ammonia is fed to the CBR daily to mimic nutrient loading in a municipal treatment plant. Phosphorus removal in the CBR is not statistically significant; however, ammonia is oxidized through nitrification in the CBR. Ammonia is converted to nitrite and then to nitrate in the CBR and some is then removed from the CBR. Significant phosphorus and total inorganic nitrogen removal was seen in the PBR, indicating that these nutrients can be removed by algae in a PBR without the addition of methanol or iron sulfate which are regularly used in denitrification and chemical phosphorus removal.

Over all 10 individual PBR cycles, complete phosphorus removal was observed and nitrogen was reduced to a minimum of 1.9 mg-N/L, 75% reduction in the PBR. The change in phosphorus, total inorganic nitrogen, and nitrate concentration going into and out of the PBR

were statistically significant at a p-value of 0.05, while the change in ammonia and nitrite concentration in the PBR were not statistically significant (Table 2.5).

Table 2.5. Reduction in nutrient content between CBR effluent going into the PBR and effluent coming from the PBR.

Cycle	Phosphorus (mg-P/L)	Total Inorganic Nitrogen (mg-N/L)	Ammonia (mg-N/L)	Nitrite (mg-N/L)	Nitrate (mg-N/L)
1	--	--	--	--	--
2	26.3	21.9	1.9	0.01	16.1
3	6.0	17.2	-0.7	0.06	15.7
4	1.6	4.1	-0.9	-0.01	4.0
5	6.0	14.5	-0.2	-0.01	12.7
6	6.9	6.9	-0.7	0.00	5.9
7	2.7	5.5	-0.4	0.00	4.0
8	10.4	10.0	-0.7	-0.02	9.6
9	1.7	14.2	-0.7	0.02	14.8
10	0.9	9.0	-1.6	0.01	10.5
Average	7.0	11.5	-0.4	0.01	10.4
p-value (Δ>0)	0.015	0.000	0.110	0.236	0.000

The nutrient concentration of water in the PBR was measured at the beginning (Initial) and end (Final) of each cycle in water coming from the PBR. Initial measurements were made after wastewater was added to the PBR and mixed for an hour to ensure dispersion of the wastewater throughout the PBR. Total inorganic nitrogen content was determined by adding the ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen concentrations. Nutrient concentration for Cycles 1-10 as well as the average and standard deviation for Initial and Final readings are shown in Table 2.6.

Table 2.6. Nutrient concentration in water in the PBR

	Phosphorus (mg-P/L)		Total Inorganic Nitrogen (mg-N/L)		Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
Cycle	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	5.8	2.7	9.3	2.2	2.0	2.2	0.28	0.01	7.0	0.0
2	13.7	1.8	7.7	1.9	2.4	1.9	0.28	0.00	5.0	0.0
3	1.6	1.8	7.1	3.7	2.1	2.7	0.03	0.01	5.0	1.0
4	2.2	0.5	4.0	2.4	2.0	1.9	0.01	0.01	2.0	0.5
5	1.6	1.8	6.9	2.2	1.8	2.2	0.04	0.01	5.0	0.0
6	5.1	2.2	10.0	4.4	2.0	2.4	0.02	0.00	8.0	2.0
7	4.0	5.1	10.3	2.2	2.3	2.2	0.02	0.00	8.0	0.0
8	5.5	0.0	6.2	3.4	2.2	1.8	0.04	0.03	4.0	1.6
9	0.0	0.0	3.4	1.9	0.3	0.7	0.25	0.01	2.9	1.3
10	0.0	0.2	5.0	2.8	0.8	1.6	0.02	0.00	4.2	1.2
Average	4.0	1.6	7.0	2.7	1.8	2.0	0.10	0.01	5.1	0.8
St. Dev.	4.0	1.6	2.4	0.8	0.7	0.6	0.12	0.01	2.0	0.8

Nitrification in the CBR accounts for the discharge of nitrate into the PBR, but the data show that nitrate was removed in this microalgal system. This finding is important because activated sludge denitrification typically requires chemical (e.g. methanol) addition. The absence of nitrite in the system shows proper nitrification is occurring in the system. The reduction of nitrogen and phosphorus in the coupled system indicates the feasibility of removing nutrients in the system without the addition of chemicals for denitrification or chemical phosphorus removal.

In addition to testing water quality at the beginning and end of each cycle, the nutrient content of the water and optical density of the samples were taken each day for one complete cycle, cycle 9, in order to observe daily nutrient removal and growth. During cycle 9 only, a second low biomass concentration PBR was built in the lab. The low biomass concentration

PBR was built and operated identically to the original PBR; the initial concentration of the PBR was the only difference between the two PBRs. Previous observations had shown cell death and poor nutrient removal in the original reactor when the concentration became too high in the reactor; therefore, the second PBR was created to compare nutrient removal and algae growth in a PBR with a high concentration of algal biomass at the beginning of the cycle to one with a lower concentration of algal biomass. It was hypothesized that faster nutrient removal and growth kinetics would be seen in the low biomass concentration PBR than in the original PBR. The low biomass PBR was inoculated using 250 mL of algae from the original *C. vulgaris* reactor and was diluted with 500 mL of wastewater effluent from the CBR. Daily nutrient content and approximate TSS (approximated through an optical density measurement, see Figure 2.5) in the PBRs are shown in Figure 2.6.

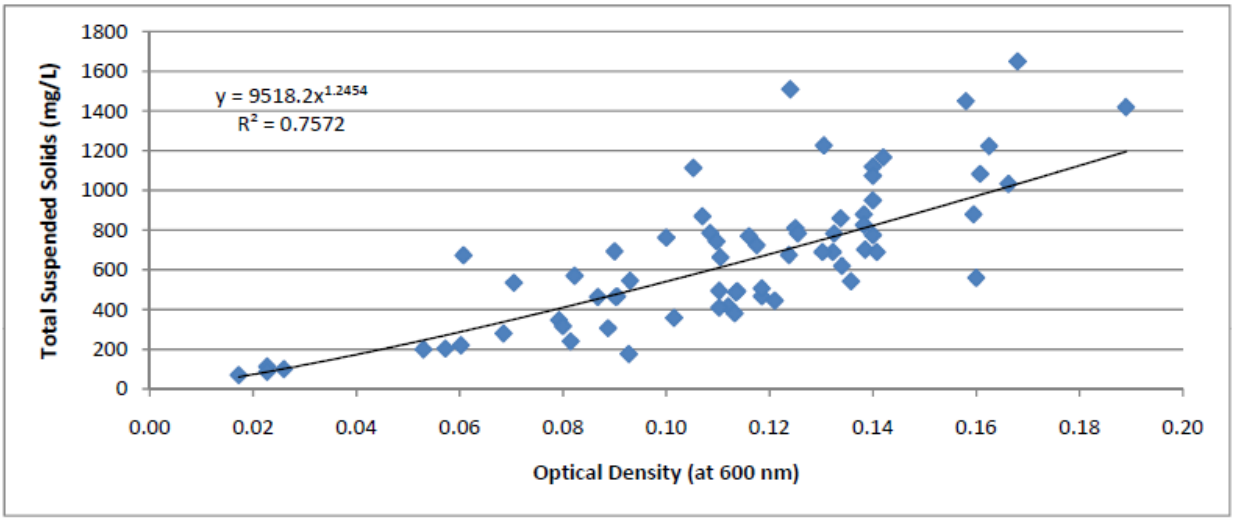
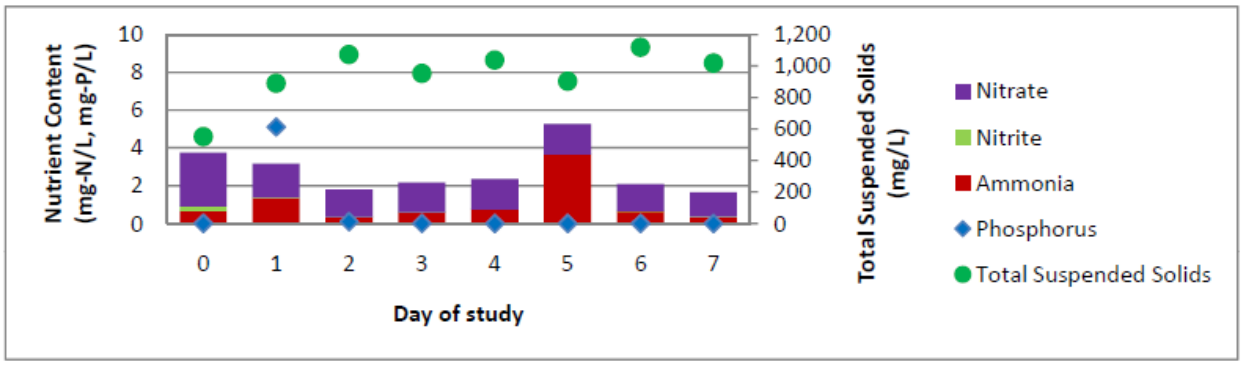
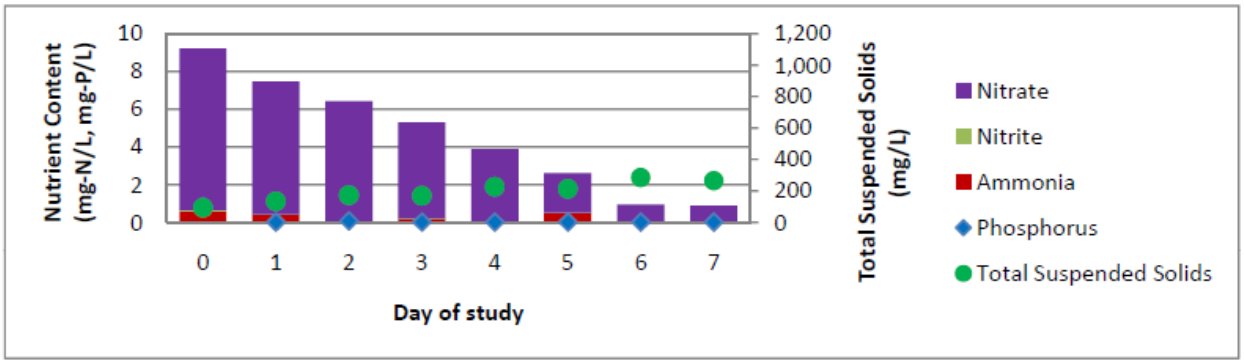


Figure 2.5. Relationship between optical density and total suspended solids (TSS) in the PBR. A correlation between TSS and OD in the system was found from measurements taken during the study period and also for other measurements taken from the PBR before the study period began so that OD could be used as a non-destructive test to estimate the TSS in the reactor.



a) Original, high biomass concentration PBR



b) New, low biomass concentration PBR

Figure 2.6. Daily nutrient content and TSS in algae cultivation cycle.

TSS and nutrient content in a) high biomass concentration PBR and b) low biomass concentration PBR.

In both PBRs, soluble orthophosphate was removed within two days of the start of the cycle, while the concentration of nitrogen species continued to change over the entire cycle. Nutrient removal and solids growth in the high concentration PBR varied over the cycle with periods of increase in nutrient concentration and loss of TSS, while the low concentration PBR showed a gradual removal of nutrients with an associated increase in TSS over the cycle. Both

reactors showed instances where phosphorus or ammonia content increased during the cycle. While the cause is unknown, the increases may be associated with cell death and subsequent release of stored nutrients back into the water (Fong, Foin et al. 1994; McMillan, Piehler et al. 2010).

Both PBRs showed an overall reduction in nutrient content and increase in TSS and associated biomass. The high biomass concentration PBR removed 2.2 mg-N/L, a 56% reduction, and produced 470 mg/L of additional algae, while the low biomass concentration PBR removed 8.3 mg-N/L, a 90% reduction, and produced 170 mg/L of additional algae. These results show that there may be a trade-off between nutrient removal and biomass production in a high biomass concentration vs. low biomass concentration reactor.

2.3.2 Value Added Products

Algal biomass is made up of lipids and carbohydrates, which are intermediate products capable of forming microalgal biodiesel and bioethanol, respectively. The composition and growth rate of the algae biomass coming from the PBR were analyzed to determine the coupled system's capability of producing intermediate products which have the potential to become value-added products. The growth rate of the algae was determined by testing the TSS in the PBR at the beginning and end of each cycle.

2.3.2.1 Lipid Content. The lipid content is based on the percentage of lipids per TSS (%Lipid/TSS). The final lipid content for each cycle ranged from 14 to 38%Lipid/TSS, while the average over all cycles was 24±3%Lipid/TSS. These values are greater than values reported by Woertz et al. (4.9-11.3% by weight) for algae grown on municipal wastewater in an outdoor

growth tank (Woertz, Feffer et al. 2009), but are below the average of 28-32% lipids by weight for *Chlorella* species (Chisti 2007).

The lipid content of all algal biomass cells measured is shown in Figure 2.7. The minimum, maximum, average, and median lipid content of the algae cells were recorded at the end of each cycle (Table 2.7). There was no statistical significance that the lipid content for any cycle was different than the average; therefore, the average lipid content was assumed to be consistent throughout all PBR cycles.

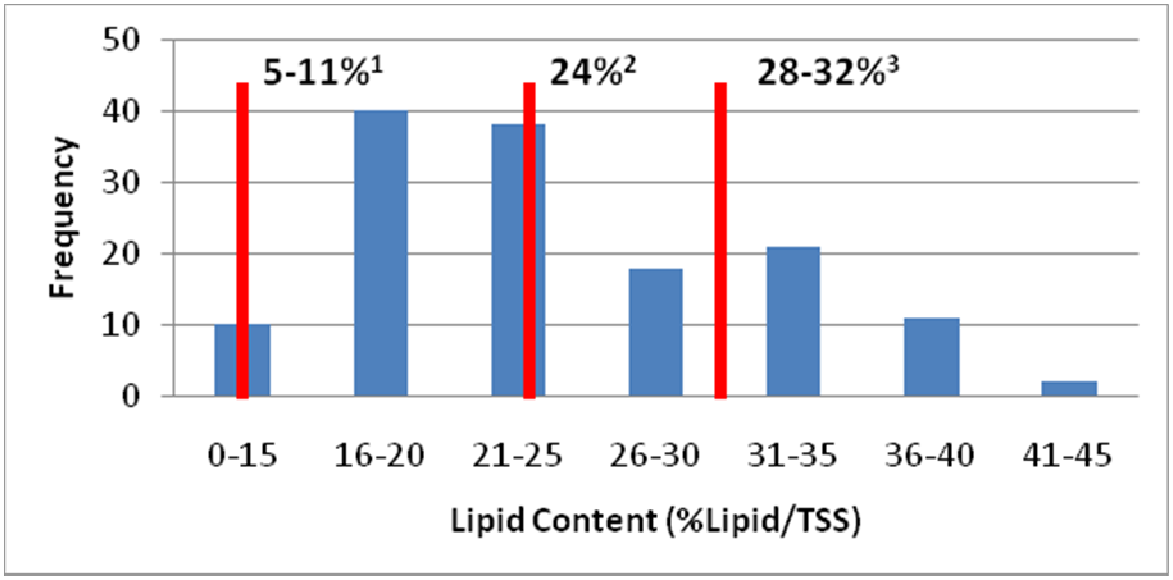


Figure 2.7. Lipid content in algae cells.

Frequency distribution of lipid content in algae cells, define by percent lipid per total suspended solids (%Lipid/TSS) of analyzed algae cells. 1) Average, algae grown on municipal wastewater (Woertz, Feffer et al. 2009), 2) Average, this study, 3) Average, C. vulgaris (Chisti 2007).

Table 2.7. Lipid content in algae cells

Cycle	Minimum (%L/TSS)	Maximum (%L/TSS)	Average (%L/TSS)	Median (%L/TSS)
1	15	40	31	32
2	13	43	26	25
3	17	40	25	25
4	13	36	20	17
5	13	33	22	20
6	17	33	26	25
7	14	43	21	17
8	13	33	21	20
9	--	--	--	--
10	--	--	--	--
Average	14	38	24	23
St. Dev.	2	4	3	5

2.3.2.2 Carbohydrate Content. Carbohydrate content in the algal biomass was tested in triplicate at the end of each cycle. The average carbohydrate content for each cycle is shown in Table 2.8. and Figure 2.8. Average carbohydrate content of algal biomass in the PBR.. It was assumed that the average carbohydrate content was the same over all 10 cycles and was equal to the overall average carbohydrate content.

Table 2.8. Average carbohydrate content of algal biomass in the PBR.

Cycle	Carbohydrates (%CH/TSS)
1	31
2	38
3	22
4	29
5	25
6	20
7	26
8	--
9	16
10	--
Average	26
St. Dev.	7

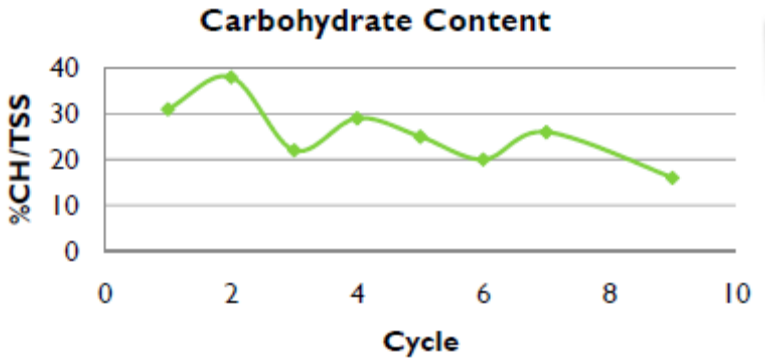


Figure 2.8. Average carbohydrate content of algal biomass in the PBR.

The carbohydrate content is based on the percentage of carbohydrates per TSS (%CH/TSS). The final carbohydrate content for each cycle ranged from 16 to 38%CH/TSS, while the average over all cycles was 26±7%CH/TSS. The average carbohydrate content was higher in this study than the average of 12-17% for chlorella species reported by Becker (Becker 1994).

2.3.2.3 Biomass Growth. Biomass growth was measured using the total suspended solids (TSS) of the samples to represent the algal biomass in the reactors. TSS was measured in duplicate at the beginning and end of each cycle. The optical density (OD) of the samples was measured at the beginning and end of each cycle in duplicate. Initial and Final TSS and Initial and Final OD are shown in Table 2.9.

Table 2.9. Initial and Final total suspended solids (TSS) and optical density (OD) measurements of algal biomass in the PBR.

Cycle	Initial TSS (mg/L)	Final TSS (mg/L)	Initial OD (abs)	Final OD (abs)
1	200.0	466.7	0.053	0.091
2	240.0	786.2	0.082	0.109
3	545.0	770.0	0.093	0.116
4	463.7	1120.0	0.087	0.140
5	492.9	1073.9	0.114	0.140
6	724.4	762.5	0.118	0.100
7	693.3	702.5	0.090	0.139
8	810.7	783.3	0.125	0.133
9	466.7	690.0	0.119	0.132
10	743.3	542.9	0.110	0.136

2.3.2.4 Useful Product Potential of Coupled System. The average lipid and carbohydrate content of the cells was used to calculate an average expected energy yield from the system. The energy content of lipids and carbohydrates was assumed to be 38.3 MJ/kg_{oil} and 13 $MJ/kg_{carbohydrate}$, respectively (Lardon, Hélias et al. 2009). To avoid making assumptions about downstream extraction and conversion processes, the energy content was reported as the potential energy contained in the biomass at the end of the harvesting cycle. This amount of energy could be obtained from burning the biomass directly or through 100% extraction and conversion processes downstream.

For the reactor with a diameter of 0.075 m and height of 0.15 m:

- The reactor volume was calculated as: $V = 6.63x10^{-4}$ m^3.
- The footprint area of the reactor is: $A = 4.42x10^{-3}$ m^2.
- The surface area of the reactor, including the top and sides of the reactor, is: $SA = 3.53x10^{-2}$ m^2.

The potential productivity of the reactor is shown in Table 2.10.

Table 2.10. Algae productivity based on area and volume of the PBR.

Biomass productivity	Algae growth	$A = 4.42x10^{-3}$ m^2	$SA = 3.53x10^{-2}$ m^2	$V = 6.63x10^{-4}$ m^3
Average	0.033 g/day	7.46 $g/m^2/day$	0.935 $g/m^2/day$	49.77 $g/m^3/day$
Maximum	0.138 g/day	31.22 $g/m^2/day$	3.91 $g/m^2/day$	208.1 $g/m^3/day$

The growth rate of algae in the PBR ranged from -47 mg/day to 138 mg/day, with an average growth rate of 33 mg/day in the PBR (0.05 $kg/m^3/day$ average, 0.2 $kg/m^3/day$

maximum). Negative growth rates occurred during cycles in which the TSS concentration was higher at the beginning of the cycle than at the end of the cycle. The cause of this reduction in concentration is unknown, but we hypothesize that it is a result of cell decay in the PBR due to high concentration or it is a function of a bench-scale system.

In each 7-day cycle, 56 mg microalgal oil is produced in the PBR. To produce 1,000 MJ (one functional unit) of microalgal diesel energy, 4.7×10^5 PBR cycles would be needed. To produce 1 gallon of microalgal diesel, 6.0×10^4 cycles would be needed. For each 1,000 MJ of microalgal diesel produced from microalgal oil, 370 MJ of energy from carbohydrates in the algal biomass is also available.

Assuming an energy content of 38.3 MJ/kg_{oil} and 100% efficiency in conversion and extraction, 26 $kg_{microalgal\ diesel}$ can produce 1,000 MJ of microalgal diesel. Additionally, a density of 870 $kg_{microalgal\ diesel}/m^3$ (Sander and Murthy 2010) is assumed to calculate that 7.9 gallons of microalgal diesel can be created from each 1,000 MJ of microalgal diesel. The additional energy available from the carbohydrate content of the algae was calculated by assuming 26% carbohydrate content in the algae with an energy content of 13 $MJ/kg_{carbohydrate}$. This energy is considered to be a by-product in the study; however, it was not considered to replace corn-based ethanol in the current life cycle study.

2.3.3 Life Cycle Assessment of Algae Cultivation and Harvesting in a Coupled System

2.3.3.1 Interpretation and Improvement Analysis. The life cycle impact assessment revealed that the energy use, global warming potential (GWP), and eutrophication potential (EP) of algae cultivation could be reduced by coupling a microalgal PBR with a conventional WWTP. These reductions are a result of avoiding further nutrient removal in a conventional wastewater treatment plant and avoiding fertilizer production for nutrient feed in the PBR (See Figure 2.3). Although environmental impact reductions were seen, these reductions were minimal when compared to the entire cultivation and harvesting stages (Figure 2.9). The avoidance of further wastewater treatment accounted for less than 0.2% in all impact categories, and the avoidance of fertilizer production accounted for less than 2% in all impact categories.

The use of waste CO_2 from other industrial processes would be more effective for reducing global warming and eutrophication impacts of algae cultivation and harvesting. However, the direct energy use (DEU), energy being consumed during the cultivation and harvesting phases of the life cycle, was higher when waste CO_2 was used due to the additional energy used for collection and injection of the waste CO_2 into the PBR. GWP, EP, and DEU could all be reduced by using a less intensive harvesting method such as filtration instead of centrifugation. The low-density polyethylene (LDPE) used to construct the PBR contributed a great amount to EP. Although LDPE has a lower EP than other materials including glass and high-density polyethylene, the vast amount of material needed for PBR construction increases the EP. PBRs must be engineered to have a higher productivity per amount of material in order to reduce EP. Separation of the algae from the wastewater results in the highest DEU in the

system. The DEU can be reduced by other methods of separation, such as filtering by 26% as shown in

Figure 2.9, however harvesting the algae still accounts for 54 to 97% of the energy use in the systems (W-CO_2, Filt.; I-CO_2, Cent., respectively), regardless of separation method. Other separation techniques, such as settling, auto-flocculation, and micro-screening (Amin 2009) might provide additional DEU savings. Although solids in the water would have to be effectively reduced in order for the water to be reused after cultivation and harvesting, the energy requirements of harvesting algae are not specific to algae cultivated with wastewater.

Because no full-scale coupled systems currently exist, various assumptions related to a hypothetical full-scale system along with the scale-up of laboratory data cause some error to exist in the study. The assumptions made in this study are reasonable based on the review of similar studies; however, completely accurate life cycle impacts of a full scale system can only be assessed after a full-scale system is built.

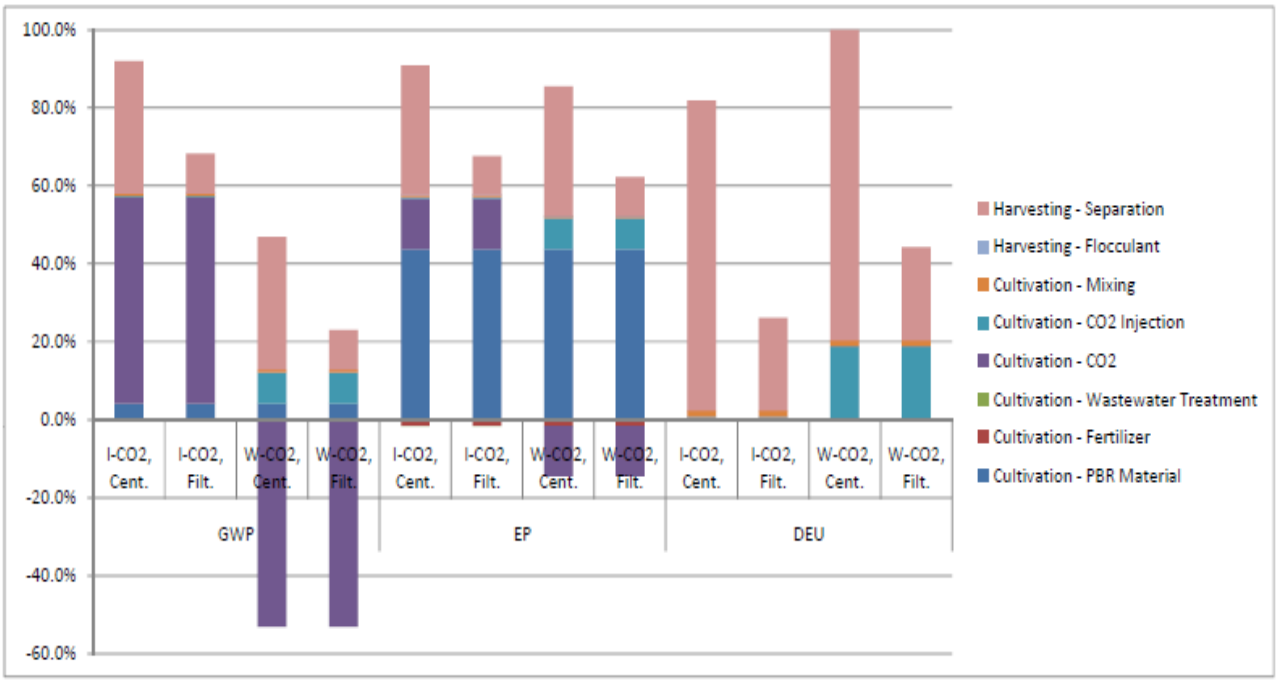


Figure 2.9. Environmental Impacts from the coupled wastewater-algae production system. Percentage of cultivation and harvesting phases contributing to Global Warming Potential (GWP), Eutrophication Potential (EP), and Direct Energy Use (DEU) in a scaled-up coupled system; Impacts from industrial CO_2 (I-CO_2), waste CO_2 (W-CO_2), Centrifugation (Cent.), and Filtration (Filt.).

2.3.3.2 Comparison to Other Studies. Few life cycle assessments of proposed large scale systems for algae cultivation have been completed, and results from these studies vary due to inconsistent system boundaries and assumptions. The results from these studies are shown in Table 2.11, and have been extrapolated to represent the same functional unit, i.e. 1,000 MJ of energy without accounting for differences in system boundaries, therefore these studies should be consulted directly for further information. The calculations and assumptions used to compare various LCA studies are shown in Table 2.12.

Table 2.11. Life cycle impacts during algae cultivation and harvesting from various studies. Direct energy use (DEU), global warming potential (GWP), and eutrophication potential (EP) are compared from various studies. Studies are compared based on a functional unit of 1000 MJ of microalgal diesel. System boundaries and assumptions used in each study may differ; consult individual studies for more information.

Cultivation method	**Open Pond**		**PBR**	
Nutrient Source	**Fertilizer**	**Wastewater**	**Fertilizer**	**Wastewater**
Direct Energy Use (MJ)/1000 MJ	8.93×10^{-1} (b) – 9.46×10^{2} (a)	7.57×10 (a) – 5.76×10^{3} (c)	5.48 (b)	1.3×10^{4} (d) – 5.0×10^{4} (d)
GWP (kg CO_2-eq)/1000 MJ	5.36×10 (a) – 3.23×10^{2} (b)	3.47×10 (a) – 3.98×10^{2} (c)	3.23×10^{2} (b)	-2.4×10^{3} (d) – 7.4×10^{3} (d)
Eutrophication Potential (kg N-eq)/1000 MJ	2.48×10^{-2} (a)	2.33×10^{-2} (a) – 2.40×10^{-2} (a)	--	3.6×10^{-1} (d) – 6.7×10^{-1} (d)

a. (Clarens, Resurreccion et al. 2010)
b. (Stephenson, Kazamia et al. 2010)
c. (Sander and Murthy 2010)
d. This study

Table 2.12. Conversions to compare other studies.
Assumptions and calculations made to compare LCA studies (Cu – Cultivation, H – Harvesting, OP – Open Pond, Fl – Flocculation, Ce – Centrifugation, FP – Filter Press, Fe – Fertilizer, BNR – Nutrients from WW treated with Biological Nutrient Removal, CAS – Nutrients from WW treated with Conventional Activated Sludge, DB – End result dry biomass, AI – avoided impacts from WW treatment) Eutrophication impacts reported in Clarens et al. (Clarens, Resurreccion et al. 2010) (as kg PO_4-eq) were multiplied by a factor of 2.38 to convert to kg N-eq based on characterization factors from TRACI (Bare 2002).

Study	Parameters	Functional Unit	Assumptions	Conversion Factor	Direct Energy Use (MJ)	GWP (kg CO_2-eq)	Eutrophication Potential (kg PO_4)
Clarens et al. [12]	Cu (OP), H (Fl, Ce), Fe, DB	317 GJ	24 GJ/Mg	1000 MJ/317,000 MJ (0.003)	$30x10^4$	$1.8x10^4$	3.3
Stephenson et al. [13]	Cu (OP), H (Fl, Ce), Fe	1170 kg lipids	38.3 MJ/kg lipids	1000 MJ/ (1170kg_{lipid}*38.3 MJ/kg_{lipid}) (0.022)	(30+10)	(1900+500)	--
Stephenson et al. [13]	Cu (PBR), H (Fl, Ce), Fe	1100 kg lipids	38.3 MJ/kg lipids	1000 MJ/ (1100kg_{lipid}*38.3 MJ/kg_{lipid}) (0.024)	231	13,600	--
Clarens et al. [12]	Cu (OP), H (Fl, Ce), DB, BNR, AI	317 GJ	24 GJ/Mg	1000 MJ/317,000 MJ (0.003)	$29x10^4$	$1.7x10^4$	3.2
Clarens et al. [12]	Cu (OP), H (Fl, Ce), DB, CAS, AI	317 GJ	24 GJ/Mg	1000 MJ/317,000 MJ (0.003)	$2.4x10^4$	$1.1x10^4$	3.1
Sander and Murthy [2]	Cu (OP), H (FP), DB, CAS	1000 MJ	1000 MJ/24 kg (42 GJ/Mg)	1000 MJ/1000 MJ (1.0)	(15.43+2915.27)	241.87	--
Sander and Murthy [2]	Cu (OP), H (Ce), DB, CAS	1000 MJ	1000 MJ/24 kg (42 GJ/Mg)	1000 MJ/1000 MJ (1.0)	(15.43+5743.32)	398.48	--

Environmental impacts from this study are greater than those reported in other studies. Harvesting impacts from this study account for the majority of the increase in energy use, while the use of industrial CO_2 increases the GWP and the PBRs themselves account for the higher EP. The GWP is negative when considering the use of waste CO_2 to feed the reactors in the cultivation process, but the CO_2 would be released into the atmosphere during combustion of microalgal diesel. The resulting life-cycle GWP would be positive over the entire life cycle, even when waste CO_2 is used.

Of the three studies evaluated in this work (shown in Table 2.11), Stephenson et al. was the only one to evaluate the energy consumption for microalgae cultivation and extraction in a PBR using synthetic fertilizers and found that most of the energy consumption results from the cultivation stage, while little comes from harvesting (Stephenson, Kazamia et al. 2010). This study shows the energy use for cultivation only in a PBR is 1100 MJ/functional unit using wastewater for nutrients, while Stephenson et al. attributes about 5 MJ/functional unit to cultivation using fertilizers as a nutrient source in a PBR. The large difference in these findings is caused by variations in assumed PBR designs. Large scale PBR implementation scenarios must be implemented in order to improve the accuracy of these findings.

3.0 CONCLUSIONS AND FUTURE WORK

3.1 CONCLUSIONS

Other studies have shown that the use of wastewater to supply nutrients for algae cultivation reduces GWP and EP in the cultivation stage when compared to using synthetic fertilizers (Clarens, Resurreccion et al. 2010; Soratana and Landis 2011). From a life cycle perspective, this study shows that GWP and EP may be reduced through a coupled system, but the cultivation and harvesting of algae for biofuel production may be limited due to the high energy requirements associated with harvesting the treated water by centrifugation. Harvesting techniques are well developed; but they often result in the highest energy use and environmental impacts in the process (Oswald and Golueke 1960; Bich, Yaziz et al. 1999; Sander and Murthy 2010). The impacts of replacing other advanced nutrient removal technologies with algae cultivation for nutrient removal may reveal a greater energy savings.

This study shows that nutrients can be removed from wastewater while simultaneously producing carbohydrates and lipids for biofuels in an algal PBR. In general, the nutrient concentration in the system decreases while the concentration of algal biomass increases throughout the cycle; however, there was no statistical significance between nutrient removal, algae growth, or lipid and carbohydrate content in the system. On average, the system is capable of producing algal biomass with lipid contents less than theoretical yields for *C. vulgaris* but

greater than biomass grown in other studies using wastewater to supply nutrients to the algae. The system results in a net-negative energy balance, in large part due to the high energy requirements for the harvesting stage. A harvesting method which uses less energy and effectively separates solids from water must be developed for a coupled system which creates useful products while treating wastewater to be feasible.

3.2 FUTURE WORK

Future work is needed in this project area to determine the life cycle impacts under different operating systems and scenarios as well as the impacts of an expanded system paradigm which would include environmental offsets for avoided impacts due to beneficial product production. A system expansion LCA needs to be performed to compare the life cycle impacts of value-added products from this system to the conventional products such as other forms of diesel.

The impacts from carbon dioxide recycling, oxygen exchange and methane gas capture need to be assessed to determine the potential environmental impacts of a large scale water treatment and resource recovery system. Variations in reactor design and the use of other microalgal species including algae cultivated from the wastewater sludge should be evaluated to determine the potential productivity of the system as well as the life cycle impact of various reactor designs. Other precursors to value-added products need to be quantified in a laboratory setting, and testing of coupled wastewater and algae cultivation systems must continue under additional bench scale and pilot scale systems. If results are promising, full-scale systems must be implemented so that the process can be assessed at a large scale.

To reduce the impacts of biofuel production from algae, Sander and Murthy has suggested that the removal of water is not necessary for further conversion of products from algae (Sander and Murthy 2010); however, the avoided impacts of wastewater treatment could not be taken into account in this scenario. The impacts of water removal from algae during the harvesting phase could be compared with impacts from advanced nutrient removal processes to determine the best strategy for overall energy and environmental impact reduction. Other life cycle environmental impacts such as those coming from variations in wastewater treatment design, variability in nutrient removal and biomass production, system shutdowns, and variations in algae harvesting must be taken into account in future studies.

APPENDIX A

LABORATORY DATA

Table A 1. TSS vs. OD

		PBR1		*PBR2*	
Cycle	Sample	OD	TSS (mg/L)	OD	TSS (mg/L)
1	Initial	0.160	880.0	0.138	880.0
	Final	0.168	1650.0	0.124	1510.0
2	Initial	0.161	1083.3	0.107	870.0
	Final	0.139		0.105	1113.3
3	Initial	0.131	1226.7	0.082	570.0
	Final	0.189	1420.0	0.126	783.3
4	Initial	0.160	560.0	0.089	306.7
	Final	0.163		0.111	663.3
5	Initial	0.158	1450.0	0.079	346.7
	Final	0.026	100.0	0.069	280.0
6	Initial	0.017	70.0	0.057	203.3
	Final	0.023	113.3	0.090	463.3
7	Initial	0.023	86.7	0.080	316.7
	Final	0.060	220.0	0.140	950.0
8	Initial	0.053	200.0	0.134	860.0
	Final	0.091	466.7	0.163	1223.6
9	Initial	0.082	240.0	--	552.0
	Final	0.109	786.2	0.142	1166.7
10	Initial	0.093	545.0	0.124	675.0
	Final	0.116	770.0	0.130	690.0
11	Initial	0.087	463.7	0.119	506.7
	Final	0.140	1120.0	0.141	690.0
12	Initial	0.114	492.9	0.114	486.7

	Final	0.140	1073.9	0.134	620.0
13	Initial	0.118	724.4	0.110	495.0
	Final	0.100	762.5	0.071	535.0
14	Initial	0.090	693.3	0.061	673.8
	Final	0.139	702.5	0.121	445.0
15	Initial	0.125	810.7	0.112	416.7
	Final	0.133	783.3	0.113	381.9
16	Initial	0.119	466.7	0.102	358.3
	Final	0.132	690.0	0.166	1033.3
17	Initial	0.110	743.3	0.140	775.0
	Final	0.136	542.9	0.138	826.7

Table A 2. Lipid Content of *C. Vulgaris*

Date	Sample	% Lipid
2/23/2011	1	31%
	2	17%
	3	25%
	4	25%
	5	33%
	6	20%
	7	25%
	8	25%
	9	20%
	10	17%
	11	25%
	12	33%
	13	22%
	14	40%
	15	22%
	16	25%
3/2/2011	1	27%
	2	30%
	3	35%
	4	15%
	5	35%
	6	38%
	7	29%
	8	29%

	9	25%
	10	22%
	11	33%
	12	40%
	13	31%
	14	36%
	15	33%
	16	36%
	17	24%
	18	30%
	19	33%
	20	33%
3/9/2011	1	20%
	2	38%
	3	33%
	4	22%
	5	29%
	6	20%
	7	25%
	8	29%
	9	17%
	10	20%
	11	25%
	12	13%
	13	20%
	14	25%
	15	17%
	16	40%
	17	43%
	18	40%
	19	29%
	20	22%
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3/23/2011	1	23%
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3/30/2011	1	13%
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4/6/2011	1	22%
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	15	29%
4/13/2011	1	27%
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4/20/2011	1	20%
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	6	33%
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